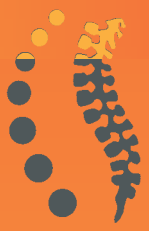
1ST asia-pacific bone and mineral research meeting

AND THE

AUSTRALIAN & NEW ZEALAND BONE & MINERAL SOCIETY

22ND ANNUAL SCIENTIFIC MEETING

PERTH 2012



ANZBMS

PLATINUM SPONSORS

AMGEN® gsk GlaxoSmithKline MSD

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. Department of Health and Ageing. Schedule of Pharmaceutical Benefits available www.pbs.gov.au/medicine/item/5457F. Accessed 1st August 2012.

AMGEN® gsk GlaxoSmithKline Supported by Amgen in collaboration with GSK

PLEASE REVIEW PRODUCT INFORMATION BEFORE PRESCRIBING.
Product Information is available from the trade display.

PBS Information: Authority required (STREAMLINED) as treatment for postmenopausal osteoporosis. Refer to PBS Schedule for full 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 EFFECT**: Hypocalcaemia, skin infections (predominantly cellulitis), pancreatitis, rarely jaw osteonecrosis. **DOSAGE AND ADMINISTRATION**: Single subcutaneous injection of 60mg, once every 6 months. ensure adequate intake of calcium and vitamin D. No dosage adjustment required in the elderly or in renal impairment. **PRESENTATION**: Pre-filled syringe with automatic needle guard. PBS price $298.79. Product Information is available at http://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.

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 Melbourne VIC • www.gsk.com.au
DMB-AUS-AMG-466-2012 Approved 18th July 2012 • AM5141/7/12

SPONSOR ACKNOWLEDGEMENTS

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

PLATINUM SPONSORS

AMGEN

gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

MSD

SILVER SPONSORS

sanofi aventis
Because health matters

WC WARNER CHILCOTT

SERVIER

WELCOME RECEPTION SPONSOR

Lilly
Answers That Matter.

DELEGATE NAME BADGE SPONSOR

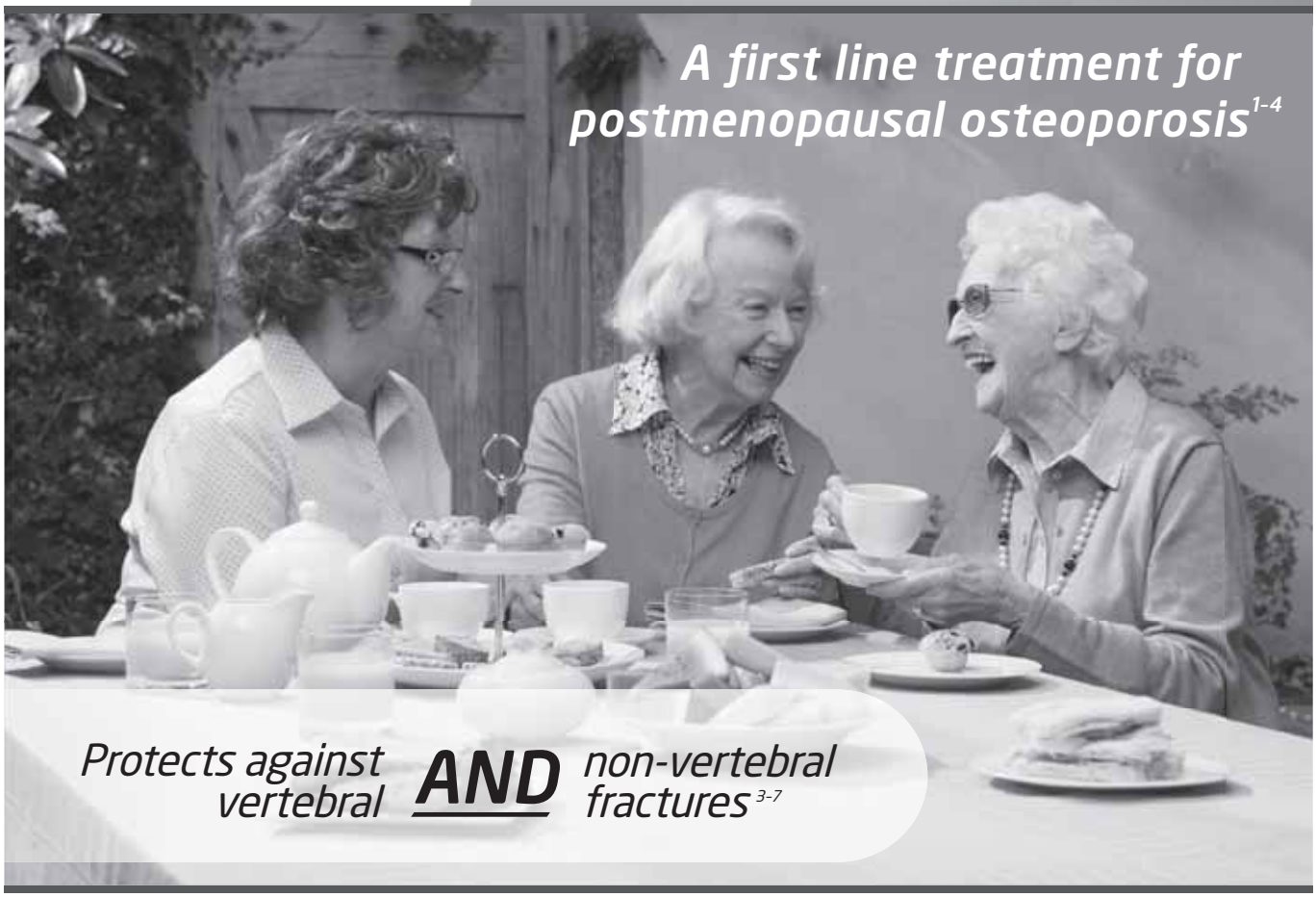
mesoblast
the regenerative medicine company

EXHIBITORS

Australian Institute of Health and Welfare
Hologic Australia Pty Ltd
Immuno
Medtel
Osteoporosis Australia
Thomson Scientific Instruments

PROTOS®

strontium ranelate



PBS listed for both primary# and secondary fracture prevention[8]

#age ≥70, T-Score ≤-3.0

PROTOS® Minimum Product Information. Please review Product Information before prescribing. To request a copy of the Product Information please telephone 1800 153 590.

Indications: Treatment of postmenopausal osteoporosis to reduce the risk of fracture. *Treatment of 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). **Precautions:** *Men with osteoporosis should be investigated for secondary osteoporosis eg. hypogonadism and treated for that condition if necessary. 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. 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. *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. **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, 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:** 4 May 2012. Servier Laboratories (Australia) Pty. Ltd. 8 Cato Street Hawthorn, VIC.

*Please note changes in Product Information.

1. Prevent the next fracture. Health Professional Guide. Osteoporosis Australia 3rd edition, 2010. 2. Protos Approved Product Information. 3. Meunier et al. N Eng J Med 2004; 350:459-468. 4. Meunier P et al. Osteoporos Int 2009; 20(10):1663-1673. 5. Reginster et al. J Clin Endocrinol Metabolism 2005; 90:2816-2822. 6. Reginster et al. Arthritis & Rheumatism 2008; 58:1687-1695. 7. Reginster JY, Kaufman JM, Goemaere S et al. Long-term treatment of postmenopausal osteoporotic women with strontium ranelate: Results at 10 years. Osteoporos Int 2010; 21(Suppl.5):S663-S674. 8. www.pbs.gov.au (accessed August 2012)

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

contents

ACKNOWLEDGEMENTS
Sponsors and Exhibitors 1

INTRODUCTION
ANZBMS Council Members 4
ANZBMS Program Committee 4
ANZBMS Local Organising Committee 4
ANZBMS Award Winners 5
Welcome 6
International Faculty Members 6

Keynote Speaker Profiles 7

PROGRAM INFORMATION
Speaker Support Centre 8
Posters 8

GENERAL INFORMATION
Venue 8
Registration Desk 8
Social Events 8

EXHIBITION
Exhibition Opening Hours 9
List of Exhibitors/Booth Numbers 9
Exhibition Floorplan 9

PRE-CONFERENCE WORKSHOPS
Advanced Skeletal Tissue and Cell Imaging 10
Clinical Update for GP's and HP's 11

SCIENTIFIC PROGRAM
Timetable 12
Plenary Poster Listing 18
Poster Listing 20
Invited and Oral Presentations 28
Plenary Poster Abstracts 68
Poster Abstracts 79

Author Index 132

INTRODUCTION

ANZBMS Council Members 2012

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 – 2011*

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

ANZBMS PROGRAM ORGANISING COMMITTEE

Ming-Hao Zheng – Chair
Emma Duncan
Paul Baldock
Charles Inderjeeth
Allison Pettit
Mark Forwood
Graeme Jones
Gustavo Duque
Nathan Pavlos
Dorit Naot
Rebecca Mason

LOCAL ORGANISING COMMITTEE

Charles Inderjeeth – Chair
Kathy Briffa
Paul Glendenning
Peter Pivonka
Roger Price
Jiake Xu
Ming-Hao Zheng

ANZBMS Secretariat

Ivone Johnson – Secretariat
Melissa Dupavillon
Tel: 02 9256 5405
Email: anzbms@racp.edu.au
Web: www.anzbms.org.au

ANZBMS Meeting Manager

Lara Birchby
The Meeting People Pty Ltd
PO Box 882, Unley South Australia 5061
Tel: 08 8272 7005 Fax: 08 8272 7006
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*

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*

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*

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*

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*

SOL POSEN RESEARCH AWARD

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

ANZBMS President's Welcome

The Australian and New Zealand Bone and Mineral Society is delighted to host this inaugural Asia-Pacific Bone and Mineral Research Meeting, which aims to highlight our achievements and provide opportunities for establishing collaborations in our global region.

I welcome each of you, and hope that you actively participate in the clinical / scientific program and provide your on-going support for this initiative, such that this becomes a focal point meeting for the Asia – Pacific region in years to come.

Professor Matthew Gillespie
President ANZBMS

Welcome

The Australian and New Zealand Bone and Mineral Society welcome you to the inaugural Asia-Pacific Bone and Mineral Research Meeting to be held in conjunction with the 22nd ANZBMS Annual Scientific Meeting at the Pan Pacific Hotel, Perth, Australia. The 2012 scientific program will host a wealth of local and internationally acclaimed experts in the field of bone and mineral research with a central theme of evidence-based translational skeletal medicine.
The key objectives of the meeting are to:
1) Better align with the National Health Form Agenda and translation into clinics as best practise for clinics and to provide clinicians with an update on metabolic bone diseases;
2) Foster research collaboration and cultural awareness in bone and mineral research across the Asia-Pacific region;
3) Elevate the international profiles and facilitate networking of Early Career Researchers.

Professor Ming Hao Zheng, PhD, DM, FRCPath
Chair of Program Organising Committee

International Faculty Members

Ho-Yeon Chung (Korea)
Alan Grodzinsky (USA)
James Hui (Singapore)
Zang-Hee Lee (Korea)
Lan T Ho-Pham (Vietnam)
PC Leung (Hong Kong)
Qin Ling (Hong Kong)
Toshio Matsumoto (Japan)
Chatlert Pongchaiyakul (Thailand)
Jiang Qin (Nanjing)
Nikhil Tandon (India)
Toshi Yoneda (Japan)

Keynote Speaker Profiles

Professor Nancy E. Lane, Director of UC Davis Centre for Healthy Aging, USA, is an internationally recognized leader in the clinical management of osteoporosis and osteoarthritis. Professor Lane is an Associate Editor for *Seminars in Rheumatic Diseases*, *Rheumatology and, Arthritis Research and Therapy*; an editorial board member for *Journal of Bone and Mineral Research*, *Journal of Rheumatology* and Consultant Editor for *Annals of Internal Medicine* and the *Journal of Bone and Joint Surgery*. Professor Lane has had a distinguished career, publishing more than 200 articles in relation to epidemiology, genetics and the treatment of musculoskeletal diseases.

Professor Shiro Ikegawa, Head of Laboratory for Bone and Joint Diseases, SNP Research Center, RIKEN, Tokyo, Japan, is a leading international authority in the field of molecular genetics of osteoarthritis and osteoporosis with a distinguished record of scientific achievement. Professor Ikegawa holds multiple publications in top-tier journals including *Nature* and *Science,* has published more than 160 publications since 1990, and was recently named among the list of global experts and leaders in scientific and clinical practice by the “Faculty of 1000 Medicine”.

Professor Stavros C. Manolagas is Professor of Medicine, the Thomas E. Andreoli, MD, MACP, Clinical Scholar Chair in Internal Medicine, Director of the Division of Endocrinology and Metabolism and the UAMS/VA Center for Osteoporosis and Metabolic Bone Diseases, Vice Chair for Research, Department of Internal Medicine, University of Arkansas for Medical Sciences, Chief of the Endocrinology Section, Central Arkansas Veteran’s Healthcare System. Professor Manolagas has published more than 200 original articles. He is a prominent member of several professional societies, editorial boards, peer review panels and numerous national and international scientific committees. In 2000, he was recognized by the American Society of Bone and Mineral Research for his outstanding contributions to basic research in bone and mineral metabolism with the Inaugural Louis V. Avioli Founders Award.

Professor Hiroshi Takayanagi is Professor of Tokyo Medical and Dental University and a leading expert in the fields of bone biology and immunology. In 2000, Professor Takayanagi contributed a seminal paper to *Nature* on T-cell-mediated regulation of osteoclastogenesis, which pioneered the field of Osteoimmunology. More recently, his work has focused on elucidating the mechanisms of bone destruction in autoimmune arthritis and mapping the intracellular signalling of bone cells. In particular, he has contributed tremendously our understanding of the molecular regulation of osteoclast differentiation through the discovery of the master transcription factor NFATc1 and ITAM-associated co-stimulatory receptors. With over 15 publications in *Nature Medicine*, Professor Takayanagi has firmly established the field of Osteoimmunology and identified several promising therapeutic targets for the treatment of bone and immune diseases.

PROGRAM INFORMATION

Speaker Support Centre
A fully equipped and staffed Speaker Support Centre will be located in the Boardroom on the conference floor of the Pan Pacific Perth. 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, Mr Mark Stevens in the room or by telephoning on 0417 809 647.

Posters
Plenary posters will be up for the duration of the meeting.
Attended authors discussions for **all posters** will take place on both **Monday 3rd September from 12:40 – 13:20 and Tuesday 4th September from 12:30 – 13:10**. Note that odd numbered posters will be presented on Monday and even-numbered ones Tuesday.
The odd posters will be on display for Monday 3rd of September and can be put up from 5.00 pm on Sunday 2nd of September but must be removed by 5 pm on Monday 3rd of September.
The even posters will be on display for Tuesday 4th of September and can be put up from 5.00pm on the 3rd of September but must be removed by the end of morning tea on Wednesday 5th 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 Golden Ballrooms at the Pan Pacific Hotel on the conference level.

207 Adelaide Terrace, Perth WA 6000

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

Sunday 2 September	14:00 - 19:00
Monday 3 September	07:00 - 17:30
Tuesday 4 September	07:00 - 18:00
Wednesday 5 September	07:30 - 14:00

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.

Car Parking
The nearest car park is located off Hill Street, Perth.

CONFERENCE EVENTS
Wednesday 8 September
Women in Science
07:30 – 08:30
Hamersley Room, Pan Pacific Perth
An additional networking event held prior to the sessions on Wednesday morning prior to the first session.

SOCIAL EVENTS
Sunday 2 September
Welcome Reception - 17:30 – 19:00
Sponsored by Eli Lilly
Exhibition, Grand River Ballroom, Pan Pacific
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 $65 per person may be purchased from the registration desk.

Monday 3 September
Fun Run – 17:30-18:30
Swan River, Perth
Run or walk a 5 km circuit along the Swan River, starting on the river directly below the Pan Pacific Hotel. The loop track runs towards McCallum Park, Victoria Park before returning back to the starting point.

Monday 3 September
Young Scientists & Students Networking Function 18:30 – 22:30
Offsite – The Generous Squire, Shafto Lane, 397 Murray Street, Perth
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.

Conference Dinner – 19:00 for 19:30 – 23:30
Coaches depart from the Pan Pacific at 18:45
Frasers Restaurant, Kings Park, Perth
Dinner will be held in Fraser's Restaurant and will include a three-course dinner, drinks and entertainment provided by the band *The Filth.* This function is included in the full registration fee. Extra tickets $130 per person available from the registration desk until lunchtime on Monday. Admission is by ticket only. Dress code: Smart casual

ANZBMS 2012 EXHIBITION

The Trade Exhibition will be located in the Grand River Ballroom of the conference level of the Pan Pacific Perth. 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	1700 – 1900 (Welcome Reception and Exhibition Opening)
Monday 3 September 2012	0800 – 1700
Tuesday 4 September 2012	0800 – 1700
Wednesday 5 September 2012	0800 – 1400

Exhibitors (see floor plan for location of booths)

Booth No	***Company***
A	Amgen
B	Servier Laboratories
C	Merck Sharp & Dohme
1	Thomson Scientific Instruments
2	Osteoporosis Australia
3	Eli Lilly Australia
5	Hologic Inc.
8	Immuno
9	Australian Institute of Health and Welfare
10	Medtel
11	sanofi-aventis/Warner Chilcott

Pre-Conference Workshops
Sunday 2nd September 2012

There are three workshops held concurrently at different venues on Sunday 2nd September.

ANZBMS Asia-Pacific Advanced Skeletal Tissue and Cell Imaging Course

Venue: FJ Clark Lecture Theatres Complex, QEII Medical Centre Campus, Nedlands (Entrance from Monash Avenue). **Run in conjunction with, and in co-location with the ANZBMS Clinical Bone Densitometry Course.**
Time: 08:30-16:30 with morning tea and lunch.
Convenor: Roger Price
Chair: Damian Myers

PROGRAM OUTLINE

08:15-08:30	**REGISTRATION & COFFEE**
08:30-08:35	Workshop opening by Roger Price (Convenor) and Introduction of Workshop Chair Damian Myers
08:35-10:15	**Keynote Lectures - Chairs: Damian Myers & Roger Price**
08:35-09:00	Quantitative imaging of skeletal tissues & structures: challenges at the frontier - Damian Myers (Melbourne)
09:00-09:25	Recent advances in skeletal tissue imaging from macro to nano - Qin Ling (Hong Kong)
09:25-09:50	Interpretation of histomorphometry data in preclinical drug development - David Ke (USA)
09:50-10:15	3D-imaging of human vertebrae with micro-CT: towards whole organ histomorphometry - Egon Perilli (Adelaide)
10:15 - 10:45	**MORNING TEA**
10:45 – 12:45	**Frontier Presentations, Chair: Damian Myers**
10:45 - 11:00	Advances on microCT; what is next? - Phil Salmon (Belgium) *(to be confirmed)*
11:00 - 11:15	Assessing subchondral bone in mouse models of arthritis by microCT – Nicole Walsh (Melbourne)
11:15 - 11:45	Advanced techniques *in vivo* & *ex vivo*: Xtreme CT and Fourier transform infrared spectroscopy - Roger Zebaze, Ali Ghasem Zadeh & Yohann Bala (Melbourne & Lyon)
11:45 – 12:00	QCT of the hip to determine biomechanical fracture prediction outcomes - J.Keenan Brown (USA)
12:00 - 12:15	QCT of the hip; from laboratory to the clinic - Richard Prince & Ben Khoo(Perth)
12:15 - 12:40	Confocal & OCT imaging of living skeletal tissues & cells - Paul Rigby & Nathan Pavlos (Perth)
12:40 -12:45	Final questions & general acknowledgments - Roger Price & Damian Myers
12:45-13:35	**LUNCH, served in Foyer of FJ Clarke Complex**
13:35-15:30	Wet-Lab Group 1 UWA Orthopaedic Research Laboratories. Techniques & technology; resin embedding of skeletal tissue; light-microscopic histomorphometry
13:30-15:30	Wet-Lab Group 2 UWA UWA Orthopaedic Research Laboratories & CMCA. Confocal imaging of living cells, confocal arthroscopy, confocal microscopy & OTC
13:30-15:30	Wet-Lab Group 3 FJ Clarke Complex. pQCT in paediatrics: techniques & clinical applications

Pre-Conference Workshops
Sunday 2nd September 2012

ANZBMS Clinical Update for GP's & HP's
Venue: Golden Ballroom, Pan Pacific Hotel, Perth

08:00	Welcome course overview & WA Osteoporosis MOC *A/Prof Kathy Briffa and Prof Charles Inderjeeth*
08:40	Bone turnover, how to assess it and why understanding it is important for best management of osteoporosis *Prof Samuel Vaskiaran*
09:15	Identifying patients at risk and determining who should be treated *Prof Markus Seibel*
09:50	Assessment of osteoporosis prior to and during treatment *A/Prof Nicholas Pocock*
10:25	Morning tea & displays
10:55	The calcium and vitamin D conundrum *Dr Ee Mun Lim*
11:30	Pharmacological management of osteoporosis *Prof Graeme Jones*
12:05	Aging, falls and fractures *Prof Keith Hill*
12:40	Lunch and displays
13:45	Grand rounds of clinical management *Dr Paul Glendanning, Prof Gustavo Duque, A/Prof Emma Duncan and Dr Ee Mun Lim*
15:15	Close *A/Prof Kathy Briffa and Prof Charles Inderjeeth*

Sunday 2nd September 2012

14:00		**Registration opens**	*Pan Pacific Foyer*
		Joint symposium with the Australia and New Zealand Orthopaedic Research Society ***Chairs: Graeme Jones and David Smith***	*Golden Ballroom*
16:00-17:20		**Cartilage and bone basic science**	
16:00-16:25		Signalling through BMPR2 controls bone mass in the adult skeleton- Vicki Rosen (USA)	
16:25-16:50		Genetics of Bone and Joint Shape – Nancy E Lane (USA)	
16:50-17:10		Bone remodelling of OA - David Findlay (Australia)	
17:10-17:20		Concluding remarks	
17:30		**Welcome and Opening ceremony – Matthew Gillespie /Minghao Zheng /Charles Inderjeeth**	
17:30-19:00		**Welcome Reception – *Sponsored by Eli Lilly***	*Grand River Ballroom*
		Exhibition Opening and Plenary Poster viewing	

Monday 3rd September 2012

07:00		**Registration opens**	*Pan Pacific Foyer*
07:00-08:20		**CONCURRENT MEET THE PROFESSOR BREAKFAST SESSIONS**	
		Breakfast Session 1: Bone cell communications Hiroshi Takayanagi, *Chair: Natalie Sims*	*Goldsworthy Room*
		Breakfast Session 2: Cochrane review of skeletal medicine Rachelle Buchbinder, *Chair: Peter Ebeling*	*Hamersley Room*
08:30 – 10:14		**Session 1: Age quake**	*Golden Ballroom*
		Chairs: Rebecca Mason and Peter Ebeling	
08:30	**IS1**	What old means to bone – Stavros Manolagas (USA)	
09:10	**IS2**	Impact of osteoporosis in aged people – Lyn March (Australia)	
09:30	**IS3**	The common links of osteoporosis and dementia – Charles Inderjeeth (Australia)	
09:50	**OR1**	**Outstanding Abstract Award (Clinical)** The transitory PTH increase following denosumab administration is associated with reduced intracortical porosity: a distinctive characteristic of denosumab therapy - Ego Seeman (Australia)	
10:02 – 10:30		**Refreshment Break and Exhibition**	*Grand River Ballroom*
10:30- 12:14		**Session 2: Translational skeletal medicine**	*Golden Ballroom*
		Chairs: Richard Prince and David Little	
10:30	**IS4**	Skeletal dysplasia in mice and human – Shiro Ikegawa (Japan)	
11:00	**IS5**	Autologous human mesenchymal stem cells for cartilage repaire: from bench to bedside – James Hui (Singapore)	
11:25	**IS6**	Anti-bone resorptive effects of a Vitamin E analogue, Trolox - Zang-Hee Lee (Korea)	
11:50	**OR2**	Contribution of myogenic and vascular cell lineages to fracture repair – Aaron Schindeler (Australia)	
12:02	**OR3**	Low bone mass in Down Syndrome humans and mice – Larry Suva (USA)	
12:14 - 12:40		**Lunch, poster sessions and Trade Display**	*Grand River Ballroom*
12:40 - 13:20		**Poster sessions (odd numbers)**	
13:20 - 14:20		**Industry symposium – MSD**	*Golden Ballroom*

Monday 3rd September 2012 continued...

14:20 – 15:40		**Session 3: Evidence-based care in musculoskeletal disorders**	
		Chairs: Gustavo Duque and Ho-Yeon Chung	*Golden Ballroom*
14:20	**IS7**	Dose and duration of action of zoledronate: an examination of the evidence – Mark Bolland (New Zealand)	
14:40	**IS8**	Inflammatory Bone Loss: mechanism and treatment – Nancy Lane (USA)	
15:10	**IS9**	Vertebroplasty – Rachelle Buchbinder (Australia)	
15:40 – 16:00		**Refreshment Break and exhibition**	*Grand River Ballroom*
16:00 – 17:10		**CONCURRENT SESSIONS**	
16:00 – 17:10		**Session 4A: Early career researchers (basic science) and oral presentation**	*Golden Ballroom North*
		Chairs: Allison Pettit and Nathan Pavlos	
16:00	**IS10**	Osteoprotection by semaphorin 3A – T Nakashima (Japan)	
16:20	**IS11**	The role of V-ATPase in bone homeostasis – T Cheng (Australia)	
16:40	**IS12**	Auto-amplification of IL-6 and RANKL signalling fuels metastatic growth in bone – Yu Zheng (Australia)	
17:00	**OR4**	EphrinB2 signaling in osteoblasts and chondrocytes is required for their differentiation and support of osteoclast formation - Stephen Tonna (Australia)	
16:00 – 17:10		**Session 4B: Early career researchers (translational and clinical science)**	
		Chairs: Lyn March and Ling Qin	*Golden Ballroom Central*
16:00	**IS13**	Beyond GWAS: gene mapping in the era of next-generation sequencing – Emma Duncan (Australia)	
16:20	**IS14**	The skeletal effects of tyrosine kinase inhibitors – Susannah O'Sullivan (New Zealand)	
16:40	**OR5**	Improving the definition of osteoporosis: cortical porosity identifies women with distal forearm fractures **-** Yohann Bala (Australia)	
16:52	**OR6**	Suggestive loci for osteoporosis: a multipoint variance component linkage analysis of extended pedigrees - Sing Nguyen (Australia)	
17:30-18:30		**Swan River Walk/Run**	*Starting point: Swan River below Pan Pacific*
18:30		**Early Career Researchers and Student's Dinner**	*The Generous Squire*
19:00		**President's Dinner (Invite only)**	*UWA Club*

Tuesday 4th September 2012

07:00		**Registration opens**	*Pan Pacific Foyer*
07:00-08:20		**CONCURRENT MEET THE PROFESSOR BREAKFAST SESSIONS**	
		Breakfast Session 3: Estrogen and bone Stavros Manolagas, *Chair: Richard Prince*	*Hamersley Room*
		Breakfast Session 4: Industry collaboration in translational skeletal medicine David Ke, *Chair: David Little*	*Goldsworthy Room*
08:30 – 10:30		**Session 5: Molecular targets of bone cells**	*Golden Ballroom*
		Chairs: Markus Seibel and Toshio Matsumato	
08:30	**IS15**	Osteoclast-derived osteoblast anabolic factors – Hiroshi Takayanagi (Japan)	
09:10	**IS16**	Choreography from the tomb: the role of osteocytes in bone remodelling – Stavros Manolagas (USA)	
09:40	**IS17**	Targeting sclerostin for anabolic therapy of bone disorders – David Ke (USA)	
10:10	**IS18**	Osteoblast suppression in multiple myeloma: opportunities for therapeutic intervention – Peter Croucher (Australia)	
10:30 – 10:50		**Refreshment Break and Trade visit**	*Grand River Ballroom*
10:50- 11:50		**CONCURRENT SESSIONS**	
10:50- 11:50		**Session 6A: Bone and cartilage (ANZORS Joint Session)**	*Golden Ballroom North*
		Chairs: Tania Crotti and Evange Romas	
10:50	**IS19**	The subchondral bone of osteoarthritis – David Hunter (Australia)	
11:10	**IS20**	Osteoporotic agents for osteoarthritis – Graeme Jones (Australia)	
11:30	**IS21**	Overloading verses underloading – two sides of the coin for the cause of OA – David Lloyd (Australia)	
10:50- 11:50		**Session 6B: Stem cells**	*Golden Ballroom Central*
		Chairs: Michelle McDonald and Liza Raggatt	
10:50	**IS22**	The biology and clinical application of skeletal stem cells – Stan Gronthos (Australia)	
11:10	**IS23**	The application of allogenic mesenchymal progenitor cells (MPC) in the reconstitution of bone and cartilage – Peter Ghosh (Australia)	
11:30	**IS24**	Circulating mesenchymal stem cells in fracture healing – Gang LI (Hong Kong)	
11:50- 12:30		**Lunch, poster sessions and Trade Display**	*Grand River Ballroom*
12:30 – 13:10		**Posters (even numbers)**	
13:10 – 14:10		**INDUSTRY SYMPOSIUM – AMGEN** **Imaging insights: Cortical porosity and anti-fracture efficacy of antiresorptives in postmenopausal osteoporosis** ***Speakers: Prof Charles Inderjeeth, Prof Ego Seeman and Prof John Eisman***	*Golden Ballroom*
14:10 – 15:46		**Session 7: Young investigator award session**	*Golden Ballroom*
		Chairs: Jill Cornish and Mark Forwood	
14:10	**OR7**	The cathepsinK inhibitor KK1-300-01 prevents bone destruction and resumes bone formation in myeloma osteolytic lesions - Keiichiro Watanabe (Japan)	
14:22	**OR8**	Small GTPase Cdc42 is essential for chondrocyte differentiation and interdigital programmed cell death during limb development – Ryo Aizawa (Japan)	
14:34	**OR9**	Direct effect of sclerostin on the mechanical loading response in bovine bone – Kamarul Khalid (Australia)	
14:46	**OR10**	Profiling SNX proteins in bone identifies sorting nexin 27 (SNX27) as a crucial modulator of skeletal homeostasis – Audrey Chan (Australia)	

Tuesday 4th September 2012 continued...

14:58	**OR11**	Hypertension and fracture risk in postmenopausal women - Shuman Yang (Australia)	
15:10	**OR12**	Rapid loss of cortical bone occurs in paretic limbs within six months of stroke – Karen Borschmann (Australia)	
15:22	**OR13**	EphrinB2/EphB4 signalling is required for anabolic actions of parathyroid hormone – Farzin Takyar (Australia)	
15:34	**OR14**	Depletion of macrophages significantly impairs endochondral fracture healing - Andy Wu (Australia)	
15:46 – 16:10		**Refreshment Break and Trade visit**	
16:10 – 17:00		**Session 8: Philip Sambrook Lecture**	*Golden Ballroom*
		Chairs: Matthew Gillespie and Rebecca Mason	
16:05	**IS25**	The Philip Sambrook Lecture - Translating clinical bone research into positive health outcomes - Peter Ebeling (Australia)	
17:00		**ANZBMS AGM**	*Golden Ballroom*
18:30		**International Faculty Meeting**	*Hamersley Room*
19:00		**Conference Dinner**	*Fraser's Kings Park*
(Coaches depart from outside the Pan Pacific at 18:45)			

Wednesday 5th September 2012

07:30		**Women in Science**	*Hamersley Room*
		Chair: Kathy Briffa	
		Professor Lyn Beazley- Chief Scientist of Western Australia State	
08:30 – 09:15		**Session 9: Greg Mundy Lecture**	*Golden Ballroom North*
		Chairs: Toshi Yoneda and TJ Martin	
08:30	**IS26**	Greg Mundy, a personal perspective – Toshi Yoneda (Japan)	
08:35	**IS27**	Sex steroids and cortical versus trabecular bone accrual and maintenance: a tale of two cities – Stavros Manolagas (USA)	
09:15 – 10:15		**CONCURRENT SESSIONS**	
		Session 10A: Genetic susceptibility of skeletal diseases	*Golden Ballroom North*
		Chairs: Emma Duncan and Rob Will	
09:15	**IS28**	High resolution genome analyses for studies of genetic regulation of bone mass and structure – Scott Wilson (Australia)	
09:35	**IS29**	Susceptibility of osteoarthritis – Jiang Qin (China)	
09:55	**IS30**	Peripheral arterial disease is a risk factor for hip fracture in older men – Paul Norman (Australia)	
		Session 10B: Translational oral presentation	*Golden Ballroom Central*
		Chairs: Robin Daly and Nicole Walsh	
09:15	**OR15**	Seminal Vesicle Secretion (SVS) 7 is expressed by mature osteoclasts and acts to regulate osteoclast precursor proliferation, differentiation and bone homeostasis - William Cundawan (Australia)	
09:27	**OR16**	Sclerostin stimulation of osteocytic osteolysis involves expression of carbonic anydrase II - Masakazu Kogawa (Australia)	
09:39	**OR17**	Central control of bone resorption: Neuropeptide Y, Y6 receptor signalling in the regulation of bone homeostasis - Ee-Cheng Khor (Australia)	
09:51	**OR18**	The relationship between cumulative lifetime UV exposure, BMD and vertebral fracture in older adults – Michael Thompson (Australia)	
10:03	**OR19**	Evidence for a specific uptake and storage mechanism for 25 hydroxyvitamin D (25OHD) in skeletal muscle cells - Myriam Abboud (Australia)	
10:15 – 10:35		**Refreshment Break and Trade visit**	*Grand River Ballroom*
10:35- 12:35		**CONCURRENT SESSIONS**	
10:35-12:35		**Session 11A: Clinical science oral presentations**	*Golden Ballroom North*
		Chairs: John Walsh and Julie Pasco	
10:35	**OR20**	Treatment with the cathepsin K Inhibitor odanacatib in postmenopausal women with low bmd: 5 year results of a phase 2 trial – John Eisman (Australia)	
10:47	**OR21**	6 years of treatment with denosumab in postmenopausal women with osteoporosis: Results from the first 3 years of the FREEDOM open-label extension study – Cae Tolman (Australia)	
11:59	**OR22**	Effects of a specialized school physical education program on bone structure and strength in primary school children: A 4-year cluster randomised controlled trial - Robin Daly (Australia)	
11:11	**OR23**	Australian fracture prediction models do not improve hip fracture prediction compared to model using hip DXA aBMD T score values alone – Satvinder Dhaliwal (Australia)	
11:23	**OR24**	Greater reduction of intracortical porosity seen with denosumab than alendronate in the compact-appearing cortex and outer transitional zone – Roger Zebaze (Australia)	
11:35	**OR25**	The effects of pioglitazone, a PPAR-γ agonist, on bone turnover markers, bone mineral density, and vertebral fractures in type 2 diabetes mellitus – Ippei Kanazawa (Japan)	

Wednesday 5th September 2012 continued...

11:47	**OR26**	Microarchitectural decay and microdamage accumulation in vertebral cancellous bone: a comparative analysis of the iliac crest, proximal femur and vertebral body in the aged postmenopausal skeleton – Julia Kuliwaba (Australia)
11:59	**OR27**	Contribution of re-fracture to early fracture-associated mortality - Dana Bliuc (Australia)
12:11	**OR28**	Strontium ranelate reduces the number of patients with radiological or radioclinical progression in primary knee osteoarthritis – Lyn March (Australia)
12:23	**OR29**	Bone density before and after heart or lung transplantation – a longitudinal study – Susannah O'Sullivan (New Zealand)

10:35-12:35 **Session 11B: Basic science oral presentations** *Golden Ballroom Central*

Chairs: Hong Zhou and Gerald Atkins

10:35	**OR30**	Notch/Rbpj/Hes1 signal in chondrocytes modulates osteoarthritis development - Shurei Sugita (Japan)
10:47	**OR31**	**Outstanding Abstract Award (Basic)** Osteoblastic lineage deletion of gp130 has divergent effects on trabecular and cortical bone – Rachelle Johnson (Australia)
11:59	**OR32**	Anabolic actions of fasting-induced adipose factor (FIAF) on bone cells – Jian-ming Li (New Zealand)
11:11	**OR33**	Quantitative analysis of the Osteoclastome - Dylan Del Frate (Australia)
11:23	**OR34**	Generation and analysis of the mature osteoblast/osteocyte deletion of VDR: evidence for the direct activity of vitamin D activity in osteoblasts to regulate bone turnover - Paul Anderson (Australia)
11:35	**OR35**	Wnt5a-Ror2 signalling boosts bone destruction in arthritis – Yasuhiro Kobayashi (Japan)
11:47	**OR36**	The potential role of vascular endothelial growth factor (VEGF) in the faulty bony repair of injured growth plate - Rosa Chung (Australia)
11:59	**OR37**	The endoplasmic reticulum stress transducer BBF2H7 suppresses apoptosis by activating the ATF5-MCL1 pathway in chondrogenesis - Soutarou Izumi (Japan)
12:11	**OR38**	RelB attenuates the activation of the classical NF-kB pathway to facilitate osteoblastogenesis - Masahiro Hiasa (Japan)
12:23	**OR39**	AR replacement specifically in mineralising osteoblasts of androgen receptor knockout mice partially restores trabecular bone but does not restore bone size - Rachel Davey (Australia)

12:35-14:00 **Lunch and conclusion** *Grand River Ballroom*

PLENARY POSTERS – BASIC SCIENCE

P1
The role of RANK in breast and prostate cancer growth in murine models of bone metastasis
Zheng Y, Chow S, Kim S, Kelly J, Dunstan CR, Sutherland RL, Zhou H and Seibel MJ

P2
Sphingomyelin phosphodiesterase 3 negatively regulates expression of chondrogenic marker genes and hyaluronan synthase 2 in ATDC5 chondrocytes
Kakoi H, Maeda S, Kawamura I, Imamura K, Yokouchi M, Ishidou Y and Komiya S

P3
***In situ* imaging of the autonomous intracellular Ca^{2+} oscillations of osteoblasts and osteocytes in bone**
Ishihara Y, Sugawara Y, Kamioka H, Kawanabe N, Naruse K and Yamashiro T

P4
Blockade of PTH/PTHrP signaling inhibits invasive capacity of genetically engineered mouse osteosarcoma in vitro and *in vivo*
Ho PWM, Russell M, Goradia A, Kocovski P, Martin TJ and Walkley CR

P5
Thymosin β_4 administration enhances the biomechanical properties of healing mouse fractures
Brady RD, McDonald SJ, Schuijers JA, Ward AR and Grills BL

P6
Activation mechanisms of mutant ALK2 found in fibrodysplasia ossificans progressiva by type II BMP receptor
Ohte S, Fujimoto M, Tsukamoto S, Miyamoto A, Sasanuma H, Shin M, Katsumi

P7
Changes in tibial bone and cartilage structure in a mouse surgical model of osteoarthritis
Tonkin BA, Romas E, Sims NA and Walsh NC

P8
Differential regulation of osteoclast precursor migration by Activin A and RANKL
Fowler TW, Kurten RC, Suva LJ and Gaddy D

P9
The transcription factor FoxC1 regulates chondrogenesis together with Gli2 through induction of PTHrP
Yoshida M, Hata K, Takashima R, Iseki S, Takano-Yamamoto T, Nishimura R and Yoneda T

P10
Completing the bone/brain circuit: Osteocalcin signals within the hypothalamus to inhibit bone formation
Lin S, Enriquez RE, Herzog H and Baldock PA

PLENARY POSTERS – CLINICAL SCIENCE

P11
Long term high dietary calcium intake and its association with fractures and cardiovascular events in a population based prospective cohort study
Khan BK, English D, Nowson C, Daly R and Ebeling PR

P12
Effect of treatment with denosumab on bone mineral density (BMD) in men with low BMD
Hall JW, Gruntmanis U, Orwoll E, Teglbjaerg CS, Langdahl BL, Chapurlat R, Czerwinski E, Kendler DL, Reginster J-Y, Kivitz A, Lewiecki EM, Miller PD, Bolognese MA, McClung MR, Bone HG, Ljunggren Ö, Abrahamsen B, Yang Y-C, Wagman RB, Siddhanti S, Grauer A and Boonen S

P13
High-dose oral vitamin D_3 administered once a year: Increased fracture risk is associated with 1,25-dihydroxyvitamin D level 3-months post dose
Sanders KM, Duque G, Ebeling PR, McCorquodale T, Shore-Lorenti C, Herrmann M and Nicholson GC

P14
Transformation of trabecular bone plates to trabecular rods accounts for the bone loss in inter-trochanteric cancellous bone of hip fracture patients
Thomas CDL, Parkinson IH, Zhou B, Wang J, Liu XS, Guo XE, Fazzalari NL and Clement JG

P15
Taller persons have thinner and more porous cortices to fall harder upon and fracture
Bjørnerem Å, Zebaze R, Ghasem-Zadeh A, Bui M, Wang XF, Hopper JL and Seeman E

P16
Individual derived quality of life changes over 12-months following fracture: The AusICUROS study
Sanders KM, Nicholson GC, Iuliano S, Seeman E, Prince R, Duque G, Winzenburg T, Cross M, March L, Ebeling PR and Borgstrom F

P17
Models of care for the secondary prevention of osteoporotic fractures: a systematic review and meta-analysis
Ganda K, Puech M, Chen JS, Speerin R, Bleasel J, Center JR, Eisman JA, March L and Seibel MJ

P18
Vertebral body strength: prediction using subregional areal bone mineral density from DXA compared to subregional bone microarchitecture from micro-CT
Perilli E, Briggs AM, Codrington JD, Kantor S, Parkinson IH, Reynolds KJ, Fazzalari NL and Wark JD

P19
Bisphosphonate-induced changes in bone mineral density depend on the degree of osteoporosis in older men and women
Zhang JTW and Seibel MJ

P20
The role of dairy intake on muscle health in older community-dwelling women
Radavelli-Bagatini S, Zhu K, Lewis JR, Dhaliwal SS and Prince RL

POSTER PRESENTATIONS

Gene and functions

P21
Analysis of high turnover type bone loss due to haploinsufficiency of Cnot3, a subunit of Ccr4-not complex(mRNA seadenylase)
Watanabe C, Ezura Y, Nakamoto T, Hayata T, Notomi T, Moriyama K and Noda M

P22
Cytotoxic therapies significantly alter the composition of the cells comprising murine hematopoietic stem cell niches
Quach J, Askmyr M, Jovic T, Baker EK, King H, White KE, Nombela-Arrieta C, Walsh N, Silberstein LE and Purton LE

P23
Extracellular calcium-induced phosphorylation of CREB in calcium-sensing receptor expressing HEK293 cells, human parathyroid cells and osteoblasts
Avlani VA, Ma W, Bracken AM, Mason RS, Delbridge L, Christopoulos A and Conigrave AD

P24
Histone deacetylase (HDAC) 1 as a target for suppressing both inflammation and bone loss in chronic inflammatory diseases
Cantley MD, Fairlie DP, Bartold PM and Haynes DR

P25
Homozygous deletion of Dickkopf-1 results in a high bone mass phenotype due to increased bon formation
McDonald MM, Morse A, Baldock PA , Peacock L , Aiken A, Tam PPL and Little DG

P26
Molecular basis of allosteric coupling in the calcium-sensing receptor: roles of conserved cysteine residues
Brown AP, Baddock HT, Goolam MA and Conigrave AD

P27
Monosodium urate crystals inhibit tenocyte viability and function: implications for periarticular involvement in chronic gout
Chhana A, Callon KE, Pool B, Naot D, Gamble G, Coleman B, McQueen FM, Cornish J and Dalbeth N

P28
Potential roles of metallothionein-I and II in protecting against acute methotrexate chemotherapy-induced damage to endochondral ossification
Fan CM, Garcia M, Scherer M, Tran C and Xian CJ

P29
The effects of long-chain saturated fatty acids on the differentiation of cells of the marrow stromal line Kusa4b10
Watson M, Costa JL, Musson D, Callon KE, Choi A, Lin JM, Cornish J, Naot D and Grey A

P30
The migration of host cells towards osteogenic differentiated donor mesenchymal stromal cells in ectopic and orthotopic osteogenesis
Zhou YH, Crawford R and Xiao Y

P31
The role of calcium-sensing receptor intracellular loops and C-tail in differential signalling
Goolam MA, Ward JH and Conigrave AD

P32
Transcriptional regulation of endochondral ossification by PLZf during longitudinal bone growth
Lin Z, Shee S, Qin A, Abel T, Thien C, Langdon W, Xu J and Zheng MH

P33
Transgenic disruption of glucocorticoid signalling in mature osteoblasts and osteocytes does not affect murine antigen-induced arthritis
Wiebe E, Spies CM, Tu J, Gaber T, Li A, Huscher D, Buttgereit F, Seibel MJ and Zhou H

P34
Wnt signalling inhibition as a potential therapeutic in ankylosing spondylitis
Haynes KH, Pettit AP, Duan R, Tseng H, Kniessel M, Glant TT, Brown MA and Thomas GP

Biomaterials

P35
An injectable scaffold for bone tissue engineering
Cheng TL, Valchev P, Dehghani F, Little DG and Schindeler A

Bone quality and mechanical properties

P36
Analysis of bone quality in smoking model rats by FTIR imaging and Raman spectroscopy
Kimura-Suda H, Ueno H, Yamato H, Kubo K, Tomoda K and Kimura H

P37
Anisotropy can be measured from clinical-level computed tomography images
Kersh ME, Wolfram U, Zysset P and Pandy MG

P38
Cortical bone loss and porosity predate menopause
Bjørnerem Å, Ghasem-Zadeh A, Zebaze R, Bui M, Wang XF, Hopper JL and Seeman E

P39
Differences in distal radius intracortical porosity in Chinese and Caucasian premenopausal women
Wang XF, Ghasem-Zadeh A, Wang Q, Teo J, Zebaze R and Seeman E

P40
Distal radial and tibal cortical and trabecular microarchitecture and BMI in premenopausal women
Teo JW, Wang X, Ghasem-Zadeh A, Wang Q and Seeman E

P41
Heterogeneity in femoral neck structure and its relationship to strength
Kersh ME, Zebaze R, Jones AC, Arns CH, Knackstedt MA, Pandy MG and Seeman E

P42
Musculoskeletal interactions in Neurofibromatosis type 1
Deo N, El-Hoss J, Sullivan K, Little D and Schindeler A

P43
Nutritional factors influence bone microarchitecture during growth
Shahmoradi N, Iuliano-Burns S, Wang XF, Ghasem-Zadeh A, Zebaze R, Wang Q and Seeman E

P44
Quantitative ultrasound estimates of volume fraction and structure of cancellous bone
Wille M-L, Flegg MB, and Langton CM

P45
The effect of silicate ions on proliferation, osteogenic differentiation and cell signalling pathways (Wnt and Shh) of bone marrow stromal cells
Pingping HAN, Chengtie WU and Yin XIAO

Cells and Tissues

P46
17β-Estradiol promotes extracellular calcification of adipose tissue-derived stem cells during osteogenesis
Wang JZ, Lewis JR, Liew JL, Tan J, Adams L and Prince RL

P47
β-adrenergic signaling directly stimulates osteoclastogenesis via reactive oxygen species
Kondo H and Togari A

P48
A novel role for glucocorticoid-mediated osteoblast-fibroblast crosstalk in inflammatory disease
Hülso C, Hardy RS, Liu Y, Tu J, Stoner S, Cooper MS, Seibel MJ and Zhou H

P49
Alg2, identified as a downstream mediator of Schnurri-3, inhibits function of *Runx2* and osteoblast differentiation
Imamura K, Maeda S, Kawamura I, Ishidou Y, Yokouchi M and Komiya S

P51
Annexin A8 is a prototypical substrate-induced gene involved in OC polarization and function and; is regulated by calcineurin-NFATc1 signalling
Crotti TN, Pavlos N, Zawawi M, OSullivan RP, Flannery MR, Goldring SR, Purdue PE and McHugh KP

P52
Bisphosphonates bound to bone inhibit growth of epithelial cells and primary rat osteoblastic cells
Bava U, Cornish J, Callon KE, Bai J, Naot D and Reid IR

P53
Butoxamine, a selective β2-adrenergic antagonist, prevents bone loss and fragility in spontaneously hypertensive rat
Arai M, Sato T Takeuchi S, Goto S and Togari A

P54
Characterisation of inflammatory murine fibroblast-like synoviocytes
Hardy RS, Hülso C, Liu Y, Tu J, Stoner S, Cooper MS, Seibel MJ and Zhou H

P55
Comparison of β-adrenergic and glucocorticoid signaling on clock gene and osteoblast-related gene expressions in human osteoblast and osteosarcoma cell
Komoto S, Kondo H, Fukuta O and Togari A

P56
Cord blood-derived macrophage-lineage cells rapidly stimulate osteoblastiv maturation in mesenchymal stem cells in a glycoprotein-130 dependent manner
Fernandes TJ, Hodge JM, Singh PS, Collier FM, Ebeling PR, Nicolson GC and Quinn JMW

P57
Cytotoxic mechanism involved with H_2O_2-induced oxidative stress in Human Bone Marrow Stem Cell (hBMSC)
Kim JH, Chung MH, Leem YH, Kim JW, Chung HY and Chang JS

P58
Determinants of adipose-derived stem cell biology and responsiveness to osteogenic stimulation
Lewis JR, Wang JZ, Adams L, Tan J, Hamdorf G and Prince RL

P59
Effects of bisphenol A on osteoclasts and osteoblasts
Chung HY, Min KH, Hwang YC, Jeong IK, Ahn KJ, Byun DW, Min YK, Park HM and Chang JS

P60
ENU-induced chemical mutagenesis reveals that choline kinase beta is an important regulator of osteoporosis
Kular J, Tickner J, Pavlos N, Viola HM, Abel T, Lim B, Hool LC, Zheng MH and Xu J

P61
Evidence for a role for serotonin in human osteoclast function
Hodge JM, Williams LJ, Collier FM, Berk M and Nicholson GC

P62
Experimental tooth movement-induced osteoclast activation is regulated by sympathetic signaling
Kondo M, Kondo H, Miyazawa K, Goto S and Togari A

P63
Expression of factors inducing osteoclast activity, receptor activator NFkappa B (RANKL), TNF-like weak Inducer of Apoptosis (TWEAK) and its receptor, Fn14, in early stages of cartilage damage in osteoarthritis
Dharmapatni AASSK, Smith MD, Chen DY, Holding CA, Atkins GJ, Findlay DM, Zheng TS, Upton AR and Haynes DR

P64
Generation of osteoclast circadian rhythm by glucocorticoid
Fujihara Y, Kondo H, Noguchi T and Togari A

P65
Glucocorticoid mediated elastin synthesis is essential for embryonic lung development and postnatal survival in mice
Li A, Hardy RS, Stoner S, Tuckermann J, Zhou H and Seibel MJ

P66
Inhibitor of dynamin rapidly disrupts actin rings of osteoclasts
Uehara S, Nakayama T, Mizoguchi T, Yamashita T, Kobayashi Y, Udagawa N and Takahashi N

P67
Involvement of down-regulating HGF synthesis in glucocorticoid-mediated growth inhibition of human osteoblasts
Togari A, Niwa Y, Tsunashima Y and Matsuda T

P68
RANKL induced elevated bone turnover a model of coupled or uncoupled remodeling?
Raggatt LJ, Wu A, Chang MK, Alexander KA, Walsh NC, Gravellese EM and Pettit AR

P69
RANKL-induced Myo1b localizes to sites of dynamic actin-membrane remodeling during osteoclast formation and function
Ng PY, Landao E, Coudrier E, Xu J, Knölker HJ, Zheng MH and Pavlos NJ

P70
Role of autophagy in palmitate-induced lipotoxicity in osteoblasts
Gunaratnam K, Thekkedam C, Boadle R and Duque G

P71
Roles of endogenous prostanoids and histamine in autocrine/paracrine control of PTH secretion from human parathyroid cells
Mun H-C, Ward DT, Delbridge L and Conigrave AD

P72
SIRT1, a class III histone deacetylase, regulates anti-inflammatory factors in human chondrocytes
Moon MH, Jeong JK, Lee YJ, Seol JW and Park SY

P73
Sphingosine-1-phosphate inhibits interleukin (IL)-12-induced inflammation in human articular chondrocytes
Moon MH, Jeong JK, Lee YJ, Seol JW, Xue M, Jackson CJ and Park SY

P74
The crosstalks between Wnt/2-catenin and ±5 integrin signalings in the articular superficial cell functions
Yasuhara R, Enomoto-Iwamoto M and Mishima K

P75
The effects of 1,25-dihydroxyvitamin D on *in vitro* mineral deposition depend on the stage of osteoblast maturation and extracellular calcium concentration
Yang D, Atkins GJ, Turner AG, Anderson PH and Morris HA

P76
The expression of osteoblast transcription factors RUNX2, osterix, TWIST1 and MSX2 in adult human bone and their putative post-developmental roles in osteocytes
Ormsby RT, Kumarasinghe DD and Atkins GJ

P77
The role of the cytoskeleton in osteoblast and osteoclast function: a study of RhoA inhibition
Musson DS, Lin J-M, Matthews BG, Watson M, Bai J-Z and Cornish J

P78
The S349T mutation of *SQSTM1* links Keap1/Nrf2 signalling to Paget's disease of bone
Rea SL, Wright T, Goode A, Bennett AJ, Ratajczak T, Long JE, Searle MS, Goldring C, Park K, Copple IM and Layfield R

P79
Development and optimization of *ex vivo* bioreactor system for tendon tissue engineering
Wang T, Zhen L, Landao E and Zheng MH

Clinical metabolic bone diseases

P80
Prevalence and risk factors for bone loss in patients treated with diabetes related foot complications
Tsimos S, Wraight PR, Kantor S and Wark JD

P81
Secondary hyperparathyroidism and reduced bone turnover during long-term treatment with Imatinib
O'Sullivan S, Horne A, Wattie D, Gamble G, Browett P and Grey A

P82
The correlation of serum levels of Wnt antagonists with inflammatory markers and the daily dose of prednisolone in patients with rheumatoid arthritis
Hashimoto J, Hirao M, Shi K, Ebina K, Kaneshiro S, Nampei A, Tsuboi H, Akita S, Ohshima S, Saeki Y and Yoshikawa H

P83
The link between nephrolithiasis, bone density and fractures in transfusion-dependent thalassaemia
Wong P, Fuller PJ, Gillespie MT, Kartsogiannis V, Strauss B, Bowden D and Milat F

Clinical osteoporosis

P84
Bone density changes following addback therapy with different GnRH agonist in endometriosis patients
Lim Y-T, Song J-Y and Park H-M

P85
Bone surface availability as a potential driver of endocortical bone loss in osteoporosis
Buenzli PR, Pivonka P, Thomas CDL and Clement JG

P86
Defining the relationship between fatness and fracture
Chan MY, Nguyen ND, Center JR, Eisman JA and Nguyen TV

P87
Intra and inter fracture risks correlation using GARVAN and FRAX tools in patients aged over 70 years old
Foo B and Inderjeeth CA

P88
The prevention and treatment of osteoporosis: who is interested?
Kim JW, Jeon YJ, Byun DW and Chang JS

P89
The prevalence of vitamin D deficiency in hospitalised elderly people and associated comorbidities
Kurusumuthu P

Cytokine, growth factor and hormone mechanisms of action

P91
EGFL7 is differentially expressed in osteoclasts and osteoblasts and mediates endothelial cell migration through the activation of extracellular signal-regulated kinase
Chim SM, Chow ST and Xu J

P92
Expression of angiogenic related cytokines in cartilage of osteoarthritis
Zhang X, Prasadam I, Crawford R and Xiao Y

P93
Expression of neurotrophic factors and receptors during growth plate cartilage bony repair in rats and skeletal cell formation *in vitro*
Su YW, Chung R, Zhou F, King T, Georgiou K, Foster BK, Zhou XF and Xian CJ

P94
Impact of allosteric modulators of the extracellular calcium-sensing receptor on calcitonin-secreting human thyroid C-cells
Mun H-C, Christopoulos A and Conigrave AD

P95
Inhibition of Wnt/β -catenin signalling promotes cartilage repair at injured growth plate in young rats
Wong DSK, Chung R, Macsai CE and Xian CJ

P96
Interleukin (IL)-11 is required for mechanical stress-induced bone formation
Kondo T, Omatsu T, Dong B, Ohnishi Y, Aizawa S-I, Endo I and Matsumoto T

P97
Interleukin-33 expression, regulation and actions in bone
Eeles DG, Singh P, Saleh H, Grills BL, Schuijers JA, McDonald SJ, Gillespie MT and Quinn JMW

P98
Limb- and sternum-specific inactivation of Dullard gene causes severe defects in skeletal development via alteration of TGF-ß signaling
Hayata T, Ezura Y and Noda M

P99
MicroRNAs targeting non-canonical Wnt/$Ca2^{+}$ pathway potentially mediate osteo-inhibitory effects of pro-inflammatory cytokines
Chakravorty N, Jaiprakash A, Crawford R, Oloyede A, Ivanovski S and Xiao Y

P100
Preconditioning with TNFα promotes osteogenic differentiation of adipose-derived mesenchymal stem cells by modulating the BMP-2 signalling pathway
Lu ZF, Wang GC, Dunstan CR and Zreiqat H

P101
Sclerostin mediates pro-osteoclastogenic effect through the LRP4 receptor on osteocytes
Wijenayaka AR, Kogawa M,Dharmapatni A, Haynes DR, Findlay DM and Atkins GJ

P102
Sex-specific action of calcitonin on osteocytic gene expression
Chia LY, Walsh NC, Gagel RF, Martin TJ and Sims NA

P103
SnoN mediates a crosstalk between TGF-β and BMP signaling to suppress hypertrophic maturation of chondrocytes
Kawamura I, Maeda S, Imamura K, Yokouchi M, Ishidou Y and Komiya S

P104
Synergistic regulation of BMP2 mediated osteogenesis of human cranial suture mesenchymal cells by glypicans: Functional and physical interaction of GPC1 and GPC3 with BMP2
Dwivedi PP, Grose RH, Hii CST, Filmus J, Anderson PJ and Powell BC

P105
Transgenic CYP27B1 expression within mature osteoblasts is anabolic
Turner AG, Hanrath M, Yang D, Anderson PH and Morris HA

Densitometry

P106
A new clinical approach to quantitative CT (QCT) bone densitometry with asynchronous calibration
Bodeen GR, Brown JK and Brett AD

P107
Analysis of four determinants of both QCT-derived and DXA-derived hip structural geometrical measurements (HSA) in elderly women
Khoo BCC, Brown K, Zhu K, Price RI and Prince RL

P108
Application of measuring subregional vertebral areal bone mineral density among individuals with glucocorticoid-induced osteoporosis
Manning L, Briggs AM, Kantor S, van Doornum S, Kale A and Wark JD

P109
Reasons for referral to DXA in men and women aged 20-49 years: Australian population-based data
Torpy AMJ, Brennan SL, Kotowicz MA and Pasco JA

P110
StrAx1.0: a non-threshold based image analysis
Ghasem-Zadeh A, Zebaze R and Seeman E

Epidemiology, public health and genetics

P111
Adult fracture and quality of life: a population-based study of Australian men
Dobbins AG, Brennan SL, Williams LJ and Pasco JA

P112
Bisphosphonate use and increased incidence of subtrochanteric fracture in South Korea: Results from the National Claim Registry
Lee Y-K, Ha Y-C, Byun DW, Park H-M, Min Y-K and Koo K-K

P113
Cardiovascular risk assessment in patients with osteoporotic fracture
Hong S, Kim SH, Nam MS, Kim YS and Moon KH

P114
Changes in General Quality of Life (QoL) after non-traditional fractures
Zhang Y, Sanders KM, Pasco JA, Lane SE and Kotowicz MA

P115
Comparison of the Australian FRAX and the Garvan hip fracture prediction models using a 10-year prospective study of elderly Australian women
Dhaliwal SS, Yu M, Josh L, Zhu K and Prince RL

P116
Consuming two additional serves of dairy food a day significantly improves protein, calcium and vitamin D intakes in ambulatory aged care residents: A feasibility study
Iuliano S, Woods J and Robbins J

P117
Explaining the sex difference in fracture risk: the role of muscle quality
Nguyen TV, Nguyen ND, Center JR and Eisman JA

P118
Depression and falls in men: the Geelong Osteoporosis Study
Stuart AL, Pasco JA, Berk M and Williams LJ

P119
GARVAN Fracture risk calculator is not an adequate substitute for need for BMD in people over 70 years of age.
Inderjeeth CA, Foo B, Van V, Chauhan A, Fisher T, Rogers J and Will R

P120
Improving outpatient osteoporosis management: performance and cost-efficacy of a 'fracture capture' service
Chauchard MA, Yates CJ, Liew D, Farrugia R, Bucknill A and Wark JD

P121
Low bone turnover in osteoporosis of the old old: a hypothesis testing pilot study
Chan K, Nair P, Lim EM, Petta A and Inderjeeth CA

P122
Morbidity and mortality in older than 50 years with hip fracture with average 6-year follow-up; A prospective cohort study
Ha YC, Lim YT, Lee YK, Chang JS, Min YK and Byun DW

P123
Normative data for total lean mass for Australian men and women: Geelong Osteoporosis Study
Gould H, Brennan SL, Kotowicz MA and Pasco JA

P124
Oral health and biochemical risk factors for bisphosphonate-associated jaw osteonecrosis
Tsao CE, Borromeo GL, Darby IB, Walsh KA , OBrien-Simpson NM, Reynolds EC and Ebeling PR

P125
Recent increases in BMI among men are accompanied by increases in body fat and decreases in lean mass and BMD
Pasco JA, Gould H, Brennan SL and Kotowicz MA

P126
Referral to DXA in an Australian population aged 50 years and over during 2003-10: Associations with sex and DXA reimbursement
Brennan SL, Kotowicz MA, Ebeling PR, Sarah B, Leslie WD and Pasco JA

P127
Selective serotonin reuptake inhibitor use and bone mineral density in men: Geelong Osteoporosis Study
Williams LJ, Pasco JA, Jacka FN, Hodge JA, Dodd S, Kotowicz MA and Berk M

P128
The association between AT_1 receptor antagonists and the risk of fragility fracture in women
Gould H, MacInnis RJ, Pasco JA, Jenkins M and Kotowicz MA

P129
Timed Up and Go Test predicts falls related hospitalization in older community-dwelling women
Zhu K, Lewis J, Radavelli-Bagatini S and Prince RL

Malignant bone disease

P130
A histomorphometric survey of breast and prostate cancer induced bone lesions in SCID mice: Breast cancer cells induce osteolytic bone lesions through a reduction in osteoblast activity
Gregory LS, Choi W and Clements JA

P131
Chemotherapy with anti-metabolite methotrexate increases expression of neurotrophins in bones of young rats
Zhou FH, Georgiou KR, King TJ, Su Y, Zhou XF and Xian CJ

P132
DNA methylation does not contribute to Wif1 epigenetic control in osteosarcoma
Baker EK, Chalk AM, Bhattacharya S, Strbenac D, Green A, Ng AJM, Gupte A, Kocovski P, Martin TJ, Purton LE, Robinson MD and Walkley CR

P133
Fish oil in comparison to folinic acid for protection against the adverse effects of methotrexate chemotherapy in bones
Raghu Nadhanan R, Fan CM, Su Y-W, Howe PR and Xian CJ

P134
Modelling distinct osteosarcoma subtypes *in vivo* using Cre:LoxP & lineage restricted transgenic shRNA
Ng AJM, Mutsaers AJ, Russell MR, Wall M, Baker EK, Ho P, Liddicoat B, Slavin J, Martin TJ, Goriada A, Purton LE, Dickins RA and Walkley CR

P136
Potential role of sFRP-1 in the attenuated Wnt/β-catenin signalling, bone loss and bone marrow adiposity following methotrexate chemotherapy in rats
Georgiou KR, King TJ, Xian CJ

P137
Trolox inhibits osteolytic bone metastasis of breast cancer due to an anti-osteoclastic activity
Lee JH and Lee ZH

Mineral regulating hormones

P138
Attenuated megalin expression in hyperfunctioning parathyroid glands possible role of vitamin D signaling
Nagata Y, Imanishi Y, Yamagata M, Kobayashi I, Ishii A, Michigami T, Yukimura T, Kato S, Arnold A and Inaba M

P139
Characterization of a murine model of tamoxifen-inducible cartilage-specific glucocorticoid receptor knockout
Tu JW, Stoner S, Zhang YQ, Kelly J, Chen D, Tuckermann J, Seibel MJ and Zhou H

P140
Fracture healing is delayed in mice with tamoxifen-induced chondrocytic glucocorticoid receptor knockout
Tu JW, Zhang YQ, Kelly J, Dunstan CR, Chen D, Tuckermann J, Seibel MJ and Zhou H

P141
Glucocorticoids regulate mineralised nodule formation in a biphasic, dose-dependent manner
Zhang YQ, Stoner S, Tu JW, Dunstan CR, Tuckermann J, Seibel MJ and Zhou H

P142
Histochemical examination on osteocytes and their lacunae after administration of parathyroid hormone in mice
Hongo H, Sasaki M, Yamada T, Hasegawa T, Nakano T, Shimoji S and Amizuka N

P143
Over-expression of osteoblastic Vitamin D Receptor (VDR) levels in a mouse model mediates anabolic or anti-anabolic activity which is associated with dietary calcium-mediated changes to circulating 1,25D levels
Triliana R, Lam NN, OLoughlin PD, Morris HA and Anderson PH

P144
Phosphate sensing in osteocytes: Extracellular phosphate induces FGF23 expression in IDG-SW3 osteocyte like cells
Ito N, Findlay DM, Ormsby R, Anderson PH, Bonewald LF and Atkins GJ

P145
Regional up-regulation of 25-hydroxyvitamin D 1alpha-hydroxylase (CYP27B1) gene is associated with the pathogenesis of ectopic calcification in the alpha klotho mutant mice
Yamamoto H, Otani A, Yokoyama N, Onishi R, Takei Y, Taketani Y and Takeda E

Orthopaedics

P146
An *in vivo* model mimicking human subchondral bone and osteocyte pathophysiological changes in osteoarthritis
Jaiprakash A, Wille M-L, Chakravorty N, Crawford R, Feng JQ and Xiao.Y

P147
Computational and experimental characterization of drug release kinetics in trabecular bone from titania nanotube implants
Kaiser J, Buenzli PR, Sinn AW M, Khalid KA, Gulati K, Atkins GJ, Pivonka P, Findlay DM and Losic D

P148
Effects of systemic administration of phenylephrine on healing of rat rib fractures
McDonald SJ, Schuijers JA, Ward AR and Grills BL

P149
HydroxyColl as a multi-drug delivery system for bone tissue engineering
Murphy CM, Yu NYC, Mikulec K, Peacock L, Aiken A, Schindeler A, OBrien FJ and Little DG

P150
Local modulation of anabolic and catabolic responses in a rat critical-sized bone defect model
Yu NYC, Gdalevitch M, Schindeler A, Mikulec K, Peacock L, Fitzpatrick J, Cooper-White JC and Little DG

P151
MEK inhibition modulates normal fracture healing
El-Hoss J, Kolind M, Mikulec K, McDonald M, Schindeler A and Little DG

P152
Reduced radiation sterilization dose of bone allografts improves surgical outcomes while retaining sterility assurance levels
Nguyen H, Morgan DAF, Gineyts E and Forwood MR

P153
Upregulation of MCP-1 gene expression following stress fracture initiation, *in vivo*, is blocked by the dominant negative mutant, 7ND
Wu AC, Kelly WL, Morrison NA and Forwood MR

Paediatrics

P154
Bone and muscle interaction in young individuals with Cystic Fibrosis (CF)
Brookes DSK, Briody JN, Hill RJ, Munns C and Davies PSW

P155
Effects of hypoxia during pregnancy on offspring bone development
Lee AMC, Morrison JL, Botting KJ, Shandala T and Xian CJ

P156
Evaluation of bone mineral density and bone/muscle geometry using pQCT in children after spinal cord injury
Biggin A, Middleton A, Ramjan KA, Briody JN, Waugh MCA and Munns CF

P157
Six monthly intravenous zoledronic acid in childhood osteoporosis
Biggin A, Ooi HL, Briody JN, Cowell CT and Munns CF

invited & oral presentations

Sunday 2nd September 2012
Joint symposium with the Australia and New Zealand Orthopaedic Research Society: Cartilage and bone basic science

Signaling through BMPR2 controls bone mass in the adult skeleton
Jonathan W. Lowery, Guiseppe Intini, Karen Cox, Sutada Lotinum, Kunikazu Tsuji, Roland Baron, Vicki Rosen

Canonical BMP signaling is required for normal skeletal development and for maintenance of a functional adult skeleton. BMP signaling occurs when heteromeric complexes form between BMP ligands and type 1 and type 2 BMP receptors. Ligand binding allows the type 2 receptor to phosphorylate the type 1 receptor, which in turn activates the canonical mediators SMAD1, 5 and 8. As such, the type II BMP receptor functions to regulate type I receptor activation and SMAD1, 5, and 8 activity. There are three known type 2 BMP receptors: BMPR2 that predominantly binds BMPs; and, ACVR2A and ACVR2B multifunctional receptors for BMPs and activin-like ligands. While ACVR2A and ACVR2B can serve as BMP receptors, they have significantly lower affinity for BMP ligands than for activin-like molecules, suggesting BMPR2 functions as the primary type 2 receptor utilized by osteoprogenitors, chondrocytes and osteoblasts during normal skeletal BMP signaling. As conditional deletion of *Bmpr2* in early limb mesenchyme with *Prx1-Cre* (*Bmpr2*-cKO mice) had no effect on BMP signaling and embryonic limb skeletal development (Gamer et al., 2011), it appears that compensation by ACVR2A/B for loss of BMPR2 is of physiological relevance. Here we report that *Bmpr2*-cKO mice develop high bone mass after birth. Micro-CT analysis of *Bmpr2*-cKO mice at 8-10 weeks of age revealed an increase in proximal tibia trabecular BV/TV in both male ($p<0.01$) and female ($p<0.001$) when compared to littermate Cre negative *Bmpr2* floxed mice. Additional analysis of this high bone mass phenotype using dynamic histomorphometry showed increased trabecular thickness ($p=0.012$), mineral apposition rate ($p=0.019$) and bone formation rate ($p=0.002$) in the proximal femur of *Bmpr2*-cKO mice. As there was no difference in osteoblast or osteoclast number between *Bmpr2*-cKO mice and littermate controls, our findings suggest that loss of BMPR2 in the Prx1-Cre expression domain leads to increased bone mass due to an overt increase in osteoblast activity. This is a distinctly different phenotype than high bone mass associated with loss of the BMP type I receptor *Alk3* in osteoblasts, where high bone mass is due to a secondary defect in osteoclastogenesis. As BMP signaling is essential for a functional skeleton, we hypothesize that the absence of BMPR2 requires ACVR2A and/or ACVR2B to transduce signals from both BMP and activin ligands, leading to an overall decrease in SMAD2/3 signaling relative to SMAD1/5/8 signaling in osteoprogenitor cells, and resulting in increased osteoblast activity. Our results are consistent with the high bone mass phenotype reported to occur when soluble ACVR2A- or ACVR2B-Fc chimeras, which sequester activin-like molecules, are administered to adult mice, and together implicate Smad2/3 phosphorylation as a negative regulator of bone mass.

Genetics of Bone and Joint Shape
Nancy E Lane (USA)

Sunday 2nd September 2012
Joint symposium with the Australia and New Zealand Orthopaedic Research Society: Cartilage and bone basic science

Bone remodelling of OA
David Findlay
Discipline of Orthopaedics and Trauma, University of Adelaide

Osteoarthritis (OA), the major disease of joints, is characterised by progressive degenerative damage to articular cartilage, but is ultimately a disease of the whole joint. In particular, changes occur in the subchondral bone of affected joints, which include cysts, thicker but less mineralised bone and osteophyte formation, and which appear to be driven by altered bone remodelling. What causes this changed remodelling remains unknown, although expression of molecules implicated in this process are elevated in both the bone and articular cartilage. In fact, expression of many of the genes that regulate bone metabolism are differentially expressed in OA bone and in osteoblasts derived from OA bone. It has been suggested that shape changes in bone, together with genetic factors that regulate cartilage material properties, interact in the initiation or progression of OA. There have been a number of studies investigating the effect of anti-resorptive agents on the progression of OA. Animal studies have shown joint protection in a number of animal models of OA, using bisphosphonates, calcitonin and osteoprotegerin, although these have yet to be convincingly translated into human disease. Recently, lesions identified in subchondral bone in OA by use of particular MRI sequences, so-called 'bone marrow lesions', have shown predictive value in the degeneration of the overlying cartilage. It is therefore of great interest to determine whether these lesions comprise zones in the bone, in which bone remodelling is additionally altered compared with the surrounding bone. The above findings, together with studies showing the interdependence between articular cartilage and subchondral bone, suggest the need to target bone in the search for disease modifying agents for OA.

Monday 3rd September 2012
Session 1: Age quake

IS1

What old means to bone

Stavros C. Manolagas, M.D., Ph.D.
Center for Osteoporosis and Metabolic Bone Diseases, Univ. Arkansas for Medical Sciences, and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA.

The hallmark of age-related osteoporosis is decreased bone formation, but the molecular mechanisms behind this change remain elusive. In the mouse model, the decline of bone mass with advancing age is associated with a progressive increase in oxidative stress (OS), decreased osteoblast number and bone formation, and increased prevelance of osteoblast apoptosis in the face of unaltered or even declining osteoclast numbers. In line with a pathogenetic role of oxidative stress on bone formation, reactive oxygen species (ROS) attenuate osteoblastogenesis and shorten the lifespan of osteoblasts. ROS, on the other hand, are required for osteoclast generation, function, and survival. Increased ROS generation leads to the activation of FoxOs – transcription factors that promote compensatory adaptations in response to OS and growth factor deprivation. Global somatic deletion of FoxOs in young mice increases OS and recapitulates the adverse effects of aging on bone. Conversely, FoxO3 over-expression in mature osteoblasts decreases OS and increases bone mass. Albeit, activation of FoxOs in response to oxidative stress or growth factor deprivation, diverts β-catenin from Wnt/TCF- to FoxO-mediated transcription. Consistent with this evidence, mice with targeted deletion of FoxO1, 3 and 4 in osteoblast progenitors expressing Osterix1 exhibit increased osteoblast numbers, high bone mass that is maintained throughout life, and decreased adiposity in the aged bone marrow. FoxO deletion from Osx1 expressing cells also increases osteoprogenitor proliferation and bone formation and up-regulates several β-catenin/Tcf-target genes, including cyclin D1. β-catenin deletion in FoxO null cells normalizes both cyclin D1 expression and proliferation, indicating that the up-regulation of Wnt signalling is responsible for the increased osteoprogenitor proliferation. The high bone mass of this model, as opposed to the osteoporotic phenotype of mice with loss of FoxO function in mesenchymal progenitors upstream from Osx1 or terminally differentiated osteoblasts, demonstrates that FoxOs have potent and distinct effects all along the osteoblast differentiation lineage. Albeit, in promoting compensatory adaptations to cellular stressors accumulating with aging, FoxOs restrain the supply of osteoblasts by attenuating Wnt signaling. Thereby, FoxOs contribute to the development of osteoporosis. FoxO3 over-expression in cells of the osteoclast lineage decreases osteoclastogenesis, increases osteoclast apoptosis, and increases bone mass, supporting the hypothesis that increased FoxO activation with age also decreases osteoclast numbers and thereby the rate of bone remodeling.

IS2

Impact of osteoporosis in aged people

Lyn March (Sydney)

Monday 3rd September 2012
Session 1: Age quake

IS3
The common links of osteoporosis and dementia
Charles Inderjeeth

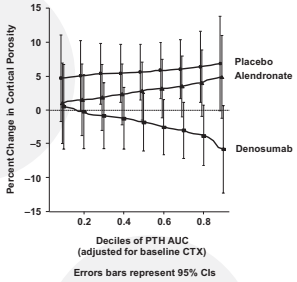
Objective: To review the common links between the aetiology, pathogenesis and treatments of osteoporosis and dementia.
Background: Both conditions are strongly associated with fracture risk and are highly prevalent in an ageing demographic with significant morbidity, mortality and cost implications. Osteoporosis often co- exists with dementia, as both disorders are strongly age related.
Dementia is an established risk factor of falls and hip fractures. The nature of the relationship between dementia and hip fractures may be complex but there seems to be an independent relationship between dementia and hip fracture. Dementia and age are independent predictors of mortality following hip fracture.
The important common risk factors include vitamin D deficiency, cardiovascular disease, oestrogen deficiency, "inflammaging" (immune dysfunction with ageing), endocrine dysfunction (eg PTH) and ageing genetics. There is an emerging body of evidence suggesting that ESR alpha, Acetylcholine, IL6, TNF and COX may be important common associations with both conditions. Evidence for the potential benefits of exercise, vitamin D, Statins, anti-inflammatory including TNF blockers, Oestrogen replacement and Acetylcholinesterase inhibitors for both conditions adds to this potential association.
To compound this, coexistence of these 2 conditions complicate the choice, initiation, compliance and potential toxicities of treatment options as evidenced by lower rates of diagnosis and treatment in this population. The significant and complex interrelationship of these 2 important conditions will be explored further.
References:

1. Curtis JR, Safford MM. Management of Osteoporosis among the Elderly with Other Chronic Medical Conditions. Drugs Aging. 2012 Jul 1;29(7):549-64.
2. Tysiewicz-Dudek M, Pietraszkiewicz F, Drozdzowska B. Alzheimer's Disease and osteoporosis: common risk factors or one condition predisposing to the other? Ortopedia, traumatologia, rehabilitacja. [Review]. 2008 Jul-Aug;10(4):315-23.
3. Acetylcholinesterase inhibitors and the risk of hip fracture in Alzheimer's Disease patients. JBMR. 2012 July 1;

OR1 Outstanding Abstract Award (Clinical)
The transitory PTH increase following denosumab administration is associated with reduced intracortical porosity: a distinctive characteristic of denosumab therapy
Seeman E[1], Libanati C[2], Austin M[2], Chapurlat R[3], Boyd SK[4], Zebaze R[1], Hanley DA[4], Zanchetta J[5], Grauer A[2] and Bilezikian JP[6]
[1]Austin Health, University of Melbourne, Melbourne, Australia; [2]Amgen Inc., Thousand Oaks, CA, USA; [3]INSERM UMR 1033, Université de Lyon, Lyon, France; [4]University of Calgary, Calgary, AB, Canada; [5]Instituto de Investigaciones Metabólicas, Buenos Aires, Argentina; [6]College of Physicians and Surgeons, Columbia University, New York, NY, USA

Objective: Denosumab reduces resorption by existing bone remodeling units (BMUs) and inhibits the birth of new BMUs accompanied by a transitory increase in PTH which may increase osteoblast longevity/activity. We hypothesized that the indirect effect on bone formation mediated by PTH in the face of inhibited osteoclastic activity will reduce cortical porosity.
Methods: Postmenopausal women (N=247), mean age 61 years and low BMD, were randomly assigned in a double-blind, double-dummy trial to denosumab 60 mg Q6M (N=83), alendronate 70 mg QW (N=82), or placebo (N=82). PTH was measured at baseline, week 1, and months 1, 3, 6, 6.25, 7, 9, and 12. An area under the curve (AUC) for PTH was derived for each subject. Porosity was evaluated in the compact-appearing cortex of the distal radius at baseline and month 12 by HR-pQCT using the Scanco methodology. Associations between PTH AUC and change in porosity were evaluated.
Results: Transitory increases in PTH were seen with denosumab and alendronate. The increase in PTH was larger with denosumab than alendronate ($P<0.05$) and was observed after each dose. At the radius by 12 months, porosity increased with placebo (+5.2%), increased less in the alendronate group (+2.9%), and was reduced by denosumab (–3.0%). With placebo and alendronate, porosity increased with increasing PTH (Figure). With denosumab, porosity decreased as PTH increased.
Conclusions: Denosumab partially reversed micro-architectural deterioration directly, by reducing remodeling intensity; and perhaps indirectly, by a PTH-dependent effect on BMU level bone formation in the setting of full suppression of osteoclast activity.

Figure: Relationship Between PTH and Cortical Porosity at 12 Months



Monday 3rd September 2012
Session 2: Translational skeletal medicine

IS4

Skeletal dysplasia in mice and human

Shiro Ikegawa, MD, PhD
Head of Laboratory for Bone and Joint Diseases, SNP Research Center, RIKEN, Tokyo, Japan

Skeleton is an organ composed by a variety of tissues, including bone, cartilage (articular and growth cartilage), tendon, and ligament. Skeletal formation is a complex process composed by multiple biological steps in which many types of cells are involved. Its maintenance is also a complex process supported by many kinds of systems. Hence, many genes are involved in formation and maintenance of the skeleton. Recent genomic studies have clarified a number of the skeletal genes; however, its total picture remains unknown.

Skeletal dysplasias, monogenic (Mendelian) diseases affecting the skeletal system tell us a lot about the mechanisms of formation and maintenance of the skeleton. They also give us a hint for understanding pathomechanism of common polygenic diseases (life-style associated diseases), including osteoarthritis, osteoporosis and lumbar disc herniation. We are tackling for skeletal dysplasia using a combine approach of human and mouse genetics. By integrating the knowledge obtained from mouse and human genetic studies, we can accelerate the steps for identification of the disease genes and clarification of their functional impact on the diseases.

IS5

Autologous Human Mesenchymal Stem Cells for Cartilage Repair: From Bench to Bedside

Head & Senior Consultant, Division of Paediatric Orthopaedics, Director, Cartilage Repair Program, Department of Orthopaedic Surgery, National University Health System, Singapore

Mesenchymal stem cell (MSC) research demonstrated therapeutic potential of the cells. They can be used to treat diversified clinical conditions, from immune modulation to tissue regeneration. We have focused on the translational development of using MSC clinically for cartilage repair. Earlier, we have demonstrated through in vitro research that human bone marrow (BM) is better source of MSC than adipose tissue (Tissue Eng A 2007). In the study, BM and adipose MSC were cultured from the same sets of donors. Results showed that BM MSC produced significantly more collagen II and s-GAG. We further demonstrated, in small and large animal, that MSC were capable of enhancing cartilage repair (Am J Sports Med 2007, Stem Cells 2007, J Bone Joint Surgery 2010). Especially in porcine, biomechanical testing showed that meniscus repaired with the addition of MSC was significantly stronger that of the control animals. A clinical trial comparing MSC and chondrocytes (Am J Sports Med 2010) showed that both cell types were capable of improving cartilage repair. However, MSC appeared to be superior in longer term follow up, work just as well in older patients (> 45 yr) and require 1 less knee surgery (cost saving with lower risk potentially). Multiple (single institution) trials (IRB approved) are now open and accruing patients at the National University Hospital in Singapore to evaluate the safety and efficiency of autologous BM MSC in cartilage repair. Harvested bone marrow samples (during knee surgery) are processed in clean room environment (cGMP cell processing facility recently re-modeled and upgraded) and cultured for 3 weeks. The cells are injected locally in the knee with hyaluronic acid. Preliminary results showed that MSC injection is a safe procedure (in terms of serious adverse events during administration of MSC) and the rate of microbial contamination is low (1 in 218 processes during a 6-year span from 2006 to 2011). Our research and development has demonstrated that, in the setting of cartilage repair, it is feasible to translate longitudinally from laboratory to clinical studies (bench to bedside), using animal models (small and large) to validate in vitro results prior to design and initiation of human trials. Efficacy of autologous MSC in cartilage repair awaits the completion of patient accrual and final analysis of the trials.

Monday 3rd September 2012
Session 2: Translational skeletal medicine

IS6
Anti-bone resorptive effects of a Vitamin E analogue, Trolox
Zang-Hee Lee (Korea)

OR2
Contribution of myogenic and vascular cell lineages to fracture repair
Schindeler A[1,2], Kolind M[1,2], Bobyn JD[1,2], Liu R[1,2] , Mikulec K[1],Peacock L[1], Morse A[1], and Little DG[1,2]
[1] *Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead.*
[2] *Paediatrics and Child Health, Faculty of Medicine, University of Sydney.*

The progenitor cells that are capable of participating in fracture and bone defect repair remain poorly understood. We have speculated that progenitors residing within the local soft tissues, particularly the myogenic or endothelial cells lineages, may make an important contribution.
To track cell lineages even after a putative transdifferentiation event, we developed conditional reporter mice using the *MyoD-cre* and *Tie2-cre* transgenic strains. We have previously shown myogenic cell contribution to open fracture healing using a cre-inducible heat-resistant alkaline phosphatase reporter, but this model lacks the capacity to double stain for current lineage markers. To overcome this deficiency, we have adopted a cre-inducible eGFP reporter (Z/EG), which allows for fluorescent cell tracking and immunofluorescent staining.
Open tibial fractures were performed on *MyoD-cre Z/EG* and *Tie2-cre Z/EG* mice and fractured limbs harvested at 4, 7, 10, and 14 day time points. This represents a range of healing stages from early cellular invasion to cartilage formation and the endochondral ossification. On cryosections, MyoD-lineage and Tie2-lineage cells were visualised by eGFO fluorescence. Immunostaining with antibodies to bone, cartilage and vascular markers (*Osx, Sox9, CD31*) were performed on the same sections to allow for eGFP+ overlays.
Our data suggest that bone repair can still occur in the absence of a functional periosteum, but that there is contribution from myogenic and endothelial progenitors from the soft tissues. These models show great utility in dissecting the cell lineages able to contribute to bone repair.

Acknowledgements: A/Prof. David Goldhamer generated and supplied MyoD-Cre mouse line. Prof. Patrick Tam supplied the Z/AP and Z/EG reporter lines with permission from Prof. Andras Nagy. This work was supported by grants from the Australian Orthopaedic Research Foundation (AORF) and from the National Health & Medical Research Council (NH&MRC grants APP457245, APP1003480). Drs Liu and Schindeler obtained salary support from the Peter Bega scholarship and CTF Young Investigator Award respectively.

Monday 3rd September 2012
Session 2: Translational skeletal medicine

OR3

Low bone mass in Down Syndrome humans and mice

Tristan W. Fowler[1,2], Nisreen S. Akel[1,2], Jaclyn Vander Schilden[2*], Robert A. Skinner[2*], William R. Hogue[2*], Frances L. Swain[2*], Galen R. Wenger[3*], Kent D. McKelvey[4*], Dana Gaddy[1,2], and Larry J. Suva[1,2]
[1] *UAMS, Department of Physiology & Biophysics*
[2] *UAMS, Department of Orthopaedic Surgery, Center for Orthopaedic Research*
[3] *UAMS, Department of Pharmacology and Toxicology*
[4] *UAMS, Department of Genetics, USA.*

Down Syndrome (DS), trisomy of chromosome 21, is one of the most common congenital disorders leading to a wide range of health problems in humans, including increased fracture risk. Low bone mineral density (BMD) has been reported in many studies of people with DS, yet the specific effects of trisomy 21 on the skeleton remain poorly defined. We measured BMD and bone biochemical markers in healthy, community dwelling, calcium-replete, euthyroid DS patients (17 males ages 19-52 and 19 females ages 18-56). DS patients consistently demonstrated low BMD at the lumbar spine, and femoral neck that was associated with decreased serum biochemical markers, indicative of low bone turnover. The low bone turnover was present despite the characteristic hypogonadism and infertility in these patients. Similarly, male Ts65Dn DS mice are hypogondal and display low bone mass that deteriorates with age. Low bone mass was correlated with significantly decreased osteoblast and osteoclast development, decreased bone turnover, bone formation rate and mechanical strength. Interestingly, low bone mass in 3 month old Ts65Dn mice was significantly increased after 4 weeks of intermittent PTH treatment. These studies demonstrate the potential of DS mouse models to improve our understanding of chromosome 21 gene dosage effects in bone, provide novel insight into the cause of bone fragility in DS and identify PTH as a potential anabolic agent in the adult low bone mass DS population.

Grant Support DK74024 (DG); HD047656 (GRW); Winthrop P. Rockefeller Chair in Clinical Genetics (KDM); Carl L. Nelson Chair in Orthopaedic Creativity (LJS).

Monday 3rd September 2012
Session 3: Evidence-based care in musculoskeletal disorders

IS7

Dose and duration of action of zoledronate: an examination of the evidence

MJ Bolland, A Grey and IR Reid
Department of Medicine, University of Auckland, Auckland, New Zealand

Annually adminstered intravenous 5mg zoledronate reduces fracture risk. However, in the initial dose-finding trial, the maximum dose studied was 4mg and the effects of a single 4mg dose on bone mineral density (BMD) and markers of bone turnover had not worn off at 1y. Therefore, we undertook 3 randomised, placebo-controlled trials exploring both lower doses of zoledronate and the persistence of its effects. 180 postmenopausal women with osteopenia were randomised to a single dose of 1mg, 2.5mg or 5mg zoledronate, or placebo. At 1y, the three zoledronate doses produced similar increases in spine (3.5-4.0%) and hip BMD (2.7-3.6%), and a dose-dependent increase in total body BMD (1.2-1.9%). The three zoledronate doses reduced bone turnover by >40%, with a dose-dependent decrease. In 43 HIV-infected men with BMD T-score <-0.5, the effects of two annual 4mg zoledronate doses persisted for at least 5y after the second dose. At 5y after the second dose, BMD was 1.5-3.5% higher and bone turnover 38-49% lower in the zoledronate group. There were no significant changes in the between-groups differences in BMD or bone turnover between 1 and 5y after the second dose. In 50 postmenopausal women with osteopenia, BMD was 2.7-5.3% higher and bone turnover 48-55% lower in the zoledronate group at 5y after a single 5mg dose of zoledronate. The between-groups differences in BMD and bone turnover remained stable between 1 and 5y after the zoledronate dose. In summary, zoledronate doses smaller than 5mg have substantial effects on BMD and bone turnover, and the effects of annual zoledronate doses on BMD and bone turnover persist for at least 5y. Trials assessing the anti-fracture efficacy of both low doses of zoledronate and dosing intervals greater than one year are justified.

IS8

Inflammatory Bone Loss: mechanism and treatment

Nancy Lane (USA)

Monday 3rd September 2012
Session 4A: Early career researchers (basic science) and oral presentation

IS9
Vertebroplasty
Rachelle Buchbinder (Australia)

IS10
Osteoprotection by semaphorin 3A
Tomoki Nakashima[1,2], Mikihito Hayashi[1,2] and Hiroshi Takayanagi[1-3]
[1]*Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University*
[2]*Japan Science and Technology Agency, ERATO, Takayanagi Osteonetwork Project*
[3]*Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo*

Bone homeostasis is achieved by the crosstalk between bone-forming osteoblasts and bone-resorbing osteoclasts. Osteoclastogenesis is strictly controlled by osteoblasts to maintain adequate bone volume since excessive osteoclastic bone resorption has been implicated in the pathogenesis of various osteopenic conditions. However, an inhibitory factor of osteoclast differentiation derived from osteoblasts was not identified except for Osteoprotegerin, a decoy receptor for RANKL. Here we show that Semaphorin 3A (Sema3A) derived from osteoblasts is a key regulator of bone formation phase in bone remodeling. Sema3A-deficient mice exhibited a severe low bone mass phenotype accompanied by enhanced osteoclast differentiation. The binding of Sema3A to Neuropilin-1 (Nrp1) inhibited RANKL-induced osteoclast differentiation through the inhibition of ITAM and RhoA signaling pathways. Notably, Sema3A-deficient mice also showed a severe defect in osteoblast differentiation and abnormally increased adipocytes in bone marrow. These findings suggest that Sema3A facilitate mesenchymal cell fate toward osteoblasts, but not adipocytes. Genome-wide screening of mRNAs expressed in Sema3A-deficient osteoblastic cells revealed that the canonical Wnt/β-catenin signaling pathway was impaired. The osteopenic phenotype in Sema3A deficient mice was recapitulated by mice in which the Sema3A-binding site of Nrp1 had been genetically disrupted. Intravenous Sema3A administration increased bone volume and prevented ovariectomy-induced bone loss. Sema3A treatment also promoted bone regeneration in a model of cortical bone defects induced by drill hole injury. Thus, Sema3A is a promising new therapeutic agent in bone and joint diseases.

IS11
The role of V-ATPase in bone homeostasis
Taksum Cheng
Centre for Orthopaedic Research, School of Surgery, The University of Western Australia QEII Medical Centre, Nedlands, WA, 6009

Vacuolar-type H^+-ATPases (V-ATPases) are multisubunit proton pumps that acidify intracellular cargos and deliver protons across the plasma membrane of a variety of specialized cells, including bone-resorbing osteoclasts. In osteoclasts, V-ATPases functions in extracellular acidification a process that initiates the dissolution of mineralized bone matrix and crucial for osteoclastic bone resorption. While the importance of V-ATPases in osteoclastic resorptive function is well-defined, whether V-ATPases facilitate additional aspects of osteoclast function and/or formation remains largely obscure. Our labs research focus has been on the identification and characterization of novel subunits of the V-ATPase which impacts osteoclast function and potential V-ATPase inhibitors for therapeutic applications. In line with this focus, we have recently reported that the V-ATPase accessory subunit Ac45 participates in both osteoclast formation and function. Using a siRNA-based approach, we demonstrate that targeted suppression of Ac45 impairs intracellular acidification and endocytosis, both are prerequisite for osteoclastic bone resorptive function *in vitro*. Interestingly, knockdown of Ac45 also attenuates osteoclast formation owing to a reduced fusion capacity of osteoclastic precursor cells. In an effort to gain more detailed insights into the functional role of Ac45 in osteoclasts, we attempted to generate osteoclast-specific Ac45 conditional knockout mice using a Cathepsin K-Cre-LoxP system. Surprisingly, insertion of the neomycin cassette in the Ac45-$Flox^{Neo}$ mice resulted in marked disturbances in CNS development leading to embryonic lethality thus precluding functional assessment of Ac45 in osteoclasts and peripheral bone tissues. More recently we have identified novel subunits of the osteoclast V-ATPase and provide new evidence that conditional knockout of these subunits results in severe osteopetrosis in mice.

Monday 3rd September 2012
Session 4A: Early career researchers (basic science) and oral presentation

IS12
Auto-amplification of IL-6 and RANKL signaling fuels metastatic growth in bone

Zheng Y[1,2]
[1] *Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia*[2] *The Kinghorn Cancer Centre and Cancer Research Program, Garvan Institute of Medical Research, Sydney, Australia*

The cytokine RANKL is produced by osteoblasts and other cells of the osteoblast lineage, and plays a key role in the 'vicious cycle' of bone metastasis by stimulating osteoclastic bone resorption. Using human breast and prostate cancer cell lines to induce tumors in the bone of immune-deficient mice, we found that recombinant RANKL up-regulates the expression and release of IL-6 by cancer cells *in-vitro*, and that RANKL derived from cells of the osteoblast lineage directly promotes IL-6 expression by cancer cells *in-vivo*. Next, we disrupted IL-6 signaling *in-vivo* either via knock-down of IL-6 in tumor cells or through treatment with specific anti-human or anti-mouse IL-6 receptor antibodies. Each method significantly reduced tumor growth in bone but not in soft tissues. Lastly, we demonstrated that activation or disruption of IL-6 signaling induces corresponding changes in RANK expression in cancer cells, and that knock-down of RANK expression in cancer cells reduces tumor growth in bone. Taken together these findings provide evidence that osteoblasts and cells of the osteoblast lineage are able to directly communicate with cancer cells via two interdependent signaling pathways involving IL-6, RANK and RANKL. Activation of these newly identified auto-amplifying mechanisms significantly enhances metastatic tumor growth in bone.

OR4
EphrinB2 signaling in osteoblasts and chondrocytes is required for their differentiation and support of osteoclast formation

Tonna S[1], Takyar F[1], Poulton IJ[1], Ho PWM[1], McGregor NE[1], Tatarczuch L[2], Mackie E[2], Martin TJ[1] and Sims NA[1]
[1]*Bone Cell Biology and Disease Unit, St Vincent's Institute, Fitzroy, Victoria, Australia.* [2]*School of Veterinary Science, University of Melbourne, Parkville, Victoria, Australia.*

Osteoblasts express EphB4 and its cognate ligand, ephrinB2. Treatment with PTH rapidly induces osteoblastic expression of ephrinB2. Blockade of ephrinB2/EphB4 signaling impedes osteoblast differentiation and mineralization *in vitro*. To investigate this signaling within osteoblasts and chondrocytes we generated mice conditional deletion of the ephrinB2 cytoplasmic domain in osteoblasts and sporadic hypertrophic chondrocytes (*Osx1.Efnb2*$^{f/f}$).
EphrinB2 cytoplasmic domain expression was reduced 90-95% in FACS-isolated *Osx1.Efnb2*$^{f/f}$ primary calvarial osteoblasts compared with *Osx1.Efnb2*$^{+/+}$ and reduced 80% in *Osx1.Efnb2*$^{f/f}$ primary chondrocytes. In FACS-sorted *Osx1.Efnb2*$^{f/f}$ osteoblasts both early and late differentiation markers were reduced by 80-90%. Similar findings were obtained when exogenous viral Cre was introduced to *Efnb2*$^{f/f}$ osteoblasts. These changes are consistent with a 50% reduction in mineralization rate in adult *Osx1.Efnb2*$^{f/f}$ mice. *Osx1.Efnb2*$^{f/f}$ osteoblasts also demonstrated reduced RANKL expression (50%) and impaired support of osteoclast formation in co-culture with wild-type bone marrow precursors.
Reduced support of osteoclast formation was also observed in *Osx1.Efnb2*$^{f/f}$ neonates, which demonstrated high trabecular bone volume, prominent cartilage remnants, and a 75% reduction in osteoclast numbers close to the growth plate. Electron microscopy revealed that the osteoclasts near the growth plate showed reduced contact with cartilage or bone, did not form ruffled borders or sealing zones and exhibited convoluted nuclei. In addition contact between osteoblasts and between osteoblasts and the bone surface was reduced and chondrocytes at all stages of maturation contained more condensed chromatin. In conclusion, these results indicate that osteoblastic ephrinB2 signaling regulates osteoblast and chondrocyte differentiation and the support of osteoclast formation by both chondrocytes and osteoblasts.

Monday 3rd September 2012
Session 4B: Early career researchers (translational and clinical science)

IS13

Beyond GWAS: gene mapping in the era of next-generation sequencing

Emma Duncan
Senior Staff Specialist in Endocrinology, Royal Brisbane and Women's Hospital
Associate Professor of Medicine, School of Medicine, Faculty of Health Sciences, University of Queensland
Senior Research Officer, UQ Diamantina Institute,
Senior Research Officer, UQ Centre for Clinical Research (adjunct)

The last twenty years have seen an unparalleled increase in our understanding of human disease through the success of gene mapping in both rare and common diseases. In the 90s, linkage approaches proved very successful for mapping Mendelian disorders; and in the last five years whole genome association studies have mapped many genes for common diseases such as diabetes and osteoporosis. Both these approaches have limitations in their ability to map disease-causing genes: linkage mapping require families with sufficiently large pedigrees to be informative; and GWAS will not discover rare variants and new mutations and depend critically on sufficiently large populations for adequate power. Over the last few years, newer approaches such as next generation sequencing (NGS) have been employed with remarkable early success. Rare Mendelian diseases have been mapped using only a few affected individuals; and further gene mapping in common diseases such as osteoporosis is just starting.
Over the last decade our laboratory has been involved in many gene mapping projects, particularly bone disorders both common (osteoporosis) and rare (skeletal dysplasias); and have used all these genetic approaches. I will present some of the highlights of this adventurous journey, and how I see gene mapping progressing in the 21st century.

IS14

The skeletal effects of tyrosine kinase inhibitors

Susannah O'Sullivan
The Bone and Joint Group, University of Auckland, Auckland, New Zealand

Imatinib mesylate and the related tyrosine kinase inhibitors (TKIs) nilotinib and dasatinib, are now standard care for the treatment of chronic myeloid leukemia (CML) and it is likely that most patients will remain on one of these agents indefinitely. These agents are also being used or trialed in the treatment of gastrointestinal stromal cell tumor (GIST) and disorders that are characterized by activated PDGF receptor signaling. "Bystander effects" due to inhibition of these TKI's target receptors in normal tissues have been observed, including effects on bone and calcium metabolism. *In vitro*, TKIs have effects on osteoblast proliferation, survival and differentiation, and osteoclastogenesis, through inhibition of the PDGFR, c-fms and possibly c-src. Some data from animal studies demonstrate reduced bone formation and bone mass after treatment with imatinib and increased bone mass after treatment with dasatinib. Humans treated with imatinib and nilotinib demonstrate secondary hyperparathyroidism and reduced levels of markers of bone turnover. In adult humans both agents have a neutral or positive effect on bone mass and data are largely reassuring regarding the safety of medium-term use for treatment of CML or GIST. In children, there may be negative effects of imatinib on growth. This presentation reviews the skeletal effects of TKIs and discusses the possible mechanisms for these effects. Given the potential for patients to receive TKIs in the long term, the implications of these effects for bone health in such patients are addressed.

Monday 3rd September 2012
Session 4B: Early career researchers (translational and clinical science)

OR5

Improving the definition of osteoporosis: cortical porosity identifies women with distal forearm fractures

Bala Y[1], Zebaze R[1], Ghasem-Zadeh A[1], Peterson J[2], Amin S[2], Melton III LJ.[2], Khosla S[2], Seeman E.[1]
[1]Endocrine Center, Austin Health, University of Melbourne, Australia, [2]College of Medicine, Mayo Clinic, Rochester, MN, USA.

Trabecular bone loss and vertebral fractures are flagships of 'osteoporosis' yet 80% of bone is cortical, 80% of fractures are non-vertebral, 70% of appendicular bone loss is cortical and occurs by intracortical remodeling. We propose the resulting porosity distinguishes women with forearm fractures better than aBMD.
Distal radius images were quantified using HRpQCT and DXA in 68 postmenopausal women with a distal forearm fracture ('cases') and 70 age-matched community controls from Olmsted County, Minnesota. Porosity of compact cortex (CC) and transitional zone (TZ) and trabecular bone volume fraction (TrabBV/TV) were measured using StrAx1.0.
Among all cases and controls, cases had lower aBMD (-0.5 SD, p=0.001), higher porosity (0.4 SD in CC p=0.02, 0.5 SD in TZ, p=0.001) and lower trabecBV/TV (-0.6 SD p=0.001). By stepwise logistic regression, TZ porosity alone satisfied the model, 1 SD higher was associated with an OR of 1.98(1.31-2.97, p=0.001).
aBMD, porosity and trabBV/TV did not differ in the 38% cases with osteoporosis from controls with osteoporosis. aBMD and trabBV/TV did not differ but CC and TZ were 0.4 SD higher ($p<0.05$) in the 62% cases without osteoporosis (38% osteopenia, 24% normal aBMD) than in controls without osteoporosis. The OR was protective 0.40(0.18-0.92, p=0.02) but elevated in cases when porosity was high in the CC: 3.13(1.14-8.77) or TZ: 4.08(1.18-14.9) and reduced when porosity was low in the CC: 0.32(0.12-0.88) or TZ: 0.25(0.07-0.85), all $p<0.02$. BV/TV did not discriminate cases from controls.
Porosity discriminates women with forearm fractures categorized at low risk by aBMD.

OR6

Suggestive loci for osteoporosis: a multipoint variance component linkage analysis of extended pedigrees

Nguyen SC, Nguyen ND, Center JR, Eisman JA and Nguyen TV
Osteoporosis and Bone Biology Research, Garvan Institute of Medical Research

Genome-wide linkage analysis using extended pedigrees is an attractive approach to search for genes for complex diseases. The primary aim of this study was to identify loci linked to bone mineral density (BMD) and quantitative ultrasound measurements (QUS).
The Dubbo Osteoporosis Genetics Study is a large multi-generational family study involving 141 pedigrees. Individuals are recruited through probands with high BMD (Z-score greater than +1.28). BMD (g/cm2) at the femoral neck, lumbar spine, and whole body were measured by DXA (GE-LUNAR). QUS measurements were obtained by a Sunlight Omnisense. Subjects were genotyped using 530 microsatellite markers. After adjusting for age, sex and other covariates, we performed a variance components linkage analysis using SOLAR.
We analysed 1007 individuals (636 women) aged 58 ± 19 (mean ± SD; range 18-98). We found suggestive linkage for BMD at the whole body (LOD score 2.02, chromosome 6) and for QUS at the tibia (LOD score 2.77, chromosome 18), but not for femoral neck (highest LOD 1.50, chromosome 6) or lumbar spine (highest LOD 1.38, chromosome 12). We also stratified by age and found suggestive linkage for femoral neck and lumbar spine BMD both on chromosome 12 (LOD scores 2.88 and 1.82, respectively) for individuals aged 50-80 years.
These data suggest the presence of multiple loci regulating bone mineral density and quantitative ultrasound measurements. The identification of specific markers or genes may lead to better prediction of fracture risk and individualised osteoporosis treatment.

Tuesday 4th September 2012
Session 5: Molecular targets of bone cells

IS15

Osteoclast-derived osteoblast anabolic factors

Hiroshi Takayanagi
Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo
ERATO, JST, Japan

Bone remodeling process is composed of bone resorption, transition and formation phases. Classically, coupling factors have been thought to mediate the transition from bone resorption to formation. There may be numerous factors working in other phases. In addition, osteocytes regulate bone remodeling cells osteoclasts and osteoblasts. Therefore, there must be various molecules involved in the communication among bone cells, which are poorly identified. IGF-1 and TGF-beta are among the coupling factors reserved in the bone matrix. Ephrin, Wnt and S1P are also proposed to underlie the coupling mechanisms. I will introduce our recent studies on the molecules mediating bone cell communication including osteocyte RANKL, osteoclast-derived Sema4D and osteoblast-derived Sema3A, which will provide novel insights into the mechanism of bone cell control as well as new therapeutic strategies.

IS16

Choreography from the tomb: the role of osteocytes in bone remodeling

Stavros C. Manolagas, M.D., Ph.D.
Center for Osteoporosis and Metabolic Bone Diseases, Univ. Arkansas for Medical Sciences, and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA.

Osteocytes, immured within the lacunar-canalicular system and mineralized matrix, are ideally located throughout bone to detect the need for, and accordingly choreograph, the bone regeneration process by independently controlling rate limiting steps of bone resorption and formation. Consistent with this role, emerging evidence indicates that signals arising from apoptotic and old/or dysfunctional osteocytes are seminal culprits in the pathogenesis of involutional, post-menopausal, steroid-, and immobilization induced osteoporosis. Osteocyte-originated signals may also contribute to the increased bone fragility associated with bone matrix disorders like osteogenesis imperfecta, and perhaps the rapid reversal of bone turnover above baseline following discontinuation of anti-resorptive treatments, like Denosumab. In support of these ideas, mice with osteoblast and osteocyte targeted deletion of both Bak and Bax, two genes indispensable for apoptosis, exhibit a 7-fold increase in cortical porosity accompanied by increased RANKL production by osteocytes, indicating that these changes are the likely culprits of the age-associated increase in cortical porosity. Further, the expression of autophagy-related genes is lower in the cortical bone of 20-month-old, compared with 6-month-old, mice. Moreover, male and female mice with osteocyte-targeted deletion of ATG7, a gene that is essential for autophagy, exhibit at 4-6 months of age all the same features observed in aged wild type mice (20 months old), including low cancellous bone volume, low cortical thickness, high cortical porosity in the femurs, as well as lower osteoclast and osteoblast numbers, bone formation rate, and wall width. Hence, loss of autophagy in osteocytes leads to low bone mass associated with low bone remodeling, suggesting that the decline in osteocyte autophagy with age contributes to the low bone mass associated with aging.

Tuesday 4th September 2012
Session 5: Molecular targets of bone cells

IS17

Targeting sclerostin for anabolic therapy of bone disorders

Hua Zhu (David) Ke
Amgen, Thousand Oaks, California, USA.

There are two classes of agents that can be used for treatment of bone disorders such as osteoporosis: anti-resorptive agents targeting osteoclast and inhibiting bone resorption and destruction, and anabolic agents targeting osteoblast and stimulating bone formation and rebuilding bone mass and structure. Although there are several agents available for the treatment of bone disorders, there are limitations for each of them such as safety, convenience of administration, compliance, etc. Therefore, there is still a great medical need for agents that have long-term efficacy, ease of administration, and minimal safety concern, particularly, agents that can stimulate bone formation, restore bone mass and rebuilt bone structure that leads to reduce the risk of skeletal fracture and improve bone healing.
Recent research reveals that the Wingless-type MMTV integration site (Wnt) pathway plays an important role in skeletal metabolism, particularly in bone formation and regeneration. For example, knockout of Lrp5/6, co-receptors for Wnts, decreased bone mineral density (BMD), while gain of function mutation of Lrp5 increased BMD in rodents and human. Secreted inhibitors of Wnt such as Dickkopf-1 (Dkk-1) and sclerostin (Scl) bind to co-receptors Lrp5/6 and inhibit Wnts from association with Lrp5/6, while secreted Frizzled-related proteins such as cerberus and Wif-1 directly interact with Wnts and Frizzled receptors to interrupt binding of Wnts to Lrp5/6. Scientific evidences showed that over-expression of Scl induced lower BMD via lower bone formation, while deletion of Scl induced higher BMD via higher bone formation in mice. Higher BMD occurred in human lacking Scl caused by the mutation in SOST gene. These evidences support the conclusion that inhibition of Scl promotes bone formation and increases BMD. Therefore, monoclonal antibody neutralizing Scl may be an attractive therapeutic agent for treatment of skeletal disorders such as osteoporosis and bone healing. We have performed a series of preclinical studies and showed that sclerostin antibody (Scl-Ab) increases bone formation, decreases bone resorption, increases bone mineral density and bone strength in animal models (mouse, rat, and nonhuman primate) of osteoporosis and fracture healing. This presentation will summarize the effects of sclerostin antibody in treatment of conditions associated with low bone mass (i.e. osteoporosis) and bone regeneration (i.e. bone fracture repair).

IS18

Osteoblast suppression in multiple myeloma: opportunities for therapeutic intervention

Peter Croucher (Australia)

Tuesday 4th September 2012
Session 6A: Bone and Cartilage (ANZORS Joint Session)

IS19

The subchondral bone of osteoarthritis

David Hunter
Professor of Medicine at University of Sydney and Staff Specialist Rheumatologist at Royal North Shore Hospital and North Sydney Orthopaedic and Sports Medicine Centre, NSW, Australia

Osteoarthritis (OA) is a condition of the whole joint – not just the cartilage – and it prominently affects the ligaments, tendons, synovium, muscle, fat and bone. As bone adapts to loads by remodelling to meet its mechanical demands, bone alterations likely play an important role in OA development. Changes in the periarticular bone in patients with OA include subchondral sclerosis, marginal osteophytosis, subchondral bone cysts, alterations in subchondral trabecular architecture, advancement of the tidemark and changes in the material properties of the subchondral bone.

Bone remodeling in the OA joint occurs preferentially in subchondral bone. A number of studies have demonstrated that bone volume fraction is increased in the subchondral bone of persons with osteoarthritis and tissue modulus decreased, which leads to reduced stiffness in the subchondral bone and, as a result, increased susceptibility to fatigue failure of the bone. Pathologically these local areas of increased remodelling have been termed bone marrow lesions which play a prominent role in symptom and disease genesis.

Understanding the pathophysiologic sequences and consequences of OA pathology will guide rational therapeutic targeting. Bone plays an incredibly important role in the etiopathogenesis of OA and its symptoms, and should be a more prominent target with regard to therapeutic developments. The above mechanisms provide a variety of potential therapeutic targets for and approaches to inhibiting subchondral bone alterations in OA.

IS20

Osteoporotic agents for osteoarthritis

Graeme Jones

It is clear that osteoarthritis is the end result of many different pathways thus a single therapy is unlikely to be effective. There is increasing evidence in recent years that bone is involved in joint health. In longitudinal studies, bone marrow lesions, tibial bone area, bone attrition and subchondral bone density all predict subsequent cartilage loss and even knee replacement. Thus, agents that affect bone may affect cartilage either directly or indirectly. Vitamin D predicts cartilage loss in longitudinal studies and is currently being evaluated in phase three trials. Multiple studies have shown a reduction in cartilage breakdown markers for hormonal agents, risedronate and strontium ranelate. Alendronate and strontium ranelate have been shown to slow down spinal osteoarthritis while both strontium and zoledronate decrease back pain independent of change in fracture risk and bone density. The key outcome measure for structural trials in knee osteoarthritis is X-ray but, unfortunately, this is very non-specific and has many limitations. Risedronate had no effect on progression but the placebo group did not progress during that trial. Using more stringent X-ray techniques, strontium ranelate was effective at decreasing both pain and X-ray progression although the clinical significance of this remains uncertain. Phenotype specific trials are the way forward. Risedronate prevents bone marrow lesion progression while zoledronate actually decreases pain and bone marrow lesion size if only subjects with bone marrow lesions are studied. Theory suggests that teriparatide may be detrimental but there is limited data. There is currently no data for denosumab but it merits further investigation.

Tuesday 4th September 2012
Session 6A: Bone and Cartilage (ANZORS Joint Session)

IS21

Overloading verses underloading – two sides of the coin for the cause of OA

Prof David Lloyd
Centre for Musculoskeletal Research, Griffith University, QLD, Australia.

Overloading of medial tibiofemoral joint loading is thought to be related to progression and onset of medial tibiofemoral osteoarthritis. But is this view valid? Common belief is that the medial tibiofemoral loading in gait is related to the knee adduction moments recorded why walking. These adduction moments have been related to thinner medial-versus-lateral tibiofemoral cartilage and loss of medial cartilage volume in people with knee osteoarthritis. However, healthy individuals with higher knee adduction moments have thicker medial-versus-lateral cartilage in the tibiofemoral joint. Why does this apparent conundrum exist? Knee adduction moments poorly represent the frequency and magnitude of loading of the tibiofemoral joint in walking. In healthy and ACL deficient people the thickest cartilage regions in the knee are loaded more frequently. Instrumented total knee replacements studies show that the magnitude of medial compartment loading is strongly influenced by knee flexion and adduction moments. Furthermore, the knee muscles in gait contribute to an additional 50% of medial compartment loading, which depends on the muscles' activation and cross-sectional-area. Greater knee muscle cross-sectional-area is related to greater cartilage thickness healthy people. However, knee muscle atrophy is evident in knee osteoarthritis that, in part, is reflected in loss of knee extension strength, which in-turn has been related to faster knee osteoarthritis progression. Further, we have recently shown that faster atrophy of vastus-medialis leads to faster loss of the medial tibiofemoral cartilage. In addition, people with mid-late stage knee OA have preferential activation of lateral-versus-medial muscles at the knee, which may drive the vastus-medialis atrophy. Atrophy and reduced activation of the vastus-medialis may both act to produce less than ideal muscular loading of the medial tibiofemoral joint in daily activities, having a negative effect on the cartilage morphology. The role of loading in aetiology of knee osteoarthritis may need to be reassessed.

Tuesday 4th September 2012
Session 6B: Stem cells

IS22

The biology and clinical application of skeletal stem cells

Gronthos S[1,2].
[1]*Mesenchymal Stem Cell Laboratory, Department of Haematology, SA Pathology Adelaide, South Australia.*
[2]*Centre for Stem Cell Research, University of Adelaide, South Australia.*

The recent impetus in stem cell research has highlighted the potential use of bone marrow stromal stem cells (BMSC) or mesenchymal stem cells (MSC), for tissue regeneration. While little is known about their properties *in situ*, BMSC are thought to possess a "plasticity" that allows them to differentiate into a variety of different stromal cell types including bone, fat and cartilage, and other lineages such as muscle cells and astrocytes. However, studies have shown that only a minor proportion of BMSC clonal cell lines can maintain a primitive multi-potential phenotype following *ex vivo* expansion. Moreover, with successive subculture, progeny of BMSC display a diminished capacity to proliferate and differentiate into various tissues such as bone. This has hampered the use of BMSC in the development of cellular therapies, in particular for skeletal tissue regeneration. Studies examining the growth of cultured BMSC have identified key regulators of stem cell self-renewal and proliferation that could lead to the development of optimal culture conditions that greatly increase the life-span of BMSC ex vivo. Furthermore, the recent finding that human BMSC exhibit immunomodulatory properties has opened up the possibility of using these populations for allogeneic tissue engineering strategies and for the treatment of autoimmune and chronic inflammatory diseases. Understanding the properties and growth requirements of BMSC is a major challenge to the development of therapeutic protocols that maintain and expand primitive MSC populations *ex vivo*, in order to effectively direct and enhance their developmental potential for a range of tissue engineering and gene therapy strategies.

IS23

The application of allogeneic mesenchymal progenitor cells (MPC) in the reconstitution of bone and cartilage

Peter Ghosh
Mesoblast Ltd, Level 39, 55 Collins Street, Melbourne, Victoria 3000, Australia.
Email Peter.Ghosh@ Mesoblast.com

Using monoclonal antibodies which recognize specific antigens on the surface of mesenchymal precursor cells (MPC) that are only expressed during the early stages of their development we have been able to obtain essentially pure cell lines with high plurogenic potential and low immunogenicity from bone marrow aspirates and other sources. These allogeneic cell lines have been evaluated as therapeutics in a wide variety of clinical indications including cardiovascular disorders, rheumatoid and osteoarthritis, degenerative disc disease, long bone fracture and bone non-unions, osteoporosis, macular degeneration and type 2 diabetes.

Here I present some of our studies with MPC on the repair of degenerate discs (DD) and describe some early stage experiments on methods for constituting new disc cartilaginous tissue within the cervical spinal column. Disc degeneration was induced in three adjacent discs (L3 – L5) of the lumbar spines of 24 adult sheep by the injection of Chondroitinase-ABC, an enzyme that depolymerises the proteoglycans of the nucleus pulposus. Three months later, low (0.5 million cells) or high (4 million cells) dose MPC, suspended in a hyaluronan (HA) carrier, were injected into the degenerate L3L4 discs, L4L5 remained untreated and L5L6 received HA alone. Animals were necropsied 3 and 6 months later. However, radiographs and MRI images were taken prior to cABC injections, 3 months post injection and just before necropsy.

At six months post treatment low and high dose MPC injections had restored disc height to within normal control levels whereas HA alone did not. For the high dose MPC-injected discs the recovery of disc height was accompanied by a significant reduction of MRI and histopathology degeneration scores. A phase 2 clinical trial is now in progress to assess the safety and efficacy of MPC for treatment of patients with chronic discogenic low back pain.

Fusion of the cervical spine is generally undertaken for DD. Is it possible to generate a new disc instead? We filled intradiscal spinal fusion cages with collagen sponges impregnated with MPC alone or MPC cryopreserved with the chondrogenic agent Pentosan Polysulfate (PPS). Cages were surgically implanted into the excised disc space of the cervical spines of sheep. Three months later the cages and adjacent vertebral bodies were examined histologically and biochemically. MPC plus collagen favored bone formation, as expected from the success of MPC in spinal fusion, but in the presence of PPS, MPC local cartilage deposition was observed. These latter findings offer a potential means of generating new cartilaginous disc tissue in the cervical spine as an alternative to vertebral fusion.

Tuesday 4th September 2012
Session 6B: Stem cells

IS24
Circulating mesenchymal stem cells in fracture healing
Prof. Gang Li, MBBS, DPhil (Oxon)
The Department of Orthopaedics and Traumatology, Stem Cell and Regeneration Program, School of Biomedical Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong, SAR, PR China.

Mesenchymal stem cells **(**MSCs) have been found in cord blood and peripheral blood (PB) of mammalian species including human, guinea pig, mice, rat, dog, horse and rabbit. The number of MSCs in PB (PB-MSCs) is rare and their biological role was not fully defined. We have found increased numbers of circulating MSCs in peripheral blood in patients with long bone fracture, non-union and in patients with cancers. The number of PB-MSCs was approximately 9 times higher in the cancer patients, suggesting there is systemic recruitment of MSCs during cancer development. We have compared the difference between the circulating MSCs and bone marrow derived MSCs and found that they share similar phenotype in vitro, but the gene expression profile between the two cell populations was significantly different. cDNA microarray analysis and quantitative RT-PCR confirmed 10 genes that are differentially expressed with more than 10 folds difference, such as cellular retinol-binding protein 1 (CRBP1), cadherin 2, bone morphogenetic protein 6 (BMP6), SRY-box containing gene 11 (Sox11), the aquaporin 1 (AQP1), et al. These genes are now being further investigated for their role in MSCs migration, homing and multiple-differentiation potential. In terms of potential clinical implications of PB-MSCs, we have demonstrated that allogenic PB-MSCs enhanced bone regeneration in rabbit ulna critical-sized bone defect model. We also demonstrated that BM-MSCs can be recruited via circulation toward the sites of bone fracture and participate fracture healing in rabbits. We have demonstrated that systemically administrated allogenic MSCs could home to fracture sites and promote fracture healing. In conclusion, PB-MSCs are new cell source of cells that may play very important roles in development, repair and disease progression.

Tuesday 4th September 2012
Session 7: Young Investigator Award Session

OR7

The cathepsinK inhibitor KK1-300-01 prevents bone destruction and resumes bone formation in myeloma osteolytic lesions

Watanabe K[1,2], Abe M[1], Mori H[3], Amachi R[1,2], Hiasa M[1,2,4], Harada T[1], Fujii S[1], Nakamura S[1], Miki H[1], Kagawa K[1], Endo I[1], Tanaka E[2], Matsumoto T[1]
[1]Department of Medicine and Bioregulatory Sciences, the University of Tokushima. [2]Department of Orthodontics and Dentofacial Orthopedics, the University of Tokushima. [3]Discovery Research Laboratories, Minase Research Institute, Research Headquarters, Ono Pharmaceutical Co., LTD. [4]Department of Biomaterials and Bioengineerings, the University of Tokushima.

Multiple myeloma (MM) enhances osteoclastogenesis while suppressing osteoblastogenesis to develop devastating bone destruction. Unlike other anti-resorptive agents, cathepsin K inhibitors potently suppress bone resorption while sparing a cytotoxic damage in osteoclasts (OCs). In the present study, we aimed to clarify the therapeutic impact of the cathepsin K inhibitor KK1-300-01 (KK1) on a bone disease in MM. KK1 potently suppressed the pit formation enhanced in the cocultures of rabbit bone cells with MM cells. However, KK1 did not impair the OC viability, and allowed OCs to enhance mineralized nodule formation by cocultured MC3T3-E1 cells. We next examined the therapeutic effects of KK1 oral dosing in human INA6 MM-bearing SCID-rab models, which exhibit tumor progression with osteolytic lesions in implanted rabbit bones. KK1 prevented bone destruction with marked increase in bone trabecular size in the rabbit bones and tumor reduction within the bone marrow cavity but not outside the bones. Histological analyses also showed increased bone volume/total volume with a marginal change in OC numbers in the treated mice. Terminally differentiated bone-forming osteoblasts have been demonstrated to directly induce apoptosis in MM cells in contrast to their precursor bone marrow stromal cells. Given OC-derived “coupling factors”, KK1 is suggested to spare the damage in OCs while inhibiting bone resorption to retain the “coupling factors” for bone formation together with reducing the release from bone of anti-anabolic factors such as TGF-beta, leading to robust bone formation and resultant tumor contraction in bone.

OR8

Small GTPase Cdc42 is essential for chondrocyte differentiation and interdigital programmed cell death during limb development

Aizawa R[1, 2], Yamada A[1], Suzuki D[1], Iimura T[3], Yamamoto G[4], Yamaguchi A[3], Yamamoto M[2] and Kamijo R[1]
Departments of [1]Biochemistry, [2]Periodontology and [4]Oral Pathology and Diagnosis, School of Dentistry, Showa University.
[3]Global Center of Excellence (GCOE) Program; International Research Center for Molecular Science in Tooth and Bone Diseases, Section of Oral Pathology, Tokyo Medical and Dental University.

Cdc42, a member of the Rho subfamily of small GTPases, has multiple roles in cellular functions, including cytoskeletal organization, proliferation, and apoptosis. However, its tissue-specific roles, especially in mammalian limb development, remain unclear. To investigate the physiological function of Cdc42 during skeletal development, we generated limb bud mesenchyme-specific inactivated Cdc42 mouse model (*Cdc42$^{fl/fl}$; Prx1-Cre*), since *Cdc42* null embryos (*Cdc42$^{-/-}$*) die before embryonic day 7.5.
Although most of the *Cdc42$^{fl/fl}$; Prx1-Cre* neonates were viable at birth, more than 90% died within a few days. Anatomical analyses demonstrated that they had short limbs and body, abnormal calcification of the cranium, cleft palate, disruption of the xiphoid process, and syndactyly. Severe defects were also found in long bone growth plate cartilage, characterized by loss of columnar organization of chondrocytes, as well as thickening and massive accumulation of hypertrophic chondrocytes, resulting in delayed endochondral bone formation associated with reduced bone growth. *In situ* hybridization analysis revealed *Col10* and *Mmp13* expressions were reduced in non-resorbed hypertrophic cartilage, indicating that deletion of Cdc42 inhibited terminal differentiation of chondrocyte. Syndactyly in *Cdc42$^{fl/fl}$; Prx1-Cre* mice was caused by metacarpal fusion and a failure of interdigital programmed cell death. Whole mount *in situ* hybridization analysis of limb buds revealed that *Sox9* was ectopically expressed in the region between the second and third digits, while expressions of *Bmp2*, *Msx1*, and *Msx2*, known to promote apoptosis in interdigital mesenchyme, were down-regulated.
These results demonstrate Cdc42 is essential for chondrogenesis and interdigital programmed cell death during limb development.

Tuesday 4th September 2012
Session 7: Young Investigator Award Session

OR9

Direct effect of sclerostin on the mechanical loading response in bovine bone

Khalid KA[1,2] and Kogawa M[1], Wijenayaka AR[1], Findlay DM[1] and Atkins GJ[1]
[1]*Bone Cell Biology Group, Discipline of Orthopaedics & Trauma, School of Medicine, Faculty of Health Sciences, University of Adelaide, South Australia, Australia*
[2]*Faculty of Medicine, International Islamic University Malaysia, Kuantan, Malaysia*

Sclerostin is expressed exclusively by mature osteocytes in bone. Our recent findings indicate that sclerostin targets pre-osteocytes/osteocytes to regulate bone mineralisation(1), osteoclast activity(2), and, potentially, osteocytic osteolysis(3). Sclerostin expression *in vivo* is associated with the osteocyte response to mechanical loading/unloading. The aim of this study was to examine the effects of sclerostin on loading-induced bone growth *ex vivo*. For this, 10x5mm bovine sternum 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 (300 cycles, 4000 μstrain, 1 Hz/day), b) identical loading regime 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. Histomorphometric assessment, including fluorochrome labelling analysis, was made at the end of the experiment.
Bone stiffness increased with mechanical loading but this was blocked by the addition of sclerostin. Media pH decreased and ionic calcium concentrations increased in the presence of sclerostin. Sclerostin also completely abrogated loading-induced calcium/calcein uptake, together suggesting that an osteocyte/osteoclast response to sclerostin was responsible for these effects. Our results are the first direct evidence for a negative effect of sclerostin on the anabolic response to mechanical loading.

1. *Atkins et al. J Bone Miner Res. 2011 26(7):1425-36.*
2. *Wijenayaka et al. PLoS One. 2011;6(10):e25900.*
3. *Kogawa M. et al. Abstract at this meeting.*

OR10

Profiling SNX proteins in bone identifies sorting nexin 27 (SNX27) as a crucial modulator of skeletal homeostasis

Chan A[1], Landao E[1], Loo LS[2], Zheng MH[1], Hong WJ[2] and Pavlos NJ[1]
[1] *Centre for Orthopaedic Research, Schools of Surgery, The University of Western Australia, Nedlands, Western Australia.*
[2] *Institute of Molecular and Cell Biology, A*STAR, Proteos Building, Singapore*

During bone growth and remodelling resident bone and cartilage cells are required to rapidly synthesize and transport proteins, macromolecules and other endocytosed materials from membrane-delimited organelles to their correct destinations in order to maintain their functional differentiation and preserve skeletal homeostasis. In eukaryotes, protein trafficking is governed by sets of evolutionary conserved protein families among which members of the Phox (PX) domain-containing sorting nexins (SNXs) are rapidly emerging as key regulators of endocytic transport. However, the importance of SNX proteins in bone cell function and skeletal development remains unknown. To begin to address the contribution of SNX proteins in bone we have systematically screened the expression and subcellular localization profiles of SNX proteins in major bone and cartilage cells. By combining quantitative-PCR with immunoanalyses we demonstrate that SNX proteins are highly yet differentially expressed in osteoclasts, osteoblasts, osteocytes and chondrocytes. Consistent with their role in facilitating endosomal trafficking, SNX proteins co-localize with prototypical markers of early endosomes and the retromer-complex. Moreover, genetic ablation of SNX27 in mice results in severe growth retardation and lethality by 4-weeks attesting to the vital importance of this trafficking protein family in skeletal homeostasis. Micro-computed tomography (CT) scans of 4-week-old SNX27-/- mice revealed drastic reductions in bone mineral density and total bone volume, consistent with a phenotype of chondrodysplasia. Histological assessment showed growth-plate disorganization, chondrocyte hyperprofileration and reduced proteoglycan levels as revealed by safranin-O staining. Collectively, these data posit an essential role for SNX proteins in the maintenance of bone cell function and skeletal homeostasis.

Tuesday 4th September 2012
Session 7: Young Investigator Award Session

OR11

Hypertension and fracture risk in postmenopausal women

Yang S[1,2], Nguyen ND[1], Center JR[1,2,3], Eisman JA[1,2,3] and Nguyen TV[1,2]
[1]*Osteoporosis and Bone Biology Research Program, Garvan Institute of Medical Research;*
[2]*School of Public Health & Community Science, Faculty of Medicine, University of New South Wales;*
[3]*St Vincent's Hospital, Sydney, Australia*

Although hypertension has been suggested to be associated with increased fracture risk, it is not clear whether the association translates into increased fracture risk independent of BMD. The present study sought to examine the interrelationships between hypertension, BMD and fracture risk in postmenopausal women.
The study included 2285 women aged 50 years who were participants in the Dubbo Osteoporosis Epidemiology Study. Baseline characteristics of participants were obtained at the initial visit (1989-1993). Bone mineral density (BMD) at the femoral neck and lumbar spine was measured by dual energy X-ray absorptiometry (GE-LUNAR Corp, Madison, WI). The presence of hypertension was ascertained by direct interview and verification through clinical history. The incidence of fragility fractures was ascertained during the follow-up period (1989-2008). The Cox proportional hazards models were used to assess the association between hypertension and fracture risk.
Overall, 308 women (19%) had a current and past history of hypertension. Compared with those without hypertension, hypertension was associated with lower BMD at the femoral neck (0.80 versus 0.85 g/cm^2, P<0.0001) and lumbar spine (1.06 versus 1.10 g/cm^2, P=0.0004), and higher fracture risk (hazard ratio [HR]:1.23; 95%CI:1.01-1.50). Hypertension has stronger effect in determining hip fracture (HR:1.54; 95%CI:1.01-2.37) than vertebral fracture (HR:0.95; 95% CI:0.69-1.30). However, the association between hypertension and fracture risk was no longer statistically significant after adjusting for femoral neck BMD (HR: 1.20; 95% CI: 0.93-1.54), age and other risk factors.
These results suggested that hypertension is associated with increased fracture risk in postmenopausal women and the association was likely mediated through lower bone mineral density. Given previous findings that the use of beta-blockers reduced fracture risk, the present results also suggest that a link between hypertension and osteoporosis is evidently possible.

Tuesday 4th September 2012
Session 7: Young Investigator Award Session

OR12

Rapid loss of cortical bone occurs in paretic limbs within six months of stroke

Borschmann K[1, 2], Ghasem-Zadeh A[3], Iuliano-Burns S[3], Pang MYC[4] and Bernhardt J[1,2]
[1] Stroke Division, Florey Neuroscience Institutes, [2] School of Physiotherapy, LaTrobe University, [3] Department of Endocrinology, University of Melbourne and [4] Department of Rehabilitation Sciences, Hong Kong Polytechnic University.

Background and aims: Substantial bone loss and structural deterioration occurs, and risk of fracture increases 7-fold 12 months after stroke. However, acute changes to bone structure have not been described. The aim of this ongoing prospective study is to determine the timing and magnitude of loss of bone mass and structure soon after stroke.
Methods: Twenty patients (median age 65.4 years, IQR 61-76, 45% female) without previous stroke or diabetes, who were medically stable and unable to walk within one week of hemispheric stroke were recruited from the Austin Hospital, Heidelberg. Total body bone densitometry and distal radii and tibiae micro-architectural assessments were undertaken by DXA and HR-pQCT (Xtreme, Scanco) respectively, within two weeks and six months after stroke.
Results: Total bone mineral density (BMD) in paretic and non-paretic legs (n=15) was reduced by 3.5% (95% CI: -5.9 to -1.1, p= 0.006) and 2.4% (-3.4 to -1.4, p < 0.001) respectively. In paretic tibiae (n=14), reductions in total vBMD (-2.3%, CI:-3.8 to -0.9 p=.007), cortical vBMD (-1.9%, CI: -3.1 to 0.6, p =.009), and cortical thickness (-6.5%, CI: -10.4 to -2.6, p=0.005) were observed. In the paretic radii (n=7), cortical vBMD decreased by 1.6% (-2.6 to -0.6, p=0.022). Non-significant losses (p>0.05) were observed in paretic radii total vBMD (-1.9%), trabecular vBMD (-0.6%) and cortical thickness (-3.9%). No changes to bone were observed at non-paretic arms.
Conclusion: Significant loss of cortical bone occurs in the paretic limbs within six months of stroke. Interventions to prevent this loss are warranted.

Tuesday 4th September 2012
Session 7: Young Investigator Award Session

OR13

EphrinB2/EphB4 signalling is required for anabolic actions of parathyroid hormone

Takyar FM[1,2], Tonna S[1,2], Ho PWM[1], Crimeen-Irwin B[1], Brennan HJ[1], Baker EK[1,2], Martin TJ[1,2] and Sims NA[1,2]
[1]*St. Vincent's Institute of Medical Research, Melbourne, Australia*
[2]*Department of Medicine at St. Vincent's Hospital, University of Melbourne, Australia*

Previous reports indicate that ephrinB2 expression by osteoblasts is stimulated by parathyroid hormone (PTH) and its related protein (PTHrP), and that ephrinB2/EphB4 signalling between osteoblasts and osteoclasts stimulates osteoblast differentiation while inhibiting osteoclast differentiation.
To determine the role of the ephrinB2/EphB4 interaction in the skeleton, we used a specific inhibitor, soluble EphB4 (sEphB4) *in vitro* and *in vivo*. 8-week-old male C57Bl/6 mice were treated with sEphB4 (10mg/kg; 3/week), either alone or with PTH (30ug/kg/day; 5/week) for 4 weeks. Femora, vertebrae and tibiae were analyzed by micro-CT and histomorphometry.
sEphB4 inhibited EphB4 and ephrinB2 phosphorylation in osteoblasts *in vitro*, and reduced mRNA levels of late markers of osteoblast/osteocyte differentiation (osteocalcin, sclerostin) while substantially increasing RANKL. sEphB4 treatment *in vivo* in the presence and absence of PTH increased osteoblast formation and mRNA levels of early osteoblast markers (alkaline phosphatase, collagen 1α1), but surprisingly, did not increase bone formation rate. Rather, in the presence of PTH, sEphB4 treatment significantly increased osteoclast formation (by 50%, $p<0.05$), an effect that prevented the anabolic action of PTH, causing instead a 20% decrease in trabecular number. This effect of sEphB4 on osteoclast formation was reproduced *in vitro*, but only in the presence of osteoblasts either because of increased RANKL or by preventing ephrinB2 inhibition of osteoclasts.
These data indicate that ephrinB2/EphB4 signaling within the osteoblast lineage is required for late stages of osteoblast differentiation and restricts the ability of osteoblasts to support osteoclast formation. These findings indicate a key role for ephrinB2/EphB4 interaction within the osteoblast lineage.

OR14

Depletion of macrophages significantly impairs endochondral fracture healing

Wu AC[1], Alexander KA[1], Kaur S[1], Wullschleger ME[2], Steck R[3], Gregory L[3], Raggatt LJ[1], Pettit AR[1]
[1.] *UQ-Centre for Clinical Research, The University of Queensland, Herston, Australia*
[2.] *Royal Brisbane and Women's Hospital, Herston, Queensland, Australia.*
[3.] *Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia*

We have previously demonstrated that osteal macrophages (osteomacs) are vital participants during *in vivo* intramembranous bone healing in a tibial injury model. We investigated macrophage and osteomac participation in a clinically relevant fracture model that repairs via endochondral callus formation using an internally fixed femoral fracture (MouseFix). Immunohistochemistry over a healing time course demonstrated that macrophages are widely distributed throughout the fracture site during all healing phases, and include osteomacs at collagen type I deposition sites. Mafia transgenic mice were used to conditionally deplete myeloid/macrophage cells by synthetic AP20187 ligand delivery. Fractured Mafia mice were injected with the depleting ligand or vehicle at the time of surgery or delayed until 5 days post surgery, coinciding with the transition from inflammatory to anabolic healing stages. Tissues and bone marrow were collected 10-13 days post surgery, a time at which robust periosteal soft callus had formed with partial conversion to hard callus in control animals. Flow cytometry of contralateral limb bone marrow confirmed significant $F4/80^+$ macrophage depletion. In macrophage depleted mice callus formation was either abolished when ligand was injected at the time of surgery (n=6) or significantly reduced when ligand delivery was delayed (n=8). A significant positive correlation was observed between callus size and residual $F4/80^+$ macrophage number. These data confirm the essential role of myeloid/macrophage cells in the generation of appropriate inflammatory events essential for initiating fracture repair. They also implicate macrophages/osteomacs as important participants in promoting endochondral ossification during periosteal callus formation in a clinically relevant fracture model.

Wednesday 5th September 2012
Session 9: Greg Mundy Lecture

IS27

"Sex steroids and cortical versus trabecular bone accrual and maintenance: a tale of two cities"

Stavros C. Manolagas, M.D., Ph.D.,
Center for Osteoporosis and Metabolic Bone Diseases, Univ. Arkansas for Medical Sciences, and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA.

The detection of the estrogen receptor (ER) α in osteoblasts and osteoclasts over twenty years ago suggested that cell autonomous effects of estrogens on both of these cell types are responsible for their beneficial effects on the skeleton. Nonetheless, the role of the ERα in cells of the osteoblast lineage has remained elusive. In addition, evidence from mice with targeted deletion of ERα from cells of the osteoclast lineage indicates that activation of the ERα in osteoclasts by estrogens can only account for the protective effect of estrogens on the cancellous, but not the cortical bone compartment that represents 80% of the entire skeleton. More recent results from mice with targeted ERα deletion at different stages of the osteoblast lineage, show that the ERα in osteoblast progenitors expressing Osterix1 potentiates Wnt/β-catenin signaling, thereby increasing proliferation and differentiation of periosteal cells, and is required for optimal cortical bone accrual at the periosteum. Strikingly, however, this function does not require estrogens. In addition, the osteoblast progenitor ERα mediates a protective effect of estrogens against endocortical, but not cancellous, bone resorption. ERα in mature osteoblasts or osteocytes does not influence cancellous or cortical bone mass. In support of the evidence that different mechanisms are involved in cancellous *versus* cortical bone loss following loss of estrogen, deletion of RANKL in B cells (but not T cells) prevents cancellous, but not cortical, bone loss in ovariectomized mice. Furthermore, an estrogen dendrimer conjugate (EDC), incapable of stimulating nuclear-initiated actions of ERα, is as potent as 17β-estradiol in preventing the effects of ovariectomy on cortical, but not cancellous, bone; without affecting the uterus. These findings reveal that the ERα plays essential roles in the accumulation and maintenance of bone mass via cell-autonomous actions in both osteoblast progenitors and osteoclasts. However, the ERα in osteoblast progenitors promotes bone formation at the periosteal surface of the cortex and prevents resorption at the endocortical surface, whereas the ERα in osteoclasts prevents resorption of cancellous bone. The role of ERα in osteoblast progenitors is further characterized by ligand-mediated (both genotropic and non- genotropic) as well as ligand-independent actions. The latter is likely important for the increased bone formation in response to strains from surrounding tissues and gravity, and thus the mechanical adaptation of the skeleton.

Wednesday 5th September 2012
Session 10A: Genetic susceptibility of skeletal diseases

IS28

High resolution genome analyses for studies of genetic regulation of bone mass and structure

Wilson SG[1,2,3]
[1]*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia.* [2]*School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia.* [3]*Department of Twin Research and Genetic Epidemiology, King's College London, London, United Kingdom.*

Genome-wide association studies have been highly successful in identifying loci associated with traits relevant to complex genetic diseases, including BMD and fracture. However, a substantial portion of the genetic variance for osteoporosis-related phenotypes remains unexplained. There is growing evidence that aside from common single nucleotide polymorphisms, other types of genetic variation such as genomic structural variants (including copy number variation (CNV)), sequence insertions and deletions (InDel) and rare single nucleotide variants may contribute to the aetiology of complex diseases, including osteoporosis. Furthermore, recent genome-wide studies using exome or whole genome sequencing have revealed a surprisingly large number of variants that are predicted to disrupt the function of protein-coding transcripts, even in apparently healthy individuals. Many of these nonsynonymous coding changes and frame shift InDels occur at low frequencies and so have previously been difficult to study. In comparison, CNV can be abundant in apparently healthy individuals, but nevertheless may strongly affect dosage-sensitive genes. We characterised a variety of genetic variants including nonsynonymous coding changes, InDels and CNVs through a series of genome-wide studies. Techniques used for these high resolution genome analyses included CNV detection using fluorescence intensity data from high density arrays, exome sequencing and low coverage whole genome sequencing. These experiments inform on patterns of rare variant diversity observed amongst study subjects, optimal approaches for statistical analysis of rare variants and possible roles of these genomic variants in the regulation of bone mass and structure.
In summary, rare variants have been hypothesized to explain a substantial fraction of the heritability of common complex diseases and early data provides evidence that these variants are likely to be relevant. However as whole genome sequencing has become a practical reality there is also an indication that even utilising sophisticated analytical approaches such as rare variant super-locus collapsing or kernel association methods which improve statistical power by pooling rare variants within a gene, large sample sizes are still likely to be required to deliver robust associations.

Wednesday 5th September 2012
Session 10A: Genetic susceptibility of skeletal diseases

IS29

Susceptibility of osteoarthritis

Jiang Qin
Director of Joint Center Drum Tower Hospital Medical School of Nanjing University Nanjing, P.R China

Osteoarthritis (OA) is a polygenic skeletal disease. By using association studies, researchers all over the world have investigated many OA susceptibility genes. Large scale of study subjects, multiple population replication and functional assay was important for identifying the real OA susceptibility gene. Genome wide association study (GWAS) is a powerful tool to find the susceptibility gene of OA. But few global OA susceptibility genes were identified. The odds ratio of OA susceptibility gene found in GWAS was relatively low. Potential patients in control subjects and secondary OA patients make the association study difficult. Multiple etiology of OA and variant recruiting criteria also make the association study difficult, especially in replication studies. Strict recruiting criteria of study subjects and stratification of OA patients will be helpful to increase the odds ratio and significance of the association study. It will also be helpful in replication of the OA susceptibility genes.

IS30

Peripheral arterial disease is a risk for hip fracture in older men

Zoë Hyde, Kurian J Mylankal, Graeme J. Hankey, Leon Flicker, Paul E Norman

Purpose: It is uncertain whether peripheral arterial disease is associated with an increased risk of subsequent hip fracture. The aim of the present study was to assess this in a large cohort of men aged 65 years and over.
Methods: Claudication was assessed by means of the Edinburgh Claudication Questionnaire in 12,094 men, and the Ankle Brachial Index (ABI) was measured in 4,321 of these men. Hospitalisations with hip fracture were identified by record linkage. The association between both claudication and an ABI <0.9 and subsequent hip fractures was assessed using survival curves and Cox regression models.
Results: Amongst the 12,094 men, the baseline prevalence of claudication according to the ECQ was 5.3%. Amongst the 4,321 men with ABI results, the prevalence of an ABI<0.9 was 11.7%. Of the 506 men with an ABI <0.9, 129 (25.5%) also had claudication. Over a median (range) follow-up of 10.8 (0.3-12.7) years, 343 (2.8%) of the 12,094 men were admitted to hospital with a hip fracture. There was no association between claudication and subsequent hip fractures (HR=0.95; 95% CI 0.60, 1.52). Over a median (range) follow-up of 11.1 (0.06-12.3) years 135 (3.1%) of the 4,321 men with ABI data were admitted to hospital with hip fractures. There was a significant association between an ABI<0.9 and subsequent hip fracture (HR=1.69; 95% CI 1.08, 2.63).
Conclusion: Older men with peripheral arterial disease defined as ABI<0.9 are at increased risk of hip fracture, whereas the symptom of claudication is not an independent predictor of hip fracture.

Wednesday 5th September 2012
Session 10B: Translational oral presentations

OR15

Seminal Vesicle Secretion (SVS) 7 is expressed by mature osteoclasts and acts to regulate osteoclast precursor proliferation, differentiation and bone homeostasis

Cundawan W[1], Tickner J[1], Abel T[3], Chim SM[1], Pavlos N[2], Zheng MH[2] and Xu J[1]
[1]School of Pathology and Laboratory Medicine, The University of Western Australia. [2]Centre for Orthopaedic Research, School of Surgery, The University of Western Australia. [3]Centre for Microscopy, Characterization, and Analysis, The University of Western Australia.

Bone is remodeled throughout life by complementary activities of bone-forming osteoblasts and bone-resorbing osteoclasts. The intercellular communication between osteoblasts and osteoclasts is achieved by the production of various cytokines, growth factors, and proteins produced by these cells. Using subtractive hybridization-based differential screening, SVS7, also known as calcium transport inhibitor (caltrin), was identified to be expressed by mature osteoclasts, not by their precursors or osteoblasts. SVS7 expression is upregulated during RANKL-induced osteoclastogenesis. To investigate the role of SVS7 in bone, we generated global SVS7 knockout mice. By µCT we find that SVS7 KO mice exhibit an osteopenic phenotype. *In vitro* osteoclastogenesis assays revealed that KO bone marrow produced significantly more osteoclasts than that of WT following RANKL stimulation. There was a trend of larger resorption pit/osteoclast in KO osteoclasts compared to WT in bone resorption assays. No significant differences in mineralization and alkaline phosphatase activity between WT and KO bone marrow-derived osteoblasts were observed. Alamar blue proliferation assays on WT and KO bone marrow osteoclast precursors showed a significantly increased proliferation rate after 72hrs of M-CSF stimulation in KO compared to WT, whereas the proliferation rates of WT and KO calvarial osteoblasts remained constant. Intracellular calcium assays revealed that there were more KO osteoclast precursors undergoing calcium oscillation than WT after 24hrs RANKL stimulation. These results indicate that SVS7 is expressed by mature osteoclasts and acts to regulate precursor cell proliferation and differentiation within bone marrow. We posit SVS7 as a novel regulator of bone homeostasis.

OR16

Sclerostin stimulation of osteocytic osteolysis involves expression of carbonic anydrase II

Kogawa M, Wijenayaka AR, Ormsby R, Findlay DM, Atkins GJ
Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide

We previously reported Sclerostin (SCL) has an anti-anabolic action on the pre-osteocyte/osteocyte(1), and a catabolic action through osteoclast formation and activity by osteocytes, via RANKL expression (2). To test whether SCL has a direct catabolic activity on osteocyte itself, we examined the effect of recombinant human SCL (rhSCL) on bone resorptive marker gene expression in human primary pre-osteocytes and mouse osteocyte-like cell line, MLO-Y4. We found rhSCL stimulated the mRNA and protein expression of carbonic anhydrase II (CA2), an enzyme that produces protons. Consistent with this, rhSCL significantly lowered intracellular pH in both cell types and a similar trend was observed for extracellular pH (pHo). To further investigate whether SCL-induced acidification resulted in decalcification of bone, mineralised cultures of human osteocyte-like cells were generated and then treated with rhSCL. Medium from cells treated with rhSCL had higher total calcium compared with untreated cells; this effect was inhibited with the CA inhibitor, acetozolamide. To investigate the specific importance of CA2, we knocked down its expression in MLO-Y4 cells using siRNA. rhSCL treatment had no effect on pHo in CA2 siRNA transfected cells. In addition, media levels of ionised calcium did not change with rhSCL in CA2 siRNA transfected cells on a calcium coated plate, unlike control siRNA transfected cells. Taken together, these results show that SCL can stimulate CA2 expression in osteocyte-like cells, resulting in acidification of extracellular media and calcium release from a bone-like matrix. We conclude that SCL may have a direct catabolic action via induction of osteocytic osteolysis.

1. Atkins GJ, Rowe PS, Lim HP, Welldon KJ, Ormsby R, Wijenayaka AR, et al. Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism. J Bone Miner Res. 2011 Jul;26(7):1425-36.
2. Wijenayaka AR, Kogawa M, Lim HP, Bonewald LF, Findlay DM, Atkins GJ. Sclerostin Stimulates Osteocyte Support of Osteoclast Activity by a RANKL-Dependent Pathway. PLoS One. 2011;6(10):e25900.

Wednesday 5th September 2012
Session 10B: Translational oral presentations

OR17

Central control of bone resorption: Neuropeptide Y, Y6 receptor signalling in the regulation of bone homeostasis

Driessler F[1], Yulyaningsih E[1], Khor EC[1], Enriquez RF[1], Xu J[2], Sainsbury A[1], Eisman JA[1], Herzog H[1] and Baldock PA[1]
[1]*Neuroscience Program, Osteoporosis and Bone Biology, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, Australia.*
[2]*School of Pathology and Laboratory Medicine, University of Western Australia, Perth, Australia.*

The past decade has seen the Neuropeptide Y system identified as a novel regulatory axes between the nervous system and bone cells. Gene knockout studies have shown that signalling through neuropeptide Y receptors in the hypothalamus and osteoblast are required for normal bone formation.
Here, we demonstrate that the lesser characterised Y6 receptor is also an important regulator of bone resorption.
Y6R-deficient mice displayed a significant decrease in whole body and femoral BMD and BMC. In addition, micro-CT analysis revealed that Y6R KO mice showed a significant decrease in trabecular bone volume due to reduced trabecular number. Cortical thickness was also reduced in Y6R KO. Interestingly, Y6R KO mice had reduced body weight associated with increased energy expenditure. Nevertheless, the restoration to WT body weight levels due to fat-feeding failed to rescue the bone phenotype. Bone histomorphometry revealed no changes in osteoblast activity, whereas osteoclast activity was significantly increased in Y6R KO. Despite the lack of Y6R gene expression in osteoclast precursors and mature osteoclasts, *ex vivo* Y6R KO osteoclast cultures showed increased osteoclast number similar to the *in vivo* phenotype.
The deletion of Y6R has uncovered an additional pathway by which the NPY system controls bone homeostasis. Moreover, the Y6R is expressed solely in the hypothalamus and is activated by a different member of the NPY family, pancreatic polypeptide (PP), which is produced by F-cells in the gut. Thus the role of Y6R in bone highlights the multi-level regulation of bone homeostasis by peripheral factors through central relay mechanisms.

OR18

The relationship between cumulative lifetime UV exposure, BMD and vertebral fracture in older adults

Thompson MJW, Dore D, Winzenberg T, Otahal P, Cicolini J and Jones G.
Menzies Research Institute Tasmania, University of Tasmania

Background and aim: While low 25-hydroxyvitamin D levels are associated with increased fracture risk, this reflects only recent sun exposure. The Beagley-Gibson (BG) method utilises microtopographical skin changes to assess cumulative, lifetime UV exposure, with higher BG Grades representing greater exposure. This study aimed to describe the relationship between BG Grade, BMD and vertebral fracture in older adults.
Methods: 868 community-dwelling adults aged 53-83 years had silicon casts taken from the dorsum of both hands and graded by the BG method. BMD was quantified using DXA. Subclinical vertebral wedge deformities and symptomatic vertebral fractures were assessed by DXA and questionnaire, respectively. Associations between BG Grade, BMD and vertebral fracture were determined using linear, Poisson and logistic regression models and adjusted for age, sex, BMI, smoking and BMD.
Results: The relationship between BG Grade, spine and total body BMD and vertebral fracture varied depending upon sex (p=0.019, 0.022 and 0.023, respectively for interactions). In males, increasing Grade was associated with greater total body (β=0.014, p=0.04) and spine (β=0.02, p=0.042) BMD and *more* DXA-detected vertebral deformities (RR=1.19, p=0.014), but not symptomatic vertebral fractures. In females, increasing Grade was associated with a reduction in symptomatic vertebral fractures (OR=0.38, p=0.008), but not BMD or vertebral deformity.
Conclusions: Higher BG Grade was associated with greater BMD and, surprisingly, more vertebral deformities in males, but fewer symptomatic vertebral fractures in females. This reflects an association between outdoor activity, BMD and fracture risk that is most likely modified by sex-specific type of activity and associated trauma risk.

Wednesday 5th September 2012
Session 10B: Translational oral presentations

OR19

Evidence for a specific uptake and storage mechanism for 25 hydroxyvitamin D (25OHD) in skeletal muscle cells

Abboud M[1], Puglisi DA[1], Davies BN[1], Cole L[1], Gordon-Thomson C[1], Fraser DR[2] and Mason RS[1]
[1]*Department of Physiology and the Bosch Institute, University of Sydney.*
[2]*Faculty of Veterinary Science, University of Sydney.*

Relatively little is known about storage of 25OHD and the mechanism for its prolonged residence time in blood. Several lines of evidence led us to propose that skeletal muscle could function as a storage site for 25OHD. In vitro studies investigated (a) the capacity of differentiated C2 murine muscle cells to take up and release 25OHD, in comparison to other cell types and (b) the involvement of the membrane protein megalin in these mechanisms. When C2 cells are differentiated into myotubes, time-dependent uptake of labelled 25OHD is 2- 6 times higher than in undifferentiated myoblasts or non-muscle osteoblastic MG63 cells ($p<0.001$). During *in vitro* release experiments (following 25OHD uptake), myotubes released only 21 ± 6% stored 25OHD after 8h, whereas this figure was 47 ± 8% for myoblasts and 95 ± 2% for osteoblasts ($p<0.01$). Using immunofluorescence, C2 myotubes and primary rat muscle fibres were, for the first time, shown to express megalin and cubilin, endocytotic receptors for the vitamin D binding protein (DBP), which binds nearly all 25OHD in the blood. DBP has a high affinity for actin in skeletal muscle. Incubation of C2 myotubes (for 24h) with receptor associated protein, a megalin inhibitor, led to a 40% decrease in 25OHD uptake ($p<0.01$). A time-dependent uptake of labelled DBP into mature muscle cells was observed by confocal microscopy. These data support the proposal that 25OHD, after uptake into mature muscle cells, is held there by DBP, which has been internalised via membrane megalin and is retained by binding to actin.

Wednesday 5th September 2012
Session 11A: Clinical Science Oral Presentations

OR20

Treatment with the cathepsin K inhibitor odanacatib in postmenopausal women with low bmd: 5 year results of a phase 2 trial

Eisman JA[1,2,3,4], Binkley N[5], Bone H[6], Gilchrist N[7], Langdahl B[8], Resch H[9], Portales JR[10], Denker A[11], Lombardi A[11], Le Bailly De Tilleghem C[12], DaSilva C[11], Rosenberg E[11] and Leung A[11].

[1]Osteoporosis & Bone Biology Program, Garvan Institute of Medical Research, [2]St Vincent's Clinical School, University of New South Wales, [3]School of Medicine, Sydney, The University of Notre Dame Australia, [4]Department of Endocrinology, St Vincent's Hospital, Sydney, [5]University of Wisconsin, Madison, WI, [6]Michigan Bone and Mineral Clinic, Detroit, Michigan, [7]Princess Margaret Hospital, Christchurch, New Zealand, [8]Aarhus University Hospital, Aarhus, Denmark, [9]Medical University Vienna, Vienna, Austria, [10]Pontificia Universidad Católica de Chile, Santiago, Chile, [11]Merck, Sharp, and Dohme, Whitehouse Station, NJ and [12]Merck, Sharp, and Dohme, Brussels, Belgium.

The selective cathepsin K inhibitor odanacatib progressively increased spine and hip BMD over 4 years. Here we report results of 5 years and of cessation. Women (mean age 63 yrs, BMD T-scores -2.0 to -3.5) received weekly placebo or odanacatib 3, 10, 25, or 50mg for 2 years, plus vitamin D3 and calcium. In year 3, women were re-randomized to odanacatib 50mg or placebo. For years 4-5, women receiving placebo or odanacatib 3mg in years 1-2 and placebo in year 3 switched to odanacatib 50mg; others continued year-3 treatments. Assessments were BMD at lumbar spine (primary endpoint) and hip sub-regions, safety and bone turnover markers (BTM). Women who received odanacatib 50mg continuously year 1-5 (n=13) had mean % BMD changes (SE) from baseline of lumbar spine 11.9 (2.1), femoral neck 9.8 (1.9), and total hip 8.5 (1.0). In women switched from odanacatib 50mg to placebo after 2 years (n=14), BMD mean % changes (SE) from baseline were: lumbar spine -0.4 (1.3), femoral neck -1.6 (1.0) and total hip -1.8 (0.8). BTM had geometric mean % changes from baseline (SE): urine NTX/creatinine -67.4 (10.1) and serum BSAP -15.3 (5.9) in women continuously receiving odanacatib 50 mg (n=9-10), and 6.0 (7.6) and -11.9 (3.9) in women switched to placebo after 2 yrs (n=10). Odanacatib was well tolerated. Women who received odanacatib 50mg for 5 years had progressive gains in mean spine and hip BMD and sustained reduction in NTX, with smaller reduction in BSAP. Discontinuation resulted in reversal of BMD gains.

OR21

6 years of treatment with denosumab in postmenopausal women with osteoporosis: Results from the first 3 years of the FREEDOM open-label extension study

Franchimont N[4], Papapoulos S[1], Brown JP[2], Chapurlat R[3], Brandi ML[5], Czerwiński E[6], Krieg M-A[7], Man Z[8], Mellström D[9], Radominski SC[10], Reginster J-Y[11], Resch H[12], Román JA[13], Roux C[14], Daizadeh NS[4], Geller ML[4], Smith S[4], Wagman RB[4], Cummings SR[15] and Bone HG[16]

[1]Leiden University Medical Center, Leiden, The Netherlands; [2]Laval University and CHUQ Research Centre, Quebec City, QC, Canada; [3]INSERM UMR 1033 and Université de Lyon, Lyon, France; [4]Amgen Inc., Thousand Oaks, CA, USA; [5]University of Florence, Florence, Italy; [6]Krakow Medical Center, Krakow, Poland; [7]University Hospital of Lausanne, Lausanne, Switzerland; [8]Centro TIEMPO, Buenos Aires, Argentina; [9]Sahlgrenska University Hospital, Göteborg, Sweden; [10]Universidade Federal do Paraná, Curitiba, Brazil; [11]University of Liège, Liège, Belgium; [12]St. Vincent Hospital, Vienna, Austria; [13]Hospital Universitario La Fe, Valencia, Spain; [14]Paris Descartes University, Paris, France; [15]San Francisco Coordinating Center, CPMC Research Institute, and UCSF, San Francisco, CA, USA; [16]Michigan Bone and Mineral Clinic, Detroit, MI, USA

Aims: The FREEDOM open-label extension trial evaluates denosumab's long-term efficacy and safety for up to 10 years. We report results from the first 3 years, representing up to 6 years of denosumab exposure.

Methods: The extension schedules each subject to receive 60mg denosumab SC Q6M and calcium and vitamin D daily. For these analyses, women from the FREEDOM placebo group received denosumab for 3 years (cross-over) and women from the FREEDOM denosumab group received denosumab for 6 years (long-term).

Results: 4550 (77%) eligible women enrolled. During the first 3 years of the extension, the cross-over group (N=2207) had significant gains in bone mineral density (BMD) (lumbar spine, 9.4%; total hip, 4.8%), similar to those observed in the denosumab group in FREEDOM (lumbar spine, 10.1%; total hip, 5.7%). In the long-term group (N=2343), further significant increases in BMD occurred (cumulative 6-year gains: lumbar spine, 15.2%; total hip, 7.5%). sCTX was rapidly and similarly inhibited after the 1st (cross-over) or 7th (long-term) injection with characteristic attenuation observed at the end of the dosing period. Fracture incidences in the cross-over group were lower than in the FREEDOM placebo group and in the long-term group remained low. Incidences of adverse events (AEs) and serious AEs did not increase over time with denosumab. Two subjects in each group had ONJ per adjudication.

Conclusions: Denosumab treatment for 6 years remained well tolerated, maintained reduced bone turnover, and continued to significantly increase BMD, with a low fracture incidence. The cross-over group reproduced observations in FREEDOM.

Wednesday 5th September 2012
Session 11A: Clinical Science Oral Presentations

OR22

Effects of a specialized school physical education program on bone structure and strength in primary school children: A 4-year cluster randomised controlled trial

Daly RM[1], Ducher G[1], Cunningham RB[2], Hill B[1], Telford RM[3], Eser P[4], Naughton G[5], Seibel MJ[6], Javaid A[7] and Telford RD[8]

[1] Centre for Physical Activity and Nutrition Research, Deakin University, Australia [2] Fenner School of Environment and Society, Australian National University, Australia [3] Centre for Research and Action in Public Health, Department of Health, University of Canberra, Australia [4] Swiss Cardiovascular Centre Bern, University Hospital (Inselspital), Switzerland, [5] Centre of Physical Activity Across the Lifespan, Australian Catholic University, Australia, [6] Bone Research Program, ANZAC Research Institute, The University of Sydney, Australia, [7] The Canberra Hospital, Australia; [8] Clinical Trials Unit, The Canberra Hospital and Medical School, Australian National University, Australia

The aim of this study was to investigate the effects of a specialist taught school physical education (PE) program on bone strength and its determinants in primary school aged children. This was a 4-year cluster RCT involving 365 boys and 362 girls in grade 2 aged 8 years from 29 primary schools in Canberra, Australia. All children received 150 min/week PE from classroom teachers but in 13 schools 100 min/week was replaced by two specialized PE classes (SPE) emphasizing more vigorous exercise and games. Radial and tibial (pQCT, 4% and 66% sites) vBMD, structure and strength and muscle CSA and pubertal status were assessed in grades 2, 4 and 6. In girls, the 4-year gains in mid-radius and mid-tibia cortical area were on average, 9.6% ($P<0.05$) and 5.0% ($P=0.08$) greater in the SPE versus common-practice PE program. In boys, the only positive effect of SPE was on mid-tibia cortical vBMD (2.4% vs 1.3%, $P<0.05$). However, neither of these benefits translated into significant improvements in bone strength in the SPE group. There was also no effect of SPE on trabecular vBMD at any site or muscle CSA. In conclusion, a generalized school-based PE program conducted twice weekly through grades 3-6 (age 8-12) of primary school can have some positive effects on cortical area and density, but these benefits did not translate into significant gains in bone strength. This suggests that targeted bone loading activities may need to be incorporated into PE classes to maximize the skeletal benefits during growth.

OR23

Australian fracture prediction models do not improve hip fracture prediction compared to model using hip DXA aBMD T score values alone

Dhaliwal SS[1], Yu M[2,4], Josh L[2,3], Zhu K[2,3] and Prince RL[2,3]

[1]School of Public Health, Curtin University; [2]Department of Endocrinology & Metabolism, School of Medicine and Pharmacology, University of Western Australia; [3]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [4]Shanghai Zhongshan Hospital, Fudan University, China.

Background: Fracture risk calculators have been developed to improve DXA aBMD structural measures as predictors of future fracture risk. We compared Australian FRAX and the Garvan hip fracture prediction models with a simple hip aBMD T score model using a 10-year cohort study of older women.
Methods: The study population was the CAREES cohort, an ongoing population based cohort study of 1500 women mean age 75 years in 1998. In this analysis we report hip fracture risk prediction in a sub population of 1127 women who had a hip aBMD measurement in 1999, and in whom complete ascertainment of hip fracture incidence over 10 years is available.
Results: The median 10-year hip fracture risk in the in the 68 participants who sustained a hip fracture compare to the 1059 who did not were: FRAX without BMD 4.9% vs 4.1%; FRAX with BMD 2.9% vs 1.9%; Garvan without BMD 6.6% vs 5.6% and Garvan with BMD 6.5% vs 3.9%. The total Hip aBMD T score in the in the 68 participants who sustained a hip fracture compare to the 1059 who did not were -1.65± 0.97 and -1.04 ± 1.01 ($P < 0.001$).
The table below shows the ROC areas under the curve (AUC) and diagnostic measures for each risk model. The Youden index was used to determine the best sensitivity and specificity cut off for each ROC.

Variable	ROC AUC (95% CI)	Cut-off	Sensitivity	Specificity	Likelihood Ratio (+)	Youden Index
FRAX + BMD	0.64 (0.57 - 0.71)	2.65%	55.9%	68.3%	1.76	0.24
GARVAN + BMD	0.64 (0.57 - 0.71)	6.65%	50.0%	73.8%	1.91	0.24
FRAX - BMD	0.56 (0.49 - 0.64)	4.45%	60.3%	56.2%	1.38	0.17
GARVAN - BMD	0.55 (0.47 - 0.63)	7.35%	48.5%	66.3%	1.44	0.15
Total Hip T-score	0.67 (0.60 - 0.73)	-1.60	57.4%	69.4%	1.88	0.27
Femoral Neck T-score	0.65 (0.58 - 0.72)	-1.55	69.1%	53.8%	1.50	0.23
Age	0.58 (0.50 - 0.66)	77.5 yrs	38.2%	81.3%	2.04	0.20

Conclusions: These data show that neither calculator improves on the hip fracture prediction compared to the model using the total hip or femoral neck T score alone.

Wednesday 5th September 2012
Session 11A: Clinical Science Oral Presentations

OR24

Greater reduction of intracortical porosity seen with denosumab than alendronate in the compact-appearing cortex and outer transitional zone

Zebaze RM[1], Libanati C[2], Austin M[2], Bilezikian JP[3] and Seeman E[1]
[1]Austin Health, University of Melbourne, Melbourne, Australia; [2]Amgen Inc., Thousand Oaks, CA, USA; [3]College of Physicians and Surgeons, Columbia University, New York, NY, USA

Objective: Significantly greater gains in volumetric bone mineral density (vBMD) and reductions in modeling and cortical porosity have been reported with denosumab than alendronate.[1,2] Remodeling is surface dependent. Denosumab can inhibit osteoclasts throughout the skeleton without binding to bone matrix. Alendronate's affinity for bone mineral may limit its distribution to deeper skeletal compartments distant from bone surfaces, so that intracortical remodeling may be less inhibited. We hypothesised that denosumab will have greater benefits than alendronate in compact cortical bone but not in trabecular bone.
Methods: Postmenopausal women aged 61±5 years were randomised double-blind to denosumab 60 mg Q6M (N=83), alendronate 70 mg QW (N=82), or placebo (N=82). All received calcium and vitamin D. Trabecular bone volume fraction (BV/TV) and porosity in the compact-appearing and trabecularised cortex (transitional zone) were measured at baseline, 6 and 12 months from distal radius HRpQCT images using software (Strax1.0) that quantifies porosity.
Results: At 12 months, both treatments reduced porosity and increased BV/TV from baseline (Table). Denosumab's effect was twice that of alendronate in the compact cortex (P=0.039) and the outer transitional zone (P=0.008). In the inner transitional zone, a similar trend was observed (P=0.063). The treatments were equally beneficial in trabecular bone (Table).
Conclusions: Superiority of denosumab in cortical bone (80% of the skeleton) could be partly due to its accessibility to intracortical surfaces. Improvements in the outer cortical bone may be associated with improved resistance to bending and compressive strength.

Table: Changes (percentage points difference) from baseline at 12 months

		Porosity		
	Trabecular BV/TV	Inner transitional zone	Outer transitional zone	Compact cortex
Alendronate	+0.19*	–0.81*	–0.86†	–0.54$^{\wedge}$
Denosumab	+0.24*	–1.15*	–1.95*$^{\#}$	–1.22*‡

P value relative to baseline, * <0.001, †<0.005, $^{\wedge}$<0.03
P value relative to alendronate, $^{\#}$0.008, ‡0.039

References:

1. Seeman E *et al. J Bone Miner Res* 2010;25:1886–94; 2. Kendler DL *et al. J Bone Miner Res* 2010;25:72–81

Wednesday 5th September 2012
Session 11A: Clinical Science Oral Presentations

OR25

The effects of pioglitazone, a PPAR-γ agonist, on bone turnover markers, bone mineral density, and vertebral fractures in type 2 diabetes mellitus

Kanazawa I, Yamaguchi T, Yamauchi M, Yamamoto M, Yano S and Sugimoto T
Internal medicine 1, Shimane University Faculty of Medicine, Japan

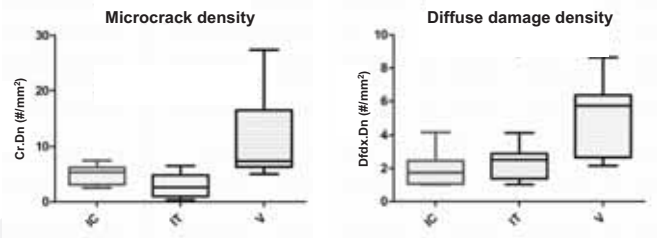
Aim: Although it has been reported that PPAR-γ agonists increase a risk of fractures, it is unclear whether pioglitazone decreases bone mineral density (BMD) and is associated with vertebral fractures in Asian patients with type 2 diabetes as well as what parameter can predict the risk of BMD reduction.
Methods: We conducted a cross-sectional study with 494 Japanese male and 344 female patients and a longitudinal study with 55 patients (pioglitazone n=22 or metformin n=23). BMD at the lumbar spine, femoral neck (F), and one-third of the radius (1/3R), bone markers, and an atherosclerosis parameter, intima-media thickness (IMT), were measured.
Results: In the cross-sectional study, multiple logistic regression analysis adjusted for confounding factors showed that, in postmenopausal women, treatment with pioglitazone was positively associated with the presence of vertebral fractures (odds ratio=3.4, $p<0.04$). In the longitudinal study, serum osteocalcin, F-BMD, and 1/3R-BMD decreased at 6 months in the pioglitazone group ($p<0.05$), while bone markers or BMD at any site were not changed in the metformin group. Multiple regression analysis showed that, in the pioglitazone group, %changes in F-BMD were negatively correlated with baseline IMT ($\beta=-0.7$, $p<0.02$), and %changes in 1/3R-BMD were negatively correlated with baseline urinary NTX and IMT ($\beta=-0.8$, $p<0.01$ and $\beta=-0.5$, $p<0.02$, respectively), and positively with insulin-like growth factor-I (IGF-I) ($\beta=0.7$, $p<0.04$).
Conclusion: These findings suggest that postmenopausal women treated with pioglitazone have a high risk of vertebral fractures and that baseline IMT, urinary NTX, and IGF-I could assess the risk of BMD reduction in the patients.

OR26

Microarchitectural decay and microdamage accumulation in vertebral cancellous bone: a comparative analysis of the iliac crest, proximal femur and vertebral body in the aged postmenopausal skeleton

Kuliwaba JS[1,2,3], Muratovic D[2,3], Parkinson IH[2,3], Carpentier VT[4] and Fazzalari NL[3]
[1]*Adelaide Centre for Spinal Research, SA Pathology and Royal Adelaide Hospital, Adelaide, Australia.*
[2]*Bone and Joint Research Laboratory, Directorate of Surgical Pathology, SA Pathology and Hanson Institute, Adelaide, Australia.*
[3]*Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, Adelaide, Australia.*
[4]*Harvard School of Dental Medicine, USA.*

Aim: The general assumption that changes in bone microstructure and material properties at the iliac crest are representative of skeletal sites that are susceptible to osteoporotic fracture has not yet been addressed. Therefore, our study aim was to perform a comparative analysis of bone microarchitecture, accumulated microdamage and osteocyte morphology between the iliac crest, proximal femur and vertebral body.
Methods: Cancellous bone cores were obtained from the iliac crest, proximal femur (intertrochanteric region) and T12 vertebral body (central region) from seven postmenopausal female cadavers, aged 70-98 years (mean 79±9 years), with no history of disease/medication that may have affected bone turnover. All bone cores were micro-CT imaged for 3D microarchitecture, then divided lengthwise for histomorphometric assessment of microdamage and osteocyte morphology.
Results: BV/TV, Tb.N and DA were lower for vertebral bone compared to iliac crest ($p<0.05$). Other architectural parameters were not different between sites. Microcrack, crack surface and diffuse damage density were higher in vertebral vs. iliac crest and proximal femur bone ($p<0.01$; Figure). Crack lengths were similar between vertebral and femoral bone, with both sites higher vs. iliac crest ($p<0.05$). Osteocyte, empty lacunar and total lacunar densities were not different between sites. Percent empty lacunae was increased in iliac crest vs. femoral bone ($p<0.05$).
Conclusions: There is variability in cancellous bone microarchitecture, damage accumulation and osteocyte morphology between skeletal sites. Vertebral cancellous bone is typified by microarchitectural decay and microcrack and diffuse damage accumulation, suggesting that the vertebral body is structurally and functionally compromised in the aged postmenopausal skeleton.



IC = iliac crest; IT = intertrochanteric region of the proximal femur; V = vertebral body

Wednesday 5th September 2012
Session 11A: Clinical Science Oral Presentations

OR27

Contribution of re-fracture to early fracture-associated mortality

Bliuc D [1], Nguyen ND [1], Nguyen TV [1,2], Eisman JA [1,2] and Center JR [1,2]
Osteoporosis and Bone Biology, Garvan Institute of Medical Research, and St Vincent's Hospital Clinical School, University of New South Wales, Victoria St, Darlinghurst, Sydney, NSW 2010 Australia

Background: Following initial fracture, there is increased risk of re-fracture and premature mortality. The role and timing of the re-fracture in relation to excess mortality is unknown. This study examined premature mortality associated with re-fracture following hip, vertebral and non-hip non-vertebral (NHNV) fractures.
Methods: 1295 fracture subjects aged 60+ from the Dubbo Osteoporosis Epidemiology Study were followed for re-fracture and mortality (1989-2010) using competing risk analyses.
Results: There were 358 re-fractures and 487 deaths in women over 5779 p-yrs and 90 re-fractures and 206 deaths in men over 1886 p-yrs. Following NHNV fracture, half of all subsequent fractures were either hip or vertebral. Most re-fractures and premature mortality occurred in the first 5 years post initial fracture. Five year excess mortality (above that expected for age and sex) was highest for hip fractures (8.1/100 p-yrs for women and 16.7/100 p-yrs for men) followed by vertebral (2.8/100p-yrs for women and 8.4/100 p-yrs for men) and NHNV (1.8/100 p-yrs in women and 3.7/100 p-yrs in men). For hip fracture, 25% (women) and 20% (men) of excess deaths occurred after re-fracture. For vertebral and NHNV fractures, 25-30% of excess deaths followed re-fracture. Population attributable risk of mortality was similar for all fracture types due to larger number of NHNV fractures (13-18% in women and 25-29% in men).
Conclusion: There is high early premature mortality associated with all osteoporotic fractures with 20-30% of excess deaths occurring after re-fracture. This highlights the importance of re-fracture and the urgency for early intervention for all fracture types.

OR28

Strontium ranelate reduces the number of patients with radiological or radioclinical progression in primary knee osteoarthritis

March L[1] on behalf of the SEKOIA study group
[1]*Institute of Bone and Joint Research, Royal North Shore Hospital, St Leonards, NSW, Australia*

Aim: Patients with joint space width narrowing (JSN) ≥0.5mm over 3 years have a higher risk of undergoing any osteoarthritis related surgery(1). Both radiological progression and lack of improvement in symptoms are considered clinically relevant endpoints (2). The objective of this pre-planned analysis was to describe the efficacy of SrRan on radiological and radioclinical progression in osteoarthritis.
Methods: Double-blind, placebo-controlled, randomised, international, 3 year trial. 1683 patients with symptomatic primary knee osteoarthritis were randomly assigned to SrRan 1 or 2 g/day or placebo. Radiological progressors (patients with JSN≥0.5mm between baseline and last observation) and radioclinical progressors (patients with JSN≥0.5mm and lack of clinical improvement in symptoms e.g. ≤20% improvement in WOMAC pain sub-score) were compared in the ITT population.
Results: The ITT population included 1371 patients. Radiological progressors were less frequent in the SrRan 1g/day (22.3%, p<0.001) and 2g/day group (25.6%, p=0.012) versus placebo (33.1%). The RRR (and NNT) compared to placebo were 32.7% (NNT=10) for 1g/day and 22.7% (NNT=14) for 2g/day. Likewise, less radioclinical progressors were observed in the SrRan 1g/day (7.7%, p=0.049) and 2g/day group (6.5%, p=0.008) compared to placebo (11.6%). The RRR (and NNT) compared to placebo were 34.0% (NNT=26) for 1g/day and 44.1% (NNT=20) for 2g/day.
Conclusions: Strontium ranelate reduced the number of knee osteoarthritis patients with a radiological and radioclinical progression, and may have a positive effect in decreasing lower limb surgery.

References:

1 Bruyere et al, Ann Rheum Dis (2005);64:1727-1730
2 Altman et al, Osteoarthritis and cartilage (2005);13:13-19

Wednesday 5th September 2012
Session 11A Clinical Science Oral presentation

OR29

Bone density before and after heart or lung transplantation – a longitudinal study

Wang T[1], O'Sullivan S[2], Gamble G[2] and Ruygrok P[1].
[1]*Green Lane Cardiovascular Service, Auckland City Hospital.*
[2] *Bone and Joint Group, Department of Medicine, University of Auckland.*

Background: Osteoporosis is prevalent amongst heart and lung transplant (HLT) candidates. Bone loss is common following transplant, predominantly in the first year, and associated with an increased fracture risk. There is a lack of consensus regarding optimal management of bone health in HLT recipients. We report data relating to bone health in a cohort of HLT recipients before and after transplant and make recommendations for their management.
Methods: Patients over the age of 20 who had heart or lung transplant between 2000 and 2011 were identified from the New Zealand HLT Service database, and demographic data, immunosuppressive regimens, bisphosphonate use and serial BMD data were obtained from clinical records.
Results: Fifty-two heart and 72 lung transplant recipients had pre-transplant BMD data; 30 and 42 respectively also had post-transplant BMD measurements. The percentages of osteopenia and osteoporosis at any site pre-transplant were 23% and 8% for heart candidates and 36% and 31% for lung candidatesPost-transplant these percentages were similar. Risk factors for reduced BMD pre-transplant were low BMI, low weight, male gender and younger age. Pre-transplant BMD was the major predictor for developing osteopenia or osteoporosis after transplantation.
Conclusion: A significant proportion of HLT recipients have osteopenia or osteoporosis pre- transplant, and this persists after post-transplant. All patients should be screened pre-transplant, and intervention considered for those with osteopenia and osteoporosis. Pre-transplant BMD is an important predictor of subsequent osteopenia or osteoporosis development, allowing risk stratification and targeted interventions in those without osteopenia or osteoporosis.

Wednesday 5th September 2012
Session 11B: Basic Science Oral Presentation

OR30

Notch/Rbpj/Hes1 signal in chondrocytes modulates osteoarthritis development

Sugita S[1], Saito S[1], Hosaka Y[1], Akiyama H[2], Ung-il C[1], and Kawaguchi H[1]
[1] *Sensory and Motor System Medicine, University of Tokyo.*
[2] *Department of Orthopaedic Surgery, Kyoto University.*

Here we examined the mechanism underlying regulation of the physiological and pathological endochondral ossification by the Notch signaling. In cultures of ATDC5 cells and primary chondrocytes, Hes1 was strongly expressed during their differentiation with Notch1, Notch2, Rbpj, while other Hes/Hey family members were little expressed. To further investigation, we conditionally inactivated Rbpj in chondrocytes by creating *Sox9-Cre;Rbpj$^{fl/fl}$* mice. They died shortly after birth and exhibited dwarfism with impaired matrix degradation of the cartilage primordia due to decrease of Mmp13 expression. Overexpression of Notch1 ICD in ATDC5 cells induced expressions of *Mmp13*, and *Hes1*. The Notch1-ICD-induced *Mmp13* expressions were suppressed by the Hes1 knockdown through the siRNA transfection. In promotor of MMP13, deletion and mutagenesis analyses of the luciferase assay identified the core responsive region of Hes1 as an E-box at intron2. To examine the role of Hes1 in physiological endochondral ossification, we conditionally inactivated Hes1 in chondroprogenitor cells by creating *Sox9-Cre;Hes1$^{fl/fl}$* mice, and found that the mice died shortly after birth without skeletal abnormality. We next examined the contribution of Hes1 to OA development by generating tamoxifen inducible conditional knockout mice (*Col2a1-Cre^{ERT};Hes1$^{fl/fl}$*) that grew normally under physiological conditions. When we inactivated Hes1 in the articular cartilage of the *Col2a1-Cre^{ERT};Hes1$^{fl/fl}$* mice (7-week-old) by tamoxifen injection and created the surgical OA model, the knee OA development was suppressed as compared to the control littermates. These results demonstrate that Hes1 is a transcriptional inducer of endochondral ossification during OA development. Hes1 may represent a promising therapeutic target of OA without physiological side effects.

OR31 Outstanding Abstract Award (Basic)

Osteoblastic lineage deletion of gp130 has divergent effects on trabecular and cortical bone

Johnson RW[1], Brennan HJ[1], Poulton IJ[1], McGregor NE[1], Koh T[1], Zainuddin MZ[1], Walker EC[1], Martin TJ[1,2] and Sims NA[1,2]
[1]*Bone Cell Biology and Disease Unit, St. Vincent's Institute of Medical Research, Fitzroy, Victoria, AUSTRALIA*
[2]*Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Melbourne, Victoria, AUSTRALIA*

Glycoprotein-130 (gp130) is a cytokine co-receptor that stimulates osteoblastic RANKL expression, osteoclast formation and bone formation in response to IL-6 family cytokines. Surprisingly, global gp130 deletion enhanced osteoclast formation, but its effects on bone formation was complicated by neonatal lethality and systemic defects. To determine its role in osteoblasts, gp130 was deleted conditionally from early osteoblasts (Osx1Cre) and late differentiated osteoblasts/osteocytes (DMP1Cre) in C57BL/6 mice. Gp130 recombination was verified by real-time PCR.
MicroCT and histomorphometry on 12wk-old male mice revealed a significant reduction in trabecular bone volume (~50%) in tibiae, femora and vertebrae of Osx1gp130f/f and DMP1gp130f/f mice compared with Cre+wt/wt controls. Both KO phenotypes had significant reductions (22-51%) in trabecular number, mineralizing surface and bone formation rate, indicating that osteocytic gp130 signaling is essential for normal bone formation. Although gp130-binding cytokines stimulate osteoclast formation, KO mice had no difference in osteoclast number, surface or size compared to controls, suggesting that osteoblastic gp130 is not required for osteoclast formation.
Cortical bone analysis by microCT revealed significantly increased cortical thickness (4,12%), marrow volume (18,29%) and periosteal surface area (10,17%) in Osx1 and DMP1gp130 KOs (respectively) with no change in bone length, indicating that osteoblastic gp130 may function differently in trabecular and cortical bone.
In conclusion, while osteoblastic gp130 is not required for osteoclast formation, its deletion may allow for compensatory signaling to support osteoclast development. Furthermore, the similarity between Osx1 and DMP1gp130 KO phenotypes indicates that gp130 signaling in late osteoblasts/osteocytes, but not early osteoblasts, is critical for normal bone formation.

Wednesday 5th September 2012
Session 11B: Basic Science Oral Presentation

OR32

Anabolic actions of fasting-induced adipose factor (FIAF) on bone cells

Lin JM, Naot D, Watson M, Costa JL, Grey AB and Cornish J
Department of Medicine, University of Auckland, Auckland, New Zealand

Fat mass and bone mass are positively correlated and adipocyte hormones or adipokines, such as leptin, likely mediate this relationship. This study aims to further explore the underlying mechanism by looking at the bone cell effects of another adipokine, fasting-induced adipose factor (FIAF) and its naturally truncated product coiled-coil domain (CCD).

Our results show that CCD is a potent osteoclast inhibitor as seen in osteoclastogenesis assays using mouse bone marrow and RAW264.7 cell cultures, and in the resorption assay using isolated primary mature osteoclasts. The inhibitory rates by CCD (500 ng/mL) were approximately 90%, 50% and 90% respectively in the above models. It also stimulated osteoblast proliferation by ~30% at this concentration. In comparison, intact FIAF (500 ng/mL) inhibited osteoclastogenesis by ~50% in bone marrow cultures, but was not active in osteoclast resorption and osteoblast proliferation assays.

Consistent with its inhibition on the osteoclasts, CCD greatly reduced the expression of macrophage colony-stimulating factor (M-CSF), nuclear factor of activated T-cells c1 (NFATc1) and dendritic cell-specific transmembrane protein (DC-STAMP), and mildly suppressed the expression of connective tissue growth factor (CTGF) in bone marrow cells. However, it did not change RANKL and OPG levels in the ways supporting its osteoclastic effect.

In conclusion, FIAF/CCD is likely an anabolic factor coupling fat mass and bone mass by indirectly or directly acting on osteoclasts. CCD's action on osteoclasts is independent of RANKL/OPG system, but dependent of M-CSF, NFATc1, DC-STAMP and CTGF pathways.

OR33

Quantitative analysis of the 'Osteoclastome'

Del Frate D[1], Grønborg M[2], Ng PY[1], Cheng TS[1], Zheng MH[1] and Pavlos NJ[1]
[1] *Centre for Orthopaedic Research, Schools of Surgery, The University of Western Australia, Nedlands, Western Australia.*
[2] *University of Copenhagen, NNF Center for Protein Research, Panum Institute, Blegdamsvej 3B, 2200 København, Netherlands*

Osteoclasts are large multinucleated cells whose exclusive function is the resorption of bone. These polarised polykaryons are formed by the fusion of mononuclear precursor cells of the monocyte-macrophage lineage under the aegis of M-CSF and RANKL. During their RANKL-induced differentiation, osteoclasts are endowed with a unique set of specialised machinery which enables them to fuse and resorb bone. While several molecules regulating osteoclast formation and function have been identified in recent years by transcriptional and proteomic profiling, a quantitative and accurate survey of the osteoclast proteome has not been previously achieved. The aim of this study was to quantitatively map the osteoclast proteome (osteoclastome) during its RANKL-driven differentiation. By combining high resolution mass spectrometry with chemical labelling (isobaric tag for relative and absolute quantitation: iTRAQ) at specific time-points of osteoclastogenesis, we identified some 1352 proteins in mature osteoclasts. Of the 1193 quantifiable proteins, 699 were differentially expressed (253/446 up/down-regulated) during the progression and fusion of precursor cells into functionally mature osteoclasts. Functional cluster analysis identified several proteins families who showed distinct expression patterns throughout osteoclast differentiation, including established osteoclast markers (e.g. V-ATPase proton pump subunits) and others whose function has yet to be assigned to the osteoclast. The expression profiles and subcellular localizations of several candidate clusters including rab GTPases and voltage-dependant anion channels (VDAC1-3) were verified by quantitative immunoanalysis and confocal microscopy. Collectively, this study provides the first quantitative map of the osteoclast proteome with insight into the molecular machinery that governs osteoclast formation, fusion and bone resorptive function.

Wednesday 5th September 2012
Session 11B: Basic Science Oral Presentation

OR34

Generation and analysis of the mature osteoblast/osteocyte deletion of VDR: evidence for the direct activity of vitamin D activity in osteoblasts to regulate bone turnover

Anderson PH[1], Atkins GJ[2], Morris HA[1], Davey RA[3]
[1]Musculoskeletal Biology Research, School of Pharmacy and Medical Sciences, University of South Australia, Australia; [2]Bone Cell Biology Group, Discipline of Orthopaedics & Trauma, University of Adelaide, Australia. [3]Department of Medicine, Austin Health, University of Melbourne, Australia

The global VDR deletion mouse model (VDRKO) has previously been shown to demonstrate features typical of vitamin D-dependent type II rickets. It is, however, difficult to use this model to address the direct actions of VDR in bone given that impaired bone remodelling in VDRKO mice may be caused, at least in part, by secondary hyperparathyroidism. Thus, we genetically inactivated the VDR in the mature osteoblastic lineage (ObVDRKO), by crossing floxed-VDR (flVDR) mice with Osteocalcin-Cre mice (Ocn-Cre). Young (6w) male and female ObVDRKO mice demonstrate significant increases in metaphyseal femoral bone volume (1.23xfold, male ObVDRKO $p<0.05$, 1.28xfold, female ObVDRKO, $p<0.05$), due to an increase in trabecular number (Tb.N). ObVDRKO mice also have increased femoral cortical bone volume relative to flVDR controls. In male ObVDRKO animals cortical bone was 11% thicker ($p<0.05$) than those in flVDR controls, whereas in females increased cortical bone is due to an overall 11% increase ($p<0.05$) in tibial cortical area. While whole bone RANKL:OPG mRNA ratio and metaphyseal osteoclast surface (OcS/mm) were unchanged, the levels of RANKL (2-fold) and OPG mRNA levels were markedly (5-fold) reduced. Furthermore, ALP and OCN mRNA levels were all reduced by 4-fold suggesting that bone turnover is markedly reduced, favouring increases in trabecular number and cortical bone volume. Thus, the present study demonstrates that VDR directly functions in mature osteoblasts and/or osteocytes, to control key vitamin D responsive genes involved in regulating bone turnover.

OR35

Wnt5a-Ror2 signaling boosts bone destruction in arthritis

Kobayashi Y[1], Maeda K[2], Udagawa N[3], Uehara S[3], Takada I[4], Kato S[7], Marumo K[2], Nishita M[9], Martin TJ[10], Minami Y[9] and Takahashi N[1]
[1]Institute for Oral Science, [3]Department of Biochemistry, Matsumoto Dental University. [2]Department of Orthopedic Surgery, The Jikei University School of Medicine. [4] Laboratory of Cell and Tissue Biology, School of Medicine, Keio University. [7]Laboratory of Nuclear Signaling, Institute of Molecular and Cellular Biosciences, University of Tokyo. [9]Department of Physiology and Cell Biology, Graduate School of Medicine, Kobe University. [10]St. Vincent Institute of Medical Research.

We have explored roles of the noncanonical Wnt pathway in osteoclastogenesis and found that 1) Wnt5a, a noncanonical Wnt ligand, enhanced RANKL-induced osteoclast formation through receptor Ror2 in osteoclast precursors; 2) Wnt5a promoted RANK expression via Ror2-JNK signaling axis; 3) Wnt5a secreted from osteoblasts enhanced $1,25(OH)_2D_3$-induced osteoclast formation in cocultures of osteoblasts and osteoclast precursors. However, sources of Wnt5a *in vivo* and roles of Wnt5a in arthritis remain to be elucidated. We generated osteoblast-specific Wnt5a conditional knockout mice ($Wnt5a^{DOb/DOb}$) and analyzed their bone phenotype. $Wnt5a^{DOb/DOb}$ exhibited low bone mass due to the reduction of bone formation. Osteoclast number was lower in $Wnt5a^{DOb/DOb}$ mice like $Wnt5a^{+/-}$ mice. These results suggest that Wnt5a secreted from osteoblasts is crucial for osteoclastogenesis in physiological bone remodeling. It has been reported that Wnt5a and Wnt4 are expressed in synovial cells from arthritis. We therefore analyzed roles of Wnt5a in arthritis using GST-sRor2, a soluble decoy receptor of Wnt5a. GST-sRor2 strongly bound Wnt5a and negated the stimulatory effect of Wnt5a on RANKL-induced osteoclastogenesis. GST-sRor2 was administered into type II collagen-induced arthritis (CIA) model mice. mCT analysis showed that the bone destruction was significantly suppressed in hind paws of CIA mice treated with GST-sRor2. Histological analysis revealed that RANK-positive cell number and osteoclast number were significantly reduced in ankle joints of those CIA mice. These results suggest that excess amounts of Wnt5a secreted from synovial tissues boosts osteoclastogenesis in arthritis. Together Wnt5a-Ror2 signaling represents a therapeutic target in inflammatory bone diseases including arthritis.

Wednesday 5th September 2012
Session 11B: Basic Science Oral Presentation

OR36

The potential role of vascular endothelial growth factor (VEGF) in the faulty bony repair of injured growth plate

Chung R[1], Foster BK[2] and Xian CJ[1]
[1]*Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia;* [2]*Orthopaedic Surgery, Women's and Children's Hospital; Adelaide, South Australia 5001, Australia*

In children, the growth plate is responsible for bone growth; yet its injuries can result in unwanted bony repair causing orthopedic problems requiring invasive corrective surgeries. Currently, little is known of underlying mechanisms for the faulty repair. While VEGF-induced angiogenesis is vital in bone fracture repair, its role is unknown in growth plate bony repair. Using a rat tibial growth plate injury model, the potential role of VEGF-mediated angiogenesis during growth plate bony repair was investigated. Immunohistochemical analysis of the growth plate injury site saw VEGF immuno-positive cells present as early as Day5 post-surgery in a rat growth plate injury model. Furthermore, cells positive for endothelial marker isolectin-B4 were also observed, suggesting involvement of angiogenesis starting from the early stage of repair. To investigate roles of VEGF in the growth plate bony repair, rats were administered 2.5mg/kg of clinically used anti-VEGF antibody Bevazicumab or vehicle immediately after growth plate injury and culled on Days 6 and 14 post-surgery for analysis. Following treatment with anti-VEGF, micro-CT results of Day 14 samples revealed a significant reduction in bone volume within the growth plate injury site of anti-VEGF-treated rats compared to vehicle controls ($P<0.05$). In addition, gene expression analysis revealed a decrease in all bone related genes (Runx2, osteocalcin) and interestingly a slight increase in levels of chondrogenic genes (Sox-9, Collagen-II) in treated rats compared to vehicle controls. These data demonstrated the importance of VEGF-mediated angiogenesis during the unwanted bony tissue repair following growth plate injury.

OR37

The endoplasmic reticulum stress transducer BBF2H7 suppresses apoptosis by activating the ATF5-MCL1 pathway in chondrogenesis

Izumi S[1,2], Saito A[1], and Imaizumi K[1]
[1]*Department of Biochemistry, Institute of Biomedical & Health Sciences, University of Hiroshima*
[2]*Department of Orthopedic Surgery, Faculty of Medicine, University of Hiroshima*

A number of cellar stress condition leads to the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), constituting a fundamental threat to the cell. This type of the stress is called ER stress. Previously, we identified the novel ER stress transducer BBF2H7. This molecule is a basic leucine zipper transmembrane transcription factor belongs to CREB/ATF family transcription family. *Bbf2h7*-deficient (*Bbf2h7*-/-) mice exhibited the severe chondrodysplasia caused by down-regulation of Sec23a, which is responsible for protein transport from the ER to Golgi and one of the BBF2H7 target gene in chondrocytes (Saito et al, Nat. Cell Biol., 2009). We found a decrease in the number of proliferating chondrocytes in the cartilage of *Bbf2h7*-/- mice. TUNEL staining of the cartilage showed that apoptosis was promoted in *Bbf2h7*-/- chondrocytes. *Activating transcription factor 5* (*Atf5*), another member of the CREB/ATF family and an antiapoptotic factor, was found to be another target of BBF2H7 in chondrocytes. ATF5 activated the transcription of Myeloid cell leukemia sequence 1 (Mcl1), belonging the antiapoptotic B-cell leukemia/lymphoma 2 family, to suppress apoptosis. Finally, we found that the BBF2H7-ATF5-MCL1 pathway specifically suppressed ER stress-induced apoptosis in chondrocytes. Taken together, our findings indicate that BBF2H7 is activated in response to ER stress caused by synthesis of abundant ECM proteins, and plays crucial roles as a bifunctional regulator to accelerate ECM protein secretion and suppress ER stress-induced apoptosis by activating the ATF5-MCL1 pathway during chondrogenesis. (235/250字)

Wednesday 5th September 2012
Session 11B: Basic Science Oral Presentation

OR38

RelB attenuates the activation of the classical NF-kB pathway to facilitate osteoblastogenesis

Hiasa M[1, 2, 3], Abe M[1], Arakaki R[2], Yamamoto A[2], Asaoka K[3], Matsumoto T[1], Hayashi Y[2], and Ishimaru N[2]
[1]Department of Medicine and Bioregulatory Sciences, The University of Tokushima Graduate School. [2]Department of Oral Molecular Pathology, The University of Tokushima Graduate School. [3]Department of Biomaterials and Bioengineering, The University of Tokushima Graduate School.

Although the activation of the classical NF-kB pathway suppresses bone formation, the role of the non-classical NF-kB pathway in osteoblastogenesis remains largely unknown. In the present study, we therefore explored the role of the non-classical NF-kB subunit relB in the classical NF-kB signaling activation and osteoblastogenesis. TNF-a suppressed mineralized nodule formation by MC3T3-E1 preosteoblastic cells and bone marrow stromal cells at 100 pg/ml but not at 10 pg/ml. Interestingly, TNF-a was able to suppress mineralized nodule formation even at 1 pg/ml by these cells upon treatment with relB siRNA or by bone marrow stromal cells from $relB^{-/-}$ mice, suggesting the potentiation of the suppressive effects of TNF-a on bone formation in the absence of relB. Furthermore, the relB knockdown facilitated the nuclear localization of the classical NF- NF-kB subunits p65/p50 and their DNA binding in MC3T3-E1 cells in the presence of TNF-a at 10 pg/ml. However, inhibition of the classical NF-kB pathway by IMG-2001 or SN50, inhibitory peptides for the nuclear translocation of p65/p50, restored the mineralized nodule formation under the relB knockdown in the presence of TNF-a, and was also able to resume fracture healing in $relB^{-/-}$ mice in which the fractured bones were otherwise not united. Interestingly, the mRNA expression of *relB* but not other NF-kB subunits, *p50*, *p52*, *p65* and *c-Rel*, was up-regulated in fractured bones in wild-type mice. There results collectively demonstrate that relB is indispensable for bone repair, and play a critical role in osteoblastogenesis at least in part through suppressing classical NF-kB signaling.

OR39

AR replacement specifically in mineralising osteoblasts of androgen receptor knockout mice partially restores trabecular bone but does not restore bone size

Davey RA[1], Russell PK[1], Clarke MV[1], Wiren KM[2,3] and Zajac JD[1].
[1]Department of Medicine, Austin Health, University of Melbourne, [2]Bone and Mineral Research Unit, Portland Veterans Affairs Medical Center, Oregon, USA and [3]Department of Medicine, Oregon Health and Science University, Portland, Oregan, USA.

Androgens are essential for skeletal growth and bone accrual during puberty and for bone maintenance post-puberty in males. We and others have shown that global knockout of the androgen receptor in mice (ARKO) results in bones of reduced size, cortical thickness and volume compared to control males. To determine the relative contribution of androgens acting directly via the AR to stimulate and maintain bone size and volume in proliferating versus mineralizing osteoblasts, we replaced the AR in ARKOs at either the a) proliferative (pOBLAR:ARKOs) or b) mineralisation (mOBLAR:ARKOs) stage of osteoblast development.
The bones of mOBLAR:ARKO replacement mice and littermate controls were analysed by μCT at 6 and 12 weeks of age (n=7-15/grp). Replacement of the AR in mineralising osteoblasts did not restore bone size or thickness in ARKOs as measured by periosteal circumference and cortical thickness. A partial restoration in trabecular bone was observed in adult mOBLAR:ARKO replacement mice at 12 weeks of age with an increase in trabecular number ($P<0.05$) and decrease in trabecular separation ($P<0.05$) compared to ARKOs, while BV/TV was unaffected.
These data are consistent with the hypothesis that the action of androgens to increase bone size and cortical thickness during growth is mediated via the AR in proliferating osteoblasts, while androgen action via the AR in mineralizing osteoblasts plays a partial role in maintaining trabecular bone in adult mice. This hypothesis is being further tested in pOBLAR:ARKOs in which the AR has been replaced in proliferating osteoblasts.

plenary poster abstracts

Plenary Posters – Basic Science

P1

The role of RANK in breast and prostate cancer growth in murine models of bone metastasis

Zheng Y[1,2], Chow S[1], Kim S[1], Kelly J[1], Dunstan CR[1,3], Sutherland RL[2], Zhou H[1] and Seibel MJ[1,4]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney*
[2]*Cancer Research Program, Garvan Institute of Medical Research*
[3]*Department of Biomedical Engineering, University of Sydney*
[4]*Dept of Endocrinology and Metabolism, Concord Hospital, University of Sydney*

Background: We previously proposed that direct cross-talk between osteoblasts and cancer cells via RANKL and IL-6 enhances the growth of skeletal cancer metastases. In this study we determined whether RANK knockdown in breast and prostate cancer cells affects tumor growth in bone and soft tissues.
Methods, Results: RANK expression was knocked down by 80% (real-time RT-PCR, Western blot) in human breast (MDA-MB-231) and prostate (PC3) cancer cell lines. Non-target (NT) sequences served as controls. RANK knockdown in either cell line had no direct effect on cell proliferation *in-vitro. In-vivo*, intratibial implantation of $MDA^{RANK\text{-}KD}$ or $PC3^{RANK\text{-}KD}$ cells resulted in a significant reduction in osteolytic lesions at all time points, compared to NT controls ($p<0.05$). Histologic analysis at endpoint demonstrated significantly smaller total tumor areas ($p<0.05$) characterised by lower mitotic activity ($p<0.01$) and higher rates of apoptosis ($p<0.01$), reduced cortical bone destruction ($p<0.01$) and fewer osteoclast-lined bone surfaces at the bone/tumor interface ($p<0.05$) in mice injected with RANK-KD cells compared to controls. When RANK-KD cells were implanted orthotopically into soft tissue, no differences were seen in any of the above parameters. In the intratibial models, serum RANKL levels were significantly lower ($p<0.05$) in animals injected with RANK-KD cells compared to NT controls, while there was no difference between groups in the subcutaneous models.
Conclusions: RANK expression by tumor cells enables RANKL-expressing cells of the osteoblast lineage to *directly* communicate with cancer cells in bone. Targeting the components of this novel feed-forward loop may offer potential new treatment strategies to control bone metastases.

P2

Sphingomyelin phosphodiesterase 3 negatively regulates expression of chondrogenic marker genes and hyaluronan synthase 2 in ATDC5 chondrocytes

Kakoi H[1,2], Maeda S[1], Kawamura I[1,2], Imamura K[1,2], Yokouchi M[2], Ishidou Y[1] and Komiya S[1,2]
[1] *Department of Medical Joint Materials, Kagoshima University, Japan.*
[2] *Department of Orthopaedic Surgery, Kagoshima University, Japan.*

Sphingomyelin phosphodiesterase 3 (*Smpd3*) encodes a membrane-bound enzyme neutral sphingomyelinase 2 (nSMase2), which generates ceramid, the lipid second messenger, by cleaving sphingomyelin. Both the two nSMase2-deficient mouse lines, targeted deletion of *Smpd3* gene and a chemically induced loss-of-function mutation of *Smpd3* known as *fragilias ossium* (*fro*), showed chondrodysplasia. However, the cell-autonomous roles of *Smpd3*/nSMase2 in chondrocyte differentiation remain largely unknown. Here, we investigated the expression of nSMase2 in developing bone of mice by immunohistochemistry, and detected the signal of nSMase2 protein not only in osteoblasts of bone collars and primary ossification center, but also in columnar proliferating chondrocytes and prehypertrophic chondrocytes. In ATDC5 chondrocytes, *Smpd3* was gradually up-regulated upon BMP-2 treatment in parallel with expression of the chondrocyte-specific gene *Col2a1*. siRNA-mediated loss of *Smpd3* enhanced the expression of BMP-2-induced *Col2a1*, whereas overexpression of *Smpd3* by adenovirus suppressed it. In addition, hyaluronan synthase 2 (*Has2*), a crucial enzyme in hyaluronan production by chondrocytes, was increased or decreased by knockdown or overexpression of *Smpd3*, respectively, in ATDC5 chondrocytes. Because ceramides had been shown to inhibit the activity of AKT in fibroblasts, we applied the ATDC5 chondrocyte lysate on a receptor tyrosine kinase (RTK) signaling antibody array, to find that phosphorylation of S6 ribosomal protein was enhanced by *Smpd3* knockdown. The increment of phosphorylation of AKT, mTOR, and S6 protein by loss of *Smpd3* was confirmed by immunoblotting. These results suggest that Smpd3 negatively regulates not only chondrocyte differentiation, but also production of hyaluronan in cartilage, by suppressing AKT signaling axis.

Plenary Posters – Basic Science

P3

In situ imaging of the autonomous intracellular Ca^{2+} oscillations of osteoblasts and osteocytes in bone

Ishihara Y[1], Sugawara Y[1], Kamioka H[1], Kawanabe N[1], Naruse K[2] and Yamashiro T[1]
[1]Department of Orthodontics, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences.
[2]Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences.

Bone cells form a complex three-dimensional network consisting of osteoblasts and osteocytes embedded in a mineralized extracellular matrix. Ca^{2+} acts as a ubiquitous secondary messenger in various physiological cellular processes and transduces numerous signals to the cell interior and between cells. However, the intracellular Ca^{2+} dynamics of bone cells have not been evaluated in living bone. In the present study, we developed a novel *ex-vivo* live Ca^{2+} imaging system that allows the dynamic intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) responses of intact chick calvaria explants to be observed without damaging the bone network. Our live imaging analysis revealed for the first time that both osteoblasts and osteocytes display repetitive and autonomic $[Ca^{2+}]_i$ oscillations *ex vivo*. Thapsigargin, an inhibitor of the endoplasmic reticulum that induces the emptying of intracellular Ca^{2+} stores, abolished these $[Ca^{2+}]_i$ responses in both osteoblasts and osteocytes. We also investigated the role of gap junctions in the maintenance of the autonomic $[Ca^{2+}]_i$ oscillations observed in the intact bone. Treatment with three distinct gap junction inhibitors, 18α-glycyrrhetinic acid, oleamide, and octanol, significantly reduced the proportion of responsive osteocytes, indicating that gap junctions are important for the maintenance of $[Ca^{2+}]_i$ oscillations in osteocytes, but less in osteoblasts. Taken together, we found that the bone cells in intact bone explants showed autonomous $[Ca^{2+}]_i$ oscillations that required the release of intracellular Ca^{2+} stores. In addition, osteocytes specifically modulated these oscillations via cell-cell communication through gap junctions, which maintains the observed $[Ca^{2+}]_i$ oscillations of bone cells.

P4

Blockade of PTH/PTHrP signaling inhibits invasive capacity of genetically engineered mouse osteosarcoma in vitro and _in vivo_

Ho PWM, Russell M, Goradia A, Kocovski P, Martin TJ and Walkley CR
Bone Cell Biology and Disease, At Vincent's Institute, Victoria, Australia

Predisposition to osteosarcoma (OS) is conferred by mutation of p53 with concurrent mutations in the Rb pathway. We used an Osterix promoter transgene to direct Cre expression to committed osteoblast progenitors and crossed with conditional alleles for both p53 ($p53^{fl/fl}$) and pRb ($Rb^{fl/fl}$). These mice developed OS with a mean latency of ~ 4.5 months, with complete penetrance in Osx-Cre $p53^{fl/fl}$ $pRb^{fl/fl}$ animals (Walkley et al, Genes Devel 22:1662, 2008), mimicking faithfully the human disease.
Parathyroid hormone (PTH) responsiveness in OS is long recognized. Tumours and cell lines from primary and metastatic OS from Osx-Cre $p53^{fl/fl}$ $pRb^{fl/fl}$ mice showed production of PTHrP and receptor (PTHR1) mRNA and protein, and functional PTHR1 by assaying cAMP responsiveness. Two shRNA's against PTHR1 were selected that reduced PTHR1 mRNA and protein (western blotting, cAMP response) by more than 80%. Specificity was tested by showing ablation of the cAMP response to PTH but retention of β-adrenergic and PGE2 responsiveness. Knock-down of PTHR1 profoundly reduced invasive capacity of the OS cells through collagen. *In vivo* growth was studied by explanting OS cells (vector control) in one flank of nude mice and OS (shRNA PTHR1) in the other, and evaluation by tumor mass, micro CT and gene expression. Knockdown of PTHR1 resulted in 70% reduction in tumour size ($p<0.001$), and substantial increase in mineralization, indicating that PTHR1 knockdown resulted in less invasion and growth, and a promotion of a more differentiated phenotype of tumour cells.

Plenary Posters – Basic Science

P5

Thymosin β_4 administration enhances the biomechanical properties of healing mouse fractures

Brady RD, McDonald SJ, Schuijers JA, Ward AR and Grills BL
Department of Human Biosciences, La Trobe University

Thymosin β_4 ($T\beta_4$) is a multifunctional regenerative peptide that has been shown to improve healing of several tissues. We hypothesised that treatment with $T\beta_4$ may also promote healing of fractured bone. The major aim of this study was to determine whether systemic administration of $T\beta_4$ enhanced the biomechanical properties of healing mouse fibular fractures.

Sixteen week-old male mice received bilateral fibular fractures. Mice were given an I.P. injection of either $T\beta_4$ (6 mg/kg) or saline at the time of fracture and subsequently at 3, 6, 9 and 12 days post-fracture. Fractures from saline and $T\beta_4$-treated mice were analysed 42 days post-fracture for their biomechanical properties using a 3-point bending apparatus. Expression of genes associated with bone remodeling, chondrogenesis and angiogenesis were compared between saline- and $T\beta_4$-treated fractures at 7, 14 and 21 days post-fracture.

When compared with fractures from saline-treated mice, fractures from $T\beta_4$-treated mice had an increased peak force to failure (44% increase, $P < 0.01$), higher ultimate tensile stress (129% increase, $P < 0.01$) and were smaller in area (26% decrease, $P < 0.05$). Furthermore, mice treated with $T\beta_4$ had increased expression of VEGF mRNA within callus at 21 days post-fracture (2-fold increase, $P < 0.05$), and decreased RANKL mRNA expression in callus at 7 days post-fracture (2.5-fold decrease, $P < 0.05$).

Our finding of enhanced biomechanical properties of fractures in mice treated with $T\beta_4$ provides novel evidence of the therapeutic potential of this peptide for treating bone fractures. At this stage, the precise mechanism for the promotion of fracture healing by $T\beta_4$ is unclear. Changes in VEGF and RANKL expression may partly explain the enhanced healing in $T\beta_4$-treated mice, as previous studies have described either an increase in expression of VEGF (Eckhardt *et al.,* 2005) or a decrease in expression of RANKL (Gerstenfeld *et al.,* 2009) improving fracture healing in other animal models.

References

1. Eckhardt H *et al.,* (2005). *J Bone Joint Surg Br*. 87:1434-8.
2. Gerstenfeld LC *et al.,* (2009). *J Bone Miner Res* 24: 196-208.

Activation mechanisms of mutant ALK2 found in fibrodysplasia ossificans progressiva by type II BMP receptor

Ohte S[1], Fujimoto M[1], Tsukamoto S[1], Miyamoto A[1], Sasanuma H[1], Shin M[1], Yoneyama K[1], Fukuda T[1], Kokabu S[1] and Katagiri T[1]
[1] *Saitama Medical University, Research Center for Genomic Medicine*

Bone morphogenetic proteins (BMPs) induce heterotopic bone formation in skeletal muscle. Intracellular signaling of BMPs is activated by binding to typeI and typeII transmembrane serine/threonine kinase receptors. In the ternary complex, typeII receptor phosphorylates an intracellular domain of the typeI receptor, and then the activated typeI receptor phosphorylates Smad1/5. Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant congenital disorder characterized by progressive heterotopic endochondral bone formation in skeletal muscle. Muscle injury induces acute heterotopic bone formation in FOP. Several mutations in the intracellular domain of ALK2, a BMP typeI receptor, have been found in patients with FOP. We found that these mutant ALK2 moderately activated phosphorylation of Smad1/5 and a BMP specific Id1WT4F-luc reporter. We examined effects of BMP typeII receptors on ALK2 because the expression level of BMPR-II was increased at the site induced muscle regeneration in vivo. In the absence of BMPs, co-transfection of BMPR-II with one of mutant ALK2 strongly induced phosphorylation of Smad1/5 and osteoblastic differentiation in C2C12 cells. BMPR-II phosphorylated ALK2, and the phosphorylation level was higher in wild-type ALK2 than that in mutant ALK2. Substitution mutation of the Ser/Thr residues by Ala/Val completely destroyed the activity of ALK2 even in the presence of typeII receptors. These results suggest that mutant ALK2 are further stimulated by BMP typeII receptors via hyper phosphorylation of GS domain. Inhibitors of BMP typeII receptors may aid in the establishment of novel treatments to prevent heterotopic ossification in FOP.

Plenary Posters – Basic Science

P7

Changes in tibial bone and cartilage structure in a mouse surgical model of osteoarthritis

Tonkin BA[1], Romas E[2], Sims NA[1,3] and Walsh NC[1,3]
[1]*St Vincent's Institute of Medical Research, Melbourne, Australia.*
[2]*Dept. of Rheumatology, St Vincent's Hospital, Melbourne, Australia.*
[3]*Dept of Medicine, St Vincent's Hospital, University of Melbourne Melbourne, Australia.*

The destabilisation of the medial meniscus (DMM) mouse osteoarthritis (OA) model is commonly used to study OA joint degeneration. In DMM-OA, the knee is destabilised by transecting the medial-meniscotibial ligament, resulting in increased loading on the medial tibial compartment. Similar to human OA, this leads to articular cartilage damage, subchondral bone accrual and osteophyte formation. We conducted a longitudinal study to define temporal changes in tibial bone structure and cartilage integrity in this model.
DMM or sham surgery was performed on right knees of male C57Bl/6 mice; left knees served as contralateral controls. *In vivo* micro-CT analyses at baseline and 4, 8,12 weeks post-surgery demonstrated focal increases in medial subchondral bone in DMM-OA tibiae; bone volume/tissue volume (BV/TV) and bone mineral density were significantly increased at this site compared to sham from 4 weeks post-surgery ($p<0.001$). Histologic assessment demonstrated proteoglycan loss in medial articular cartilage in DMM-OA tibiae from 4 weeks post-surgery, with significant cartilage erosion evident by 8 weeks post-surgery. Interestingly, medial subchondral bone BV/TV was similar between DMM-OA tibiae and their contralateral tibiae, suggesting an effect of altered gait on subchondral bone structure, as observed in human OA. Articular cartilage was intact in these contralateral tibiae.
In summary, changes in subchondral bone structure occur early in DMM-OA and precede articular cartilage erosion. Altered bone structure in contralateral tibiae of DMM mice, suggests that increased subchondral bone alone, does not impact overlying articular cartilage integrity, and also highlights the need to include sham-operated mice when using this model.

P8

Differential regulation of osteoclast precursor migration by Activin A and RANKL

Fowler TW[1,2], Kurten RC[1], Suva LJ[1,2] and Gaddy D[1,2]
[1] *University of Arkansas for Medical Sciences, Department of Physiology & Biophysics*
[2] *University of Arkansas for Medical Sciences, Department of Orthopaedic Surgery, Center for Orthopaedic Research*

The early recruitment of osteoclast (OC) precursor cells involves cell migration, an important regulatory step in bone resorption. RANKL and Activin A (ActA) are potent stimulators of osteoclastogenesis in murine bone marrow cultures, yet ActA does not induce osteoclastogenesis in isolated murine bone marrow macrophages (BMM) but inhibits RANKL-induced OC development. We hypothesized that RANKL and ActA differentially regulate OC precursor motility and migration, and tested this *in vitro* using time-lapse video microscopy. BMMs were cultured with 25 ng/ml mCSF alone for 24hrs, prior to the addition of RANKL (100 ng/mL) or ActA (50 ng/mL), alone or together, for up to 4d. BMMs cultured with mCSF alone migrated at a constant velocity and distance from d1-d4. RANKL treatment significantly increased motility rate, cumulative track length, and maximum instantaneous velocity on days 2 & 3, an effect that was lost by d4. In contrast, ActA treatment caused a significant decrease in all measured parameters on d1-4. Surprisingly, when added together, ActA treatment completely blocked RANKL stimulated OC precursor motility. To determine if the effects on OC precursor migration were consistent with motility, cells were treated for 6hr with different combinations of mCSF, ActA, and RANKL in transwell dishes, and the number of migrating cells determined. Although RANKL significantly increased migration, ActA alone had no effect. However, ActA prevented any RANKL-induced increase in precursor migration. Together, these data provide the first evidence of ActA inhibition of RANKL action and demonstrate the dominant inhibitory affect of ActA over the pro-OC activity of RANKL.

Plenary Posters – Basic Science

P9

The transcription factor FoxC1 regulates chondrogenesis together with Gli2 through induction of PTHrP

Yoshida M[1, 2],Hata K[1], Takashima R[1], Iseki S[3], Takano-Yamamoto T[2], Nishimura R[1] and Yoneda T[1]
[1]*Biochemistry, Osaka University Graduate School of Dentistry, Japan*
[2]*Division of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Tohoku University, Japan and*
[3]*Section of Molecular Craniofacial Embryology, Graduate School, Tokyo Medical and Dental University, Japan.*

Endochondral ossification, an essential event for mammalian skeletal development, is harmoniously regulated by various transcription factors. Identification of a novel transcription factor and its functional role in chondrogenesis would advance our understanding of the molecular basis of endochondral ossification. To approach this, we generated transgenic mice carrying Venus gene driven by Col2a1 promoter. Venus-positive chondrogenic cells were isolated by sorting using the FACS Aria. We identified FoxC1 (Forkhead Box C1) as a candidate transcription factor by differential microarray between Venus-positive and –negative cells. FoxC1 mutation is known to cause Axenfeld-Rieger syndrome (ARS) characterized by skeletal abnormalities in human. Whole mount *in situ* hybridization demonstrated FoxC1 expression in the developing limbs. Overexpression of FoxC1 induced alcian blue-positive chondrogenesis. These results suggest a positive regulatory role of FoxC1 in chondrogenesis. To further determine the role of FoxC1, we searched for a transcriptional target of FoxC1. FoxC1 transcriptionally induced PTHrP expression through direct binding to the FoxC1 binding element in the PTHrP gene promoter determined by ChIP assay. Notably, FoxC1 increased PTHrP expression induced by Gli2. IP-Western analysis revealed a physical interaction between Gli2 and FoxC1. Pathogenic missense mutation (Ile126Met) of FoxC1, which is responsible for ARS, attenuated FoxC1 transcriptional activity. Moreover, the Ile126Met mutant failed to functionally interact with Gli2 and reduced PTHrP expression by IHH. In conclusion, our results suggest FoxC1 up-regulates PTHrP expression together with Gli2, thereby leading to chondrogenesis. Characterization of FoxC1 would deepen our insights into the mechanism of transcriptional control of chondrogenesis.

P10

Completing the bone/brain circuit: Osteocalcin signals within the hypothalamus to inhibit bone Formation

Lin S[1], Enriquez RE[1], Herzog H[2] and Baldock PA[2]
[1]*Neuroscience Research Program, Garvan Institute of Medical Research.*
[2] *Clinical Medicine, University of New South Wales.*

Osteocalcin (Ocn) regulates energy/glucose homeostasis. However, a major role for Ocn in bone has yet to be identified. Ocn null mice display a mild increase in bone mass. Thus the fundamental action of Ocn in bone homeostasis remains unclear.
We hypothesised that Ocn may form a feedback loop to the brain, to regulate bone mass.
First we examined whether Ocn could activate hypothalamic neurons. An i.p. bolus of Ocn (1ug) induced c-fos in neurons of the arcuate nucleus, indicating that serum Ocn activates hypothalamic neurons. Injection of Ocn directly into the CSF circulation also activated neurons in the arcuate, indicating direct Ocn signalling in the brain.
To model chronic Ocn supply to the hypothalamus, we injected a viral vector expressing Ocn (AAV-Ocn) into the arcuate of 10 week old mice and examined bone 12 weeks later. AAV-Ocn significantly reduced cancellous bone volume of the distal femur (40%), with a significant reduction in mineral apposition rate (33%). This was associated. These changes occur despite a fall in serum Ocn (AAV-empty 137±7 ng/ml vs AAV-Ocn 115±6 $p<0.05$), highlighting the importance of central signalling to the anti-anabolic effect.
This anti-anabolic Ocn effect requires intact NPY signalling, as AAV-Ocn injection did not alter bone in NPY KO or Y2 Receptor KO mice, both of which act in the arcuate.
Ocn signals directly in the arcuate to inhibit bone formation and bone mass. This study defines, for the first time, a central feedback mechanism for bone mass, completing the bone-brain circuit.

Plenary Posters – Clinical Science

P11

Long term high dietary calcium intake and its association with fractures and cardiovascular events in a population based prospective cohort study

Khan BK[1], English D[2], Nowson C[3], Daly R[3] and Ebeling PR[1]
[1]*Department of Medicine, North-West Academic Centre, University of Melbourne.*
[2]*Melbourne School of Population Health, University of Melbourne.*
[3]*School of Exercise and Nutrition Sciences, Deakin University.*

Objective: To measure the association between dietary calcium intake and risk of all fractures, fragility fractures, cardiovascular events and mortality.
Design: Prospective cohort study (Melbourne Collaborative Cohort Study).
Participants: 41,514 men and women followed for an average of 13-14 years for mortality, incident fractures and cardiovascular events. 12,528 were eligible for fracture analysis, and 37,253 for cardiovascular and mortality analyses.
Outcome measures: Self-reported incident fractures of any type (including fragility fractures) and non-fatal cardiovascular events, and mortality. Diet was assessed at baseline using a validated food frequency questionnaire and quartiles of dietary calcium intake were defined. Cox regression and logistic regression was used to estimate hazard ratios for mortality and odds ratios for self reported events.
Results: 824(10.4%) participants reported incident fractures, including 96(1.2%) fragility fractures and 80(1%) wrist fractures. 2,674(12.9%) had incident non-fatal cardiovascular events; 2,045(9.9%) and 765(3.3%) were IHD and stroke, respectively. 3,445(9.25%) deaths occurred; of which 647(1.74%) were from cardiovascular disease. After adjusting for potential confounders, the odds of an incident fracture were 34% lower for the highest compared with lowest calcium intake quartile (CI 0.47-0.93; p=0.02). Mortality from myocardial infarction was 49% lower in the highest quartile (CI 0.26-1.00; p=0.05), cardiovascular-related mortality was 37% lower (CI 0.44-0.90; p=0.01) and all-cause mortality was 20% lower (CI 0.69-0.93; p<0.005). The odds for fragility fractures and non-fatal cardiovascular events were not lower in the multivariate analyses, although the trend was still protective.
Conclusion: Increasing dietary calcium intake was associated with decreased risk of fractures, cardiovascular and all-cause mortality.

P12

Effect of treatment with denosumab on bone mineral density (BMD) in men with low BMD

Hall JW[17], Gruntmanis U[1], Orwoll E[2], Teglbjaerg CS[3], Langdahl BL[4], Chapurlat R[5], Czerwinski E[6], Kendler DL[7], Reginster J-Y[8], Kivitz A[9], Lewiecki EM[10], Miller PD[11], Bolognese MA[12], McClung MR[13], Bone HG[14], Ljunggren Ö[15], Abrahamsen B[16], Yang Y-C[17], Wagman RB[17], Siddhanti S[17], Grauer A[17] and Boonen S[18]
[1]*Dallas Veterans Affairs Medical Center and University of Texas Southwestern Medical Center, Dallas, TX, USA;* [2]*Oregon Health and Science University, Portland, OR, USA;* [3]*Center for Clinical and Basic Research, Ballerup, Denmark;* [4]*Aarhus University Hospital, Aarhus, Denmark;* [5]*Hôpital Edouard Herriot, Lyon, France;* [6]*Krakow Medical Center, Krakow, Poland;* [7]*University of British Columbia, Vancouver, BC, Canada;* [8]*University of Liège, Liège, Belgium;* [9]*Altoona Center for Clinical Research, Duncansville, PA, USA;* [10]*New Mexico Clinical Research & Osteoporosis Center, Albuquerque, NM, USA;* [11]*Colorado Center for Bone Research, Lakewood, CO, USA;* [12]*Bethesda Health Research Center, Bethesda, MD, USA;* [13]*Oregon Osteoporosis Center, Portland, OR, USA;* [14]*Michigan Bone and Mineral Clinic, Detroit, MI, USA;* [15]*Uppsala University, Uppsala, Sweden;* [16]*Copenhagen University Hospital Gentofte, Hellerup, Denmark;* [17]*Amgen Inc., Thousand Oaks, CA, USA;* [18]*Leuven University, Leuven, Belgium*

Aims: To evaluate the effect of denosumab on BMD in men with low BMD.
Methods: This double-blind, placebo-controlled study randomised subjects 1:1 to denosumab 60mg or placebo Q6M for 12 months; all subjects received calcium and vitamin D daily. Subjects were male, aged 30–85 years, with BMD T-score ≤-2.0 and ≥-3.5 at the lumbar spine (LS) or femoral neck (FN), or T-score ≤-1.0 and ≥-3.5 at the LS or FN and prior major osteoporotic fracture. Primary endpoint was percent change from baseline in LS BMD at 12 months. Secondary endpoints included percent change from baseline in total hip (TH), FN, trochanter (TR) and forearm (1/3R) BMD at month 12. Efficacy on LS BMD was assessed in different subgroups (grouped by testosterone level, minimum BMD T-score, and 10-year major osteoporotic fracture risk). Safety analysis included all randomised subjects who received ≥1 dose of investigational product.
Results: 242 subjects were randomised; 94.2% completed 1 year of treatment. Mean (SD) age was 65 (9.8) years; 15% of subjects had baseline testosterone concentration <250mg/dL. After 12 months' denosumab treatment, BMD increased from baseline by 5.7%, 2.4%, 2.1%, 3.1% and 0.6% at the LS, TH, FN, TR and 1/3R, respectively (all $p<0.01$ versus placebo, adjusted for multiplicity). Denosumab treatment resulted in significant increases in LS BMD overall and in all subgroups ($p<0.005$). Adverse event incidence was similar between groups.
Conclusions: 1 year's denosumab therapy in men with low BMD was well tolerated and resulted in significant increases in BMD at all measured skeletal sites.

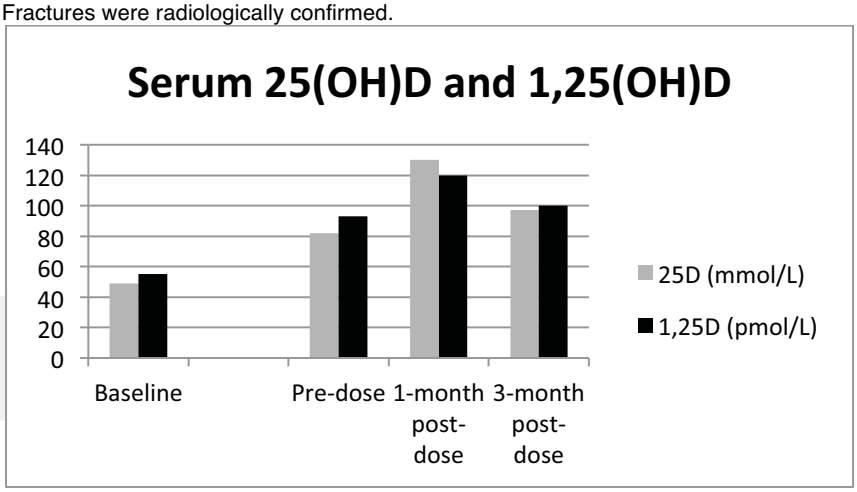
Plenary Posters – Clinical Science

P13

High-dose oral vitamin D_3 administered once a year: Increased fracture risk is associated with 1,25-dihydroxyvitamin D level 3-months post dose

Sanders KM[1], Duque G[1,2], Ebeling PR[1], McCorquodale T[1,2] Shore-Lorenti C[1], Herrmann M[1,2] and Nicholson GC[3]
[1]Department of Medicine, NorthWest Academic Centre University of Melbourne: [2]Nepean Hospital, University of Sydney, Australia. [3]Rural Clinical School, School of Medicine, The University of Queensland, Queensland, Australia.

We have reported increased falls and fracture rates in a RCT using single annual doses of 500,000IU cholecalciferol administered orally to 2,256 older women[1]. The increased falls rate in the vitamin D group was higher in the first 3 months following dosing (p=0.017) suggesting an adverse mechanism in the immediate post-dose period. Serum 1,25-dihydroxyvitamin D levels (1,25D) have been assessed in 65 sub-study participants using DiaSorin immunoassays. Fractures were radiologically confirmed.



Our *post-hoc* analysis used logistic regression with fracture (yes/no) as the outcome. The model included age and 1,25D at 3-months post-dose as the covariates. There was a 10% increased risk of fracture with increasing 1,25D levels (odds ratio {OR}: 1.10; 95% C.I: 1.02, 1.18, p<0.000). The increased risk of falls was not associated with 1,25D levels (OR: 1.02, 95% C.I: 0.98, 1.06, p=0.34). The normal range for 1,25D is 47 to 203pmol/L. We have previously reported increased bone turnover in the vitamin D group

These findings suggest high 1,25D levels following a 500,000IU dose cholecalciferol are associated with an increased fracture risk in older women. The finding does not explain the increased risk of falls but progresses our understanding relating to increased fracture risk following annual high-dose vitamin D supplementation.

[1]Sanders KM et al, JAMA 2010

P14

Transformation of trabecular bone plates to trabecular rods accounts for the bone loss in inter-trochanteric cancellous bone of hip fracture patients

Thomas CDL[1], Parkinson IH[2], Zhou B[3], Wang J[3], Liu XS[4], Guo XE[3], Fazzalari NL[2] and Clement JG[1]
[1] Melbourne Dental School, University of Melbourne
[2] Directorate of Surgical Pathology, SA Pathology, Adelaide
[3] Department of Biomedical Engineering, Columbia University
[4] Department of Orthopaedic Surgery, University of Pennsylvania

There is significant cancellous bone loss at or near the fracture site in hip fracture patients. This bone loss has been characterised, morphologically, as loss of trabeculae, greater separation between the trabeculae and thinner trabeculae. The development of individual trabecular segmentation (ITS) morphological technique to decompose micro-CT imaging datasets of the cancellous structure into individual rods and plates has enabled the study of how these trabecular elements change in osteoporotic individuals.

Inter-trochanteric cores, 10mm in diameter were obtained from 23 patients undergoing hip replacement surgery for inter-trochanteric hip fracture and from 22 cadaveric controls. Micro-CT imaging was performed at 15 micron isotropic spatial resolution (Skyscan 1174). In addition to conventional 3D morphometric analysis, the datasets were analysed with ITS technique to provide morphological analysis of individual rods and plates.

Bone volume fraction was significantly lower in the fracture group compared to the controls (8.5±2.6 versus 11.6±4.1, p<0.004). Trabecular number (Tb.N) was lower (0.86±0.27 versus 1.09±0.42, p<0.04), trabecular separation (Tb.Sp) was higher (987±186 versus 865±169, p<0.03) and trabecular thickness (Tb.Th) was lower (155±12 versus 179±26, p<0.02) in the fracture group. ITS analysis showed a decrease in plate bone volume (6.2±0.2 versus 9.2±4.3, p<0.004) but not rod bone volume (2.1±0.08 versus 2.0±0.6, p=0.6) and the rods were shorter (327±35 versus 352±35, p<0.02) in the fracture group compared to controls.

This novel ITS analysis shows that the cancellous bone loss associated with osteoporotic fracture of the hip is due to loss of trabecular plates. While the volume of trabecular rods within the sample is unchanged, the average length of the rods is shorter. This suggests that longer trabecular rods may have been lost but shorter rods, which have arisen from resorption-mediated perforation of trabecular plates, now constitute the majority of rods in the structure. These observations provide evidence as to how trabecular plates transform to trabecular rods in cancellous bone adjacent to the site of hip fracture, with a concomitant increase in the risk of fracture during falls.

Plenary Posters – Clinical Science

P15

Taller persons have thinner and more porous cortices to fall harder upon and fracture

Bjørnerem Å[1], Zebaze R[2], Ghasem-Zadeh A[2], Bui M[3], Wang XF[2], Hopper JL[3] and Seeman E[2]
[1]*Department of Clinical Medicine, University of Tromsø, Norway.*
[2]*Endocrine Centre, Austin Health, University of Melbourne, Australia.*
[3]*Centre for MEGA Epidemiology, University of Melbourne, Australia.*

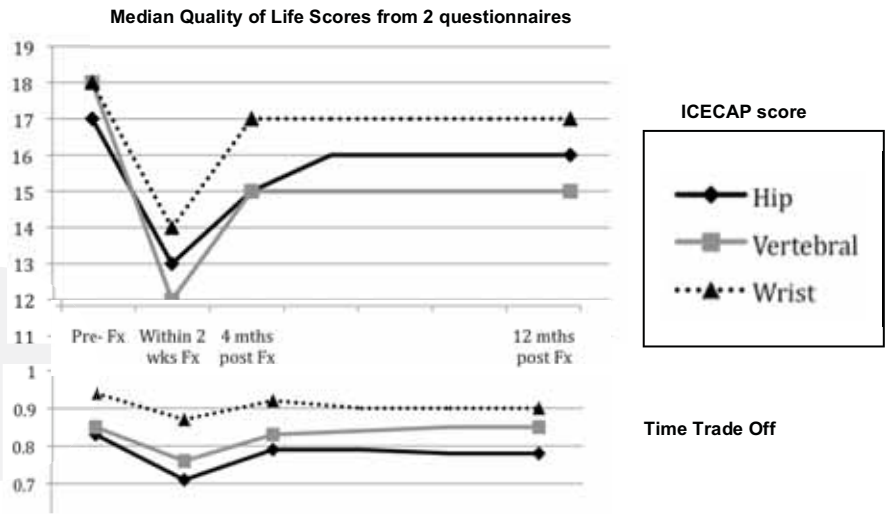
Introduction: Taller women are at increased fracture risk even though their long bones have a larger total cross sectional area (CSA) which increases resistance to bending. Mass is minimized in the larger bone by assembling a thinner cortex (relative to total CSA) by greater endocortical resorption relative to periosteal apposition so cortical area and compressive strength are conserved. We proposed that as the intracortical (haversian canal) surface is contiguous with the endocortical surface, mass is also minimizing by assembling the thinner cortices with higher porosity.
Methods: We measured distal tibial microarchitecture using high-resolution peripheral quantitative computed tomography (Scanco Medical) and calculated cortical porosity using Strax1.0 in 185 pairs of female twins aged 40 to 61 years, 93 with fractures. Results were age and BMI adjusted.
Results: Each standard deviation (SD) greater height was associated with a 0.71 SD larger tibia total CSA, 0.68 SD larger medullary CSA, 0.47 SD larger medullary area/TCSA area (thinner cortices) and 0.37 SD higher porosity (all $p<0.001$). For each SD larger medullary CSA/total CSA, porosity was 0.53 SD higher ($p<0.001$). These results were confirmed in a within-twin pair analysis (not shown). Each SD greater porosity was associated with 36-79% higher risk of prevalent fracture; compact-appearing cortex (OR 1.36; 95% CI 1.03-1.80), outer (OR 1.38; 95% CI 1.01-1.88) and inner (OR 1.79; 95% CI 1.06-3.02) transitional zones.
Inference: Taller women have thinner and more porous cortices which contribute to fracture risk because cortical stiffness is proportional to the 7th power of its apparent density.

P16

Individual derived quality of life changes over 12-months following fracture: The AusICUROS study

Sanders KM, Nicholson GC, Iuliano S, Seeman E, Prince R, Duque G, Winzenburg T, Cross M, March L, Ebeling PR and Borgstrom F

Eight centres across Australia are participating in an international health economics study on fragility fractures. Prospective data are collected from patients with recent fracture (Fx) at four timepoints: Phase 1 collects information within two weeks of Fx and documents QoL before (recollected) and immediately after Fx and phases 2 to 4 collect data 4-, 12- and 18-months post-fracture. Eligibility includes a low-energy Fx and age 50+ years. Recruitment occurs largely through emergency departments and orthopaedic wards.
QoL changes following Fx are assessed using time trade-off (TTO: 1=perfect health, 0-death) and visual analogue scale (VAS; 'thermometer' 100=perfect health, 0=death) and ICECAP index (score out of 20). The ICECAP provides an estimate of 'non health-specific' QoL, assessing attributes of friendship, sense of purpose, enjoyment, emotional security and control of decision-making.
Results from 564 participants (80% women, 95% living at home) are presented with 12-month data for 312 (25% unable to answer the TTO).
At 12-month post Fx many aspects of QoL including those not directly health-related, have not returned to pre-Fx status in patients with hip and vertebral Fx. Throughout the 12-month post-fracture period, self-rated health (VAS) and ICECAP attributes were similar in vertebral and hip Fx patients (p value for difference range 0.2 to 0.8). Although there is a recruitment bias towards more serious vertebral Fx patients, the impact of vertebral Fx on QoL is likely to be currently underestimated compared to hip Fx. The work is novel in addressing both the health and non-health related attributes of QoL following Fx.



Plenary Posters – Clinical Science

P17

Models of care for the secondary prevention of osteoporotic fractures: a systematic review and meta-analysis

Ganda K[1], Puech M[2], Chen JS[3], Speerin R[4], Bleasel J[5], Center JR[6], Eisman JA[6], March L[3] and Seibel MJ[1]
[1]Bone Research Program, ANZAC Research Institute, The University of Sydney. [2]Public Health Unit - Hornsby Office, Hornsby Ku-ringai Hospital, Hornsby NSW Australia.
[3]Institute of Bone & Joint Research, The University of Sydney.
[4]Agency for Clinical Innovation, Chatswood, NSW Australia.
[5]Royal Prince Alfred Hospital, Camperdown NSW Australia.
[6] Bone and Mineral Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, University of New South Wales, Sydney, Australia.

Introduction: Most people presenting with incident osteoporotic fractures are not being assessed or treated for osteoporosis, despite the availability of effective treatments. We evaluated the effectiveness of published systematic approaches for the secondary prevention of fractures in people with osteoporosis.
Methods: We searched eight medical literature databases to identify reports published between 1996 and 2011, describing models of care for secondary fracture prevention. Information extracted from each publication included study design, patient characteristics, identification strategies, assessment and treatment initiation strategies, and outcome measures (rates of BMD testing, osteoporosis treatment initiation, re-fractures, and cost-effectiveness). Meta-analyses of studies with valid control groups were conducted for two outcome measures: BMD testing and osteoporosis treatment initiation.
Results: Out of 574 references, 41 studies were identified as analysable. These studies were grouped into four general models of care: Type A, involving identification, assessment and treatment of patients as part of the service; Type B, similar to A, without treatment initiation; Type C, involving alerting patients plus primary care physicians; Type D, involving patient education only. Meta-analyses revealed increased rates of BMD testing ($p = 0.06$) and treatment initiation ($p = 0.03$) with increasing intensity of intervention (table). One Type A service with a valid control group showed a significant decrease in re-fractures. Type A and B services were described as being cost-effective.
Conclusion: Fully coordinated models of care for secondary fracture prevention are more effective in improving patient outcomes than approaches involving alerts and/or education only.

P18

Vertebral body strength: prediction using subregional areal bone mineral density from DXA compared to subregional bone microarchitecture from micro-CT

Perilli E[1,2], Briggs AM[3,4], Codrington JD[5], Kantor S[4], Parkinson IH[2,6], Reynolds KJ[1], Fazzalari NL[2,6] and Wark JD[4]
[1]Medical Device Research Institute, School of Computer Science Engineering and Mathematics, Flinders University, Adelaide, SA.
[2]Bone and Joint Research Laboratory, SA Pathology and Hanson Institute, Adelaide, SA.
[3]Curtin Health Innovation Research Institute, Curtin University, Perth, WA. [4]Department of Medicine, University of Melbourne, Bone & Mineral Service, Royal Melbourne Hospital, Melbourne, VIC.
[5]School of Mechanical Engineering, The University of Adelaide, Adelaide, SA. [6]Discipline of Anatomy and Pathology, The University of Adelaide, Adelaide, SA, Australia.

Areal Bone Mineral Density (aBMD) measured by Dual X-ray Absorptiometry (DXA) remains suboptimal in providing an individualized risk of vertebral fracture. Bone distribution, microstructure, and strength, vary within the vertebra. Thus, vertebral subregional aBMD measurements from lateral-projection DXA might be more informative about vertebral fragility, than the commonly-used posterior-anterior (PA) projection approach. Micro-computed tomography (micro-CT) allows three-dimensional micro-structural characterisation of entire vertebrae. Study aim: to measure vertebral subregional aBMD (lateral DXA) and trabecular bone volume fraction (BV/TV, micro-CT), and to assess their capability to predict whole vertebra failure load assessed experimentally.

Eight human cadaver spines were scanned by DXA in PA and lateral projections; subregional aBMD was measured from lateral images (L2, L3), dividing the vertebral area into three subregions (superior, central, inferior). The vertebrae were then scanned by high-resolution micro-CT; vertebral volume was divided into three subregions, on which BV/TV was measured. The vertebrae were then tested in compression to determine failure load.

Statistically significant differences between subregions were observed for both aBMD and BV/TV. aBMD from the superior subregion was a better predictor of failure load (R^2=0.79, $p<0.01$), compared to other subregions and to whole-vertebra aBMD in lateral- and PA projection (R^2=0.70, $p<0.01$, and R^2=0.30, $p<0.05$). Similarly, BV/TV assessed by micro-CT in the superior subregion was a better predictor of failure load (R^2=0.58, $p<0.01$), compared to other subregions and to BV/TV of the entire vertebra (R^2=0.52, $p<0.01$).

These novel findings highlight the capability of subregional aBMD assessed using lateral-projection DXA to predict vertebral strength, and provide basis for further exploring the clinical application of lateral-projection DXA analysis.

Plenary Posters – Clinical Science

P19

Bisphosphonate-induced Changes in Bone Mineral Density Depend on the Degree of Osteoporosis in Older Men and Women.

Zhang JTW and Seibel MJ
Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, and Bone Research Program, ANZAC Research Institute, Sydney

Aims: The effects of bisphosphonate treatment on bone mineral density (BMD) vary greatly between patients. We analysed bisphosphonate-induced changes in BMD in patients with baseline T-scores within the 'osteoporotic range', comparing subjects with extremely low bone mass (T≤ 4.0) to patients with T-scores above this cut-off but below the WHO threshold for osteoporosis.
Methods: Retrospective analysis of serial BMD measurements in 189 patients with confirmed osteoporosis on long-term bisphosphonate therapy. Patients were stratified into three groups according to baseline lumbar spine (LS) or femoral neck (FN) BMD T-scores: **A**, -2.5 ≥ T-score > -3.5; **B**, -3.5 ≥ T-score > -4.0; **C**, T-score ≤ -4.0. Changes in BMD were compared to baseline values at intervals of ~12 months, using standard statistics.
Results: Baseline characteristics as well as absolute and relative changes in LS and FN BMD during bisphosphonate treatment are summarised in the table below. Repeated measures analysis confirmed significant gains in LS & FN BMD in all groups ($p< 0.05$). However, absolute (g/cm^2) and relative (%) changes in LS BMD were significantly greater at 36 months in patients with extremely low baseline BMD (group C) compared to patients in group A (Mann-Whitney, $p = 0.014$). Similar effects were observed at the FN, although numbers in group C were too small to allow for reliable comparisons.
Conclusion: Changes in bone mass in response to bisphosphonate therapy are greatest in osteoporotic patients with extremely low BMD at baseline.

Baseline Characteristics	Lumbar Spine			Femoral Neck		
	Group A (N = 40)	Group B (N = 23)	Group C (N = 22)	Group A (N = 81)	Group B (N = 14)	Group C (N = 9)
Female	38 (95%)	19 (83%)	17 (77%)	64 (79%)	9 (64%)	4 (44%)
Age (yrs)	66 ± 10	68 ± 9	66 ± 10	67 ± 11†	77 ± 9†	70 ± 12
Baseline BMD (mg/cm^2) ± SD	0.831 ± 0.037	0.755 ± 0.036	0.673 ± 0.070	0.648 ± 0.038	0.565 ± 0.036	0.492 ± 0.046
Change in BMD from baseline (mg/cm^2 ± SD)						
12 months	0.022 ± 0.036	0.026 ± 0.046	0.030 ± 0.033	0.004 ± 0.018	0.016 ± 0.021	0.024 ± 0.036
24 months	0.032 ± 0.040	0.033 ± 0.046	0.051 ± 0.036	0.006 ± 0.026#	0.022 ± 0.023	0.042 ± 0.047#
36 months	0.028 ± 0.041*	0.043 ± 0.052	0.057 ± 0.040*	0.012 ± 0.027^	0.024 ± 0.024	0.052 ± 0.058^
Percentage change in BMD from baseline (% ± SD)						
12 months	2.7 ± 4.4	3.4 ± 6.3	4.5 ± 5.4	0.6 ± 2.8	2.9 ± 3.8	5.1 ± 7.5
24 months	3.8 ± 4.8	4.3 ± 6.2	7.5 ± 5.6	1.0 ± 3.9 #	3.9 ± 4.3	9.3 ± 10.4#
36 months	3.4 ± 5.0*	5.4 ±6.9	8.4 ± 6.1*	1.6 ± 3.9^	4.2 ± 4.4	11.5 ± 12.9^

† p = 0.001, Group A vs. B.
* p = 0.014, LS Group A vs. C at 36 months.
\# p = 0.046, FN Group A vs. C at 24 months.
^ p = 0.021, FN Group A vs. C at 36 months.

P20

The role of dairy intake on muscle health in older community-dwelling women

Radavelli-Bagatini S[1,2], Zhu K[1,2], Lewis JR[1,2], Dhaliwal SS[3] and Prince RL[1,2]
[1]Bone and Vascular Research Group, Department of Endocrinology and Diabetes, Sir Charles Gardiner Hospital; [2]School of Medicine and Pharmacology, University of Western Australia; [3]School of Public Health, Curtin University of Technology.

Impaired muscle function has been demonstrated to be an important predictor of fracture in the elderly. There are limited data on dairy intake and muscle health in older women. The aim of this study is to evaluate the association between dairy intake and lean body mass, physical performance and risk of falls in older women aged 70-85. 1,456 older women were assessed for dairy consumption by a validated food frequency questionnaire and had physical performance tested including hand grip strength and timed up and go (TUG) test and self-reported number of falls. Body composition by DXA (dual-energy x-ray absorptiometry, n=493) was also performed. Women were dichotomised into those consuming ≥ or <1.5 serves of dairy/day. The mean age was 75 years and BMI 27.2 kg/m^2. Women consuming ≥1.5 serves of dairy/day had significantly greater lean whole body mass (34.0 ± 4.0 vs. 33.1 ± 4.1, p=0.009) and appendicular skeletal muscle mass (15.1 ± 2.2 vs. 14.6 ± 2.2, p=0.011) compared to those with a lower dairy intake. A greater hand grip strength was seen in those with higher dairy consumption (21.3 ± 4.2 vs. 19.7 ± 4.4, p=0.001) as well as a better physical performance in the TUG test (9.4(8.2-10.7) vs. 9.7(8.5-11.3), p=0.027), and fewer falls in the previous three months (10.6% vs. 14.6%, p=0.029) before and after adjustments for age, BMI, energy intake and physical activity level. In conclusion consumption of ≥1.5 serves of dairy/day is associated with greater lean body mass, better physical performance and reduced risk of falling in older women.

poster presentations

P21

Analysis of high turnover type bone loss due to haploinsufficiency of Cnot3, a subunit of Ccr4-not complex(mRNA seadenylase)

Watanabe C [1,2,3], Ezura Y[1], Nakamoto T[1,2], Hayata T[1], Notomi T[1,2], Moriyama K[2,3] and Noda M[1,2]
[1] *Department of Molecular Pharmacology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.* [2] *Global COE program, Tokyo Medical and Dental University, Tokyo, Japan.* [3] *Department of Maxillofacial Orthognathics, Tokyo Medical and Dental University, Tokyo, Japan.*

Gene expression is regulated in an integrated manner by post-transcriptional and transcriptional control. Recently, post-transcriptional control such as degradation of mRNA has been focused on as a highly adaptive control system rather than transcriptional control. We focused on Ccr4-not complex which is deadenylase and major degradation system of mRNAs. Our aim is to analyze whether Cnot3, one of the elements of this complex, is involved in the control of bone metabolism and the following investigation was carried out. We found Cnot3 was expressed in bone and osteoblastic cells in mice. Then we analysed Cnot3 knockout mice to determine bone phenotype using Cnot3+/- mice since -/- is embryonic lethal. At 14 weeks old, Cnot3+/- mice showed lower BMD and BV/TV when compared to wild-type mice. From the data of bone histomorphomerty and TRAP staining, mineralized surface and osteoclast number were upregulated in Cnot3+/- mice. These data indicates that Cnot3+/- mice exhibits high-turnover type low bone mass. Cortical bone thickness was more reduced in aged Cnot3+/-mice in comparison to wild-type mice. These results suggest that there is a relationship between aging and Cnot3. Expression levels of genes related to bone resorption were up-regulated in osteoblasts(M-CSF and Opg) and osteoclasts(Rank) where Cnot3 was knocked down. In conclusion, our data indicate that Cnot3 is required for the osteoclast function and maintain the bone mass.

P22

Cytotoxic therapies significantly alter the composition of the cells comprising murine hematopoietic stem cell niches

Quach J[1], Askmyr M[1], Jovic T[1], Baker EK[1,2], King H[1], White KE[1], Nombela-Arrieta C[4], Walsh N[2,3], Silberstein LE[4], Purton LE[1,2]
[1]*Stem Cell Regulation Unit, St Vincent's Institute, Melbourne, Victoria, Australia*
[2]*Dept of Medicine, The University of Melbourne, Melbourne, Victoria, Australia*
[3]*Bone Cell Biology and Disease Unit, St Vincent's Institute, Melbourne, Victoria, Australia*
[4]*Joint Program in Transfusion Medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA*

Haematopoietic stem cells (HSCs) are regulated within the bone marrow in locations termed HSC niches. Cancer therapies significantly impair haematopoiesis, however, their effects on HSC niche cells are largely unknown. Using adult male mice, we have extensively analysed the effects of irradiation or chemotherapy on HSC niche cells. Our studies have focused on putative HSC niche cell types that are also important in regulating bone: endothelial cells (ECs), adipocytes, osteoblasts (Obs) and osteoclasts (Ocs).
In all treatment groups the earliest histologic change observed was to the vasculture, with altered sinusoid morphology apparent as early as day 2 post-therapy whereas arteries were not affected. Sinusoid vessels were dilated whilst vessel number remained largely unchanged. Significant increases in adipocytes were also seen early post-therapy, which appeared to be due to altered fate commitment of osteoblast lineage cells. Furthermore, all treatments resulted in a net bone loss with an associated increase in bone turnover (as indicated by increases in the numbers of Obs and Ocs). Inhibition of Ocs by a single dose of the bisphosphonate, zoledronic acid (ZA) prevented this bone loss, but in contrast to recent reports, did not significantly impair HSC numbers or function.
In conclusion, niche cells that are important in regulating both HSCs and bone remodelling are highly susceptible to cancer therapies. Reducing damage to these niche cells may in turn improve blood cell recovery in cancer patients.

P23

Extracellular calcium-induced phosphorylation of CREB in calcium-sensing receptor expressing HEK293 cells, human parathyroid cells and osteoblasts

Avlani VA[1], Ma W[1], Bracken AM[1], Mason RS[2], Delbridge L[3], Christopoulos A[4] and Conigrave AD[1]
[1] *School of Molecular Bioscience, University of Sydney, NSW 2006*
[2] *School of Medical Sciences and Bosch Institute, University of Sydney, NSW 2006*
[3]*Department of Surgery, Sydney Medical School, University of Sydney, NSW 2006*
[4]*Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences & Department of Pharmacology, Monash University, VIC 3052 Australia*

The calcium-sensing receptor (CaSR) is a class C G Protein-Coupled receptor that signals via activation of $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$ heterotrimeric G-proteins. The CaSR is expressed in parathyroid cells, where it mediates Ca^{2+}_o-dependent feedback regulation of PTH secretion. It is also expressed in osteoblasts and osteoclasts, and participates in bone remodelling via transcriptional-dependent control of cell survival, proliferation and differentiation. The CaSR activates transcription downstream of ERK1/2, JNK, NFAT and the serum response factor. In the current study, we investigated the impact of elevated Ca^{2+}_o (1.5-5 mM) on Ser-133 phosphorylation of another key transcription factor, cAMP response element binding protein (CREB) in HEK293 cells stably expressing the CaSR (HEK-CaSR), human adenomatous parathyroid cells and human fetal osteoblasts. Exposure of all three cell-types to high Ca^{2+}_o resulted in time and concentration-dependent increases in CREB phosphorylation (pCREB). The positive and negative modulators of the CaSR, cinacalcet and NPS-2143 respectively promoted and suppressed pCREB levels in HEK-CaSR cells and parathyroid cells but not in osteoblasts. Inhibitor studies revealed that CREB phosphorylation was dependent on PI-PLC and PKC in HEK-CaSR cells and PI-PLC, PKC and $G_{i/o}$ in parathyroid cells but not osteoblasts. Taken together, the data indicate that Ca^{2+}_o-induced pCREB is mediated by CaSR homodimers in HEK-CaSR and parathyroid cells but not osteoblasts. These findings suggest a mechanism by which elevated Ca^{2+}_o concentrations may control the local generation of 1,25-dihydroxyvitamin D in parathyroid cells and osteoblasts, via CRE sites on the CYP27B1 gene, encoding 1a-hydroxylase.

P24

Histone deacetylase (HDAC) 1 as a target for suppressing both inflammation and bone loss in chronic inflammatory diseases

Cantley MD[1], Fairlie DP[2], Bartold PM[3] and Haynes DR[1]
[1]*Discipline of Anatomy and Pathology, School of Medical Sciences, University of Adelaide, South Australia.*
[2]*Institute for Molecular Bioscience, University of Queensland, Brisbane.*
[3]*Colgate Australian Clinical Dental Research Centre, School of Dentistry, University of Adelaide, South Australia.*

Histone deacetylase inhibitors (HDACi) are emerging as promising treatments for a variety of diseases including inflammatory disorders. Until recently HDACi have been broad acting but novel specific HDACi are now available. The aim was to assess expression of HDAC1 in human tissues and investigate effects of a specific inhibitor of HDAC1 on inflammation and bone resorption *in vitro.*

Expression of HDAC1 in human periodontitis (PD) and peri-prosthetic (PP) osteolysis tissues was assessed using real time PCR and immunohistochemistry. PP tissues were obtained during revision surgery (n=10) and compared with osteoarthritic (OA) tissues (n=5). Gingival tissues were obtained from patients with chronic PD (n=9) and compared with normal gingival tissues (n=8). The effect of a HDACi (NW-21) that targets HDAC1 on human osteoclast activity was assessed *in vitro*. Anti-inflammatory effects of NW-21 were also investigated in LPS and TNF-α stimulated monocytes *in vitro*.

HDAC 1 mRNA expression was significantly upregulated in both human PD and PP tissues. Immunohistochemistry revealed strong expression of HDAC1 by inflammatory cells in PD. In PP tissues HDAC1 was expressed by multinucleated "osteoclast-like" cells with little or no expression in the OA control tissues. NW-21 significantly suppressed human osteoclast mediated bone resorption at concentrations higher than 0.8nM *in vitro*. NW-21 also significantly suppressed chemokines monocyte chemotactic protein (MCP1) and macrophage inflammatory protein (MIP1-α) but not TNF-α or IL-1β in stimulated monocytes.

Inhibition of HDAC1 results in both anti-resorptive and anti-inflammatory effects *in vitro*. HDAC1 is therefore a promising target for treating inflammatory and bone loss.

P25

Homozygous deletion of Dickkopf-1 results in a high bone mass phenotype due to increased bon formation

McDonald MM[1,2], Morse A[1,3] , Baldock PA[2], Peacock L[1], Aiken A[1], Tam PPL[4] and Little DG[1,3]
[1]*Orthopaedic Research and Biotechnology Dept. The Kid's Research Institute, The Children's Hospital Westmead, NSW Australia*
[2] *Bone Program, The Garvan Institute of Medical Research, NSW Australia.*
[3] *The University of Sydney, NSW Australia*
[4] *Developmental Embryology Dept. The Children's Medical Research Institute NSW*

Dickkopf-1 (*Dkk1*) is an antagonist of osteoblast differentiation through blockade of Wnt signaling via the LRP5/6 co-receptor. Recently, homozygous *Dkk1* KO mice were generated by modifying Wnt3 levels[1]. We examined the bone phenotype in *Dkk1*-/-; *Wnt3*+/- (HOM/HET) compared to *Dkk1*+/+;*Wnt3*+/+ (WT/WT).

Analysis of calvarial RNA confirmed no postnatal expression of Wnt3 in either genotype. Body mass did not differ. Whole body BMC was increased 11% in HOM/HET mice ($p<0.05$ vs WT/WT). Interestingly female HOM/HET mice showed a 24% increase in body fat percentage ($p<0.02$ vs WT/WT). MicroCT analysis of metaphyseal BV/TV revealed a 3-fold increase in female and a 2-fold increase in male HOM/HET mice ($p<0.01$ vs WT/WT). This was associated with large increases in trabecular number ($p<0.05$) but no alteration in trabecular TMD. Cortical BV was increased in both male (15%) and female (19%) HOM/HET mice ($p<0.02$ vs WT/WT). Cortical thickness also increased 13% in females, leading to a 35% increase in moment of inertia ($p<0.05$ vs WT/WT). Vertebral trabecular BV/TV was also increased in female (94%) and male (79%) in HOM/HET mice ($p<0.01$ vs WT/WT).

Histologically, increases in trabecular MAR of 52% and BFR/BS 58% were noted in female HOM/HET mice compared to WT/WT ($p<0.05$) with no alterations in osteoclast parameters.

In conclusion, our findings to date have revealed that the absence of DKK1 results in a robust high bone mass phenotype due to enhanced bone formation. Further analyses include examination of fat mass and other soft tissues, *in vitro* analyses of primary cells and mechanical testing.

[1]Lewis SL *et al.*Dkk1 and Wnt3 interact to control head morphogenesis in the mouse.
Development. 2008 May;135(10):1791-801. Epub 2008 Apr 9.

P26

Molecular basis of allosteric coupling in the calcium-sensing receptor: roles of conserved cysteine residues

Brown AP, Baddock HT, Goolam MA and Conigrave AD
School of Molecular Bioscience, University of Sydney, NSW, 2006, Australia

The calcium-sensing receptor (CaR) is a Class C GPCR that responds to Ca^{2+} and positive allosteric modulators including cinacalcet and amino acids. Ligand binding in the CaR's extracellular Venus Flytrap Domain (VFTD) induces signalling by the heptahelical domain (HHD) in the plasma membrane via an intervening Cys-rich domain (CRD). Although the mechanism of this interdomain coupling is unknown, an interdomain disulfide is predicted to directly couple conformational changes in the VFTD to the effector HHD.

In this study, we investigated whether C561 in the CRD participates in an interdomain disulfide with C236 in the VFTD. HEK 293 cells were transiently transfected with the CaR mutants, C236S and C561S and evaluated for receptor surface expression and signalling via Ca^{2+}_i mobilisation (Fura-2AM). Mutant receptors failed to respond to Ca^{2+}_o in the presence of the VFTD-binding modulator L-Phe but retained near normal responses to cinacalcet, which binds in the HHD.

These results demonstrate that C236 and C561 are essential for allosteric coupling between the VFTD and HHD, but not for direct activation of the HHD itself. C542 and C562 at the dimer interface also participate in receptor signalling.

P27

Monosodium urate crystals inhibit tenocyte viability and function: implications for periarticular involvement in chronic gout

Chhana A[1], Callon KE[1], Pool B[1], Naot D[1], Gamble G[1], Coleman B[2], McQueen FM[3], Cornish J[1] and Dalbeth N[1]
[1]*Bone & Joint Research Group, Department of Medicine, University of Auckland, Auckland, New Zealand.*
[2]*Department of Orthopaedic Surgery, Middlemore Hospital, Auckland, New Zealand.*
[3] *Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand.*

Aim: Recent advanced imaging studies have demonstrated that urate deposition in periarticular structures is common in gout. Urate deposition has been observed both adjacent to and within tendons, suggesting that monosodium urate monohydrate (MSU) crystals are likely to be in direct contact with tenocytes, the stromal cells of tendons. The aim of this study was to determine the effects of MSU crystals on tenocyte viability and function.
Methods: Cultures of primary rat tenocytes were prepared from Wistar rat tails, and primary human tenocytes from patients undergoing orthopaedic surgery. MTT and flow cytometry assays were used to assess tenocyte viability following culture with MSU crystals. Real-time PCR was used to determine changes in gene expression and Sirius red staining was used to determine changes in collagen deposition in tenocytes cultured with MSU crystals.
Results: MSU crystals rapidly reduced viability in a dose-dependent manner in both primary rat and human tenocytes. Differing MSU crystal lengths and increased serum levels in cultures did not alter this effect. The reduction in tenocyte viability was specific to MSU crystals, as soluble uric acid did not reduce cell viability. Culture with MSU crystals reduced mRNA expression of collagen types 1 and 3; and tenocytic markers, including tenomodulin, scleraxis and tenascin-C. Collagen deposition was inhibited in tenocytes cultured with MSU crystals in a dose dependent manner.
Conclusions: These data indicate that MSU crystals directly interact with tenocytes to reduce cell viability and function. These interactions may contribute to tendon damage in patients with chronic gout.

P28

Potential roles of metallothionein-I and II in protecting against acute methotrexate chemotherapy-induced damage to endochondral ossification

Fan CM [1], Garcia M[1], Scherer M [1], Tran C[2] and Xian CJ[1]
[1]*Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia;* [2]*Department of Gastroenterology, Women's and Children's Hospital, North Adelaide, SA, Australia.*

Chemotherapy-induced bone growth arrests are significant problems in paediatric cancer patients. Several studies have examined the cellular and molecular mechanisms by which the major childhood chemotherapy antimetabolite methotrexate (MTX) affects bone integrity through the folate-dependent pathway. However, whether folate-independent pathway such as oxidative stress can contribute to chemotherapy-induced bone damages remain unclear. Hence, this study aims to investigate the roles of metallothioneins (MT) in MTX-induced bone damages in the growing skeleton of young mice. Three weeks old male MT-I&II knockout ($MT^{-/-}$) and wild type ($MT^{+/+}$) male mice were injected with saline or MTX at 12.5mg/kg once daily for three consecutive days, with mice sacrificed on days 5, 8 and 14. The effects on bone growth were analysed for structural and cellular changes in the growth plate and metaphysis- two important regions responsible for bone lengthening. This study found that 3 doses of MTX at 12.5mg/kg did not significantly affect bone growth in $MT^{+/+}$ mice. In $MT^{-/-}$ mice, however, significant endochondral ossification was observed after MTX treatment when compared to the untreated mice, as revealed by thinning of growth plate, reduction of proliferating chondrocytes, induction of chondrocyte apoptosis and reduced metaphysis heights. Moreover, analysis of total liver glutathione (GSH) levels also revealed significant lower GSH levels in all $MT^{-/-}$ mice when compared to $MT^{+/+}$ mice. Thus, more severe MTX chemotherapy-induced damages to endochondral bone formation were present in metallothionein-I/II null mice which may be associated with oxidative damage.

P29

The effects of long-chain saturated fatty acids on the differentiation of cells of the marrow stromal line Kusa4b10

Watson M, Costa JL, Musson D, Callon KE, Choi A, Lin JM, Cornish J, Naot D and Grey A
Bone Research Group, Department of Medicine, University of Auckland, New Zealand.

Total body fat mass is positively related to bone density and inversely related to fracture risk. Feeding experiments showed that fat ingestion acutely influences bone turnover, thus raising the possibility that ingested nutrients have direct bone effects and could function as a link between fat and bone. We have previously reported that the long-chain, saturated fatty acids palmitic acid (C18) and stearic acid (C16) inhibit osteoclastogenesis *in vitro*. In the current study, we investigate the effects of palmitic and stearic acids on the murine marrow stromal cell line KUSA4b10 differentiation into osteoblastic or adipogenic lineage.
In KUSA 4b10 cells cultured in osteogenic medium, palmitic and stearic acids increased mineralisation at 10 and 50μg/ml while at 75μg/ml only stearic acid had a positive effect. Under adipogenic conditions, both fatty acids increased adipocyte formation in all concentrations tested. Gene expression analysis by quantitative RT-PCR showed that levels of the osteoblast markers alkaline phosphatase and collagenIα1 were not significantly different in comparison to controls in either osteogenic or adipogenic media. Palmitic and stearic acids significantly induced the expression of the adipocyte markers Fabp4 and adiponectin in KUSA4b10 cells cultured in adipogenic medium and unexpectedly these genes were induced in osteogenic medium as well.
Palmitic and stearic acids promote progression of multipotent Kusa4b10 cells into more differentiated phenotype, along the osteogenic and the adipogenic pathways. These results confirm that marrow stromal cells are a target of palmitic and stearic acids.

P30

The migration of host cells towards osteogenic differentiated donor mesenchymal stromal cells in ectopic and orthotopic osteogenesis

Zhou YH[1], Crawford R[1,2] and Xiao Y[1]
[1]*Bone Group, Medical Device Domain, Institute of Health and Biomedical Innovation, Queensland University of Technology.*
[2]*Prince Charles Hospital, Brisbane, Queensland, Australia.*

Aims: Cell-cell interaction is believed to play an important role in osteogenesis. However, it is still unclear how donor osteogenic cells behave and interact with host cells *in vivo*. The purpose of this study was to investigate the interactions between transplanted donor cells and host cells during osteogenesis.
Methods: *In vitro* migration assay was carried out to investigate the ability of osteogenic differentiated human mesenchymal stromal cells (O-hMSCs) to recruit MSCs. At the *in vivo* level, O-hMSCs were implanted subcutaneously or into skull defects in severe combined immunodeficient (SCID) mice. Tissue mineralization and new bone formation were assessed by micro-CT, histological and immunohistochemical methods. *In situ* hybridization (ISH) against human Alu sequences was performed to distinguish donor cells from host cells.
Results: *In vitro* migration assay revealed an increased migration potential of MSCs while co-culturing with O-hMSCs. Significantly enhanced tissue mineralization was detected in both ectopic and orthotopic sites when O-hMSCs were transplanted *in vivo*. ISH against human Alu sequences showed that host mouse cells migrated in large numbers into the transplantation site in response to donor O-hMSCs. Interestingly, host cells recruited by O-hMSCs were the major cell populations in newly formed bone tissues, indicating that O-hMSCs can trigger and initiate osteogenesis when transplanted in orthotopic sites.
Conclusion: The present study demonstrated that *in vitro* induced O-hMSCs were able to attract host cells *in vivo* and were involved in the osteogenesis together with host cells, which is important for bone tissue engineering applications.

P31

The role of calcium-sensing receptor intracellular loops and C-tail in differential signalling

Goolam MA, Ward JH and Conigrave AD
School of Molecular Bioscience, University of Sydney, NSW, 2006, Australia

The extracellular Ca^{2+}-sensing receptor (CaR) is a class C GPCR that in addition to Ca^{2+}, responds to various extracellular agonists and modulators in tissues including the parathyroid, kidney and bone. The CaR mediates cell responses by differential intracellular signalling, inducing changes in intracellular enzymes and metabolites like phospholipases, MAP kinases, Ca^{2+}_i and cAMP. In this study, we investigated the role of residues in intracellular loops (iL) and the CaR C-tail in mediating signalling by distinct pathways.
Three iL mutants, F706A, L797A and E803A, and a receptor truncated after residue 865, $CaR_{865}X$, lacking most of the C-tail, were studied. The mutants were transiently transfected into HEK 293 cells and evaluated for their effect on expression, Ca^{2+}_i mobilisation and changes in cAMP levels in response to various extracellular Ca^{2+} concentrations.
None of the four mutants impaired the level of total or plasma membrane CaR expression. While the three iL mutants exhibited significantly impaired maximal responses for Ca^{2+}_i mobilisation by around 40%, $CaR_{865}X$ abolished the response. In contrast, F706A (iL2) and L797A (iL3) abolished CaR-mediated inhibition of forskolin-stimulated cAMP levels, whereas E803A and $CaR_{865}X$ maintained cAMP inhibition at levels comparable to wild-type CaR.
These results suggest that F706 and L797 may play a general role in CaR G-protein coupling, while E803 and the C-tail are important for coupling to $G_{q/11}$ and/or PLC activation pathways but not for $G_{i/o}$ coupled pathways. Future studies will involve investigating the role of these mutants in the activation of other important signalling events including ERK1/2 phosphorylation and IP turnover.

P32

Transcriptional regulation of endochondral ossification by PLZf during longitudinal bone growth

Lin Z[1], Shee S[1], Qin A[1], Abel T[2], Thien C[3], Langdon W[3], Xu J[3], Zheng MH[1]
[1]*Centre for Translational Orthopaedic Research,* [2]*Centre for Microscopy, Characterisation and Analysis The University of Western Australia,* [3]*School of Pathology and Laboratory Medicine, The University of Western Australia Nedlands, 6009, WA, Australia*

Endochondral ossification is an essential process for longitudinal bone growth and development of osteoarthritis. Chondrocyte proliferation and differentiation, formation of secondary ossification, cartilage matrix degradation, and vascular invasion are all central steps of endochondral ossification during normal long growth, we have observed the deformity of hind limbs and defect of long bone development in Promyelocytic Leukaemic Zinc Finger (plzf) gene mutated mice. In this study, we aimed to characterize the skeleton development of the plzf mutated mice at embryonic and postnatal stage, and to investigate the role of plzf in the process of endochondral ossification and its effect on bone development. The mutation of the plzf protein consisted of an 8bp deletion (△nt1176-1183), which resulted in a truncation of plzf protein missing 9 of the zinc finger motifs. This mutation repress the nuclear translocation of plzf gene, and thus hidden its binding to subsequence target genes. The plzf mutated mice exhibited absence of vascular invasion at the epiphysis end of long bone around 1 week of age as the first noted difference during bone growth. The delay of secondary ossification center formation last till 3 week of age, and resulted in the early appearing of osteoarthritis in week 11. However, genes associated with vasculization in endochondral ossification centre, such as MMP-14, VEGF, HIF-1alpha, HIF-2alpha and HIF-3alpha, did not show significant reduction in the mutant. Time course culture of mesenchymal stem cells in differentiation medium revealed that the mutation of plzf gene abolished the upregulation of COMP, col10a1 and osteocalcin gene expression. Hence, plzf is an important regulator that targets several crucial genes for endochondral ossification and chondrocyte differentiation, and the regulation may due to its transcriptional activity via the zinc finger domain.

P33

Transgenic disruption of glucocorticoid signalling in mature osteoblasts and osteocytes does not affect murine antigen-induced arthritis

Wiebe E[1,2], Spies CM[1,2], Tu J[1], Gaber T[2,3], Li A[1], Huscher D[2,3], Buttgereit F[2], Seibel MJ[1,4] and Zhou H[1]
[1]Bone Research Program, ANZAC Research Institute, The University of Sydney, Sydney, Australia; [2]Department of Rheumatology and Clinical Immunology, Charité - University Medicine Berlin, Berlin, Germany, [3]German Rheumatism Research Centre (DRFZ), Berlin, Germany, [4]Dept of Endocrinology & Metabolism, Concord Repatriation Hospital, The University of Sydney, Sydney, Australia.

Objective: Endogenous glucocorticoids appear to play an immunomodulatory role in the initiation and maintenance of auto-immune arthritis. We previously demonstrated that disruption of GC signalling in osteoblasts attenuates K/BxN mouse serum-induced arthritis, a model critically driven by innate immune system players. By using the model of T cell-dependent antigen-induced arthritis (AIA), we now tested whether osteoblast-specific GC effects modulate both, innate and adaptive immune responses, further characterising this novel aspect of osteoimmunology.
Methods: GC signalling was disrupted in osteoblasts by transgenic overexpression of 11β-hydroxysteroid dehydrogenase type 2 controlled by a collagen type I promoter. AIA was induced by intra-articular injection of methylated BSA (mBSA) into one knee joint of pre-immunised mice. Controls received PBS. Prolonged arthritis was achieved through three repeated intravenous injections of mBSA. Knee joint swelling was measured continuously until day-14 and day-28, respectively. Disease extent was assessed by histology, histomorphometry and micro-CT.
Results: Both transgenic and wild-type mice treated with mBSA developed acute and significant arthritis with maximum knee joint swelling on day-1 post injection and abatement thereafter, with no significant difference between transgenic and wild-type mice. These findings were corroborated by corresponding histological indices of inflammation and cartilage damage. Similar observations were made in the prolonged AIA model.
Conclusion: In contrast to K/BxN serum-induced arthritis, disruption of GC signalling in osteoblasts does not affect murine AIA. This suggests that osteoblasts do not modulate the adaptive immune response (T cells, B cells) but the innate immune response (complement, Fc-receptors, neutrophils, mast cells) via a GC-dependent pathway.

P34

Wnt signalling inhibition as a potential therapeutic in ankylosing spondylitis

Haynes KH[1], Pettit AP[2], Duan R[1], Tseng H[1], Kniessel M[3], Glant TT[4], Brown MA[1] and Thomas GP[1]
[1]University of Queensland Diamantina Institute, Brisbane, Australia; [2]University of Queensland Centre for Clinical Research, Brisbane, Australia; [3]Novartis Institutes for BioMedical Research, Basel, Switzerland; Section of Rheumatology, Rush University Medical Center, Chicago, USA.

The mechanism driving the progression from inflammation to osteoproliferation and excessive bone formation in ankylosing spondylitis (AS) is poorly understood. In the proteoglycan-induced spondylitis model (PGISp) inflammation leads to intervertebral disc destruction which is then followed by ectopic cartilage formation that eventually ossifies resulting in ankylosis.
Recently several studies have proposed that altered levels of the Wnt inhibitors SOST and DKK1 may play a role in AS and we have reported decreased levels of SOST and DKK1 in PGISp mice. In this study we have tested the therapeutic potential of treatment with recombinant SOST (rSOST) to inhibit Wnt signalling in this model.
PGISp mice were treated with rSOST (2.5μg/day/sub-cut) for 8 weeks from week 8 when inflammation commences. ELISA showed rSOST was stable in circulation for between 8-24hrs after injection. Serum SOST levels in PGISp mice were decreased 4 weeks post disease induction but after 8 weeks of rSOST treatment serum SOST levels had partially recovered. Vertebral SOST levels were decreased in PGISp mice by IHC but rSOST–treated PGISp mice showed increased SOST suggesting the rSOST was targeting the joints. No changes were seen in BMD by DEXA. Vertebral disease was assessed histologically at the termination of the study and 8 weeks of rSOST treatment showed no effects on disease possibly due to SOST levels not regaining normal values.
This pilot study has demonstrated stability of rSOST *in vivo* and activity in affected joints. Future studies will utilise higher rSOST doses and a longer time course of treatment.

P35

An injectable scaffold for bone tissue engineering

Cheng TL[1,2], Valchev P[3], Dehghani F[3], Little DG[1,2] and Schindeler A[1,2]
[1] Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead.
[2] Paediatrics and Child Health, Faculty of Medicine, University of Sydney.
[3] Chemical Engineering Department, University of Sydney.

Abstract not able to be published.

P36

Analysis of bone quality in smoking model rats by FTIR imaging and Raman spectroscopy

Kimura-Suda H[1], Ueno H[1], Yamato H[2], Kubo K[3], Tomoda K[4] and Kimura H[4]
[1]Department of Bio- and Material Photonics, Chitose Institute of Science and Technology, [2]Biomedical Research Laboratories, Kureha, [3]Laboratory Animal Research Center, Nara Medical University, [4]Second Department of Internal Medicine, Nara Medical University

Smoking has been known to cause low bone mineral density (BMD), femoral neck fracture, and osteoporosis in addition to an increased risk for the overall decline in health status. BMD in humans has been reported to depend on smoking status, non-smokers > the ex-smokers > smokers, and smokers have about 1.3-1.8 times the risk of fracture compared with non-smokers. On the other hand, the mechanism of bone loss associated with smoking remains unclear, since there are direct and indirect impacts of smoking on bone health. In previous works, we have characterized bone qualities in rats and mice by Fourier transform infrared (FTIR) imaging and microscopic Raman spectroscopy. Bone strength reflects the integration of two main features: bone density and bone quality. Although each of high turnover bone due to secondary hyperparathyroidism caused by chronic kidney disease or the bone of the rickets shows all low bone density, the bone qualities such as bone structure, bone turnover, calcification, cross-linking of collagen were different. In this works, we have focused on bone quality in smokers who have lower BMD, and describe the complementary analysis by FTIR imaging and Raman spectroscopies to characterize bone quality in smoking model rats. Ten-week-old male SHE rats were exposed to cigarette smoke twice daily 40min for 8 weeks. The femurs of smoking rats were characterized by x-ray, bone density measurement, FTIR imaging and microscopic Raman spectroscopy (1064nm), and each data was compared to that of control rats. The X-ray image and bone density indicate that the bone density of smoking rat is clearly reduced. FTIR images of the calcification of femur in smoking rat show trabecular bone was decreased rather than cortical bone, and the carbonate-substituted hydroxyapatite, i.e. carbonated hydroxyapatite was increased, and the crystallinity was reduced. From the above results, we conclude that bone quality of smoking rat is different from that of other low density bone due to some disease.

P37

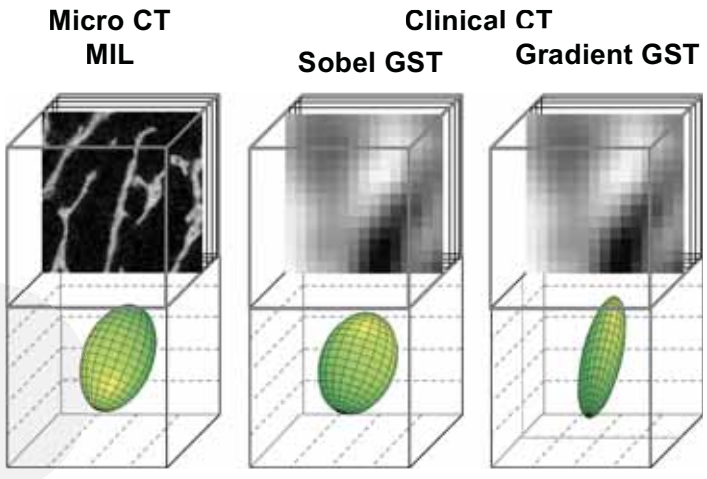
Anisotropy can be measured from clinical-level computed tomography images

Kersh ME[1], Wolfram U[2], Zysset P[2] and Pandy MG[1]
[1]Department of Mechanical Engineering, University of Melbourne
[2]Institute of Surgical Technology and Biomechanics, University of Bern

The question remains if the gradient structure tensor (GST) can be effectively used to calculate anisotropy in 3D in clinical-level computed tomography (CT) data. We compared the principal anisotropic direction and degree of anisotropy (DA) using the GST to the mean intercept length (MIL) in 100 trabecular cubes (side length = 5.4mm) from a human proximal femur. The cubes were scanned in a micro-CT scanner (18 μm spatial resolution) and in a clinical QCT scanner (360 μm). Two methods for calculating the GST in the clinical QCT were evaluated: 1) Sobel kernel; and 2) central difference gradient. The sensitivity to edge artifacts was analyzed.

There were no significant differences in the principal direction of bone using either GST method when edge voxels were ignored, and the average error was 28 ± 19º. Including voxel edges increased the error in the Sobel-based principal direction to 35º but did not change the gradient results. No differences in measures of DA were found when edge voxels were ignored, and the root mean square (RMS) error was 0.43. The error in DA using the Sobel-based GST (RMS error = 0.16) was significantly lower (alpha = 0.05) than that obtained using the gradient method (0.42) when edge voxels were included.

While edge voxels are usually ignored in morphological calculations, their exclusion is more detrimental to calculations of DA due to the sparse nature of clinical QCT data. Our results reveal that the gradient method is sensitive to these edge effects and the Sobel-based GST is a more robust estimator of anisotropy. The inclusion of anisotropic parameters may be useful in finite element models based on clinical level QCT data, and improve their predictive capabilities.



Measures of anisotropy visualized within identical perfect cubes as the shape (DA) and orientation of an ellipsoid using the gold standard (left), a Sobel –based tensor (middle), and gradient tensor (right).

P38

Cortical bone loss and porosity predate menopause

Bjørnerem Å[1], Ghasem-Zadeh A[2], Zebaze R[2], Bui M[3], Wang XF[2], Hopper JL[3] and Seeman E[2]
[1]*Department of Clinical Medicine, University of Tromsø, Norway.*
[2]*Endocrine Centre, Austin Health, University of Melbourne, Australia.*
[3]*Centre for MEGA Epidemiology, University of Melbourne, Australia.*

Introduction: In young adulthood, remodeling is balanced; no bone loss occurs. Remodeling imbalance appears before menopause; less bone is replaced than resorbed producing trabecular bone loss. Cortical bone loss is held to begin after menopause even though the imbalance is global. An alternative explanation is that cortical bone loss occurs by intracortical remodeling creating small pores requiring sensitive methods of detection.
Methods: Images of the distal tibia were obtained using high-resolution peripheral quantitative computed tomography (HR-pQCT; Scanco Medical) in 200 pre- and 83 post-menopausal women in a cross-sectional study in Melbourne. Cortical porosity was quantified using StrAx1.0.
Results: In premenopausal women, cortical porosity correlated positively with age; each standard deviation (SD) greater age was associated with 0.20 SD higher porosity ($p = 0.004$) in the cortical transitional zone ($p = 0.01$). These deficits were not detected using DXA. In postmenopausal women, each SD greater age was associated with 0.38 SD higher porosity now in compact appearing cortex as well as in the transitional zone where porosity was 0.40 SD higher; twice that in premenopausal women (all $p < 0.001$). Higher porosity was associated with high levels of remodeling markers in pre- and post-menopausal women.
Inference: Intracortical remodeling produces bone loss and porosity before menopause. As the slow loss of the four-fold larger cortical than trabecular bone volume accounts for 70% of all appendicular bone loss and porosity reduces cortical stiffness to the 7^{th} power, this finding has implications regarding the timing of intervention.

P39

Differences in distal radius intracortical porosity in Chinese and Caucasian premenopausal women

Wang XF, Ghasem-Zadeh A, Wang Q, Teo J, Zebaze R and Seeman E
Department Endocrinology and Medicine, Austin Health, University of Melbourne.

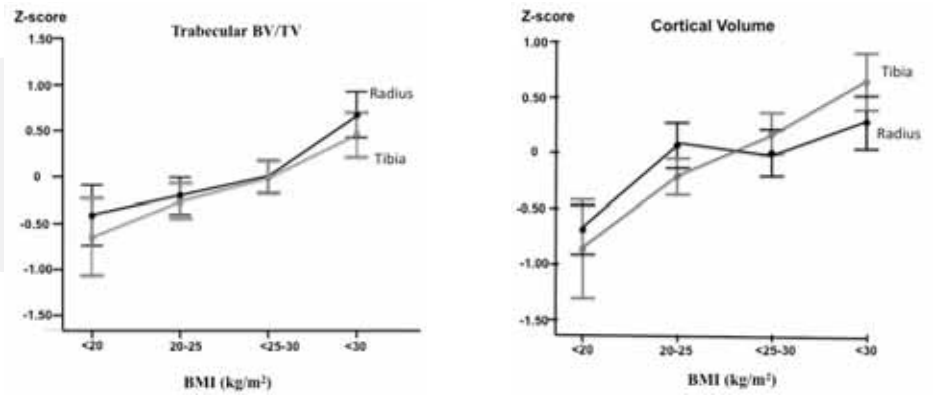
Chinese women have lower fracture rates, smaller appendicular bones with thicker cortices, thicker but fewer trabeculae. The endocortical and intracortical surface (haversian canals) are contiguous and so we speculated that reduced excavation of the (smaller) medullary canal producing the thicker cortex also results in less porosity than in Caucasians.
Distal radius images from high-resolution peripheral quantitative computed tomography (HR-pQCT, XTreme CT, Scanco) were processed in 23 healthy premenopausal Chinese and 59 Caucasian women (18-46 years) using Strax1.0, a non-threshold based image analysis algorithm that segments the mineralized matrix volume and void volumes of the compact-appearing cortex, the transitional and trabecular regions. The proportion of void was quantified as the average of void spaces in each voxel.
Chinese women were shorter. Porosity of the compact cortex and outer transitional zone both were lower in Chinese than Caucasians (21.2 vs. 23.3%; 31.6 vs. 33.6%. both $p<0.05$). The porosity of the inner transitional zone was similar by race (77.0 vs. 77.9%, NS). The relatively larger cortical area within a smaller bone in Chinese was attributed to a larger compact mineralized cortex than Caucasians (25.5% vs 20.3%, $p<0.001$), not due to lower void volume.
Lower fracture risk in Chinese women may be partly due to less porous but thicker cortex in a smaller bone – more bone within the bone than in Caucasians.

P40

Distal radial and tibal cortical and trabecular microarchitecture and BMI in premenopausal women

Teo JW, Wang X, Ghasem-Zadeh A, Wang Q and Seeman E
Department of Medicine, University of Melbourne, Melbourne, Australia

Body mass index (BMI) is protective against fragility fractures, perhaps, in part, due to loading. We hypothesized that BMI will be more strongly associated with tibial than radial microstructure.
In 138 Caucasian premenopausal women, a mean age 35 yrs (range 19-50), images of the distal metaphyses of radius and tibia of non-dominant side were scanned using high-resolution pQCT (XtremeCT, Scanco). Participants were categorized according to their BMI < 20, 20-25, 25-30, and >30 without difference in age, height or bone total CSA.
Women with greater BMI had high trabecular BV/TV at both site ($p < 0.001$) and no interaction between group and bone site on trabecular parameter was found. Cortical bone volume was lower in those with the lowest BMI at both sites compared to other groups ($p < 0.001$). Higher cortical volume at distal tibia was associated with higher BMI ($p < 0.001$) while there is no difference in cortical volume at the distal radius between the normal, overweight and obese group (group x site interaction $p = 0.007$)(fig). We infer that BMI may influence cortical structure via mechanical loading and influence trabecular morphology by other mechanism, perhaps hormonal factors.



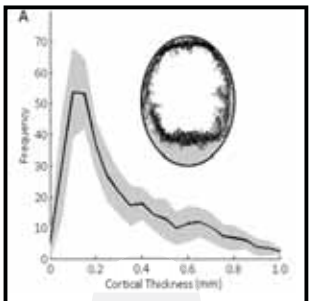
P41

Heterogeneity in femoral neck structure and its relationship to strength

Kersh ME[1], Zebaze R[2], Jones AC[3], Arns CH[2], Knackstedt MA[3], Pandy MG[1] and Seeman E[2]
[1]*Department of Mechanical Engineering, University of Melbourne*
[2]*Departments of Medicine and Endocrinology, Austin Health, University of Melbourne*
[3]*Department of Applied Maths, RSPhysSE, Australian National University, Canberra*

Structural measures of strength derived using dual energy X-ray absorptiometry or quantitative computed tomography (QCT) model the femoral neck as a cylinder with a single cortical thickness. We hypothesized that these simplifications introduce errors in estimated indices of strength and alternative surrogates that recognize the heterogeneity in bone structure will better capture femoral neck strength. Twelve postmortem femoral specimens from women aged 29-85 years were analyzed for cortical area, cortical fraction, cortical thickness and section modulus using high resolution QCT.

There was heterogeneity in the radial distribution of cortical thicknesses. These were not normally distributed but skewed towards thinner cortices in all slices (Fig) Use of the mean cortical thickness overestimated the central tendency of cortical thickness in 81% of slices and the correlation between cortical thickness and strength was higher using the median than the mean cortical thickness ($r = 0.85$ vs. 0.56 respectively, $p < 0.001$). However, neither median nor mean accurately predicted section modulus with accuracy being 45% for the median and 38% for the mean.



Distribution of normalized cortical thicknesses across all slices of the femoral neck in one specimen.

The heterogeneity of the femoral neck structure is the very feature that determines the diversity of its regional strength within and between individuals. While neither measure consistently predicted section modulus with accuracy greater than 50%, the median cortical thickness best captured the heterogeneity of the femoral neck compared to the mean cortical thickness.

P42

Musculoskeletal interactions in Neurofibromatosis type 1 (NF1)

Deo, N[1,2], El-Hoss J[1,2], Sullivan K[1], and Little DG[1,2] and Schindeler A[1,2]
[1] *Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead, NSW, Australia.*
[2] *Paediatrics and Child Health, Faculty of Medicine, University of Sydney, NSW, Australia.*

Neurofibromatosis type 1 (NF1) is a common genetic disorder that can have profound orthopaedic complications including tibial dysplasia/pseudarthrosis and scoliosis. The relationship between NF1-deficient bone and muscle is poorly understood, but there are recent anecdotal reports and limited clinical evidence for reduced muscle function in children with Neurofibromatosis type 1.

To better understand the interplay between muscle and bone we have completed a detailed study in two mouse models of NF1 deficiency. The *Nf1*$^{+/-}$ mouse has one defective copy of the NF1 gene and presents with a mild phenotype with only some of the clinical features of NF1. These mice showed no difference in overall weight or lean tissue mass and grip strength tests indicated no substantive muscle weakness. In a model of botox-induced limb disuse and recovery, both *Nf1*$^{+/-}$ mice and wild type controls showed comparable muscle degeneration/regeneration and bone loss and restoration.

Next a novel muscle-specific conditional knockout was generated crossing *MyoD-cre* transgenic mice with the *Nf1*$^{flox/flox}$ strain (*Nf1*$_{muscle}^{-/-}$ mice). These mice showed a failure to thrive and neonatal lethality. Muscle mass was reduced, as was fibre size in d6 muscle. Muscle ultrastructure was examined by electron microscopy, which revealed profuse intra-myofibre fat globules. This was confirmed by Oil Red O staining. Enzymatic studies are underway to examine the underlying biochemical deficiency.

In summary, these findings support a key role for NF1 in muscle. Further work is required to evaluate the pathobiology of muscle in NF1 patients, as well the patho-physiological relationship between muscle weakness and scoliosis progression.

P43

Nutritional factors influence bone microarchitecture during growth

Shahmoradi N, Iuliano-Burns S, Wang XF, Ghasem-Zadeh A, Zebaze R, Wang Q and Seeman E
Department of Medicine, University of Melbourne, Melbourne, Australia

Rational and Aim: The skeleton is responsive to environmental factors during growth, in part perhaps due to the intense cellular activity of modeling and remodeling which assemble its structure during the first two decades of life. High resorptive modeling (not followed by formation at the same location) and perhaps remodeling activity excavate the medullary canal determining cortical thickness together with periosteal apposition. At the ends of long bones, endochondral apposition and condensation of growth plate trabeculae form the metaphyseal cortex with varying degrees of porosity determined by the differing tempo of longitudinal and appositional growth. We hypothesized that a higher intake of calcium, vitamin D and protein during childhood are associated with attainment of a more robust appendicular skeleton; thicker and less porous cortices and a smaller medullary canal.

Material and Methods: We measured tibial macro- and micro-architecture using high-resolution peripheral quantitative computed tomography (HR-pQCT). Dietary intakes were assessed using 3-day weighed-food diaries.

Results and Conclusion: Cross-sectional study of 62 healthy boys and girls aged from 6 to 18 years (mean 11 years) of whom 53.2% were prepubertal, 27.4% peripubertal and 19.4% postpubertal. Intakes were calcium (803 mg, range 216 to 1623), vitamin D (2.89 ug, range 0.6 to 9.9) and protein (82 g, range 25.9 to 208). Results were expressed as mean, 95% confidence interval (CI).In a General Linear Model multivariate analysis, the effect size (ES) estimated from the partial beta^2 is greater between cortical volumetric bone mineral density (vBMD) and calcium intake (ES= 0.025%, 95% CI, -0.095 to 0.022) and vitamin D intake (ES= 0.054%, 95%CI, -18.5 to 0.72). Protein intakes explained the greatest effect size (ES= 0.054, 95 % CI, 0.107 to 3.206%) on total cross sectional area of the tibia compare to other bone macro- and microarchitecture results. Two factors limit the power of this study, the small sample size and the range of nutritional intakes which may be above those defining 'insufficiency'. Within these constraints we infer that ensuring adequate protein intake may benefit the attainment of peak bone microstructure during growth.

P44

Quantitative ultrasound estimates of volume fraction and structure of cancellous bone

Wille M-L[1], Flegg MB[2] and Langton CM[1]
[1]*Institute of Health & Biomedical Innovation and Faculty of Science & Engineering, Queensland University of Technology, Brisbane*
[2]*Oxford Centre for Collaborative Applied Mathematics, Mathematical Institute, University of Oxford, UK*

Aims: The Measurement of Broadband Ultrasonic Attenuation (BUA) at the calcaneus for the assessment of osteoporosis has been extensively clinically validated; there lacks however a fundamental understanding of the dependence of BUA upon the material and structural properties of cancellous bone. It has recently been proposed that the primary attenuation mechanism is phase interference due to variations in transit time as detected over the phase-sensitive surface of the receive ultrasound transducer [1]. This has subsequently led to the development of Ultrasound Transit Time Spectral Analysis (UTTSA), with the potential to quantify both the bone volume fraction and 'structure' of cancellous bone.
Methods: A 1 MHz transmission ultrasonic signal was recorded in each of the three orthogonal directions in magnified stereolithography replicas of four cancellous bone samples that had previously been microCT scanned. Through deconvolution of the input and output ultrasound signals, transit time spectra were derived, from which the bone volume fraction (BVF) and standard deviation of trabecular thickness (SD[Tb.Th]) were estimated.
Results: Coefficients of determination (R^2%) of 96.7% and 86.1% were achieved for the UTTSA estimated values of BVF and SD[Tb.Th] respectively compared to the known values derived from the microCT scans.
Conclusion: This experimental study has successfully demonstrated that UTTSA provides reliable estimates of both BVF and SD[Tb.Th] in stereolithography replicas of cancellous bone. Future work should consider in vitro and *in vivo* validation.

1. Langton C M; 2011; 25th Anniversary of BUA for the Assessment of Osteoporosis – Time for a New Paradigm?; Engineering in Medicine; 225 (2),113-125

P45

The effect of silicate ions on proliferation, osteogenic differentiation and cell signalling pathways (Wnt and Shh) of bone marrow stromal cells

Pingping HAN[1], Chengtie WU[2*] and Yin XIAO[1*]
[1]*Institute of Health & Biomedical Innovation, Queensland University of Technology, and* [2]*State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences.*

Background: Silicon (Si) is a trace element, which plays an important role in human bone growth [1]. Si has been incorporated into biomaterials for the application of bone regeneration in order to improve the osteogenic potential, both *in vitro* and *in vivo* [2, 3]. Little is still known, however, as to how Si ions elicit its biological response on bone-forming cells.
Aims and Methods: To investigate the effect of Si ions on the proliferation, differentiation, bone-related gene expression and cell signalling pathways of bone marrow stromal cells (BMSCs) by comparing BMSC response to different concentrations of NaCl and Na_2SiO_3, taking into account and excluding the effect of Na ions (Table1).
Results and Discussion: Our study showed that Si ions at a certain concentration significantly enhanced the proliferation, mineralization nodule formulation and bone-related gene expression *(OCN, OPN and ALP)* of BMSCs. Furthermore, Si ions at 0.625mM could counteract the effect of Wnt inhibitor on the osteogenic genes expression, *(OPN, OCN and ALP)*, Wnt and Shh signalling pathway-related genes in BMSCs.
Conclusions: These results suggest that Si ions by themselves play an important role in regulating the proliferation and osetogenic differentiation of BMSCs with the involvement of Wnt and Shh signalling pathways.

ACKNOWLEDGEMENTS
Funding for this study was provided by ARC Discovery DP120103697, the Prince Charles Hospital Foundation MS2011-05, and One Hundred Talent Project, SIC-CAS (Dr Wu).

REFERENCES

1. Carlisle EM. Science 1970; 167: 279-280.
2. Schwarz K, Milne DB. Nature 1972; 239: 333-334.
3. Vallet-Regi M, Arcos D. J Mater Chem 2005; 15: 1509-1516.

Table 1 Corresponding concentrations of Na and Si ions in two groups (A: NaCl; B: Na_2SiO_3) (mM)

Group A	**Na**	10	5	2.5	1.25	0.125	0.0125
	Si	0	0	0	0	0	0
Group B	**Na**	10	5	2.5	1.25	0.125	0.0125
	Si	5	2.5	1.25	0.625	0.0625	0.00625

P46

17β-Estradiol promotes extracellular calcification of adipose tissue-derived stem cells during osteogenesis

Wang JZ[1,2], Lewis JR[1,2], Liew JL[1,2], Tan J[3], Adams L[1], Prince RL[1,2]
[1]*School of Medicine and Pharmacology, University of Western Australia, Crawley, WA*
[2]*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA*
[3]*Department of General Surgery, Sir Charles Gairdner Hospital, Nedlands, WA*

Human subcutaneous adipose tissue is considered as a promising source of bone progenitor cells. The mechanisms of 17β-Estradiol enhancing the human skeleton remain uncertain. This study investigates the role of 17β-Estradiol on *ex vivo* differentiation of human female subcutaneous tissue-derived stem cells to the osteoblastic linage. Subcutaneous adipose tissue was collected from 11 patients (mean age 45.5 ± 10.7) during abdominal surgery. The MSC phenotype was confirmed prior to osteogenic differentiation using either osteogenic media (OSM), osteogenic media with low and high concentrations of 17β-Estradiol supplement (E2: 10^{-9}M and 10^{-8}M) and osteogenic media with high 17β-Estradiol plus an estrogen receptor alpha antagonist ICI 182780. Cellular staining and gene expression were used to characterise osteogenesis. Compared to control, OSM stimulated cellular proliferation from day 7 to day 28 ($P<0.001$) and increased alkaline phosphatase and collagen staining regardless of estradiol levels. Although OSM + E2 (10^{-9}M and 10^{-8}M) did not increase cellular proliferation the addition of ICI showed a significant lower proliferation at day28 ($p<0.001$). Extracellular calcification assessed by Alizarin Red was increased with OSM + HIGH E2 (10^{-8}M) (mean difference from OSM 3.84 ± 0.72%, p=0.004). This effect was removed by the addition of ICI.
Runx2, Alpl and *OMD* gene expression was increased in all OSM groups while no osteocyte markers were detected by quantitative RT-PCR in any OSM group. In conclusion, 17β-Estradiol enhances their extracellular matrix calcification during osteogenesis of human subcutaneous-derived stem cells via an ER alpha pathway however the mechanism of calcification needs further study.

P47

β-adrenergic signaling directly stimulates osteoclastogenesis via reactive oxygen species

Kondo H and Togari A
Department of Pharmacology, School of Dentistry, Aichi-Gakuin University.

The sympathetic signaling regulates bone resorption through the receptor activator of nuclear factor κB ligand (RANKL) expression via the β-adrenergic receptor (β-AR) on osteoblasts. Reactive oxygen species (ROS) are known as one type of osteoclast regulatory molecule. Here we show that sympathetic signaling directly regulates osteoclastogenesis through β2-AR expressed on osteoclasts via intracellular ROS generation. In an *in vitro* study, β-AR agonist, isoprenaline, increased intracellular ROS generation in osteoclasts prepared from bone marrow macrophages (BMMs) and RAW264.7 cells. Isoprenaline enhanced osteoclastogenesis through β2-AR expressed on BMMs and RAW264.7 cells. The anti-oxidant, α-lipoic acid (α-LA), inhibited isoprenaline-enhanced osteoclastogenesis. Isoprenaline increased the expression of osteoclast-related genes such as nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1), tartrate-resistant acid phosphatase (TRAP), and cathepsin K on osteoclasts. α-LA also inhibited isoprenaline-induced increases of these gene expressions. These *in vitro* results led to the hypothesis that β-adrenergic signaling directly stimulates osteoclastogenesis via ROS generation. In an *in vivo* study, isoprenaline treatment alone caused oxidative damage in local bone and reduced bone mass due to an increase in bone resorption. Furthermore in α-LA-treated mice, isoprenaline did not increase the number of tibial osteoclast even though the RANKL/osteoprotegerin (OPG) ratio increased. These results revealed that α-LA prevents isoprenaline-induced osteoclastogenesis independently of RANKL expression via β2-AR on osteoblasts. These *in vitro* and *in vivo* results indicate that β-adrenergic signaling, at least in part, directly stimulates osteoclastogenesis through β2-AR on osteoclasts via ROS generation.

P48

A novel role for glucocorticoid-mediated osteoblast-fibroblast crosstalk in inflammatory disease

Hülso C[1*], Hardy RS[1,2*], Liu Y[1], Tu J[1], Stoner S[1], Cooper MS[2], Seibel MJ[1,4] and Zhou H[1]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia;* [2]*Centre for Endocrinology, Diabetes and Metabolism, Institute of Biomedical Research, University of Birmingham, Birmingham, UK;* [3]*Department of Immunity and Infection, Institute of Biomedical Research, University of Birmingham, Birmingham, UK;* [4]*Department of Endocrinology & Metabolism, Concord Hospital, The University of Sydney at Concord Campus, Sydney, Australia.*
**These Authors contributed equally*

Within the synovium, crosstalk between leukocytes and local stromal populations maintain inflammation and drive destruction during joint disease. Osteoblasts have been shown to promote the inflammatory process in a glucocorticoid (GC) dependant manner. We have investigated GC regulated osteoblast signaling to better elucidate these pro-inflammatory mechanisms. MC3T3-E1 cells were differentiated for 12 days under osteogenic condition. We examined multiple pro-inflammatory secreted factors in response to endogenous GCs using real time PCR and ELISA for the effects of OB conditioned media on primary synovial fibroblasts. At 12 days calcified nodules were formed in MC3T3-E1 culture. At day 12 under differentiation conditions, OBs were treated with either vehicle, TNFa (10 ng/ml) or corticosterone (10 nmol/l) for a further16 hr. OBs increased secretion of IL-6 in response to TNFa (24.2 $\pm$ 1.3 versus undifferentiated control 7.4 $\pm$ 1.5; $p<0.05$) but were unaffected by corticosterone. mRNA expression of the pro-inflammatory factor resistin and leukotriene generating enzymes LOX5 and 15 were increased corticosterone treatment (resitin, 3.9; LOX5, 3.3; LOX15, 2.3 fold; $p<0.05$). Conditioned media from TNFa stimulated OBs resulted in strong trends towards elevated IL-6, VCAM-1 and ICAM-1 mRNA expression in synovial fibroblasts and a significant increase in CCl-2 expression (5.4 fold; $p<0.05$). This study has identified potential glucocorticoid regulated pro-inflammatory signaling factors including resistin and leukotrienes as candidates for osteoblast crosstalk with synoviocytes. We have also demonstrated that conditioned media from inflammatory cytokine stimulated OBs induces an inflammatory phenotype in primary synovial fibroblasts cultures.

P49

Alg2, identified as a downstream mediator of Schnurri-3, inhibits function of *Runx2* and osteoblast differentiation

Imamura K[1,2], Maeda S[1], Kawamura I[1,2], Ishidou Y[1], Yokouchi M[2] and Komiya S[1,2]
[1]*Department of Medical Joint Materials, Kagoshima University, Kagoshima, JAPAN and* [2]*Department of Orthopaedic Surgery, Kagoshima University, Kagoshima, JAPAN.*

Objective: Schunurri-3 (*Shn3*) was shown to promote proteasomal degradation of Runx2 protein by recruiting E3 ubiquitin ligase Wwp1, that Shn3 knockout mice exhibited increased bone formation and bone mass. However, because Shn3 is originally known as a transcriptional factor, we hypothesized that Shn3 induces *de novo* gene(s) to regulate osteoblast differentiation.
Methods: We analyzed the expression profile of *Shn3* siRNA-transfected MC3T3-E1 osteoblasts by microarray. We induced osteoblast differentiation of bone marrow stromal cell ST-2 and MC3T3-E1 with BMP-2. Gene overexpression was achieved by generating stable transfectants or infecting adenovirus. Gene expression was analyzed by real-time RT-PCR.
Results: Asparagine-linked glycosylation 2 homolog (*Alg2*) was highly expressed in MC3T3-E1 cells, and it was down-regulated by *Shn3* knockdown, whereas it was up-regulated by *Shn3* overexpression. Promotor of *Alg2* harbored a consensus binding sequence of *Shn3*. *Alg2* is a mannosyltransferase playing a role in N-linked protein glycosylation. *Alg2* knockdown in ST-2 resulted in a promoted osteoblast differentiation without affecting protein level of Runx2, while overexpression of *Alg2* inhibited osteoblast differentiation. Luciferase assay using 6xOSE2 luc reporter revealed that Alg2 inhibited the activity of Runx2 in a dose-dependent manner. An immunocytochemistry showed that combined transfection of Alg2 and Runx2 in COS7 prevented nuclear localization of Runx2. A weak physical interaction between Alg2 and Runx2 was detected by immunoprecipitation followed by immunoblotting.
Conclusion: These results suggested that Alg2 inhibited the activity of Runx2 by preventing its nuclear localization to suppress osteoblast differentiation.

P50

Altered cross-talk between chondrocytes and osteocytes in osteoarthritic joint pathophysiology

Jaiprakash A[1], Wille M-L[1], Chakravorty N[1], Crawford R[1, 2], Feng JQ[3] and Xiao Y[1]
[1]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia*
[2]*Prince Charles Hospital, Brisbane, Queensland, Australia*
[3]*Texas A&M Health Science Center, Baylor College of Dentistry, Texas, USA*

Osteoarthritis (OA) is an organ-level failure of the joint. Current pathophysiological concepts focus on OA as a disease of the whole joint, rather than a “chondro-centric” or “osteo-centric” model. Our recent studies suggest that dysregulated osteocytic proteins contribute to the pathological changes in OA subchondral bone. However, a significant knowledge gap exists in understanding the role of osteocytes and their interaction/cross-talk with articular cartilage chondrocytes (ACCs), in bone remodeling. This study investigated the effects of soluble factors from OA-ACCs on osteocyte metabolism in an in vitro model.

To study the synergistic relationship, chondrocytes from knee tissue of donors undergoing total knee replacement surgery were collected and characterized for their phenotypic stability. After 14 days of differentiation (3D culture), media was replaced with serum free media for 24 hours. Conditioned media (CM) was collected and mixed with fresh osteocyte media (1:1) and used to culture the well-established osteocyte cell line, MLOY4 for three days.

MLOY4 cells demonstrated increased proliferation when treated with OA-ACC-CM. qPCR revealed a statistically significant increase in the relative expression of the bone formation markers, ALP and OPN. RANKL, a bone resorption marker, was also found to be increased. Increased expression of E11 (immature osteocyte marker), DMP1 and OSX (osteocyte/mineralization markers) was also observed.

These findings indicate a possible role of soluble factors from OA-ACCs on osteocyte regulation which may lead to an increased subchondral bone volume and hampered mineral metabolism as observed in OA. This *in vitro* model could contribute to a better understanding of OA pathophysiology.

P51

Annexin A8 is a prototypical substrate-induced gene involved in OC polarization and function and; is regulated by calcineurin-NFATc1 signalling

Crotti TN [1,2], Pavlos N [3], Zawawi M[1], O'Sullivan RP[2], Flannery MR[2], Goldring SR [4], Purdue PE [4] and McHugh KP [2,5]
[1]*The Discipline of Anatomy and Pathology, The University of Adelaide,*
[2]*Beth Israel Deaconess Medical Center and Harvard Medical School New England Baptist Bone and Joint Institute, Boston, MA, USA*
[3]*Faculty of Medicine, Dentistry and Health Sciences School of Surgery, Centre for Orthopaedic Research University of Western Australia*
[4]*Hospital for Special Surgery, New York, NY, USA*
[5]*College of Dentistry School of Advanced Dental Sciences Department of Periodontology, University of Florida, USA*

Introduction: Profiling experiments of pre-osteoclasts (OCs) cultured on different substrates identified Annexin A8 (AnxA8) as a late stage OC gene, which is regulated by contact with the mineralised component of the bone substrate (Crotti J Cell Phys 2011). Quantitative RT PCR confirmed the super-induction of AnxA8 on bone substrate interaction, which was significantly greater than OC markers, including TRAP. AnxA8 protein was higher in bone-associated pre-OCs versus foreign body giant cells attached to biomaterial particles in peri-implant osteolysis. AnxA8, a member of the Annexin family of calcium- and phospholipid-binding proteins, plays a role in F-actin organisation and endosomal association with actin. We investigated the role of AnxA8 in actin modulation and in the organisation and function of endosomes in pre-OC/OC cells. Additionally, we investigated regulation of AnxA8 by the OC transcription factor NFATc1
Methods/Results: Dual immunofluorescence assays of human OC on bone indicated that AnxA8 is associated with punctate vesicles that are reminiscent of endosomes in bone-resorbing OCs. The subcellular localization of AnxA8 appeared membrane oriented, being largely distributed along the basolateral surface of highly-polarised OCs and partially co-distributing with F-actin and β3-integrin. *In silico* promoter analysis identified NFATc1 binding sites in the AnxA8 promoter and promoter/reporter constructs were induced by RANKL and co-transfection with NFATc1. Inhibition of NFATc1 by 11R-VIVIT in human OC assays abrogated AnxA8 expression.
onclusion: AnxA8 is likely regulated by calcineurin signaling, and more specifically, by NFATc1. AnxA8 may regulate cytoskeletal reorganisation in OCs, a process central to the establishment of osteoclastic polarisation and bone resorption.

P52

Bisphosphonates bound to bone inhibit growth of epithelial cells and primary rat osteoblastic cells

Bava U, Cornish J, Callon KE, Bai J, Naot D and Reid IR
Bone Research Group, Department of Medicine, University of Auckland, Auckland, New Zealand.

Bisphosphonates are used as effective antiresorptive agents due to their high affinity for bone and subsequent uptake by osteoclasts. Once internalised by osteoclasts, nitrogen containing bisphosphonates inhibit the enzyme farnesyl pyrophosphate (FPP) synthase within the mevalonate pathway causing disruption of osteoclast function by accumulation of unprenylated proteins. In this study we wished to determine if cells other than osteoclasts are affected by bisphosphonates with a focus on epithelial cells due to bisphosphonates being implicated in osteonecrosis of the jaw (ONJ).
Sterile bovine bone slices were pre-coated with either PBS or bisphosphonate solution. Epithelial cells (Caco-2 and CHO-S cell lines) and primary rat osteoblasts were cultured on these bone slices. Cell growth was assessed by cell counts and thymidine incorporation at 4-72 hours. Cell lysates were used to determine levels of unprenylated Rap1A, and cleavage of caspase-3 was uses as a marker of apoptosis.
Cells cultured on bisphosphonate-coated bone showed a significant reduction in cell number and thymidine incorporation compared to cells on control bone. These reductions were related to the clinical potency of the bisphosphonate used and were dose dependent. Levels of cleaved caspase-3 did not change in cells grown on bisphosphonate-coated bone but there was accumulation of unprenylated Rap1A in these cells, implying in inhibition of FPP synthase in these cells.
Our findings demonstrate that growth of epithelial cells and primary osteoblastic cells is inhibited when cultured on bisphosphonate-coated bone. Similar to osteoclasts, cells adjacent to bone bound bisphosphonate are affected via the mevalonate pathway.

P53

Butoxamine, a selective β2-adrenergic antagonist, prevents bone loss and fragility in spontaneously hypertensive rat

Arai M[1,3], Sato T[1,2], Takeuchi S[1], Goto S[2] and Togari A[1]
[1] *Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, Japan.*
[2] *Department of Orthodontics, School of Dentistry, Aichi-Gakuin University, Japan.*
[3] *Department of Dental Hygiene, Aichi-Gakuin Junior College, Japan.*

Recent studies have shown that osteoblasts and osteoclasts express β2-adrenoceptor, and increased sympathetic nervous activity causes bone loss via an increase in osteoclastic bone resorption and a decrease in osteoblastic bone formation. We previously demonstrated that non-selective β-blocker propranolol at low doses (0.1 and 1 mg/kg), but not at a higher dose (10 mg/kg), improved bone loss and bone fragility without affecting blood pressure in spontaneously hypertensive rats (SHR) with hyperactivity of the sympathetic nervous system. In the present study, the dose effects of butoxamine, a selective β2-adrenoceptor antagonist, on bone metabolism were examined in SHR by analysis of microcomputed tomography, bone histomorphometry, biomechanical testing, and plasma biochemistry. Treatment of SHR with butoxamine at 0.1, 1 and 10 mg/kg (p.o.) for 12 weeks increased bone mass indices and biomechanical parameters of strength and toughness of the lumbar vertebrae, suggesting antiosteoporotic activity. Butoxamine dose-dependently decreased osteoclast number and surface per bone surface with decreases in plasma tartrate-resistant acid phosphatase-5b level, a biochemical index of osteoclastic activity. On the other hand, histomorphometry indices of bone formation and plasma osteocalcin concentration reflecting osteoblastic activity were increased in SHR treated with butoxamine at 0.1 and 1 mg/kg, but not at 10 mg/kg. These results suggested that β-adrenoceptor antagonists at a low dose improve osteoporosis with hyperactivity of the sympathetic nervous system via β2-adrenoceptor blocking action, while they may have a somewhat inhibitory effect on osteoblastic activity at a high dose.

P54

Characterisation of inflammatory murine fibroblast-like synoviocytes

Hardy RS[1,2], Hülso C[1], Liu Y[1], Tu J[1], Stoner S[1], Cooper MS[2], Seibel MJ[1,4] and Zhou H[1]
[1] *Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia;* [2] *Centre for Endocrinology, Diabetes and Metabolism, Institute of Biomedical Research, University of Birmingham, Birmingham, UK;*
[3] *Department of Immunity and Infection, Institute of Biomedical Research, University of Birmingham, Birmingham, UK;*
[4] *Department of Endocrinology & Metabolism, Concord Hospital, The University of Sydney at Concord Campus, Sydney, Australia.*

Despite a growing use of fibroblast-like-synoviocytes (FLS) isolated from murine inflammatory models to examine their inflammatory behaviour, a robust characterisation of these cells has not previously been performed. In this study, FLS were isolated from inflamed joints of K/BxN mice and their purity in culture determined by immuno-fluorescence and RT-PCR. Their basal, TNFα and corticosterone stimulated expression of pro-inflammatory genes was determined by Real-Time PCR and ELISA and compared to other mesenchymal cell populations. FLS culture was identified by positive expression of fibronectin, prolyl-4-hydroxylase, CD90.2 and CD248. FLS isolated from K/BxN mice possessed significantly greater basal expression of the inflammatory markers IL-6, CCL-2 and VCAM-1 compared to FLS isolated from non-inflamed tissue (IL-6, 3.6 fold; CCL-2, 11.2 fold; VCAM-1, 9 fold; $P<0.05$). This was abrogated by corticosterone (100 nmol/l). TNFα increased all inflammatory markers to a greater degree in K/BxN FLS relative to other mesenchymal cell lines (K/BxN; IL-6, 40.8 fold; CCL-2, 1343.2 fold; VCAM-1, 17.8 fold; ICAM-1, 13.8 fold; $P<0.05$), with secreted IL-6 mirroring these results (K/BxN; Con, 169 $\pm$ 29.7 versus TNFα, 923 $\pm$ 378.8 pg/ml/1x10^5 cells; $P<0.05$). Dose responses demonstrated maximal effects at 100 nmol/l for corticosterone, and 10 ng/ml for TNFα. The inflammatory phenotype remained stable between passages four and seven. This study validates murine FLS as a model of inflamed human synovial fibroblasts for the investigation of their pro-inflammatory behaviour. Furthermore, we have established a well characterised inflammatory profile in FLS that provide guidelines on their inflammatory responses as well as their effective culture duration.

P55

Comparison of β-adrenergic and glucocorticoid signaling on clock gene and osteoblast-related gene expressions in human osteoblast and osteosarcoma cell

Komoto S[1,2], Kondo H[1], Fukuta O[2] and Togari A[1]
[1] *Department of Pharmacology, School of Dentistry, Aichi-Gakuin University.*
[2] *Department of Pediatric Dentistry, School of Dentistry, Aichi-Gakuin University.*

Most living organisms exhibit circadian rhythms and these oscillations are generated by endogenous circadian clocks, present in suprachiasmatic nuclei (SCN). Output signals from SCN are believed to transmit standard circadian time to peripheral tissue through the sympathetic nervous system and humoral routes. Therefore, we examined the expression of clock genes following treatments with β-adrenergic receptor agonist (isoprenaline) or synthetic glucocorticoid (dexamethasone) in cultured human osteoblast SaM-1 and osteosarcoma MG63. SaM-1 cells were treated with isoprenaline or dexamethasone for 2 hours and gene expressions were determined using real-time PCR analysis. Treatment with isoprenaline or dexamethasone induced the circadian expression of clock genes such as human period 1 (hPer1), hPer2, hPer3 and hbrain and muscle Arnt-like protein1 (hBMAL1). Isoprenaline or dexamethasone treatment immediately increased hPer1 and hPer2 and caused circadian oscillation of hPer1 and hPer2 with three peaks within 48 hours. The hPer3 expressions had one peak after isoprenaline or dexamethasone treatment. The hBMAL expressions had two peaks after isoprenaline or dexamethasone treatment, whose pattern was antiphase to those of other clock genes. Dexamethasone treatment delays oscillation for 2-6 hours compared with isoprenaline treatment in all clock genes. Similar observations were also obtained from MG63 cells. We also examined the expression of osteoblast-related genes such as hα-1 type I collagen (hCol1a1), halkaline phosphatase (hALP) and hosteocalcin (hOC) in SaM-1 cells. Isoprenaline and dexamethasone induced the oscillation of hCol1a1 and isoprenaline up-regulated hCol1a1 expression, but dexamethasone down-regulated hCol1a1 expressions in the first phase.

P56

Cord blood-derived macrophage-lineage cells rapidly stimulate osteoblastic maturation in mesenchymal stem cells in a glycoprotein-130 dependent manner

Fernandes TJ[1], Hodge JM[2], Singh PS[3], Collier FM[2], Ebeling PR[1], Nicholson GC[4] and Quinn JMW[3,5]
[1]Northwest Academic Centre, Dept of Medicine, The University of Melbourne, Footscray, Victoria, 3011, Australia; [2]Barwon Biomedical Research, The Geelong Hospital, Geelong, Victoria 3220, Australia; [3]Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia; [4]Rural Clinical School, The University of Queensland, Toowoomba, Queensland 4350, Australia; [5]Dept of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3168, Australia.

Resident osteal macrophages have been shown to stimulate bone formation and play an important role in bone dynamics. We investigated the influence of macrophage-lineage cells on the induction of osteoblastic differentiation in $CD73^+/90^+/105^+$ mesenchymal stem cells (MSC) derived from human adipose tissue. Macrophages were generated from human CFU-GM progenitors treated with M-CSF for 14 days. Media conditioned for 3 days by macrophages (CM) strongly stimulated osteoblastic differentiation of MSC, evident by elevated alkaline phosphatase (ALP) levels within 4 days, formation of mineralised matrix, and increased Runx2 mRNA expression. This occurred in the presence and absence of ascorbate/dexamethasone co-treatment. CM medium from macrophages activated by interferon-γ produced less osteoblastic differentiation than control CM whereas treatment with lipopolysaccharide, IL-4 or GM-CSF had no effect. The osteoblastic stimulus provided by macrophage CM was blocked by both anti-gp130 and anti-oncostatin M (OSM) antibodies and recombinant OSM strongly stimulated osteoblast maturation in MSC. Thus, macrophage-lineage cells may drive osteoblast differentiation of MSC in a gp130-dependent manner, although this may vary with type and activation status of the macrophage lineage cell, and supports the hypothesis that eliciting gp130-dependent signals in MSC would be a useful approach to increase bone formation.

P57

Cytotoxic mechanism involved with H_2O_2-induced oxidative stress in Human Bone Marrow Stem Cell (hBMSC)

Kim JH[1], Chung MH[1], Leem YH[1], Kim JW[2], Chung HY[3] and Chang JS[1]
[1]Department of Orthopaedic Surgery, Asan Medical Center, Ulsan University College of Medicine, Seoul, South Korea.
[2]Department of Orthopaedic Surgery, Inje University Haeundae Paik Hospital, Busan, South Korea.
[3]Department of Endocrinology and Metabolism, Kyung Hee University School of Medicine, Seoul, South Korea.

Reactive oxygen species (ROS)-induced oxidative stress can be attributed to imbalance between the production of ROS and antioxidative capacity. Bone marrow stem cell (BMSC) plays a crucial role in musculoskeletal homeostasis. There is mounting evidence on relationship between oxidative stress and pathophysiological events in malfunction and damage of BMSC. The detrimental effects of oxidative stress on BMSC haven't fully understood. Accordingly, we further investigated underlying mechanism linked to oxidative stress-induced cytotoxicity of BMSC. We measured the dose- and time-dependent effects of H_2O_2 on cytotoxicity of hBMSC using LDH assay. Based on results, we adopted a cell death experimental paradigm with 200 μM H_2O_2 for 18h, unless indicated otherwise. H_2O_2-induced cytotoxicity was completely blocked by catalase (100-300 unit), and NAC (100-500 μM) repressed 80-90% cytotoxicity. Neither ascorbate (100-500 μM) nor Trolox (100-500 μM) inhibited H_2O_2-induced cytotoxicity. Furthermore, cell death of hBMSC was dramatically inhibited by treatment with Ebselen (10-100 μM), GSH-EE (1-3 mM), and NAME (1-5 mM). In contrast, MnTBAP (30-100 μM) and DPI (1-10 μM) couldn't inhibit H2O2-induced cytotoxicity. In addition, SP60015 (10-30 μM) and SB203580 (10-30 μM) greatly inhibited H2O2-induced cell death, although PD98059 (10-30 μM) slightly repressed cytotocixity. Collectively, H2O2-induced cytotoxicity of hBMSC is involved with both hydrogen peroxide- and nitric oxide-linked ROS, and ROS producing factors is GSH dependent in this reaction. Also, H2O2-induced cell death of hBMSC is mediated via JNK/p38 pathway in this oxidative stress.

Reference
Zhu H et al. (2006) Free Radical Biology & Medicine 41, 132-143.
Linares et al. (2009) Bone 44, 795-804.

P58

Determinants of adipose-derived stem cell biology and responsiveness to osteogenic stimulation

Lewis JR[1,2], Wang JZ[1,2], Adams L[1], Tan J[3], Hamdorf G[3] and Prince RL[1,2]
[1]School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia
[2]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia
[3]Department of General Surgery, Sir Charles Gairdner Hospital, Nedlands, WA

In vitro studies have shown that adipose tissue-derived stem cells (ADSC) have multi-linage differentiation capacity. We examined the *ex vivo* growth and differentiation of 30 human ADSC from 23 individuals to the osteoblastic lineage from subcutaneous, omental and bone marrow adipose tissue collected during abdominal or orthopaedic surgery. After passage cells were characterised by CD105, CD90, CD73 and CD45 surface markers and induced using control or osteogenic media. Cell counts, alkaline phosphatase activity, collagen and extracellular calcification staining were performed at days 7, 14, 21 and 28. In omental and subcutaneous-derived cells the dominant population of cells were CD90+CD73+CD45- however the proportion of CD105+ cells in subcutaneous-derived cells was higher ($P = 0.020$).
After adjusting for tissue depot, age and body mass index were negatively associated with unstimulated cellular proliferation rate (standardized β -0.300, $P = 0.014$ and -0.407, $P = 0.001$) but not simulated cellular proliferation rate. Men had significantly lower un-stimulated ($P = 0.016$) and stimulated ($P = 0.022$) ADSC cellular proliferative rate over 28 days. Significant differences in alkaline phosphatase activity and extracellular calcification but not collagen production were observed with bone marrow-derived ADSC displaying the greatest response to osteogenic stimulation followed by subcutaneous-derived ADSC.
These data confirm large inter-individual differences in ADSC proliferation and responsiveness to osteogenic stimulation may be explained in part by donor phenotype and may account for conflicting findings from smaller studies. Bone marrow-derived ADSC are the best source of osteoprogenitor cells while omental-derived ADSC have a more limited osteogenic potential.

P59

Effects of bisphenol A on osteoclasts and osteoblasts

Chung HY[1], Min KH[1], Hwang YC[1], Jeong IK[1], Ahn KJ[1], Byun DW[2], Min YK[3], Park HM[4] and Chang JS[5]
[1]Kyung Hee University, [2]Soonchunhyang University, [3]Sungkyunkwan University, [4]Chung-Ang University, [5]University of Ulsan, Seoul, Korea

Bisphenol A, a known endocrine disruptor, is a major component of epoxy resins used in protective coatings. Moreover, this compound binds to the estrogen receptor. In this paper, we thus examine the direct effects of bisphenol A on in vitro osteoclast and osteoblast culture systems.
According to our findings, bisphenol A significantly inhibited RANKL-induced TRAP-positive MNC formation in bone marrow-derived macrophages (BMMs) and RAW 264.7 cell cultures in a dose-dependent manner (0.5 uM to 12.5 uM). The suppression of ERK, JNK, AKT, and p38 mitogen-activated protein kinases (MAPKs) engaged by RANK, were observed in Western blotting after bisphenol A treatment in RAW 264.7 cells. Furthermore, bisphenol A suppressed Bcl-2 (anti-apoptotic) and stimulated Bax (pro-apoptotic) protein expression in RAW 264.7 cells. Bisphenol A also significantly suppressed ALP activities and bone nodule formation in MC3T3-E1 cell cultures. Specifically, the expression of Bcl-2 protein was decreased, but the expressions of caspase 3, 8, 9 were increased by bisphenol A treatment in MC3T3-E1 cells. Taken together, we found that bisphenol A directly suppressed both osteoclastic and osteoblastic activities in vitro. Our data suggest that bisphenol A suppress cell differentiation and survival.

P60

ENU-induced chemical mutagenesis reveals that choline kinase beta is an important regulator of osteoporosis

Kular J[1], Tickner J[1], Pavlos N[2], Viola HM[3], Abel T[4], Lim B[1], Hool HC[3], Zheng MH[2] and Xu J[1]
[1]School of Pathology and Laboratory Medicine, The University of Western Australia, Western Australia, Australia
[2]Centre for Orthopaedic Research, School of Surgery, The University of Western Australia, Western Australia, Australia
[3]Cardiovascular Electrophysiology Laboratory, The University of Western Australia, Western Australia, Australia
[4]Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Western Australia, Australia

The maintenance of bone homeostasis requires a tight balance between bone formation and bone resorption by osteoblasts and osteoclasts. The molecular mechanism(s) underlying the fundamental activities of these cells still remains largely unclear. In search of novel molecules that potentially play an important role in bone homeostasis we screened a number of ENU-induced mutant mouse lines. We identify choline kinase beta, a kinase that phosphorylates the first reaction in the biosynthesis of phosphatidylcholine, as a novel candidate regulator of bone homeostasis. Choline kinase beta mutant mice exhibit an osteoporotic phenotype as evidenced by microCT and histological assessment. *In vivo* and *in vitro* analysis reveals elevated osteoclast numbers in the mutant mice. Furthermore, osteoclasts from choline kinase beta mutant mice exhibit increased resorptive activity compared to those of littermate controls. Interestingly, exposure to elevated extracellular calcium results in a significant increase in intracellular calcium in osteoclasts derived from control mice however, this response is significantly attenuated in osteoclasts derived from choline kinase beta mutant mice. This may account for the increased resorptive activity in osteoclasts derived from the mutant mice. Treatment with CDP-choline *in vivo* and *in vitro* reduces osteoclast numbers, thereby rescuing the osteoclast phenotype. *In vitro* assays show a reduction in bone mineralisation in osteoblast cultures derived from the bone marrow of mutant mice. Taken together, our data document, for the first time, that choline kinase beta plays an important role in bone homeostasis by regulating both osteoclasts and osteoblasts.

P61

Evidence for a role for serotonin in human osteoclast function

Hodge JM[1], Williams LJ[2], Collier FM[1], Berk M[2] and Nicholson GC[3]
[1]Barwon Biomedical Research, Department of Medicine: Barwon Health, The Geelong Hospital, Geelong, Victoria 3220, Australia
[2]School of Medicine, Deakin University, Geelong, Victoria 3217, Australia
[3]Rural Clinical School, School of Medicine, The University of Queensland, Toowoomba, Queensland 4350, Australia

Serotonin (5-HT) acts on the central nervous system to regulate functions including mood and blood pressure, while peripherally it controls cardiovascular and gut function. Actions of 5-HT on bone remain controversial, with conflicting evidence concerning the peripheral role of serum 5-HT. Support for a direct action includes expression of the 5-HT transporter (5HTT), the rate limiting enzyme in 5-HT biosynthesis (tryptophan hydroxylase; TPH_1) and 5-HT receptors in osteoblasts and osteoclasts (OC). We aimed to investigate the expression profile of serotonergic genes during human OC differentiation.
Gene expression of 5-HT receptor 5-HTR_{2B}, 5HTT and TPH_1 was assessed by real time PCR during differentiation of OC from CFU-GM precursors treated with RANKL(125ng/mL) and M-CSF(25ng/mL) for 21d.
Extensive osteoclastogenesis was present at d8. Expression of 5-HTR_{2B} was low or undetectable prior to d11, peaked (7,404-fold) at d14, and dropped to baseline levels by d18. Expression of 5HTT was negligible prior to d15, increasing at d18 and peaking at d21 (9.4-fold). TPH_1 expression was initially elevated in CFU-GM prior to RANKL and M-CSF exposure, then dropped to a low baseline by d2, but similar to the expression profile of 5-HTR_{2B}, peaked at d14 (3.6-fold compared to d2), returning to baseline by d18.
These data demonstrate key serotonergic genes are expressed during human osteoclastogenesis, in the case of 5-HTR_{2B}, this being substantial although transient. The relative delay in onset of upregulation of these genes during osteoclastogenesis correlates with late stage maturation, suggesting a potential autocrine/paracrine role for 5-HT in mature OC function.

P62

Experimental tooth movement-induced osteoclast activation is regulated by sympathetic signaling

Kondo M[1,2], Kondo H[1], Miyazawa K[2], Goto S[2], Togari A[1]
[1] *Department of Pharmacology, School of Dentistry, Aichi-Gakuin University.*
[2] *Department of Orthodontics, School of Dentistry, Aichi-Gakuin University.*

Experimental tooth movement (ETM) changes the distribution of nerve fibers in periodontal ligament (PDL) and bone architecture through the stimulation of bone remodeling. As sympathetic signaling is involved in bone remodeling, we examined whether ETM is controlled by sympathetic signaling or not. Two groups of male mice had elastic rubber inserted between the upper first molar (M1) and (M2) for 3 or 5 days respectively. Not only sensory neuromarker such as calcitonin gene-related peptides (CGRP) but also sympathetic neuromarkers such as tyrosine hydroxylase (TH) and neuropeptide Y (NPY) were increased in PDL during ETM. To elucidate the effect of sympathetic signal mediated by ETM, mice were intraperitoneally injected β-antagonist, propranolol, (PRO: 20 μg/g/day) or β-agonist, isoproterenol (ISO: 5 μg/g/day) from 7 days before ETM. PRO suppressed the amount of tooth movement by 12.9% in the 3-days ETM group and 32.2% in the 5-days ETM group compared with vehicle. On the other hand, ISO significantly increased it. ETM remarkably increased the number of osteoclasts (Oc.N) in PDL. PRO suppressed Oc.N by 39.4% in the 3-days ETM group and 29.7% in the 5-days ETM group while ISO increased it by 32.1% in the 3-days ETM group and 57.3% in the 5-days ETM group. Chemical sympathectomy by 6-hydroxy dopamine (250 μg/g) showed results similar to PRO treatment in both the amount of tooth movement and Oc.N. These data suggest that mechano-adaptive response induced by ETM is altered by sympathetic signaling through osteoclast activation.

P63

Expression of factors inducing osteoclast activity, receptor activator NFkappa B (RANKL), TNF-like weak Inducer of Apoptosis (TWEAK) and its receptor, Fn14, in early stages of cartilage damage in osteoarthritis

Dharmapatni AASSK[1], Smith MD[2], Chen DY[1], Holding CA[1], Atkins GJ[3], Findlay DM[3], Zheng TS[4], Upton AR[1] and Haynes DR[1].
[1]*Discipline of Pathology, University of Adelaide, Adelaide, SA, Australia, 5005,* [2]*Rheumatology Research Unit, Repatriation General Hospital, Daw Park, SA, Australia,* [3]*Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia, 5005 and* [4]*Immunology, Biogen Idec Inc., Cambridge, MA 02142, USA.*

Along with RANKL, TWEAK is reported to have an important role in stimulating joint destruction in various arthritidies. We have recently reported that RANKL expression is elevated in human OA cartilage showing the early signs of cartilage damage (Upton et al Rheumatol Int 2012; 32:535-40). Here we investigated the expression of TWEAK and its receptor (Fn14) in OA cartilage showing various grades of damage. Soluble TWEAK in OA synovial fluids was also measured. In addition, in order to elucidate the possible mechanisms involved *in vitro* chondrocyte expression of TWEAK, Fn14 and RANKL following TWEAK, TNF-a and alone or in combination were determined.
RANKL, TWEAK and Fn14 mRNA and protein expression was determined in cartilage of various grades of OA damage in 20 patients. TWEAK levels were measured in 15 OA synovial fluid samples. *In vitro*, chondrocytes from OA patients were tested as described above.
Significantly higher levels of RANKL, TWEAK and Fn14 protein and mRNA were observed in cartilage showing early damage (grade 2) compared to healthy (grade 0) cartilage ($p<0.05$). High levels of TWEAK were present in all OA synovial fluids tested (713 ± 134 pg/ml). TWEAK induced RANKL mRNA and TNF-a induced Fn14 mRNA in chondrocytes *in vitro*.
Stimulation of RANKL, TWEAK and Fn14 in OA in the early stages of cartilage damage in OA suggests a role for these pro-osteoclastogenic factors in the pathogenesis of OA. The TWEAK stimulation of RANKL expression in chondrocytes indicates possible mechanisms linking cartilage damage to subchondral bone changes in OA.

P64

Generation of osteoclast circadian rhythm by glucocorticoid

Fujihara Y[1,2], Kondo H[1], Noguchi T[2] and Togari A[1]
[1]*Department of Pharmacology, School of Dentistry, Aichi-Gakuin University.* [2]*Department of Periodontology, School of Dentistry, Aichi-Gakuin University, Japan.*

Circadian rhythms are prevalent in bone metabolism. The master circadian rhythms are generated by endogenous circadian clocks in suprachiasmatic nuclei (SCN). Recently we reported that output signals from the SCN are transmit from master circadian rhythms to peripheral osteoblasts through the sympathetic nervous system and glucocorticoid. In this report we examined the expression level of molecular clock on osteoclast *in vivo* and *in vitro*. Four-week old male mice were maintained under alternatively 12 hour light and dark periods at least two weeks and then sacrificed at Zeitgeber time 0, 4, 8, 12, 16, 20. mRNA were extracted from SCN and femur (cancellous bone) and analyzed the expression of osteoclast-related genes and clock genes such as Clock, Period and BMAL. Period1 expression showed circadian rhythmicity in all tissues. However, the peak of expression on cancellous bone was delayed 4 hours compared with SCN. Col1a, NFATc1 and CathepsinK expression in cancellous bone also showed circadian rhythmicity. In *in vitro* study dexamethasone treatment remarkably synchronized clock genes on cultured osteoclast whereas RANKL, LPS and isoprenaline did not. Not only clock genes but also TRAP and cathepsin K was synchronized by dexamethasone treatment. Therefore, we examined the circadian rhythm of clock genes and osteoclast-related genes *in vivo* using adrenalectomized mice. In adrenalectomized-mice bone, the circadian rhythms of clock genes were maintained. However, the circadian rhythm of Cathepsin K and NFATc1 had disappeared. These results suggest that glucocorticoid may mediate circadian rhythm from the SCN to osteoclasts and generate circadian rhythm of osteoclastic bone resorption.

P65

Glucocorticoid mediated elastin synthesis is essential for embryonic lung development and postnatal survival in mice

Li A[1*], Hardy RS[1,2*], Stoner S[1], Tuckermann J[3], Zhou H[1], Seibel MJ[1,4]
[1]Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia; [2]Centre for Endocrinology, Diabetes and Metabolism, Institute of Biomedical Research, University of Birmingham, Birmingham, UK; [3]Molecular Biology of Tissue specific Hormone Action, Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany; [4]Dept of Endocrinology & Metabolism, Concord Hospital, Sydney, Australia
**These Authors contributed equally*

Glucocorticoid signaling is essential during embryonic lung development, with both the global and epithelial glucocorticoid receptor (GR) null mice presenting with lung atelectasis and post natal lethality. In this study we have examined the role of glucocorticoid signaling within mesenchymal tissues. To study the role of the GR in mesenchymal tissues during embryogenesis we crossed GRflox mice with Dermo1-Cre mice to generate GR^{Dermo1} mice, where the GR gene was conditionally deleted within mesenchymal cells. GR^{Dermo1} mice displayed severe pulmonary atelectasis, defective abdominal wall formation and high postnatal lethality. GR^{Dermo1} mice failed to progress from the canalicular to saccular stage of lung development, as evidenced by the presence of immature air sacs, thickened interstitial mesenchyme and an underdeveloped vascular network between E14.5 and E18.5. Myofibroblasts and vascular smooth muscle cells, although present in normal numbers, were characterized by significantly reduced elastin synthesis, whilst epithelial lining cells of the immature saccules were poorly differentiated. Reduced elastin and collagen deposits were also noted in connective tissues adjacent to the umbilical hernia. This study demonstrates that eliminating the GR in the mesenchymal lineage results in marked affects on interstitial fibroblast function, including a significant decrease in the synthesis of the extracellular matrix component elastin, which is essential for normal embryonic lung development. This results in lung atelectasis and post natal lethality, as well as additional hitherto unrecognized developmental defects in abdominal wall formation. In addition, it translates directly into cell-non-autonomous effects of the differentiation of epithelial cells.

P66

Inhibitor of dynamin rapidly disrupts actin rings of osteoclasts

Uehara S[1], Nakayama T[2], Mizoguchi T[3], Yamashita T[3], Kobayashi Y[3], Udagawa N[1] and Takahashi N[3]
[1]Department of Biochemistry, [2]Department of Periodontology and [3]Institute for Oral Science, Matsumoto Dental University, Japan.

Several lines of evidence indicate that dynamin, a GTPase responsible for endocytosis, is also involved in the bone-resorbing process of osteoclasts. However, it remains to elucidate how dynamin regulates the bone-resorbing activity. Then we investigated the effects of dynasore, an inhibitor of the GTPase-activity of dynamin, on actin ring and pit formation in osteoclast in vitro and *in vivo*.
Osteoclasts were obtained from cocultures of calvarial osteoblasts and bone marrow cells and seeded on dentin slices (dentin). Osteoclasts were cultured for 48 hr in the presence or absence of dynasore and stained for F-actin and TRAP activities. After removal of cells from dentin, dentin was stained with Mayer's hematoxylin to observe resorption pits. Dynasore dose-dependently inhibited formation of actin rings and resorption pits by osteoclasts. Those dentins were stained for TRAP to observe TRAP-marks, another marker of polarized osteoclasts. TRAP-marks also disappeared by the addition of dynasore. We examined the time course of changes in the dynasore-induced disappearance of actin rings and TRAP-marks in osteoclasts that had polarized on dentin. Most actin rings and TRAP-marks disappeared within 30 min and 60 min, respectively, by the addition of dynasore. Finally, we examined whether dynasore disrupts actin rings of osteoclasts *in vivo*. Dynasore was administered i.p. to mice. After 60 min of the injection, calvariae were dissected and stained for F-actin. Actin rings disappeared in calvariae of mice injected with dynasore. Taken together, these results suggest that the GTPase-activity of dynamin is involved in the maintenance of actin rings in osteoclasts.

P67

Involvement of down-regulating HGF synthesis in glucocorticoid-mediated growth inhibition of human osteoblasts

Togari A, Niwa Y, Tsunashima Y and Matsuda T
Department of Pharmacology, School of Dentistry, Aichi-Gakuin University.

Glucocorticoids have multiple systemic effects that may influence bone metabolism but also directly affect osteoblasts by decreasing their proliferation. Using human osteoblastic SaM-1 cells, we examined whether the effects of hydrocortisone on cellular proliferation are mediated by hepatocyte growth factor (HGF). A cellular proliferation assay was performed to detect BrdU-labeled DNA using ELISA system. Gene and protein expressions were assessed by RT-PCR and ELISA analysis, respectively. Human osteoblasts constitutively express both HGF and c-Met, its receptor. Hydrocortisone decreased the gene and protein expression of HGF as well as proliferation in SaM-1 cells. These hydrocortisone (0.01-1 mM)-induced decreases in HGF synthesis and cellular proliferation occurred in a concentration-dependent manner. However, no hydrocortisone (0.01-1 mM)-induced decrease in cellular proliferation was observed in human osteosarcoma-derived cells (HOS and SaOS-2), which are not able to produce HGF. In the cellular proliferation in SaM-1 cells, the decrease was blocked concentration-dependently by exogenously applied HGF (0.01-3 ng/ml). Furthermore, SU11274 (1 mM), a highly specific inhibitor of c-Met, suppressed the proliferation of SaM-1 cells, but not HOS cells. In conclusion, the present study demonstrated the existence of an autocrine/paracrine loop for HGF in normal human osteoblast (SaM-1 cells) that supports mitogenesis. Furthermore, hydrocortisone inhibits the proliferation of SaM-1 cells by interfering with this autocrine/paracrine loop by inhibiting HGF synthesis without any accompanying change in the expression of its receptor, c-Met. This suggests a new mechanism underlying glucocorticoid-mediated growth inhibition of human osteoblasts.

P68

RANKL induced elevated bone turnover – a model of coupled or uncoupled remodeling?

Raggatt LJ[1], Wu A[1], Chang MK[1], Alexander KA[1], Walsh NC[2], Gravallese EM[2] and Pettit AR[1]
[1]*University of Queensland Centre for Clinical Research, Royal Brisbane Hospital, Herston QLD, 4029 and*
[2]*University of Massachusetts Medical School, Worcester, MA, U.S.A*

In mice we have identified a canopy structure encapsulating BMU, similar to that reported in human bone. In contrast to the human scenario, mouse BMU canopy cells are F4/80$^+$ osteal macrophages (osteomacs). BMU are rare in mouse bones so to further investigate the role of osteomacs in BMU remodeling we have used a RANKL-GST regimen, which has been reported to induce both resorption and formation. Daily treatment with RANKL-GST (1mg/kg) elevated bone marrow myeloid cells including macrophages (flowcytometry), increased TRAP$^+$ osteoclasts and increased osterix$^+$ cuboidal osteoblasts (immunohistology). At sites of bone formation osterix$^+$ cuboidal osteoblasts did not express osteocalcin, lacked an osteomac canopy and were overlaying osteoid. These features are not observed in growing mice, suggesting that in RANKL-induced heightened bone turnover there is failure to form appropriate BMUs and that this compromises osteoblast mineralizing function. These observations may help explain why bone loss predominates in disease where both resorption and formation are elevated. This model also provided the opportunity to evaluate osteomac usage as *in vivo* osteoclast precursors. F4/80$^+$ osteomacs were visible adjacent to TRAP$^+$ osteoclasts but coexpression of these markers, as would be expected in cells transitioning from an osteomac to a pre-osteoclast, was not evident. This is similar to our observations under physiologic conditions but contrasts to our results in adjuvant-induced arthritis where F4/80$^+$TRAP$^+$ transition cells were associated with resorption. While many myeloid cells (including osteomacs) can form osteoclasts *in vitro* the osteoclast precursor in a physiologic or supraphysiologic setting is more restricted than in pathological states.

P69

RANKL-induced Myo1b localizes to sites of dynamic actin-membrane remodeling during osteoclast formation and function

Ng PY[1], Landao E[1], Coudrier E[2], Xu J[3], Knölker HJ[4], Zheng MH[1] and Pavlos NJ[1]
[1]*Centre for Orthopaedic Research, School of Surgery, The University of Western Australia, Nedlands, Western Australia.* [2]*Institut Curie, Centre de Recherche, Morphogenesis and Cell Signalization CNRS, UMR144, Paris, France.* [3]*Molecular Laboratory, School of Pathology and Laboratory Medicine, The University of Western Australia, Nedlands, Western Australia and* [4]*Department of Chemistry, TU Dresden, Dresden, Germany.*

The osteoclast (OC) boasts a specialized cytoskeleton that is unique amongst all eukaryotic cells. The necessity of a highly dynamic cytoskeleton 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 the establishment of OC polarization, the nature and identity of molecules that directly link the OC plasma membrane with the underlying cytoskeleton remain largely obscure. Utilizing state-of-the-art transcriptional profiling, we have 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 employed the natural compound Pentachloropseudilin (PCIP), a specific and allosteric inhibitor of myosin-I ATPase activity. We show that blockade of myosin-I motor function dose-dependently attenuates OC formation and bone resorptive capacity *in vitro*, an effect that correlates with morphological disturbances in F-actin ring organization and membrane integrity. 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.

P70

Role of autophagy in palmitate-induced lipotoxicity in osteoblasts

Gunaratnam K[1], Thekkedam C[1], Boadle R [2] and Duque G[1]
[1]*Ageing Bone Research Program, Sydney Medical School Nepean, The University of Sydney, Penrith, NSW.*
[2] *Electron Microscope Laboratory, ICPMR, Westmead, NSW, Australia.*

Introduction: We have found that fatty acids, predominantly palmitic acid (PA), exert a lipotoxic effect on osteoblasts by inducing apoptosis. Autophagy is a defence mechanism that involves degradation of cellular components by the lysosome, during cell stress. However, the role of autophagy in PA-induced apoptosis in osteoblasts remains unknown.
Aim: To identify the role of autophagy in PA-induced lipotoxicity in normal human osteoblasts (Ob).
Methods: Ob (Lonza, Switzerland) were treated with either vehicle or PA at increasing doses (100, 250 and 500 µM) and at different timed intervals (24, 48 and 72h). Autophagy was determined using western blotting (WB), immunofluorescence and electron microscopy. Cell survival was quantified by MTS. Autophagy was also inhibited using 3MA (5mM).
Results: WB showed an increase in LC3-II/LC3-I protein ratio, a widely used marker of autophagy, in a dose and time dependant manner ($P<0.01$). These observations were confirmed by immunofluorescence with LC3-II punctate expression (corresponding to autophagosomes) significantly increased in a dose and time dependent manner ($P<0.01$). Electron microscopy showed increased presence of autophagosomes at higher doses. Finally, at 24h, treatment with 3MA effectively inhibited autophagy followed by a significant reduction in apoptosis at 48h. **Conclusion**: In this study, we have found that, additionally to apoptosis, PA also induces autophagy in Ob. Our evidence elucidates a role of autophagy as an early step in PA-induced apoptosis. Inhibition of autophagy could constitute an effective approach to prevent lipotoxicity in bone cells.

P71

Roles of endogenous prostanoids and histamine in autocrine/paracrine control of PTH secretion from human parathyroid cells

Mun H-C[1], Ward DT[2], Delbridge L[3] and Conigrave AD[1]
[1]School of Molecular Bioscience, University of Sydney, NSW 2006, Australia, [2]Faculty of Life Sciences, University of Manchester A.1025 Michael Smith Building, Oxford Road, Manchester M13 9PT UK, [3]University of Sydney Endocrine Surgical Unit, Royal North Shore Hospital, St Leonards, NSW 2065 Australia

Parathyroid hormone (PTH) is a key regulator of extracellular calcium and phosphate concentrations that is secreted spontaneously by parathyroid chief cells http://en.wikipedia.org/wiki/Parathyroid_glandvia an exocytotic mechanism whose intrinsic control is not well understood. The mechanism is subject to negative feedback regulation by Ca^{2+}_o and is positively modulated by various cAMP-linked receptors including β-adrenergic, dopamine, secretin, prostanoid and histamine H_2 receptors. Previous studies have identified intrinsic production of several PTH secretagogues in parathyroid tissue including prostanoids, histamine, serotonin and dopamine. However, the potential role(s) of these locally-produced activators is unknown. In the current study we investigated the potential roles of locally-produced prostanoids and histamine in supporting the intrinsic PTH secretion mechanism from perifused normal human parathyroid cells. We prepared the samples of normal human parathyroid tissue under guidelines established by the local hospital committees. Human parathyroid cells were prepared by collagenase digestion and perifused for analysis for PTH secretion. With respect to the possible roles of local prostanoids, the broad-spectrum cyclo-oxygenase (COX) inhibitor indomethacin (50 μM) as well as COX1-selective inhibitor II (10 – 50 μM) and the COX2-selective inhibitor NS-398 (10 – 50 μM) all markedly suppressed intrinsic PTH secretion in the presence of 1.0 mM Ca^{2+}_o. In addition, the broad-spectrum prostanoid receptor inhibitor AH6809 and an inhibitor of the IP1 receptor Ro1138452 (0.5 – 5 μM) suppressed intrinsic PTH secretion. With respect to the possible roles of locally produced histamine, cimetidine (5-50 μM) and the highly selective H_2 receptor antagonist aminopotentidine (1-20 μM) also induced acute and reversible suppression of PTH secretion. These findings support the concept that PTH secretion is supported by the local production of activators that interact with cognate cAMP-linked receptors expressed on the surface of parathyroid chief cells.

P72

SIRT1, a class III histone deacetylase, regulates anti-inflammatory factors in human chondrocytes

Moon MH, Jeong JK, Lee YJ, Seol JW and Park SY
Biosafty Research Institute, College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk 561-756, South Korea

Resveratrol is a naturally occurring polyphenol found in the grapes which has been shown to have anti-inflammatory effects. Also, resveratrol is well known for SirT1 activator. Arthritis is a common chronic inflammatory and destructive arthropathy. Current anti-inflammatory drugs have many side effects so, safe and efficacious drugs are needed. The present study was performed to elucidate a possible role of resveratrol signaling in human articular chondrocytes. Reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting were performed to detect gene products and proteins involved inflammation and cartilage degradation when human primary chondrocytes were stimulated by interleukin-1β (IL-1β). Matrix metalloproteinase (MMP)-2 activity was evaluated by gelatin zymography. To investigate whether SirT1 is a pivotal role in the anti-inflammatory effect of resveratrol in the chondrocyte, we used chondrocytes were overexpressed with SirT1. Resveratrol dose dependently inhibited IL-1β–induced COX-2, MMP-1, MMP-3 and iNOS mRNA expression in the human chondrocytes. The MMP-2 activity was increased by IL-1β, but resveratrol decreased the pro-inflammatory effect of IL-1β. Resveratrol decreased the main pro-inflammatory regulator, NF-κB protein expression increased by IL-1β. When using chondrocytes were overexpressed with SirT1, the anti-inflammatory action of SirT1 was the similar effect to the resveratrol treatment. Resveratrol have anti-inflammatory action on the chondrocytes so, we suggest that resveratrol and SirT1 could be a potential therapy for arthritis.

P73

Sphingosine-1-phosphate inhibits interleukin (IL)-1β-induced inflammation in human articular chondrocytes

Moon MH[1], Jeong JK[1], Lee YJ[1], Seol JW[1], Xue M[2], Jackson CJ[2] and Park SY[1]
[1]*Biosafty Research Institute, College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk 561-756, South Korea*
[2]*Sutton Arthritis Research Laboratories, Institute of Bone and Joint Research, Kolling Institute, University of Sydney at Royal North Shore Hospital, St. Leonards, New South Wales, Australia*

Sphingosine-1-phosphate (S1P) is a pluripotent lipid mediator that transmits signals through a family of G-protein-coupled receptors (GPCRs) to control diverse biological processes including inflammation and wound healing. In this study a novel biological activity of S1P in articular chondrocytes was identified. Human primary chondrocytes were cultured in a monolayer. Reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting were performed to detect genes and proteins involved in inflammation and cartilage degradation when human primary chondrocytes were stimulated by interleukin-1β (IL-1β). Matrix metalloproteinase (MMP)-2 and MMP-9 activity was evaluated by gelatin zymography. Glycosaminoglycan (GAG) degradation was evaluated by the dimethylene blue method. Prostaglandin E_2 (PGE_2) was measured by enzyme-linked immunosorbent assay (ELISA). By using $S1P_1$ receptor agonist and antagonist we discovered the key role played by $S1P_1$ in the S1P-dependent inhibition of IL-1β-induced inflammation in human chondrocytes. S1P dose dependently inhibited IL-1β–induced NF-κB p65, cyclooxygenase-2 (COX-2), MMP-1, MMP-3, MMP-13, and MMP-14 mRNA expression in human chondrocytes and IL-1β–induced PGE_2 synthesis and GAG degradation in human cartilage explants. W146, a known $S1P_1$ receptor antagonist, inhibited the active form of NF-κB p65 and COX-2 expression induced by IL-1β. The anti-inflammatory action $S1P_1$ receptor agonist SEW2871 was similar to that of S1P. This study shows that S1P has anti-inflammatory effects on chondrocytes via the $S1P_1$ receptor. Our data suggests that targeting S1P and $S1P_1$ could be a potential therapy for arthritis.

P74

The crosstalks between Wnt/2-catenin and ±5 integrin signalings in the articular superficial cell functions

Yasuhara R[1], Enomoto-Iwamoto M[2], and Mishima K[1]
[1]*Department of pathology and diagnosis, School of Dentistry, Show University,* [2]*Musculoskeletal and Orthopedic Research Laboratories, Division of Orthopedic Surgery, Department of Surgery, Children's Hospital of Philadelphia*

Articular surface is covered with a layer of flattened cells, called superficial layer (SFL). The SFL cells have been distinguished from chondrocytes by cell shape and functions expressing low proteoglycan and unique molecules such as lubricin and tenascin C. However, much remains unclear about the functions of SFL cells, especially in aging, articular injuies and prevention of articular cartilage degeneration. We have previously demonstrated that Wnt/β-catenin controls organization and gene expressions of SFL cells. In this study, we performed a gene array analysis to characterize SFL cells in more details and compared them to those of articular chondrocytes. There were 170 genes that showed over 2.0 fold statistical differences in gene expression levels between the SFL cells and the articular chondrcotyes. The genes include many extracellular matrix genes and cytoskeleton-related genes, indicating that the SFL cells have distinct nature involved in cell-matrix interaction and cytoskeletal regulation from articular chondrocytes. Consistently the SFL cells expressed a5 and b1 integrins at a higher level compared to chondrocytes. Interestingly, expression of the a5 integrin was dramatically reduced by β-catenin ablation while increased by treatment with rWnt3A. The neutralizing blocking antibody for α5 integrin inhibited rWnt3A-induced Wnt/b-catenin signaling activity and cell proliferation in cultured SFL cells. Furthermore, β-catenin-deficient SFL cells showed lower response to fibronectin substrate as determined by level of phosphorylated ERK. Our data indicate that á5 integrin appears to be important for β-catenin dependent regulation in cell-matrix interaction in SFL cells.

P75

The effects of 1,25-dihydroxyvitamin D on *in vitro* mineral deposition depend on the stage of osteoblast maturation and extracellular calcium concentration

Yang D[1,2,3], Atkins GJ [1,3], Turner AG [2,4], Anderson PH [2,4] and Morris HA [1,2,4]
[1]*Discipline of Medicine, University of Adelaide, Adelaide, SA, Australia 5005*
[2]*Endocrine Bone Research, Chemical Pathology, SA Pathology, Adelaide, SA, Australia 5000*
[3]*Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia, 5005*
[4]*Musculoskeletal Biology Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia, 5000*

1,25-dihydroxyvitamin D (1,25D) is known to inhibit osteoblast proliferation, enhance mineralisation and induce RANKL expression. *In vitro* mineralisation is also enhanced by increasing medium calcium. We now report the interaction between osteoblast maturation, the level of extracellular calcium and the effect of 1,25D on *in vitro* mineralisation.
Primary calvarial cells extracted from 1-3 day old C57BL/6 mouse calvariae (**Calvarial cells**) and primary cortical osteoblast-like cells grown from 4 week-old C57BL/6 femoral cortical bone (**Cortical cells**) were used for experimentation. The effects of 1.25D (10^{-9}M) with 1.8 and 2.8mM extracellular Ca^{2+} on mineralisation and gene expression were assessed.
Calvarial cells exhibited less mature properties compared to Cortical cells including a 36% ($p<0.01$) reduction of mineral deposition and the induction of RANKL mRNA by 1,25D by Day 24 (3-fold, $p<0.01$) which occurred only in Calvarial cells. In Calvarial cultures 1,25D inhibited mineral deposition at Day 24 only when media contained 1.8mM Ca^{2+} (decreased 28%, $p<0.01$) and not with 2.8mM Ca^{2+}. Consistent with this, 1,25D treatment in 1.8mM Ca^{2+}-medium increased MEPE (600%, $p<0.01$) and SOST (1300%, $p<0.01$) mRNA levels. In contrast, 1,25D enhanced mineralisation by Cortical cells only in 2.8mM Ca^{2+}-medium at Day 21 (120%, $p<0.01$), with no effect on MEPE and SOST mRNA expression.
Our data indicate that 1,25D influences mineralisation distinctly according to the stage of osteoblast differentiation and possibly skeletal origin; the effect of 1,25D is to inhibit mineral deposition by immature, calvaria-derived cells, and enhance mineral deposition by mature, cortical bone-derived cells, especially in response to increased extracellular calcium.

P76

The expression of osteoblast transcription factors RUNX2, osterix, TWIST1 and MSX2 in adult human bone and their putative post-developmental roles in osteocytes.

Ormsby RT[1], Kumarasinghe DD[1,2] and Atkins GJ[1].
[1]*Bone Cell Biology Group, Discipline of Orthopaedics & Trauma, University of Adelaide,* [2]*Bone & Joint Research Laboratory, Surgical Pathology, SA Pathology, and Discipline of Anatomy & Pathology, The University of Adelaide, Adelaide, South Australia.*

Transcription factors including RUNX2, Osterix (OSX), TWIST1 and MSX2 are key for osteoblast growth and differentiation. Our aim was to identify relationships between the expression of these transcription factors and that of osteocyte markers in human bone and human bone cell cultures to illuminate their potential roles in osteocyte biology.
Trabecular bone biopsies were taken from the intertrochanteric region of 15 patients undergoing hip replacement surgery. Total RNA was extracted from each sample for the analysis of gene expression by real-time PCR. A similar cohort of 5 bone samples was used to establish primary osteoblast cultures. These were cultured for 42d under mineralising conditions and gene expression analysed as above.
All bone samples and osteoblast cultures expressed MSX2, OSX, TWIST1 and RUNX2. In bone, strong correlations in mRNA expression existed between all transcription factors except between RUNX2 and OSX. MSX2, TWIST1 and OSX expression all correlated with that of the osteocyte marker, DMP1. Interestingly, no correlations existed between transcription factors and SOST mRNA expression. Specific relationships also existed between the transcription factors and various osteocyte markers in human primary osteoblast cultures. A number of novel relationships highlighted in the osteoblast cultures suggest that these are associated with active osteoblast differentiation and transition into an osteocyte phenotype.
This study has identified relationships between transcription factors with known roles in bone development and osteoblast differentiation in adult human bone. In particular, our findings highlight new potential roles for transcription factors in osteocytes that have not been previously described.

P77

The role of the cytoskeleton in osteoblast and osteoclast function: a study of RhoA inhibition.

Musson DS[1], Lin J-M[1], Matthews BG[2], Watson M[1], Bai J-Z[1] and Cornish J[1]
[1]*Department of Medicine, University of Auckland, New Zealand.*
[2]*Department of Reconstructive Sciences, University of Connecticut Health Center, Farmington, USA*

Cell shape is a key factor in determining cell lineage and function. Notably, mesenchymal stem cell commitment to an osteoblast lineage is increased through adherent, flattened cell morphology, while osteoclast activity is reliant on F-actin regulated podosome formation. Rho activity plays an important role in cytoskeletal formation and focal adhesion.
We investigated a RhoA inhibitor on primary rat osteoblasts (ROBs), MC3T3-E1 osteoblast-like cells, isolated mature osteoclasts and bone marrow cultures treated with increasing concentrations of Cethrin, a RhoA inhibitor, 0.01μM - 10μM.
Cethrin dose-dependently decreased ROB mitogenesis in 2D cultures (2-fold P>0.05), an effect that was reduced in 3D cultures (1.25-fold (P>0.05). Interestingly, stromal cell mitogenesis was not significantly affected by Cethrin in *ex vivo* bone marrow cultures. At lower concentrations, Cethrin significantly increased MC3T3-E1 cell mineralisation, an effect reversed at higher concentrations. Osteoclast number and activity was also dose-dependently decreased (two-fold P>0.05) following Cethrin treatment in bone marrow cultures. Phase microscopy of 2D ROB cultures demonstrated severe changes in cell shape with high concentrations of Cethrin, similar disruption of the cytoskeleton was observed with fluorescence staining following Cethrin treatment in 2D and 3D cultures.
RhoA inhibition has significant, yet differing effects on osteoblast cells cultured in 2D and 3D, indicating a possible role for accessory pathways activated by increased integrin binding in 2D cultures. Notably, RhoA inhibition significantly decreased osteoclast formation and activity, likely resulting from a lack of podosome formation. Here we have demonstrated that cytoskeletal regulation plays a key role in the function of osteoblasts and osteoclasts.

P78

The S349T mutation of *SQSTM1* links Keap1/Nrf2 signalling to Paget's disease of bone

Rea S[1*], Wright T[2*], Goode A[2], Bennett AJ[2], Ratajczak T[1], Long JE[4], Searle MS[4], Goldring C[3], Park BK[3], Copple IM[3,*], and Layfield R[2,*,*] Denotes equal contributions
[1]Centre for Medical Research, *University of Western Australia*, and Department of Endocrinology and Diabetes, *Sir Charles Gairdner Hospital*, Nedlands, WA; [2]School of Biomedical Sciences, *University of Nottingham, UK*; [3]MRC Centre for Drug Safety Science, *University of Liverpool*, UK; [4]Centre for Biomolecular Sciences, School of Chemistry, *University of Nottingham*, UK

Mutations affecting the *Sequestosome 1* gene (*SQSTM1*) commonly occur in patients with the skeletal disorder Paget's disease of bone (PDB), a condition characterised by defective osteoclast differentiation and function. Whilst most mutations cluster within the ubiquitin-associated (UBA) domain of the SQSTM1 protein, and are associated with dysregulated NFkB signalling, several non-UBA domain mutations have also been identified. Keap1 is a SQSTM1-interacting protein that regulates the levels and activity of the Nrf2 transcription factor. This in turn controls the expression of numerous cytoprotective genes that contribute to the cell's capacity to defend itself against chemical and oxidative stress, through binding to the antioxidant response element (ARE). The PDB-associated S349T mutation maps to the Keap1-interacting region (KIR) of SQSTM1, however the effects of PDB mutant SQSTM1 on Keap1 function have not been investigated. Here we show that unlike other SQSTM1 mutations, the S349T mutation results in neither impaired ubiquitin-binding function in pull-down assays, nor dysregulated NFkB signalling in luciferase reporter assays. Keap1 is expressed in differentiating osteoclast-like cells, a cell type relevant to PDB, and the S349T mutation selectively impairs the SQSTM1-Keap1 interaction in co-immunoprecipitations, which molecular modelling indicates results from effects on critical hydrogen bonds required to stabilise the KIR-Keap1 complex. Further, S349T mutant SQSTM1, but not other PDB-associated mutants, showed reduced ability to activate Nrf2 signalling as assessed by ARE-luciferase reporter assays. Thus, SQSTM1-mediated dysregulation of the Keap1-Nrf2 axis, which could potentially lead to aberrant production of oxidative response genes, may contribute to disease aetiology in a subset of PDB patients.

P79

Development and optimization of *ex vivo* bioreactor system for tendon tissue engineering

Wang T, Zhen Lin, Landao E and Zheng MH
Centre of Orthopaedic Surgery, School of Surgery, University of Western Australia, Nedlands, WA 6009 Australia

Programmable mechanical stimulation is the key component of a bioreactor system for tendon tissue engineering. The aims of the study were to design a bioreactor system with cyclic mechanical stimulation to mimic the condition that tendon undergoes *in vivo*, and to define the optimal tensile loading strain for maintaining the Achilles tendon homeostasis. An *ex vivo* bioreactor systems with programmable dynamic tensile stretch were designed and manufactured. Each bioreactor system contains 6 individual culture chambers. The mechanical loading regimes, such as frequency and loading period, can be preprogrammed through a computerized input system. Achilles tendons from New Zealand white rabbits were loaded in the bioreactor with or without cyclic tensile loading (0.25Hz for 8 hours per day). A range of tensile strains, from 0% to 9%, were applied on the rabbit Achilles tendon for 6 days. General histology, immunohistochemical staining of type III collagen, TUNEL assay and quantitative-PCR were applied to examine the structural integrity and matrix turnover of the tendons. Furthermore, the study shown that without tensile loading, Achilles tendon lost its structure integrity as evidenced by disorientated collagen fiber, increased type III collagen production and gene expression, and increased cell apoptosis. Tendons with 3% of tensile loading did not prevent matrix deterioration, whilst 6% of tensile loading was able to maintain the structural integrity and cellular function. Exceeded loading regime of 9% caused massive tendon rupture. The result indicated that an optimal cyclic tensile loading is required to maintain the tendon homeostasis. Completed/partial loading deprivation or overload results in abnormal matrix structure and cellular function in tendon, which are observed in clinical tendinopathy. Our bioreactor system could be programmed to provide a suitable dynamic culture condition to mimic the *in vivo* situation for tendon tissue engineering purposes, and moreover a study model to define the tendon-specific biological, biochemical and biomechanical profiles under physical/pathological conditions.

P80

Prevalence and risk factors for bone loss in patients treated with diabetes related foot complications

Tsimos S[1], Wraight PR[1], Kantor S[1] and Wark JD[1]
1. Department of Medicine, and Bone and Mineral Service, The Royal Melbourne Hospital, University of Melbourne, Victoria, Australia.

Foot complications are common in individuals with diabetes, requiring a complex management approach which includes pressure off-loading to the affected foot. This study aims to assess whether patients with diabetes-related foot complications (DRFCs) have reduced bone mineral density (BMD) measurements at baseline and whether this declines during their management.
Risk factors for bone loss were assessed via questionnaire, file review and baseline blood testing. Patients underwent bilateral peripheral quantitative computed tomography (pQCT) of the distal tibiae and dual X-ray absorptiometry (DXA) at the lumbar spine and both hips. Scans were performed at study entry and will be repeated at 6-months.
To date, 13 patients (7 males, 6 females) with mean age 67.61±12.21 have been recruited. Of these, 11 participants had either a soft tissue infection or osteomyelitis. Only 6 participants had an adequate dietary calcium intake, whilst only 3 had adequate serum 25-hydroxyvitamin D levels (>50 nmol/L). Sub-optimal eGFR measurements were recorded in 5 of the participants. All female participants were postmenopausal. At baseline, 4 patients had WHO criteria for osteoporosis and 3 had osteopenia. Those with higher levels of physical activity had higher BMD results ($p<0.05$). Smoking and time spent outdoors showed trends towards being significantly correlated with a reduced BMD, but the small sample size to date has limited statistical power. Sample size will be addressed by ongoing recruitment
We suggest that osteoporosis and osteopenia are highly prevalent in DRFC patients at presentation. These patients also have multiple risk factors for ongoing bone loss specific to their condition.

P81

Secondary hyperparathyroidism and reduced bone turnover during long-term treatment with Imatinib

O'Sullivan S[1], Horne A[1], Wattie D[1], Gamble G[1], Browett P[2] and Grey A[1]
Departments of [1]Medicine and [2]Molecular Medicine and Pathology, University of Auckland, New Zealand.

Imatinib mesylate is a first-line therapy for chronic myeloid leukemia (CML). We have previously reported that 2 years of treatment with imatinib causes secondary hyperparathyroidism, biphasic changes in bone turnover markers and stable or increased bone mineral density (BMD), in patients with CML. Here we report the results of 48 months follow-up from a prospective study of 7 patients receiving imatinib for treatment of CML. Between 18 and 48 months of treatment, mild secondary hyperparathyroidism persisted, but did not worsen, and biochemical markers of bone turnover remained in the low-normal range. BMD lumbar spine, proximal femur and total body remained stable between 24 and 48 months, at levels similar to those at baseline. Body weight and fat mass were higher at 48 months than at baseline, but were similar to values at 24 months, whereas lean mass declined between baseline and 48 months. Our findings show that long-term treatment with imatinib leads to stable, mild secondary hyperparathyroidism and reduced bone turnover, but does not change BMD. These data are reassuring with regards to the skeletal safety of long-term imatinib therapy.

P82

The correlation of serum levels of Wnt antagonists with inflammatory markers and the daily dose of prednisolone in patients with rheumatoid arthritis

Hashimoto J[1], Hirao M[1], Shi K[2], Ebina K[2], Kaneshiro S[2], Nampei A[3], Tsuboi H[1], Akita S[1], Ohshima S[1], Saeki Y[1] and Yoshikawa H[2]
[1] Department of Rheumatology, Osaka Minami Medical Center, Japan.
[2] Department of Orthopaedics, Osaka University Graduate School of Medicine, Japan.
[3] Department of Orthopaedics, Osaka Rousai Hospital, Japan.

Aims: Inflammation, glucocorticoid use and functional disability are major contributors to bone loss or fragility in patient with rheumatoid arthritis (RA). The present clinical study aimed to investigate how these factors associates with the serum level of sclerostin and dkk-1 which inhibits bone formation by suppression of Wnt-β-catenin signaling in patients with RA.
Methods: Ambulatory female RA patients (n=83) with normal renal function enrolled in this cross-sectional study. Mean age was 62.5 ± 12.6 (average ± SD) years old. Correlation with serum sclerostin or dkk-1 levels and inflammatory markers, age, anthropometric factor, Steinblocker's functional class and daily dose of prednisolone was analyzed using PASW statistics 18 (SPSS An IBM COMPANY, Illinois USA).
Results: There was no correlation between serum level of sclerostin and dkk-1. Both Wnt antagonists were not correlated with age, body weight and height. Serum sclerostin level was negatively correlated with CRP (Spearman's correlation coefficient and p-value, -0.289, 0.009) and positively correlated with Hb (0.221, 0.047), while it was not correlated with MMP-3 and RF. The daily dose of prednisolone was negatively correlated with the sclerostin level (-0.275, 0.024). On the other hand, serum level of dkk-1 was positively correlated with WBC count (0.320, 0.003) but not with CRP, MMP-3, RF, Hb and daily dose of prednisolone. There was no difference in sclerostin and dkk-1 level between functional class 1 and 2.
Conclusion: Our data suggested the different role of these two Wnt antagonists of sclerostin and dkk-1 in pathogenesis of bone loss occurred in patients with RA.

P83

The link between nephrolithiasis, bone density and fractures in transfusion-dependent thalassaemia

Wong P[1,2,3], Fuller PJ[1,2,3], Gillespie MT[1], Kartsogiannis V[1], Strauss B[3], Bowden D[4] and Milat F[1,2,3]
[1]*Prince Henry's Institute of Medical Research;*
[2]*Department of Endocrinology, Southern Health;*
[3]*Monash University Department of Medicine and*
[4]*Thalassaemia Service, Monash Medical Centre, Clayton, Victoria 3168, Australia*

Introduction: Thalassemia is a disorder of haemoglobin synthesis due to mutations in the globin chains (α or β). Transfusion-dependent thalassaemia is associated with reduced bone mineral density (BMD) and fractures. Many causes are implicated including hypogonadism, growth hormone deficiency, marrow expansion and iron overload. However, the relationship of nephrolithiasis to BMD and fractures has not previously been studied.
Method: A retrospective cohort study of 166 patients with transfusion-dependent thalassaemia was undertaken to determine the prevalence of nephrolithiasis, and its association with BMD and fractures. Logistic regression analysis using age and gender matches was employed to account for potential confounding factors.
Results: There were 73 (44%) male and 93 (56%) female participants aged between 4 to 66 years with a median age of 34 years. Fractures occurred in 19.9% of study participants and were more common in males than females (27.4% vs 14%). The overall prevalence of nephrolithiasis was 18.1%, occurring in 28.7% of males and 9.7% of females. Reduced Femoral neck Z scores were associated with an increased risk of nephrolithiasis (OR=1.63; 95% CI: 1.10-2.42). Furthermore, subgroup analysis showed nephrolithiasis in males was associated with an increased risk of fracture after adjusting for Femoral neck or Lumbar spine Z score (OR=5.59; 95% CI:1.16-27.03, OR=5.21; 95% CI:1.06-25.64, respectively).
Conclusion: Nephrolithiasis is common and significantly associated with reduced BMD and fractures after adjusting for potential confounders. These findings demonstrate the need for ongoing surveillance of BMD, fractures and nephrolithiasis in the management of transfusion-dependent thalassaemia.

P84

Bone density changes following addback therapy with different GnRH agonist in endometriosis patients.

Yong-Taik Lim[1], Je-Yeon Song[1] and Hyoung-Moo Park[2]
[1] *Department of Obstetrics and Gynecology, The Catholic University of Korea,*
[2] *Department of Obstetrics and Gynecology, College of Medicine, Chung-Ang University, South Korea*

Objectives: GnRH agonist was one of most useful medical therapy of endometriosis, but proven to lower bone mass density by inhibit secretion of estrogen. This study was designed to compare the clinical characteristics, changes of bone mass density among gonadotropin-releasing hormones (leuprolide (Leuprin), triptorelin (Decapeptyl) and goserelin (Zoladex)) for postoperative hormonal therapy of endometriosis.
Design & Method: For each group, thirty women were subjected to use 6 times GnRH agonists for postoperative treatment of endometriosis from January 1998 through September 2011 in Kangnam St Mary's hospital and St Mary's Hospital, Korea. All patients had add-back therapy by using estrogen plus progesterone or tibolone or alendronate. The patients who had history of smoking, alcohol, steroid therapy, previous fracture were excluded.
Results: Three kinds of GnRH agonist were applied for postoperative therapy of endometriosis, which were leuprolide, triptorelin and goserelin. Each of groups had similar age (38.3 vs 36.5 vs 36.1 years), parities (1.2 vs 1.4 vs 1.2), BMI (21.3 vs 21.6 vs 21.5 kg/m^2), presence of dysmenorrhea (80% vs 73% vs 70%) and stage of endometriosis at diagnosis (3 vs 2.87 vs 2.5). When comparing GnRH agonist by BMD, the mean changes of T score of lumbar vertebra 2-4 (-0.33±0.47), lower part of lumbar vertebra (-0.31±0.51), Rt femur neck (-0.35±0.77), lower part of Rt femur (-0.25±0.44), Lt femur neck (-0.19±0.36), lower part of lumbar vertebra (-0.19±0.31) of leuprolide were similar with those of triptorelin, which were (-0.43±0.56), (-0.33±0.95), (-0.37±0.73), (-0.39±0.84), (-0.42±0.72), (-0.38±0.61) and those of goserelin, which were (0.53±6.25), (-1.59±6.95), (-0.11±0.77), (-0.21±0.76), (-0.19±0.70), (-0.16±0.73), respectively. The differences were statistically insignificant (all $p > 0.05$).
The FSH measured after 6 times of goserelin use was lower (3.3±3.6 mIU/ml) than those of leuprolide (9.7±8.3 mIU/ml) and triptorelin (6.9±6.9 mIU/ml) ($p < 0.01$). The estradiol measured after 6 times of leuprolide use was higher (83.3±123.0 pg/ml) than those of triptorelin (42.9±63.1 pg/ml) and goserelin (21.6±33.4 pg/ml) ($p = 0.04$). The proportion of patients whose value of estradiol were checked less than 30pg/ml were 40% from leuprolide, 56% from triptorelin and 91% from goserelin.
Conclusion: Among GnRH agonist (leuprolide, triptorelin , goserelin) for postoperative hormone therapy of endometriosis, no statistically significant change on BMD were appeared. But, when we compared the potential bone protective effect by estradiol level, leuprolide could be considered the most effective and conservative choice.

P85

Bone surface availability as a potential driver of endocortical bone loss in osteoporosis

Buenzli PR[1], Pivonka P[1], Thomas CDL[2] and Clement JG[2]
[1]*Engineering Computational Biology Group, The University of Western Australia, Perth, WA, Australia*
[2]*The Melbourne Dental School, The University of Melbourne, VIC, Australia*

Age-related bone loss and postmenopausal osteoporosis are disorders of bone remodelling, in which less bone is reformed than resorbed. Yet, this dysregulation of bone remodelling does not occur equally in all bone regions. Pronounced bone loss occurs near the endocortical surface, leading to cortical wall thinning and consequently to an expansion of the medullary cavity, a process sometimes referred to as "trabecularisation". Cortical wall thinning is of primary concern in osteoporosis due to the strong reduction in bone mechanical properties that it is associated with.

In this contribution, we examine the hypothesis that the nonuniformity of bone surface availability within the tissue could explain the predominant loss of bone near the endocortical wall observed in osteoporosis. The idea that bone surface availability may influence bone loss in osteoporosis was proposed by RB Martin a few decades ago. However, this question is challenging to address experimentally. Here, we investigate this hypothesis in a spatially nonuniform context using a simple computational model of bone remodelling, and a phenomenological relationship between bone specific surface and bone porosity. This enables us to follow the evolution of the medullary cavity area in a simulated osteoporotic condition. The results of the computational model are compared to experimental data obtained from human femur midshafts of the Melbourne Femur Collection. We conclude that the microscopic availability of bone surface within the tissue, needed for resorption and formation processes to occur, is potentially a significant factor in explaining focal bone loss predominantly near the endocortical wall.

P86

Defining the relationship between fatness and fracture

Chan MY[1], Nguyen ND[1], Center JR[1,2], Eisman JA[1,2] and Nguyen TV[1,2,3]
[1]*Osteoporosis and Bone Biology, Garvan Institute of Medical Research;* [2]*St Vincent's Hospital and St Vincent's Clinical School;* [3]*School of Public Health and Community Medicine, University of New South Wales, Sydney, Australia.*

Whether increased body weight (BW) or body mass index (BMI) is associated with higher fracture risk is controversial. Also, it is unclear as whether the effect of BW or BMI on fracture risk is independent of bone mineral density (BMD). The present study was designed to test the hypothesis that association between BMI/ BW and fracture risk is mediated by BMD. A total of 3,550 individuals (2,199 women and 1,351 men) aged > 60 years old were drawn from the cohort of Dubbo Osteoporosis and Epidemiology Study (DOES). The participants were categorized into osteporotic, osteopenic and normal BMD groups based on their femoral neck BMD T-score as measured by dual energy X-ray absorptiometry (GE Lunar DPX-L densitometer). Weight and height were measured at baseline, and BMI (kg/m²) was derived. The incidence of low-trauma fracture occurred during the follow-up period (median: 6 years; ranges: 0.1- 22 years) was ascertained by radiographic reports. Approximately 35% (n=774) of women and 19% (n=258) of men had experienced low-trauma fracture during the follow-up period. In either men or women, fracture risk decreased with higher BMI. However, the association was BMD-and gender-dependent. In women with osteopenia, fracture risk was associated with increased BMI (HR=1.22; 95% CI, 1.09-1.36) or increased BW (HR=1.17; 95% CI, 1.03-1.32), and the association was independent of age and fall. Taken together, femoral neck BMD accounted for 13% of the variance of fracture risk, whereas BMI and BW could only explain approximately 2-5% of the variance. However, among women with osteoporosis, although fracture risk declined with increasing BMI, the association was not statistically significant. For normal-BMD women, no significant association was found between BMI/BW and fracture risk. In men, there was no significant association between BMI/BW and fracture risk, regardless of BMD levels. These results suggest that the association between fatness and fragility fracture is complex, because it is dependent on sex and BMD. The positive association between higher degree of fatness and greater risk of fracture in osteopenic women suggests that body mass index may provide additional prognostic information for fracture risk prediction.

P87

Intra and inter fracture risks correlation using GARVAN and FRAX tools in patients aged over 70 years old

Foo B[1] and Inderjeeth CA[1,2]
[1]*Area Rehabilitation and Aged Care, North Metropolitan Health Service, Perth*
[2]*University of Western Australia, Perth*

Bone mineral density (BMD) is a useful tool to identify people with osteoporosis. Fracture risk assessment tools, like FRAX®[1] and GARVAN[2] are available options to identify high risk patient groups.

Aims: We assessed fracture risk based on FRAX® and GARVAN scores without BMD. The main objective was to assess the intra and inter risk tools scores correlation and relation to actual measured BMD.

Methods: 935 patients aged ≥ 70 years were screened from selected Aged Care facilities/ GP Practices. All patients had osteoporosis risk assessment (BMD study and fracture risk questionnaire). Fracture risks based on FRAX® and GARVAN were analysed and degree of correlation between these two tools and with patients' BMD were assessed.

Results: Garvan (without BMD) 10 year hip fracture risk correlates best with its corresponding major osteoporotic fracture risk but correlates less well with FRAX® fracture risk scores. FRAX® hip and major osteoporotic fracture risks (without BMD) correlated less well with each other. GARVAN scores performed marginally better than FRAX® when correlated to "worst BMD" or any site BMD (weak correlation).

Correlations

		GH	GM	FH	FM	WORST	TH	FN
GH	Pearson Correlation	1	0.928	0.611	0.500	-0.363	-0.255	-0.237
	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.000	0.000
GM	Pearson Correlation	0.928	1	0.587	0.488	-0.356	-0.256	-0.237
	Sig. (2-tailed)	0.000		0.000	0.000	0.000	0.000	0.000
FH	Pearson Correlation	0.611	0.587	1	0.727	-.0330	-0.253	-0.227
	Sig. (2-tailed)	0.000	0.000		0.000	0.000	0.000	0.000
FM	Pearson Correlation	0.500	0.488	0.727	1	-.0303	-0.246	-0.245
	Sig. (2-tailed)	0.000	0.000	0.000		0.000	0.000	0.000

Legend:

1. GH- GARVAN 10 year hip fracture risk (without BMD)
2. GM- GARVAN 10 year major osteoporotic fracture risk (without BMD)
3. FH- FRAX® 10 year hip fracture risk (without BMD)
4. FM- FRAX® 10 year major osteoporotic fracture risk (without BMD)
5. WORST- worst BMD result
6. TH- total hip BMD
7. FN- femoral neck BMD

Conclusion: There is better intra- Garvan risks correlation between hip and major osteoporotic fractures than FRAX®. However, none correlated especially well with any BMD measurement.

Reference:

1. FRAX®, WHO Fracture Risk Assessment Tool, available from: www.shef.ac.uk/FRAX
2. GARVAN fracture risk calculator, available from: http://www.garvan.org.au/bone-fracture-risk/

P88

The prevention and treatment of osteoporosis: who is interested?

Kim JW[1], Jeon YJ[2], Byun DW[3] and Chang JS[4]
[1]*Department of Orthopaedic Surgery,* [2]*Department of Family Medicine, Haeundae Paik Hospital, College of Medicine, Inje University, Busan,* [3]*Department of Internal Medicine, College of Medicine, Soonchunhyang University,* [4]*Department of Orthopaedic Surgery, Asan Medical Center, College of Medicine, University of Ulsan, Seoul, South Korea*

Purpose: To evaluate the rate of management in osteopenia and osteoporosis patients after health screening and to determine factors of patient interested in the prevention and treatment of osteoporosis

Material & methods: We retrospectively reviewed 172 osteopenia patients and 73 osteoporosis patients who had been diagnosed in health screening. Telephone surveys were performed 1 year after the diagnosis. The questionnaire included consultation to specialist, life style modification and osteoporosis medication. We analyzed factors related osteoporosis treatment and risk factors were used in FRAX model.

Results: The average age was 56.2 years in osteopenia group and 59.0 years in osteoporosis. Sixty three patients (36.6%) visited to a specialist, 108 patients(62.8%) had life style modification and 8 patients (4.7%) took medication in osteopenia group. Forty seven patients (64.4%) met a specialist, 60 patients (82.2%) modified life style and 20 patients(27.4%) took medicine in osteoporosis group. The rates were higher in osteoporosis group significantly. Multivariate logistic regression analysis showed that only consultation to a specialist were strongly associated with lifestyle modification in osteopenia group ($p<0.01$), consultation to a specialist also were strongly associated with medication in osteoporosis group ($p=0.03$). The other risk factors did not affect life style modification or medication. The osteopenia patients who visited to a specialist were significantly older and had parental or previous fracture history and disease associated with secondary osteoporosis.

Conclusion: For prevention and treatment of osteoporosis, it is important to recommend consultation to a specialist after health screening in both osteopenia and osteoporosis patients.

P89

The prevalence of vitamin D deficiency in hospitalised elderly people and associated comorbidities

Kurusumuthu P
Advanced Trainee in geriatric medicine, Nepean hospital, NSW, Australia

Background: Vitamin D deficiency is becoming an increasingly recognized geriatric syndrome and has been associated with fractures, falls, osteomalacia, and functional decline in the general geriatric population. Vitamin D supplementation reduces the incidence of falls and fractures in older adults.
Objectives: To determine the: 1) prevalence of vitamin D deficiency; 2) prevalence of supplementation; 3) establishes an appropriate dose for vitamin D supplementation; 4) potential association between vitamin D deficiency and associated comorbidities.
Methods: Serum vitamin D level was checked in all patients admitted consecutively for four weeks in April 2011 to the Geriatric wards in Westmead hospital. In addition to the vitamin D level, patients' basic demographics, co-morbidities, history of falls, residential status and vitamin D supplantation status were recorded.
Results: The mean serum 25-hydroxy- vitamin D concentration for all the 228 patients was 61.67 nmol/l. Seventy five percent of participants were not on vitamin D supplements (172 of 228 total study population). The prevalence of vitamin D deficiency among the people was not taking a vitamin D supplement was 85.5 percent (146 of 171 people). Mean vitamin D level of patients not on vitamin D supplements was 51 nmol/l. The mean vitamin D levels in patients those who weren't supplemented, from nursing home is 34.5 nmol/l, from hostel is 31 nmol/l and from home is 54 nmol/l, these levels are significantly lower than currently recommended at ≥75 nmol/l (Table 2 and 3). However we also noticed that patients those who have been on vitamin D supplements also have significance vitamin D deficiency i.e. 32% patients has vitamin D level less than 75nmol/l.
Conclusion: This study showed a high level of vitamin D deficiency in this study population and only one in four in people were supplemented with vitamin D. We strongly recommend widespread screening for vitamin D deficiency and routine vitamin D supplementation should be considered especially those who have in residential aged care facilities (RACF). Furthermore it is also important to check vitamin D level after initiation of vitamin D supplements to ensure that there is an adequate increase in vitamin D level because one in three people in our study who have been on supplementation have significant deficiency.

Table 2 . Prevalence of Vitamin D deficiency

Dwellings type	Percentage of patients has Vit D level less than 25 nmol/l	Percentage of patients has Vit D level less than 50 nmol/l	Percentage of patients has Vit D level less than 75 nmol/l
Home			
Female	11%	38%	85%
Male	18%	53%	80%
Nursing Home			
Female	31%	69%	88%
Male	33%	80%	100%
Hostel			
Female	34%	71%	88%
Male	33%	67%	100%

Table 3 . Vit D level in different dwelling types

Vit D status	Home	NH	Hostel	Others
Number of Patients	161	49	16	2
Mean Vit D level (nmol/l)	61.35	51.72	61.37	83
On Vit D supplement	32 (20%)	18 (37%)	6 (38%)	1
Mean Vit D level (nmol/l)	91	95	112	46
Not on Vit D supplement	129 (80%)	31 (63%)	10 (62%)	1
Mean Vit D level (nmol/l)	54	34.5	31	120

P91

EGFL7 is differentially expressed in osteoclasts and osteoblasts and mediates endothelial cell migration through the activation of extracellular signal-regulated kinase

Chim SM, Chow ST and Xu J
School of Pathology and Laboratory Medicine, The University of Western Australia, Western Australia, Australia

Angiogenesis plays a pivotal role in bone formation, remodeling and fracture healing. The regulation of angiogenesis and bone remodeling is highly complex and orchestrated by multiple intercellular communication between bone cells and endothelial cells. However, local factors contributing to the cross-talk within the bone microenvironment is still unclear. The aim of our study was to identify novel secreted factors expressed by bone cells, which regulate angiogenesis and bone homeostasis. Here, we report that EGF-like domain 7 (EGFL7), a member of the epidermal growth factor (EGF) repeat superfamily proteins is differentially expressed in osteoclasts and osteoblasts and regulates endothelial cell activities. Microarray analysis revealed that EGFL7 is differentially expressed during osteoclast and osteoblast differentiation. The gene expression of EGFL7 in osteoclasts and osteoblasts was confirmed by semiquantitative RT-PCR. An expression construct encoding the full-length of mouse EGFL7 was generated using expression vector pcDNA3.1A-EGFL7-c-myc/his. COS-7 cells were transfected with EGFL7 expression construct to produce conditioned medium containing recombinant EGFL7 as a 32 kDa protein, confirming that the EGFL7 is a secreted protein. Using scratch wound healing assay, we found that conditioned medium containing recombinant EGFL7 potentiates SVEC (A simian virus 40-transformed mouse microvascular endothelial cell line) cell migration. Furthermore, we show that EGFL7 recombinant protein induces phosphorylation of ERK in SVEC endothelial cells. Together, these results demonstrate that osteoblasts and osteoclasts express EGFL7, a secreted factor that is capable of promoting endothelial cell migration via ERK activation. Understanding the mechanisms by which EGFL7 regulates the intercellular communication within the local bone environment might aid in advancing our knowledge of bone remodeling and angiogenesis and offer an important new target for the potential treatment of bone diseases, including osteoporosis, and bone fracture healing.

P92

Expression of angiogenic related cytokines in cartilage of osteoarthritis

Zhang X, Prasadam I, Crawford R And Xiao Y*
Institute of Health & Biomedical Innovation, Queensland University of Technology, QLD, Australia

Background: Osteoarthritis (OA) is the most common form of musculo-skeletal disorders resulting in articular cartilage degeneration and abnormal subchondral bone metabolism. Inflammation and angiogenesis are closely integrated process in OA [1,2], which involved a broad range of cytokines[3,4].
Aims and Methods: This study was aimed to investigate the difference of cytokine profiles between OA and normal cartilage, especially focusing on the expression of angiogenic factors. Human knee samples were collected from three OA patients undergoing joint replacement surgery. Proteins and RNA were extracted from normal and OA cartilages. The protein microarray which designed to detect 54 growth factors, cytokines, or chemokines were used to investigate the cytokine expression profiles in OA cartilage. Angiogenesis PCR array were performed to investigate the expression pattern of angiogenic factors in cartilage.
Results and Discussion: From cytokine array analysis, totally twenty proinflammatory cytokines or chemokines showed significantly increased levels in OA compared to normal cartilage. Several factor related to angiogenesis were highlighted which showed upregulation in OA sample, such as PECAM-1, PDGF AA, VE-Cadherin, Tie-2, MMP-9, VEGFR3, IP-10. Gene expression pattern of these angiogenic factors were also upregulated in OA samples demonstrated by PCR array.
Conclusions: These results suggest that upregulated angiogenic factors contained in cartilage may contribute to the chondrocyte hypertrophy and cartilage degradation in OA.

References

1. Suri S, et al. Bone. 2011, doi:10.1016.
2. Fransès R.E, et al. Osteoarthritis Cartilage. 2010, 18(4): 563–571.
3. Ashraf S, et al. Current Opinion in Rheumatology 2008, 20:573–580.
4. Walsh DA, et al. Rheumatology 2010;49:1852–1861.

P93

Expression of neurotrophic factors and receptors during growth plate cartilage bony repair in rats and skeletal cell formation *in vitro*

Su YW, Chung R, Zhou F, King T, Georgiou K, Foster BK, Zhou XF and Xian CJ
School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA 5001

The growth plate cartilage is responsible for bone growth in children and yet its injuries are often repaired undesirably by bony tissue causing bone growth defects, for which the mechanisms are unclear. Neurotrophic factors (NTs) have been shown to regulate fracture healing; however their potential roles in growth plate cartilage bony repair are unknown. We investigated expression of NTs and receptors in the bony repair at the injured growth plate and during skeletal cell formation *in vitro*. Using a rat model of tibial growth plate injury, the expression of NTs (NGF, BDNF, NT-3, and NT-4) and receptors (TrkA, TrkB, TrkC and p75) at the injury site was investigated by RT-PCR and immunohistochemistry at different time points post-surgical injury. Upregulated mRNA expression of NTs and their high affinity receptors (TrkA, TrkB and TrkC) was observed at different times (days 5, 8, 14 and 28) at the growth plate injury site, where NT-3 showed the highest expression by 50 to 100 folds compared to non-injured control. The localization of NTs in mesenchymal cells, chondrocytes, osteoblasts and osteocytes at the injury site was also observed by immunostaining. In addition, differentially regulated expression of these NTs and receptors was also detected during *in vitro* chondrogenesis, osteoblastogenesis, mineralisation and osteoclastogenesis in cultured bone marrow progenitor cells from normal rats, in which NT-3 mRNA displayed the greatest upregulation by 250 to 350 folds during chondrogenesis. Therefore, neurotrophic factor signalling may be involved in the skeletal cell formation and in the repair processes at injured growth plate.

P94

Impact of allosteric modulators of the extracellular calcium-sensing receptor on calcitonin-secreting human thyroid C-cells

Mun H-C[1], Christopoulos A[2] and Conigrave AD[1]
[1]School of Molecular Bioscience, University of Sydney, NSW 2006, Australia, [2]Monash Institute of Pharmaceutical Sciences and Department of Pharmacology, Monash University, Parkville, Victoria 3052, Australia.

The calcium-sensing receptor (CaSR) plays a central role in the control of calcium homeostasis. We previously demonstrated that L-amino acids and γ-glutamyl peptides are positive allosteric modulators of the CaSR that activate Ca^{2+}_i mobilization and suppress PTH secretion. In the current study, we investigated the impacts of the positive modulators cinacalcet (Sensipar), L-Phe and S-methylglutathione (SMG) as well as the calcilytic NPS 2143 on Ca^{2+}_i mobilization and calcitonin secretion from human TT C-cells. TT-cells were cultured in F12-K nutrient medium with 10% FBS in the presence of 100 ng mL^{-1} IL-1β for 48 h. For Ca^{2+}_i mobilization studies, TT-cells were cultured on coverslips and loaded with the fura-2 AM. For analysis of calcitonin secretion, TT cells were cultured in 24-well plates and incubated for various times (0 – 20 min). L-Phe (1-10 mM), SMG (1-30 μM) and cinacalcet (1 μM) all enhanced intracellular Ca^{2+} mobilization in the presence of extracellular Ca^{2+} (2.0 mM) and these effects were suppressed by 2 μM NPS-2143. In addition, cinacalcet, L-Phe and SMG all markedly stimulated calcitonin release at submaximal Ca^{2+}_o concentrations. Positive modulators of the CaSR that bind in the Venus FlyTrap domain (L-Phe and SMG) or the heptahelical domain (cinacalcet) promote intracellular Ca^{2+} mobilization and calcitonin secretion from IL-1β-treated thyroid C-cells. In addition, the calcilytic NPS-2143 markedly suppresses calcitonin secretion whether evoked by calcium or calcimimetics.

P95

Inhibition of Wnt/b-catenin signalling promotes cartilage repair at injured growth plate in young rats

Wong DSK, Chung R, Macsai CE and Xian CJ
Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5001, Australia

The growth plate cartilage holds sole responsibility for lengthening of long bones in children. However, once injured, bony tissue repair often occurs at the injury site resulting in bone growth defects such as limb length discrepancies and angulation deformity. Previous studies have identified the inflammatory, mesenchymal infiltration, osteogenesis and remodelling repair responses during the injury repair. However, molecular mechanisms for the growth plate bony repair remains unclear. While earlier studies have documented the importance of Wnt/b-catenin signalling pathway during the osteogenic differentiation of mesenchymal progenitor cells and our recent microarray study has suggested increased Wnt/b-catenin signalling at the early stage of growth plate repair (Macsai et al *Bone* 2012). This study investigated the potential roles of Wnt/b-catenin signalling in a rat growth plate injury model. After surgical drill-hole injury at the proximal tibial growth plate, rats were treated with known b-catenin inhibitor ICG-001 or vehicle control (oral gavage at 200mg/kg for 8 days, commenced at day 2 post-injury). At day 10, gene analysis of the injury site showed enhanced collagen II gene expression. Consistently, histological analysis showed an increase in cartilaginous tissue proportion and a decrease in bony trabeculae formation in treated rats compared to vehicle controls. *In vitro* study with bone marrow stromal cells showed that ICG-001 treatment suppressed osteogenic differentiation (CFU-f-ALP assay) but enhanced chondrogenic differentiation (by pellet culture). These findings suggest that the Wnt/b-catenin signalling pathway is important for the growth plate bony repair and its suppression could enhance cartilage repair.

P96

Interleukin (IL)-11 is required for mechanical stress-induced bone formation

Kondo T, Omatsu T, Dong B, Ohnishi Y, Aizawa S-I, Endo I and Matsumoto T
Department of Medicine and Bioregulatory Sciences, Tokushima University, Kuramoto Tokushima, Japan

Interleukin (IL)-11 is mainly expressed in bone marrow stromal cells, and inhibits adipocytic differentiation while stimulating osteoblastogenesis. The expression of IL-11 is enhanced by mechanical stress via the rapid induction of fosB gene transcription and Smad1/5 phosphorylation. The increased transcript, deltaFosB, forms heterodimer with JunD and up-regulates IL-11 gene transcription in cooperation with activated Smad1/5. However, the physiological role of IL-11 in the maintenance of bone mass in response to mechanical stress remains unclear. The present study was undertaken to clarify the role of IL-11 in mechanical stress-induced bone formation using IL-11 knockout (KO) mice.

IL-11KO mice exhibited low bone mineral density (BMD) in weight-bearing bones after weaning, which was aggravated with aging. In sharp contrast, BMD of non-weight bearing calvaria tended to increase in IL-11KO mice. TRAP5b, a bone resorption marker, was not different from the control, but bone formation markers including osteocalcin were reduced in IL-11KO mice. Bone histomorphometric analysis revealed that the number of osteoblasts as well as calcein double staining was markedly reduced in IL-11KO mice. In contrast, there was no significant difference in osteoclast surface and number. The expression of Runx2 and osteocalcin in the femur was significantly reduced and the enhancement of the expression of Runx2 and osteocalcin after mechanical stimulation was impaired in IL-11KO mice.

These results demonstrate that BMD is reduced due to a blunted osteoblastogenic response to mechanical stress in IL-11KO mice, and suggest that IL-11 plays an important role in the maintenance of bone formation in response to mechanical stress.

P97

Interleukin-33 expression, regulation and actions in bone

Eeles DG[1,2], Singh P[1], Saleh H[1], Grills BL[2], Schuijers JA[2], McDonald SJ[2], Gillespie MT[1,3] and Quinn JMW[1]
[1]*Prince Henry's Institute, Clayton, Australia.*
[2]*Musculoskeletal Research Centre, La Trobe University, Australia*
[3]*Dept of Biochemistry and Molecular Biology, Monash University, Australia*

Interleukin 33 (IL-33) is a secreted Th_2-stimulating cytokine and an intracellular alarmin. IL-33 is an IL-1 superfamily member, signalling through receptor ST2L. We previously showed IL-33 inhibits osteoclast formation and increases osteoblast mineralisation, and established that IL-33 was expressed by bone cells.

We have addressed the regulation of IL-33 expression by anabolic factors. IL-33 mRNA was detected in osteoblasts and chondrocytes, with little to no mRNA or protein expression in osteocytes or bone marrow cells. Anabolic factors including parathyroid hormone (PTH) and oncostatin M (OSM) increased IL-33 and ST2L mRNA and protein levels in primary osteoblasts. We also determined the actions of IL-33 upon the osteoblast. IL-33 treatment suppressed expression of the anti-anabolic factor sclerostin in osteocytic cells in long term osteoblast cultures, as well as in calvarial organ cultures. Long bones of IL-33 injected mice also showed reduced sclerostin mRNA expression. In contrast, dickkopf-1 levels were unaffected. *In vitro*, IL-33 was shown to transiently enhance Wnt-1, Wnt-3a and Wnt-10 mRNA expression, but not other Wnts.

Mechanosensory regulation of IL-33 (previously observed in other cell types) was determined in response to cyclic stretching of osteoblasts in vitro, but this did not alter IL-33 levels. In contrast, bone fracture elevated IL-33 mRNA levels in long bones, possibly resulting from local inflammatory responses rather than effects on bone loading.

Thus, IL-33 levels are altered by bone anabolic factors and fracture, and IL-33 has significant effects on osteoblast Wnt pathway components.

P98

Limb- and sternum-specific inactivation of Dullard gene causes severe defects in skeletal development via alteration of TGF-ß signaling

Hayata T[1], Ezura Y[1] and Noda M[1,2]
[1]*Department of Molecular Pharmacology, Medical Research Institute and* [2]*Global Center of Excellence Program for Molecular Science for Tooth and Bone Diseases, Tokyo Medical and Dental University, Tokyo, JAPAN*

The transforming growth factor (TGF)-ß and bone morphogenetic protein (BMP) signaling pathways play important roles in endochondral bone formation, an essential process for skeletal growth. However, it has also been shown in mouse models that limit of TGF-ß signaling by E-selectin-ligand-1, an inhibitor of TGF-ß maturation in Golgi apparatus is of importance in endochondral bone formation as well as limit of BMP signaling by BMP inhibitors including Noggin and Smad6. Although our previous in vitro studies revealed that an intracellular factor Dullard acts as an inhibitor of both TGF-ß and BMP signaling in bone cells, physiological relevance of Dullard in endochondral bone formation is unknown. In this study, we report that mice lacking Dullard in early limb and sternum mesenchyme with Prx1-Cre system exhibit retardation of ossification. Approximately 70% of the Dullard (Prx1) mutant mice died 1 day after birth presumably due to defects in suckling milk and the rest of them displayed dwarfism and defects in locomotion and died before weaning. Dullard (Prx1) mice showed reduction in length of limb long bones and retardation of ossification in metatarsal and metacarpal bones as well as in sternum. Histological analysis showed occupation of hypertrophic chondrocytes in sternum of the Dullard (Prx1) mice. Luciferase reporter assay using immature murine articular chondrocytes (iMACs) isolated from P0 mice revealed that TGF-ß signaling, but not BMP signaling was augmented in Dullard-deficient iMACs after 24-hour treatment. These results suggest that Dullard regulates endochondral bone formation via suppression of TGF-ß signaling in chondrocytes.

P99

MicroRNAs targeting non-canonical Wnt/Ca^{2+} pathway potentially mediate osteo-inhibitory effects of pro-inflammatory cytokines

Chakravorty N[1], Jaiprakash A[1], Crawford R[1,3], Oloyede A[1], Ivanovski S[2] and Xiao Y[1]
[1]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Queensland, Australia*
[2]*School of Dentistry and Oral Health, Centre for Medicine and Oral Health, Griffith University, Gold Coast Campus, Queensland, Australia*
[3]*Prince Charles Hospital, Brisbane, Queensland, Australia.*

Inflammation and immunomodulation are important regulators of osteogenesis and bone remodeling, and are known to play a crucial role in successful implantation of orthopedic and dental implants. Modified titanium implant surfaces, like the chemically modified sand-blasted, large-grit, acid etched (modSLA) surface, are associated with lower expression of pro-inflammatory cytokines, higher expression of Wnt/Ca^{2+} pathway genes, down-regulation of microRNAs potentially targeting Wnt/Ca^{2+} genes and improved osteogenic properties compared to smooth surfaces. Recent studies suggest that inhibition of the non-canonical Wnt/Ca^{2+} signaling pathway in inflammatory micro-environments is responsible for inhibition of osteogenic differentiation. The present study aimed at exploring the role of certain miRNAs during osteogenic differentiation under the influence of pro-inflammatory cytokines. SAOS-2 cells (human osteoblast-like cells) were cultured in osteogenic media in presence of Interleukin-6 (IL6) or Tumor necrosis factor-α (TNFα) (0.5ng/ml-15ng/ml) for 7 days. Cell cytotoxicity assays (MTT assay), alkaline-phosphatase (ALP) activity and Alizarin Red-S staining were performed at days 3 and 7. Relative expression of genes of the canonical (WNT3A, AXIN2, CTNNB1) and non-canonical (WNT5A, FZD2) Wnt pathways, osteogenic differentiation markers (ALP, BGLAP, SPP1, RUNX2) and miRNAs targeting Wnt/Ca^{2+} pathway were studied using qPCR. The results indicated suppression of osteogenic differentiation (decreased osteogenic markers), relatively low cytotoxicity, down-regulation of Wnt/Ca^{2+} pathway and up-regulation of the miRNAs. The findings from this study indicate that the up-regulation of miRNAs targeting members of the Wnt/Ca^{2+} family could be potentially responsible for arbitrating the osteo-inhibitory effects of pro-inflammatory cytokines and targeting miRNAs might be an interesting therapeutic option in inflammatory conditions.

P100

Preconditioning with TNF-α promotes osteogenic differentiation of adipose-derived mesenchymal stem cells by modulating the BMP-2 signalling pathway

Lu ZF, Wang GC, Dunstan CR and Zreiqat H
Biomaterials and Tissue Engineering Research Unit, School of AMME, the University of Sydney, Sydney, Australia.

There is a major medical need for developing effective approaches for repairing non-union and critical-sized bone defects. Mesenchymal stem cells (MSCs) hold great promise for tissue repair or regeneration, however current strategies to direct MSCs into the osteogenic lineage are not optimal in terms of effectiveness. The aim of this study was to investigate whether tumour necrosis factor-α (TNF-α) preconditioning is capable of directing adipose tissue-derived mesenchymal stem cells (ASCs) into osteogenic differentiation. We demonstrated that three days of TNF-α pre-conditioning significantly increased osteogenic gene expression (collagen I, Runx2, osteopontin and osteocalcin) and alkaline phosphatase (ALP) activity of ASCs after 4 and 14 days of culturing following the withdrawal of TNF-α. TNF-α pre-conditioning significantly induced bone morphogenetic protein-2 (BMP-2) protein expression in ASCs and blocking BMP-2 expression by siRNA partially inhibited the osteogenic differentiation of ASCs induced by TNF-α. Three days of BMP-2 pre-conditioning also significantly increased osteogenic gene expression and ALP activity of ASCs after 4 and 14 days of culturing but expression levels were significantly lower than those induced by TNF-α. TNF-α treatment directly inhibited the Smad1/5 signaling pathway despite elevated BMP-2 protein expression in ASCs. TNF-α promoted erk1/2 and p38 mitogen-activated protein kinase (MAPK) signaling pathways, but only inhibition of erk1/2 reduced BMP-2 and osteogenic induction. In conclusion, TNF-α preconditioning promotes osteogenic differentiation of ASCs at least in part by modulating BMP-2 signalling. TNF-α preconditioning might serve as an effective strategy to direct stem cells into osteogenic lineage for clinical applications.

P101

Sclerostin mediates pro-osteoclastogenic effect through the LRP4 receptor on osteocytes

Wijenayaka AR[1], Kogawa M[1],Dharmapatni A[2], Haynes DR[2], Findlay DM[1] and Atkins GJ[1]
[1]*Bone Cell Biology Group, Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA 5005, Australia*
[2]*Discipline of Pathology, University of Adelaide, Adelaide, SA 5005, Australia*

Sclerostin is an important regulator of bone mass and appears to exert its effect by a number of means, including an anti-anabolic effect through the regulation of matrix mineralisation and osteocyte maturation(1), and a pro-catabolic effect through osteocyte support of osteoclast activity via induced RANKL expression(2). Sclerostin has been characterised as a Wnt signalling inhibitor by binding to Wnt co-receptors LRP5/6. Recent evidence suggests that LRP4 is also a sclerostin receptor. The aims of this study were to assess the catabolic effect of sclerostin in human bone tissue cultured *ex vivo* and examine the pathways, through which sclerostin may act.
To achieve these aims, trabecular bone from patients undergoing total hip replacement surgery were obtained and cultured *ex vivo* in the presence of recombinant human sclerostin to study gene expression and histology. SiRNA knockdown of LRP4 mRNA expression was used to assess the role for LRP4 in the sclerostin response in MLO-Y4 cells.
In human bone, RANKL mRNA levels increased in response to treatment. There was also a significant release of βCTX (crosslaps) into the media consistent with osteoclastic resorption. Results also indicated that the RANKL mRNA increase in response to sclerostin treatment was ablated by knockdown of LRP4 in MLOY4 cells.
Our findings further support a role for sclerostin in the resorption of human bone. Our findings also indicate that this effect is mediated in part through the LRP4 receptor in osteocytes.

1. Atkins et al. J Bone Miner Res. 2011 26(7):1425-36.
2. Wijenayaka et al. PLoS One. 2011;6(10):e25900.

P102

Sex-specific action of calcitonin on osteocytic gene expression

Chia LY[1,2], Walsh NC[1,2], Gagel RF[3], Martin TJ[1,2] and Sims NA[1,2]
[1]*Bone Cell Biology and Disease Unit, St. Vincent's Institute of Medical Research, Melbourne, Australia*
[2]*Department of Medicine at St. Vincent's Hospital, University of Melbourne, Australia*
[3]*Department of Endocrine Neoplasia and Hormonal Disorders, University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA*

While calcitonin (CT) inhibits bone resorption, mice deficient in CT/calcitonin-gene related peptide (CT/CGRP) or calcitonin receptor (CTR) have increased bone formation, suggesting that CT inhibits bone formation. Previously, we showed that in young female mice, salmon calcitonin (sCT) increased sclerostin expression, but reduced other osteocytic genes (matrix extracellular phosphoglycoprotein (MEPE) and dentin matrix protein (DMP-1)). We also identified CTR expression in osteocytes by immunohistochemistry and by PCR of FACS-sorted calvarial osteocytes from young mice expressing GFP under the control of the DMP-1 promoter.
We have now detected a significant reduction (~35%) in sclerostin positive osteocytes in 3 month-old CT/CGRP KO female calvariae, consistent with their increased bone formation.
As observed previously in female mice, subcutaneous sCT injection in 4-week old male C57Bl/6 mice decreased CTR and DMP-1 mRNA expression in calvaria and femur. mRNA levels of other osteocytic genes, MEPE and fibroblast growth factor (FGF-23) were also reduced, but only in calvariae. In contrast to the observations in female mice, sclerostin was not increased. Subsequent qPCR of GFP+ osteocytes isolated from sCT-treated male and female DMP1-GFP mice confirmed that the reduction in FGF-23 and MEPE was specific to osteocytes. Again, up-regulation of sclerostin mRNA was only observed in FACS-sorted female calvarial osteocytes. Finally, in calvarial organ culture from 8 day old male mice, sCT treatment decreased CTR and DMP-1 mRNA levels, but did not increase sclerostin.
These data confirm that sCT influences osteocytic gene expression, but the upregulation of sclerostin mRNA expression is restricted to female mice.

P103

SnoN mediates a crosstalk between TGF-β and BMP signaling to suppress hypertrophic maturation of chondrocytes

Kawamura I[1,2], Maeda S[1], Imamura K[1,2], Yokouchi M[2], Ishidou Y[1] and Komiya S[1,2]
[1] *Department of Medical Joint Materials, Kagoshima University, Kagoshima, Japan.*
[2] *Department of Orthopaedic Surgery, Kagoshima University, Kagoshima Japan.*

Objective: TGF-β signaling inhibits maturation of hypertrophic chondrocytes and progression of osteoarthritis (OA) by largely unknown mechanism. We searched for target genes downstream of TGF-β signaling to inhibit chondrocyte hypertrophy directly.
Methods: We examined chondrocyte maturation of ATDC5 cells and mouse metatarsal bone culture with combined application of BMP-2 and a TGF-β type I receptor inhibitor SB431542 (SB).
Results: Upon treatment with SB, BMP-induced calcification of cartilage matrix was promoted in bone culture. Expression of *Col10a1* and *Mmp13* was upregulated in ATDC5 micromass culture by SB. Although the phosphorylation of BMP Smads-1/5/8 was not influenced by SB, expression of *Id1*, a downstream target gene, was enhanced by SB, suggesting that BMP signaling seemed to be inhibited by endogenous TGF-β signaling at the level downstream of the phosphorylation process of BMP-Smads. We found that *SnoN* was induced by the endogenous TGF-β signaling during chondrocyte maturation. SnoN suppressed the activity of BMP-signaling reporter, as well as expression of *Col10a1* and *Id1* in ATDC5 cells. Conversely, knockdown of *SnoN* resulted in enhanced expression of *Col10a1* and *Id1*. The immunohistochemistry revealed that the strong expression of SnoN protein, as well as that of TGF-b1 and phosphorylated Smad2, was detected in prehypertrophic chondrocytes of mouse growth plate. Similarly, in human moderate OA cartilage, SnoN was exclusively detected in prehypertrophic-like chondrocytes around the pathologically hypertrophic chondrocytes.
Conclusion: These results suggest that SnoN suppresses BMP signaling and hypertrophic conversion of chondrocytes under control of TGF-β signaling.

P104

Synergistic regulation of BMP2 mediated osteogenesis of human cranial suture mesenchymal cells by glypicans: Functional and physical interaction of GPC1 and GPC3 with BMP2

Dwivedi PP[1], Grose RH[1], Hii CST[2], Filmus J[4], Anderson PJ[1,3] and Powell BC[1]
[1]Craniofacial Research Group, Women's and Children's Health Research Institute, [2]Department of Immunology, Women's and Children's Hospital, SA Pathology, [3]Australian Craniofacial Unit, Women's and Children's Hospital, North Adelaide-5006, Australia, [4]Sunnybrook Health Sciences Centre, Toronto, Canada

The growth of the skull vault is regulated by the activity of osteoblastic cells at the four fibrous joints known as sutures which separate the bony cranial plates. Craniosynostosis, the premature bony fusion of the skull sutures, is a developmental abnormality of infancy, affecting one in 2500 live births. We have discovered that the heparan sulphate proteoglycans, glypicans (GPC1 and GPC3), are down regulated during premature suture fusion in craniosynostosis [Coussens et al. 2007]. Glypicans can regulate growth factor signalling in non-osteogenic tissues [Midorikawa et al. 2003] and have been shown to regulate the bone morphogenetic proteins (BMPs) pathway. BMPs have a central role in skull development, and we hypothesize that a Glypican-BMP axis regulates skull growth and that changes in the levels of GPC1 and GPC3 disrupt BMP signalling and lead to abnormal skull growth.
We have shown that GPC1, GPC3 and BMP receptors are expressed by primary human cranial suture mesenchymal cells. We have demonstrated that increased GPC1 and GPC3 reduce BMP2-mediated osteogenesis in a synergistic fashion and that blockade of endogenous GPC1 and GPC3 by specific antibodies increases BMP2 activity. Moreover, our data suggest that glypicans directly interact with BMP2 and that the core GPC3 protein not the HS chains are required for functional interaction. We conclude that soluble GPC1 and GPC3 are antagonists of BMP2 activity in human suture cells and regulate BMP2-mediated osteogenesis. We predict that down-regulation of GPC1 and GPC3 in the sutures increases BMP2 activity and causes the abnormal bone growth that leads to premature suture fusion.
Reference:
Coussens et al. (2007): BMC Genomics. 8,458
Midorikawa et al. (2003): Int J Cancer. 103,455

P105

Transgenic CYP27B1 expression within mature osteoblasts is anabolic

Turner AG[1,2], Hanrath M[3], Yang D[2], Anderson PH[1,2] and Morris HA[1,2]
[1]School of Pharmacy and Medical Sciences, University of South Australia. [2]Musculoskeletal Biology Research, Chemical Pathology, SA Pathology. [3]School of Pharmacy, University of Utrecht, The Netherlands.

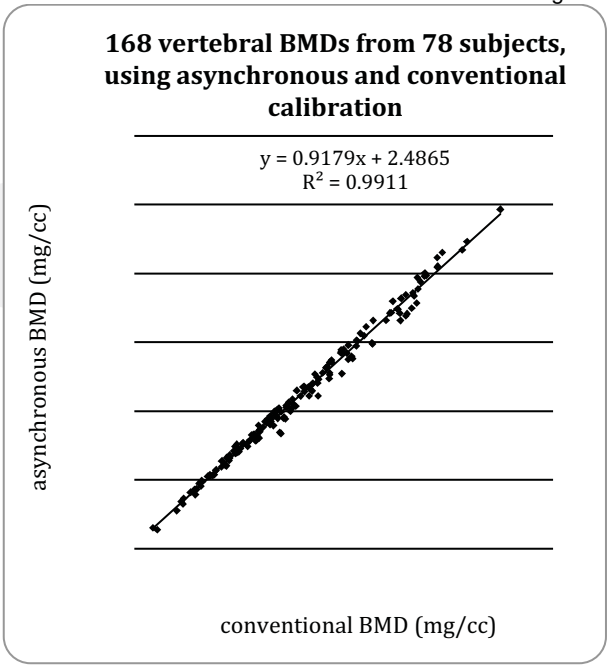
The endocrine hormone 1,25-dihydroxyvitamin D (1,25D) is best known as a regulator of serum calcium and phosphate homeostasis but intriguingly is also synthesized by bone cells by virtue of the activity of the CYP27B1 enzyme. Regulation of the Cyp27b1 gene is poorly understood, particularly in bone, and we consider this key for understanding 1,25D's hypothesised role within the framework of bone remodelling. We have created a transgenic mouse model in which transcription of the human *Cyp27b1* sequence is driven by the 3.6kb human osteocalcin promoter (OSCyp27b1) limiting transgene expression to mature osteoblast lineage cells. In adult (20wk) mice, BV/TV in the lumbar vertebra is 10.8% greater in OSCyp27b1 males than wild-type littermates ($p<0.05$, n=12), and 10.1% greater in females ($p=0.08$, n=15). Female OSCyp27b1 mice lose less femoral BV/TV than WTs with age, with BV/TV strongly correlated with bone formation rate ($R^2=0.67$, $p=0.01$). To further pursue the effect of the OSCyp27b1 transgene on osteoblast function we have isolated osteoblasts from neo-natal OSCyp27b1 calvaria. Differentiated cultures produced a mineralised extracellular matrix and expressed high levels of the human Cyp27b1 transgene, as well as osteocyte related genes such as DMP1 and SOST, suggesting that the transgene may have effects in osteocytes as well as osteoblasts. Transiently transfected deletion constructs of the human CYP27B1 promoter-luciferase reporter in osteocyte-like MLO-Y4 cells reveal an enhancer and repressive region characteristic of osteoblasts. Overall, local synthesis of 1,25D within the osteoblast lineage appears independently regulated, and yields an anabolic response in bone.

P106

A new clinical approach to quantitative CT (QCT) bone densitometry with asynchronous calibration

Bodeen GR, Brown JK, and Brett AD
Mindways Software, Austin, TX, USA

Background: Asynchronous calibration for quantitative CT bone densitometry permits extracting QCT measurements from other abdominal/pelvic CT procedures with zero additional radiation dose. Accurate CT bone densitometry has previously relied on simultaneous scanning of patient and calibration phantom. We report here assessments of systematic measurement differences between a prototype commercial QCT device using asynchronous calibration and a commercially available conventional QCT device.
Methods: Our retrospective cohort included 78 vertebral subjects and 73 femoral subjects, ages ranged 3 to 97, using multiple scanner models from each of four major manufacturers. BMD for each vertebra or femur was measured in QCT PRO Version 5.0 (Mindways Software, Austin, TX, USA) in its conventional mode and a new mode for asynchronous calibration using independently acquired, scanner-specific QA scans.
Results: Vertebral BMD ranged from 13.4 mg/cc to 262.2 mg/cc. The linear least-squares regression line between calibration conditions lay slightly off unity, showing a consistent bias wherein asynchronously calibrated BMD averaged 5.4% lower than conventional BMD. The SEE of this regression was 5.0 mg/cc.



Results were similar in the proximal femur, with a correlation above 0.98, an average decrease of 5.8% versus conventional calibration, and a SEE of 0.021 g/cm2 for BMDs ranging from 0.335 to 1.254 g/cm2.
Conclusions: The high correlations of asynchronously calibrated BMD with conventional BMD suggest this approach has substantially equivalent accuracy in reproducing T-Scores. A linear transformation suffices to correct the measurement bias without introducing significant variance. This approach may provide new clinical utility in dual-use and retrospective CT BMD screening.

P107

Analysis of four determinants of both QCT-derived and DXA-derived hip structural geometrical measurements (HSA) in elderly women

Khoo BCC[1,2], Brown K[3], Zhu K[4,5], RI Price[1,2] and Prince RL[4,5]
[1]*Medical Technology and Physics, Sir Charles Gairdner Hospital, Nedlands, WA, Australia*
[2]*School of Physics, University of Western Australia, Nedlands, WA, Australia.*
[3]*Mindways Software, Austin, TX, USA*
[4]*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia*
[5]*School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA, Australia.*

Introduction: Corresponding QCT and DXA derived planar hip structure analysis structural geometry measures of proximal femur have different numerical values. We characterised differences in HSA structural geometrical measurements at the femoral neck relative to four-basic measures that capture the complete set of information required to undertake regeneration of the 8 HSA variables currently in use. These are (i) areal bone mineral density (ii) femoral neck width (iii) SD of mineral-mass projection profile distribution and (iv) displacement between the centre-of-mass and the geometric centre of a mineral-mass projection profile induced by asymmetry of the profile.

Methods: To demonstrate the efficacy of the method QCT and DXA HSA, analyses were performed on proximal femur of 237 elderly low bone density women who were randomised into two similar cohorts of 118 (A) and 119 (B). Intercepts and gradients from linear regressions of four-basic measures of QCT versus DXA-derived measures obtained from Cohort A were used to convert Cohort B QCT-derived structural geometry measurements into corresponding DXA values.

Results: Differences between QCT and DXA-derived measures were removed by the cross-calibration process.

QCT and DXA-derived structural geometrical measurements for cohort B; mean(SD)

	Buckling-Ratio	***Cross-sectional-area(cm^2)***	***Section-modulus(cm^3)***
Before cross-calibration			
QCT-derived	15.92(3.42)	1.90(0.32)	0.83(0.17)
DXA-derived	11.63(2.18)[a]	2.64(0.39)[a]	1.34(0.23)[a]
After cross-calibration			
QCT-derived	11.42(1.78)	2.64(0.34)	1.33(0.19)
DXA-derived	11.63(2.18)	2.64(0.39)	1.34(0.23)

[a]: P<0.001

Conclusion: These data illustrate the importance of these four-basic measures that not only allow transformation of data acquired using one technology into the other but offer new insights into the computational basis of the structural assessment of bone.

P108

Application of measuring subregional vertebral areal bone mineral density among individuals with glucocorticoid-induced osteoporosis

Manning L[1,2], Briggs AM[1,2,3], Kantor S[1,2], van Doornum S[1], Kale A[1,2] and Wark JD[1,2]
[1.] *University of Melbourne Department of Medicine, Victoria, Australia*
[2.] *Bone and Mineral Service, Royal Melbourne Hospital, Victoria, Australia*
[3.] *Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia*

Despite comparable areal bone mineral density (aBMD), individuals with glucocorticoid-induced osteoporosis (GIO) experience vertebral fractures at a rate greater than individuals with primary osteoporosis. Assessing subregional vertebral aBMD from lateral-projection DXA may improve diagnostic sensitivity in GIO. The aim of this pilot study was to assess differences in subregional aBMD profiles between individuals (mean ±SD age: 61±5.5 years) with GIO (n=8), primary osteoporosis (n=27) and healthy controls (n=41). Standard postero-anterior (PA) and lateral-projection DXA of the lumbar spine were performed. The lateral scan image was used to measure aBMD in 3 sagittal (anterior, middle, posterior) and 3 transverse (superior, central, inferior) subregions at L3, based on an established protocol. There was a significant difference in whole vertebral aBMD between groups at L3 for PA-projection data ($p<0.001$) with controls having higher mean ±SD aBMD (1.11±0.11g/cm^2) than both osteoporosis groups; individuals with GIO had higher aBMD (0.91±0.16g/cm^2) than individuals with primary osteoporosis (0.77±0.07g/cm^2). In lateral-projection, there was no difference in aBMD between the two osteoporosis groups. A main effect for group ($p<0.001$) was observed in both sagittal and transverse subregional analysis, reflecting higher subregional aBMD in controls compared to the other two groups. A subregion x group interaction was observed sagittally ($p=0.029$) indicating that GIO participants had lower mean aBMD in the anterior subregion compared to individuals with primary osteoporosis (Fig.1), adjusted for vertebral area. Measurement of vertebral aBMD in the anterior subregion may offer valuable insights into vertebral fragility in patients with GIO.

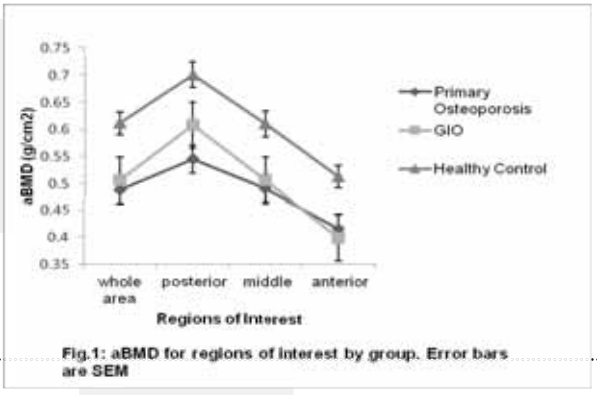


Fig.1: aBMD for regions of interest by group. Error bars are SEM

P109

Reasons for referral to DXA in men and women aged 20-49 years: Australian population-based data

Torpy AMJ[1], Brennan SL[1,2], Kotowicz MA[1,2,3] and Pasco JA[1,2]
[1]*School of Medicine, Deakin University.*
[2]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne.*
[3]*Department of Endocrinology and Diabetes, Barwon Health.*

Osteoporosis poses a significant public health problem for ageing Australians. However, approximately 25% of Australian adults aged 20-49years have osteopenia, a precursor condition to osteoporosis. Despite this, little is known about BMD testing in this age group.

Reasons for referral to DXA were examined in 2,264 patients aged 20-49years, referred 2001-10 to the Geelong Bone Densitometry Service, Geelong Hospital, Victoria. Referral reasons were determined from clinical-indication codes derived from patient-records. Age, sex, and BMD(T-scores) were ascertained for each patient.

The most common reason for referral for women reflected glucocorticoid use, and for men reflected fracture or low BMD. Compared to women, men were more likely to be referred because of minimal trauma fracture or low BMD(35.5% versus 18.0%, $p<0.001$). No further sex-differences were identified, with similar numbers of referrals observed for secondary osteoporosis, and drug therapy monitoring. Sex-differences were observed for BMD(T-score) for each referral code(all $p<0.02$, Table); the lowest BMD was observed in those who were referred to DXA due to changes in drug therapy.

Table: Sex differences in median BMD T-score(range) by clinical-indications

	Men(n=417)	Women(n=1,847)	*p*-value
Fracture/low BMD[a]	-1.5(-5.0,1.8)	-1.1(-4.3,2.1)	**0.002**
Glucocorticoid use[b]	-1.3(-6.3,2.9)	-1.0(-4.8,2.3)	**0.018**
Secondary osteoporosis[c]	-1.4(-3.3,1.6)	-0.8(-3.2,2.2)	**<0.001**
Drug therapy monitoring[d]	-3.0(-3.8,-2.2)	-1.4(-3.6, 0.4)	*

*Due to small numbers(n=19), *p*-values for between sex-differences could not be determined

These are the first data to examine reasons for referral to DXA among Australians aged 20-49years. Focused attention toward this age group informs current understanding of reasons for DXA referral; information which is integral to understanding future risk.

P110

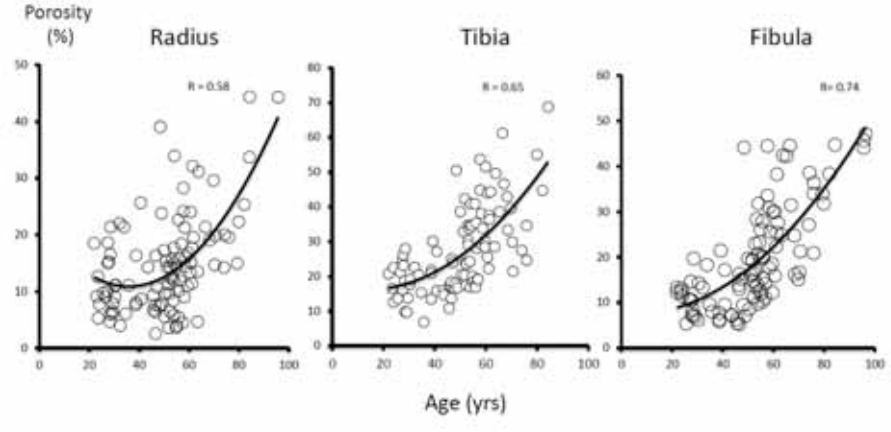
StrAx1.0: a non-threshold based image analysis

Ghasem-Zadeh A, Zebaze R and Seeman E
Dept Endocrinology and Medicine, Austin Health, University of Melbourne

Aging is associated with an exponential increase in bone fragility attributable, in part, to the rise in intracortical porosity, the source of 70% of all bone loss. Intracortical remodeling and the porosity is a diagnostic and therapeutic target. However, quantifying porosity *in vivo* is challenging because the median size of haversian canals is ~50 microns. Even at the highest image resolution achievable *in vivo* in human subjects, 82μm, ~80% of voxels are composites; they contain mineralized matrix and void volume in varying proportions.

We present a method enabling quantification of void space in these composite voxels to total porosity. The non-threshold based algorithm uses the attenuation value of a voxel relative to background (0%) and fully mineralized bone matrix (100%) to estimate void volume of a voxel, no matter how small. The sum of the void volume within each voxel quantifies total porosity of the compact and transitional zones. The method is fully automated and provides an accurate (~90%) and reproducible (CV% <2%) quantification of porosity *in vitro* and *in vivo*.

We report an age related increase in porosity of about 50% at the distal radius, tibia and fibula, the last a robust site because of its thick cortex (figure). The increase in porosity is approximately 2-3 times greater than reported using histomorphometry and other methods that either fail to detect smaller pores or disregard them due to partial volume effects and the use of segmentation by thresholding.



P111

Adult fracture and quality of life: a population-based study of Australian men

Dobbins AG[1], Brennan SL[1,2], Williams LJ[1,3] and Pasco JA[1,2]
[1]*School of Medicine, Deakin University, Geelong, Australia*
[2]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[3]*Department of Psychiatry, The University of Melbourne, Parkville, Australia*

Objective: To investigate associations between adult fracture (sustained ≥20 years of age) and quality of life (QOL) in men.
Methods: Men enrolled in the Geelong Osteoporosis Study 5-year follow-up (2007-11, participation 81%) and aged 50-85 years were included for analysis (n=448). We employed the Australian World Health Organization Quality of Life Instrument (WHOQOL-BREF) to measure QOL in the domains of physical health, psychological health, environment and social relationships. Self-reported fractures were grouped as recent (≤10 years pre-assessment) or non-recent. Logistic regression models were adjusted for age and alcohol consumption.
Results: 174 men (38.8%) sustained at least one fracture prior to assessment; hip (n=3), spine (n=8), ribs/sternum (n=30), wrist (n=39), other upper limb (n=46), lower limb (n=56), other (n=61). One-quarter (26.4%) of the fractures were considered recent. Reduced satisfaction was observed for the physical health QOL domain in association with recent (OR=0.46, 95%CI 0.20-1.04) and non-recent (OR=0.47, 95%CI 0.27-0.83) fracture. Both the psychological health (OR=0.48, 95%CI 0.24-0.97) and environmental (OR=0.52, 95%CI 0.25-1.09) domains were associated with recent fracture only. No association was observed between fracture and the social relationships domain. Results were sustained after adjustment for physical activity, smoking and BMI.
Conclusion: We present the first data examining the association between adult fracture and QOL in a population-based sample of Australian men using the WHOQOL-BREF. Fractures were associated with poorer QOL in the domains of physical health, psychological health and environment; dissatisfaction with physical health was sustained over a longer post-fracture period. These findings have important implications for rehabilitation following fracture.

P112

Bisphosphonate use and increased incidence of subtrochanteric fracture in South Korea: Results from the National Claim Registry

Lee Y-K[1], Ha Y-C[2], Byun DW[3], Park H-M[4], Min Y-K[5] and Koo K-K[1]
[1]*Department of Orthopaedic Surgery, Seoul National University Bundang Hospital*
[2]*Department of Orthopaedic Surgery, Chung-Ang University College of Medicine*
[3]*Department of Internal Medicine, Soonchunhyang University College of Medicine*
[4]*Department of Obstetrics and Gynecology, Chung-Ang University College of Medicine*
[5]*Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine*

Background: Recently, atypical hip fractures in the subtrochanteric region have been reported among patients on bisphosphonate. However, the association between atypical hip fracture and bisphosphonate is controversial. We evaluated trends in the incidences of typical and atypical hip fracture in relation bisphosphonate use in Korea from 2006 to 2010, using nationwide data obtained from the Health Insurance Review and Assessment Service (HIRA)
Methods: All new visits or admissions to clinics or hospitals for a typical and atypical hip fractures were recorded nationwide HIRA using the ICD-10 code classification. Typical and atypical hip fractures were defined as femoral neck/intertrochanteric and subtrochanteric fracture, respectively. Bisphosphonate prescription data was also abstracted from the HIRA database.
Results: The absolute number of typical and atypical hip fracture increased during the study period. Although age-adjusted incidence rates of typical hip fractures were stable in men and women, those of atypical hip fractures increased in women. Nationally, the annual numbers of prescriptions of bisphosphonate also increased during the study period.
Conclusions: The results of this study suggest a possible causal relationship between bisphosphonate use and the increased incidence of atypical hip fracture in Korea.

P113

Cardiovascular risk assessment in patients with osteoporotic fracture

Hong S[1], Kim SH[1], Nam MS[1], Kim YS[1] and Moon KH[2]
[1]*Department of Internal Medicine,* [2]*Depatment of Orthopedics, Inha University*

The prevalence of both cardiovascular disease (CVD) and osteoporosis is increasing dramatically as the worldwide population ages. Atherosclerotic disease and fractures likely share a common biological pathway and accumulating evidence links vascular factor to osteoporosis and fracture. Hip fracture causes excess mortality due to cardiovascular and infectious disease in older people. Although several studies included an assessment of fracture risk in patients with cardiovascular disease, cardiovascular risk in patients with osteoporosis has not been studied. Assessment of cardiovascular risk is not performed adequately in clinical practice and its effect on mortality has not been tested. Aim of this study is to evaluate general CVD risk and to know whether it is related to six month mortality after osteoporotic fracture. This is an observational study to assess the cardiovascular risk in patients with patients with osteoporosis. The primary endpoint in this study is the difference of Framingham risk score depending clinical fracture. 80 osteoporotic patients with or without clinical fracture were included. 10 year risk of coronary event is increased in patient with clinical fracture compared to non-fracture group. Mean A1c and blood pressure in clinical fracture group were higher than control. Vitamin D concentrations were below 20 ng/ml in 70% of patients with clinical fracture. Framingham score was correlated with FRAX ($p<0.05$). Patients who experienced osteoporotic fracture had high risk in cardiovascular disease. Its effect on mortality should be observed carefully.

P114

Changes in general Quality of Life (QoL) after non-traditional fractures

Zhang Y[1], Sanders KM[1], Pasco JA[1,2], Lane SE[3] and Kotowicz MA[1,2]

1. *NorthWest Academic Centre, Sunshine Hospital, Department of Medicine, University of Melbourne*
2. *School of Medicine, Deakin University*
3. *Department of Medicine, Barwon Health, Geelong*

General QoL associated with fractures at sites other than hip, spine and wrist has been under-investigated. The aim of this study was to investigate general QoL related to non-traditional fractures including ankle, "other weight bearing" (pelvis, femur, proximal and mid tibia/fibula, foot) humerus, and "other non-weight bearing" (rib, clavicle, forearm, and hand) fractures.

Participants aged 50+yrs with a radiologically confirmed low trauma fracture were recruited from the Geelong Hospital between June 2010 and December 2011. Using the ICEpop Capability Index (ICECAP) questionnaire, QoL immediately before and after fracture was assessed and repeated 4 months post fracture. High trauma, multiple and pathological fractures (n=92), those with cognitive disabilities (n=65) or who were unable to understand the questionnaire (n=37) and others (n=65) were excluded. Changes in QoL were investigated using the Wilcoxin test. The impact of variables on QoL (age, gender, fracture-related hospital admission, fracture sites, time between first interview and fracture, and pre-fracture QoL) was assessed using regression analysis, confidence intervals estimated using bias-corrected accelerated bootstrap method.

Table 1 shows the impact of fractures on QoL. After fracture, QoL declines ($p<0.001$), but "other non-weight bearing" fractures recovered after four months ($p=0.21$). Pre-fracture QoL had a significant impact on loss of QoL immediately (coefficient: -0.36 95%CI: -0.45,-0.28) and 4 months after fracture (coefficient: -0.52, 95%CI: -0.61,-0.43)

General QoL decreases by 11-19% immediately after fracture, with recovery only at "other non-weight bearing fractures" four months later. Pre-fracture QoL and had a substantial impact upon QoL immediately and 4 months after fracture.

Table 1: Median general QoL (IQR) immediately before and after fracture, and 4 months after fracture

Fracture sites	n	Pre-Fracture	Post-Fracture	4 Months
Ankle	64	0.93 (0.89-1.00)	0.75* (0.57-0.84)	0.85* (0.73-0.91)
Other Weight Bearing	63	0.91 (0.84-0.98)	0.70* (0.31-0.81)	0.89* (0.77-0.94)
Humerus	53	0.91 (0.83-0.98)	0.70* (0.49-0.78)	0.87* (0.77-0.92)
Other Non-Weight Bearing	47	0.90 (0.83-0.94)	0.77* (0.65-0.89)	0.89 (0.84-0.91)

*indicates significant difference in QoL to pre-fracture levels ($p<0.05$)

P115

Comparison of the Australian FRAX and the Garvan hip fracture prediction models using a 10-year prospective study of elderly Australian women

Dhaliwal SS[1], Yu M[2,4], Josh L[2,3], Zhu K[2,3] and Prince RL[2,3]

[1]School of Public Health, Curtin University; [2]Department of Endocrinology & Metabolism, School of Medicine and Pharmacology, University of Western Australia; [3]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, [4]Shanghai Zhongshan Hospital, Fudan University, China.

Background: Fracture risk calculators have been developed to improve DXA aBMD structural measures as predictors of future fracture risk. We compared Australian FRAX and the Garvan hip fracture prediction models using a long running cohort study of older women.

Methods: The study population used was the CAREES study, an ongoing population based cohort study of 1500 women with a mean age of 75 years at baseline in 1998. In this paper we report hip fracture risk prediction in a sub population of 1127 women who had a hip aBMD measurement in 1999 and in whom complete ascertainment of hip fracture incidence over 10 years is available.

Results: Hip fractures occurred in 68 (6%) participants. The median 10 year hip fracture risks were 4.1% for FRAX without BMD, 1.9% for FRAX with BMD, 5.6% for Garvan without BMD, and 3.9% for Garvan with BMD. The correlations of the predicted hip fracture risk of the two models were Pearson R = 0.721 and Spearman rank R = 0.890 for FRAX and Garvan without BMD, and Pearson R = 0.754; Spearman rank R = 0.913 for FRAX and Garvan with BMD (all $P < 0.001$). The kappa scores were FRAX and Garvan without aBMD 0.649, and FRAX and Garvan with aBMD 0.699.

Conclusions: These data show that there is reasonably good agreement between the two calculators for 10-year hip fracture risk.

P116

Consuming two additional serves of dairy food a day significantly improves protein, calcium and vitamin D intakes in ambulatory aged care residents: A feasibility study

Iuliano S[1], Woods J[2] and Robbins J[3]
[1]*Department of Endocrinology, University of Melbourne / Austin Health*
[2]*Department of Nutrition and Dietetics, Monash University*
[3]*Judy L. Robbins Consultant Dietitian to Aged Care*

Low-level aged care residents are at high risk of malnutrition. Sub-optimal intakes of protein, calcium and vitamin D contribute to fracture risk. Oral supplements and fortified foods are used to overcome malnutrition in the elderly but require special preparation and administration by staff, over and above standard food and beverages served. We proposed that increasing current dairy food intake in residents from two to four serves per day would improve intake of these nutrients. This was a 2-month prospective intervention study in 68 residents (78% female, mean age 86.5 years) in 2 low-level aged care facilities in Melbourne, Australia. Menus were modified to include at least two additional serves of dairy food per day. Mean macro- and micro-nutrient intakes before and after intervention was recorded using observed intake (food served minus waste), and comparisons made using paired t-tests. Following intervention, daily increases in mean energy intake (+900kJ, $p<0.001$), protein intake (+25g, $p<0.0001$), proportion of energy from protein (+4%, $p<0.0001$) and proportion of estimated energy requirements (+18%, $p<0.0001$) were observed, while proportion of energy from fat decreased (-3%, $p<0.0001$). Increases in mean daily micronutrient intakes were observed for calcium (+679mg, $p<0.0001$), and vitamin D (+1.4μg, $p<0.0001$), with recommended intake levels for calcium achieved on the higher dairy diet. Mean sodium intakes remained unchanged. Two additional serves of dairy food can significantly improve protein, calcium and vitamin D intakes in aged care residents and its ease of provision makes it a viable option to potentially prevent fractures.

P117

Explaining the sex difference in fracture risk: the role of muscle quality

Nguyen TV, Nguyen ND, Center JR and Eisman JA
Osteopororis and Bone Biology Program, Garvan Institute of Medical Research, Sydney, Australia

This study was designed to test the hypothesis that measures of skeletal muscle mass account for the sex difference in fracture risk.
The population based prospective study involved 779 women and 354 men aged 50 or above at baseline. Baseline fat mass, lean mass, and BMD were measured by DXA (GE-Lunar Prodigy). Appendicular muscle mass was estimated as kilograms of fat-free soft tissue in the upper plus lower extremities. Sarcopenia was defined appendicular skeletal muscle mass/height^2 less than 2SD below the mean for young, healthy reference populations. Muscle quality (MQ) was defined as muscle strength (measured using a dynamometer) per unit of mass.
During the 10 year median duration of follow-up, 238 women (31%) and 67 men (19%) had sustained a low-trauma fracture. The prevalence of sarcopenia was ~20% for both sexes. Increased fracture risk was associated with sarcopenia, but the association was not independent of BMD. Muscle quality in men was not significantly different from women. In either sex, each SD lower in MQ was associated with ~30% increased risk of fracture (95% CI: 1.10-1.56). In the model with age, BMD and sex, women had greater risk of fracture than men (1.5; 1.1-2.2). However, when MQ was added to the model, the effect was no longer statistically significant, but MQ remained an independent predictor.
These results suggest that muscle quality is a predictor of fracture risk, and that the sex difference in fracture risk could be in part explained by the association between MQ and fracture.

P118

Depression and falls in men: the Geelong Osteoporosis Study

Stuart AL[1], Pasco JA[12], Berk M[13] and Williams LJ[13]
[1]*School of Medicine, Deakin University, Geelong, Australia*
[2]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[3] *Department of Psychiatry, The University of Melbourne, Parkville, Australia*

Depression and falls are common concerns. The use of sedatives and other medications that affect the central nervous system, often taken by those suffering depression, are known to contribute to falls.
Aim: The aim of the current study is to investigate the link between major depressive disorder (MDD) and falls in an age-stratified, random, population-based sample of 952 men aged 24-97 years participating in the Geelong Osteoporosis Study.
Method: MDD was diagnosed utilising the Structured Clinical Interview for DSM-IV-TR Research Version, Non-patient edition (SCID-I/NP) and categorised as 12-month/past/never. BMI and gait were measured; falls, smoking status, psychotropic medication use and alcohol intake were self-reported.
Results: Thirty-four (3.6%) men met criteria for 12-month MDD, and 110 (11.6%) for past MDD. 175 (18.4%) men reported falling at least once during the past 12 months. Fallers were older [66 (IQR 48-79) vs 59 (45-72) yr, $p=0.001$] and more likely to use psychotropic medication (14% vs 10%, $p=0.07$) than non-fallers. Participants with 12-month MDD had a 2 -fold increased odds of falling (age-adjusted OR 2.22, 95%CI 1.03-4.80, $p=0.04$). The odds of falling were not associated with past depression ($p=0.4$). Further adjustments for psychotropic drug use, gait, BMI, smoking status, blood pressure and alcohol did not explain these associations.
Conclusion: 12-month depression was associated with increased risk of falling. As this association was not explained by age or psychotropic drug use, further investigations into interactions between depression, balance and the vestibular system are warranted.

P119

GARVAN Fracture risk calculator is not an adequate substitute for need for BMD in people over 70 years of age

Inderjeeth CA[1,2], Foo B[1], Van V[1], Chauhan A[1], Fisher T[3], Rogers J[4] and Will R[5]
[1]*Area Rehabilitation and Aged Care, North metropolitan Health Service, Perth*
[2]*University of Western Australia,* [3]*RAAFA Medical Practice, Merriwa WA* [4]*Padbury Medical Practice, Padbury WA*
[5]*Metabolic Bone and Musculoskeletal research Unit, Royal Perth Hospital, Perth*

Bone mineral density (BMD) screening is recommended for people over 70 years. However access to BMD may be a barrier in frail elderly people. This limits our ability to assess fracture risk in this high risk group.
Aim: Utilising a mobile DXA service we assessed an elderly populations who would otherwise not have access to DXA. We also assessed fracture risk based on GARVAN fracture risk calculator (without BMD). The main objective was to assess the threshold predictive value in identifying patients with osteoporosis.
Methods: 935 patients aged ≥ 70 years were screened from selected Aged Care facilities/ GP Practices. All patients had osteoporosis risk assessment (BMD study and fracture risk questionnaire). We analysed the sensitivity, specificity and positive predictive values of GARVAN 10 year major and hip fracture risks thresholds calculated without BMD in identifying patients with osteoporosis.
Results: Mean age was 79±6 years; 55% female; 246(26.3%) reported prior fracture and 38(4.1%) prior hip fracture. 84 had t ≤-2.5 (BMD criteria for diagnosis of osteoporosis). Calculated GARVAN 10 year hip fracture risk (without BMD) ≥ 4.1% aiming for a sensitivity of 90% had specificity of 30% in identifying patients with osteoporosis. Calculated GARVAN 10 year major fracture risk (without BMD) ≥ 18.6% aiming for a sensitivity of 90% had a specificity of 28%.
Conclusion: We did not identify a fracture risk threshold based on GARVAN without BMD with adequate sensitivity and specificity to provide a reliable predictive value to substitute for BMD in patients over 70 years.

References:

1. GARVAN fracture risk calculator, available from: http://www.garvan.org.au/bone-fracture-risk/

P120

Improving outpatient osteoporosis management: performance and cost-efficacy of a 'fracture capture' service

Chauchard MA[1,2], Yates CJ[1,2], Liew D[3], Farrugia R[4], Bucknill A[4] and Wark JD[1,2]
[1] *Department of Medicine, University of Melbourne.*
[2] *Bone & Mineral Service, Royal Melbourne Hospital*
[3] *Melbourne EpiCentre, University of Melbourne and Melbourne Health*
[4] *Department of Orthopaedics, Royal Melbourne Hospital*

In 2010, we established a 'Fracture Capture' service to improve osteoporosis detection and management in outpatients over 50 years old who sustained a fragility fracture. A nurse-coordinator screened patients attending orthopaedic clinics and arranged pathology and DXA testing prior to physician review. We assessed performance to April 2012 using a clinic database, patient questionnaire, the Garvan Fracture Risk Calculator, and published cost and efficacy data.

Table 1. Patient characteristics

N		203
Age		66 (12)
Female		157 (77%)
Fracture Site (n)		
	Hip	4
	Wrist	84
	Humerus	38
	Ankle	24
	Other	67
DXA T-score		
	Lumbar Spine	-1.3 (1.59)
	Total Hip	-1.4 (1.04)
	Femoral Neck	-2.0 (1.24)
25-Hydroxyvitamin D		58 (31)
5-year Fracture Risk		
	Any site	20% (15%)
	Hip	0.5% (0.6%)
10-year Fracture Risk		
	Any site	34% (21%)
	Hip	1.1% (1.1%)

Values are mean (SD) except Sex (%), Fracture Site (n)

Osteoporosis-specific medications were prescribed to 124 patients (61%): 44 risedronate, 32 alendronate, 27 strontium ranelate, 16 zoledronic acid, 2 teriparatide, 1 denosumab, 1 pamidronate, 1 testosterone. Eighty-four completed the questionnaire: 95% very satisfied/satisfied; 70% reviewed within 2 months, 79% medication compliance. We estimated that with treatment as above, 'Fracture Capture' reduced number of fractures over 5 years from 40 to 34. Over this time, quality-adjusted-life-years (QALYs) per patient were estimated to have improved by 0.043 at net cost $1879, equating to an incremental cost-effectiveness ratio of $44181/QALY gained. 'Fracture Capture' is a popular, cost-effective model to improve outpatient osteoporosis management. Because of economies of scale, cost-effectiveness will improve as referrals increase.

P121

Low bone turnover in osteoporosis of the old old: a hypothesis testing pilot study

Chan K[1], Nair P[1], Lim EM[2], Petta A[1] and Inderjeeth CA[1, 2]
[1]*Area Rehabilitation and Aged Care, North Metropolitan Health Service, Perth, WA 6009, Australia*
[2]*North Metropolitan Health Service, University of Western Australia, Hospital Avenue, Nedlands, Perth, WA 6009, Australia*

Background and Aim: The pathophysiology of osteoporosis in the old old may be different to postmenopausal osteoporosis. We aim to compare the bone turnover rate of the two groups.
Methods: Retrospective audit of all fasting metabolic bone studies (FMBS) performed by the author during the period 2002-2009 in outpatient clinics. Patients' case notes were reviewed and analyses performed on those aged 50 and above with osteoporosis. Patients aged 50-65 were grouped as 'postmenopausal' (PM), and those aged 75 and above as 'old old' (OO). Exclusion criteria were prior use of anti-osteoporosis medication (excluding calcium and vitamin D), fracture within 6 months and glucocorticoid induced osteoporosis.
Results: 488 FMBS were performed, of these, 248 patients' notes were available for review. In the PM group assessed to date, the mean age was 59 ± 4; and in the OO group, the mean age was 79 ± 3. There was a difference in urine N-Telopeptide/Creatinine between the groups (PM: 50.2 ± 18.0 vs. OO: 36.4 ± 6.7; p=0.07). There was also an expected significant difference in femoral neck BMD (PM: T – 1.57 vs. OO: T – 2.95; p=0.015). The other indices measured were comparable, including serum calcium, albumin, creatinine, alkaline phosphatase, parathyroid hormone and vitamin D.
Conclusion: The bone turnover profile of the old old with osteoporosis may be different to that of postmenopausal women, with lower bone turn over as reflected by lower bone resorption markers. This may have therapeutic implication. Further study is warranted.

P122

Morbidity and mortality in older than 50 years with hip fracture with average 6-year follow-up; a prospective cohort study

Ha Y-C[1], Lim Y-T[2], Lee Y-K[3], Chang JS[4], Min Y-K[5] and Byun DW[6]
[1] *Department of Orthopaedic Surgery, Chung-Ang University College of Medicine, Seoul, South Korea*
[2] *Department of Obstetrics and Gynecology, College of Medicine, the Catholic University of Korea.*
[3] *Department of Orthopaedic Surgery, Seoul National University Bundang Hospital, Seongnam, South Korea*
[4] *Department of Orthopedic Surgery, Asan Medical Center, Ulsan University College of Medicine, Seoul, Korea*
[5] *Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.*
[6] *Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul Korea*

Introduction: The author performed an epidemiologic study in Jeju Island, South Korea using data prospectively collected from 2002 until 2006. Jeju is geographically isolated from the main land and patients with hip fractures typically require hospitalization, which makes epidemiologic studies easier and more reliable than those on other fracture types. This prospective study was performed to estimate morbidity and mortality after hip fracture among patients more than 50 years old, in Jeju island, South Korea.
Methods: Seven hundred and ninety patients over 50 years of age that sustained a femoral neck or intertrochanteric fracture from 2002 to 2006 were followed-up for a mean of 6 years . Crude and standardized annual mortality ratios were calculated and mortalities in the cohort and the age and sex matched general population were compared. The effects of different risk factors on mortality and functional capacity before and after injury and on activities of daily living and walking ability were assessed.
Results: Accumulated mortality was 16.7% (132 patients) at 1 year, 45.8% (337 patients) at 5 years, and 60% (372 patients) at 8 years. Standardized mortality ratio at 5 years post-injury was 1.3 times that of the general population. Multivariate analysis (performed using a Cox proportional hazards model) demonstrated that age (OR = 1.074, 95% CI, 1.050-1.097, p<0.001), gender (OR = 1.893, 95% CI, 1.207-2.968, p=0.005), medical comorbidity (OR = 1.334, 95% CI, 1.167-1.524, p<0.001) were independently associated with mortality after hip fracture. Fifty-nine of 150 patients (39.3%) that were able to ambulate outdoors at pre-injury achieved normal activity levels at final follow-up.
Conclusion: Patients that had sustained a hip fracture were found to have an elevated mortality rate at 5 years after fracture and poor activity levels after a mean follow-up of 6 years. Action should be taken to ensure that the public health care system is improved to reduce mortality rates after hip fracture and to minimize mobility limitations and disabilities.
Financial support for this study was obtained from Sanofi –Aventis, South Korea.

P123

Normative data for total lean mass for Australian men and women: Geelong Osteoporosis Study

Gould H[1,2], Brennan SL[1,2], Kotowicz MA[1,2] and Pasco JA[1,2]
[1]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[2]*School of Medicine, Deakin University, Geelong, Australia*

Aim: To develop normative data for total lean mass measured using dual energy X-ray absorptiometry (DXA) for randomly selected, population-based men and women residing in south-eastern Australia.
Method: Men and women enrolled in the Geelong Osteoporosis Study were randomly selected from the Barwon Statistical Division using the electoral roll as a sampling frame in 2001-06 (67% response) and 1993-97 (77%), respectively. Participants were measured for total lean mass using DXA (Lunar DPX-L or Prodigy Pro). Means and standard deviations (SD) for young adult (20-29 yr) men and women were calculated and linear regression was used to explore the relationship between total lean mass and each of age, weight and height.
Results: For men (n=1418; 20-96 yr) and women (n=1002; 20-93 yr) the median age was 55.9 yr (interquartile range [IQR]: 39.0–73.2 yr) and 50.9 yr (IQR: 34.7–66.0 yr), respectively. Mean (±SD) total lean mass for young men (n=180) was 59.5±6.9 kg and 41.2±4.8 kg for young women (n=181); lean mass for *all* men and women was 57.8±7.2 and 39.4±4.5 kg, respectively. Lean mass decreased with age for both genders. Parsimonious models for lean mass for both genders included age, weight and height; these models explained more of the variance in lean mass in men than in women (adjusted R^2, 75.9% *vs* 65.2%).
Conclusion: These data describe reference ranges for total lean mass measured using DXA for men and women, providing normative data useful for identifying lean mass deficits in the assessment of muscle wasting and sarcopenia.

P124

Oral health and biochemical risk factors for bisphosphonate-associated jaw osteonecrosis

Tsao CE[1,2], Borromeo GL[1], Darby IB[1,2], Walsh KA[1,2], O'Brien-Simpson NM[1,2], Reynolds EC[1,2] and Ebeling PR[3]
[1] *Melbourne Dental School, The University of Melbourne*
[2] *Oral Health CRC, The University of Melbourne*
[3] *NorthWest Academic Centre, The University of Melbourne, Western Centre for Health Research and Education, Western Health*

Anti-resorptive drugs have been associated with jaw osteonecrosis (ONJ). Despite serum bone turnover markers (BTMs) being used to assess ONJ risk, the relationship between serum BTMs and bone turnover in the periodontium is uncertain. In addition, the role of oral health as a risk factor for ONJ remains unclear. Firstly, we aimed to compare levels of BTMs in GCF and serum. Twenty healthy volunteers received oral examinations, and gingival crevicular fluid (GCF) and serum sampling. Serum was tested for the amino-terminal propeptide of Type I collagen (P1NP) using automated non-isotopic immunoassays. GCF was tested for P1NP using a RIA. Secondly, we aimed to investigate oral health risk factors for ONJ. This cross-sectional study compared ONJ cases (n=22) with controls (n=41), who all received bisphosphonates. Oral examinations were conducted, and serum and GCF sampled. Serum was tested for specific IgG titres against periodontitis-associated bacteria, biochemical parameters, BTMs, and with multiplex analysis. GCF underwent multiplex analysis and P1NP RIA testing. Results: GCF PINP levels were 10-fold higher than in serum. A significant correlation was found between GCF and serum P1NP levels (r=0.629, p=0.002), suggesting that assumptions regarding bone turnover activity in the periodontium may be inferred from serum levels. Periodontitis was associated with ONJ, as measured by three clinical parameters and the surrogate markers of serum IgG titres against P*orphyromonas gingivalis* (OR 2.72, p=0.018) and GCF Interleukin-1β (OR 24.7, p=0.044). Conclusions: Bone turnover was higher in the periodontium compared with serum. Our results also suggest that periodontitis is an ONJ risk factor.

P125

Recent increases in BMI among men are accompanied by increases in body fat and decreases in lean mass and BMD

Pasco JA[1,2] , Gould H[1,2], Brennan SL[1,2] and Kotowicz MA[1,2.3]
[1]*Deakin University, School of Medicine, Geelong, Australia*
[2]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[3]*Department of Endocrinology and Diabetes, Barwon Health, Geelong, Australia*

Introduction: The obesity epidemic has been monitored in terms of BMI but this index masks relative changes in body fat mass, lean mass and bone. The aim of this study was to determine musculoskeletal changes in body composition over five years.
Methods: Body composition was evaluated for men in the Geelong Osteoporosis Study (GOS) using DXA (Lunar) during two time periods, approximately five years apart. DXA was performed for 1329 men (25-96yr) during 2001-6 and for 900 men (25-98yr), 2006-11. Fat and lean mass were divided by the square of height (kg/m^2) and bone was expressed as whole body BMD (g/cm^2). Regression models were used to determine time-related differences; models were age-adjusted and checked for interaction terms.
Results: Mean BMI increased from 26.9kg/m^2 (95%CI 26.7-27.1) in 2001-6, to 27.2kg/m^2 (27.0-27.4) in 2006-11 (p=0.04). Mean fat mass increased 9.0% from 6.98kg/m^2 (6.84-7.11) in 2001-6, to 7.60kg/m^2 (7.44-7.77) in 2006-11 (p<0.001); in contrast, mean lean mass decreased 0.9%, from 18.92kg/m^2 (18.83-19.01) to 18.75kg/m^2 (18.64-18.86) (p=0.02). Mean BMD decreased, from 1.263g/cm^2 (1.255-1.270), to 1.255g/cm^2 (1.247-1.263) (p=0.08); further adjustment for lean mass explained the apparent difference in BMD (p=0.5) and this was not observed when adjustments were made for fat mass or weight.
Conclusion: We report an increase in BMI over a period of five years, which reflects a substantial increase in body fatness. During this period, there has been a decline in both lean mass and BMD. This may have implications for future development of bone fragility and sarcopenia in the male population.

P126

Referral to DXA in an Australian population aged 50 years and over during 2003-10: Associations with sex and DXA reimbursement

Brennan SL[1,2], Kotowicz MA[1,2], Ebeling PR[1], Sarah B[2], Leslie WD[3] and Pasco JA[1,2]
[1]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[2]*School of Medicine, Deakin University, Geelong, Australia*
[3]*Department of Medicine, University of Manitoba, Winnipeg, Canada*

Osteoporosis in men has been neglected compared with women. Data from the Barwon Statistical Division (BSD), south-eastern Australia, 1991-8, show women were nine times more likely to be referred for DXA than men. This study examined sex-bias in DXA referrals (2003-10) for individuals aged ≥50years, and the impact of reimbursements introduced by Medicare (April 1st, 2007) for individuals aged ≥70years.
Patients aged ≥50years resident within the BSD, who were referred for a DXA (non-research purposes) to the major public health service provider in the BSD, 2003-10(n=14,324; 16% men) were included. Age, sex, and year of DXA were determined. Observed referrals were tabulated according to sex and calendar year. To examine the proportion of men versus women referred for DXA, expected referrals were estimated by age-standardization to the 2006 population at risk (data not shown). In patients aged ≥70years (6,096; 21% men) DXA referrals were compared pre- and post-2007.
In individuals aged ≥50years, 0.7% of men underwent DXA compared with 3.1% of women. In a subset of individuals aged ≥70years, 0.5% of men and 1.4% of women underwent DXA pre-2007, whereas 0.9% and 2.6% underwent DXA post-2007, respectively (p=0.068). The overall male:female ratio of DXA referrals was ~1:3, however was age-dependent.
Referrals to DXA in the BSD (2003-10) reflected the overall between-sex differences in fracture risk. DXA reimbursement introduced in 2007 has resulted in a doubling of the proportion of men and women aged ≥70years referred to DXA in the BSD. Nevertheless, low rates of DXA utilization persist, suggesting that osteoporosis remains under-diagnosed and therefore undertreated in both sexes.

P127

Selective serotonin reuptake inhibitor use and bone mineral density in men: Geelong Osteoporosis Study

Williams LJ[1,2], Pasco JA[1,3], Jacka FN[1,2], Hodge JA[1,3,4], Dodd S[1,2], Kotowicz MA[1,3] and Berk M[1,2]
[1]*School of Medicine, Deakin University, Geelong, Australia*
[2]*Department of Psychiatry, The University of Melbourne, Parkville, Australia*
[3]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[4]*Barwon Biomedical Research, The Geelong Hospital, Geelong, Australia*

Aim: The aim of this study was to investigate the association between SSRI use and bone mineral density (BMD) in a population-based sample of men (n=1467; 20-96yr) participating in the Geelong Osteoporosis Study.
Methods: BMD (g/cm^2) was measured at the PA-spine, hip and total body using dual energy absorptiometry (Lunar). Anthropometric measurements and socio-economic status (SES) were determined and information on medication use, depression and lifestyle was obtained via questionnaire.
Results: Fifty (3.4%) men reported using SSRIs. After adjustment for age, weight, height and glucocorticoid use, BMD among SSRI users was 4.9% lower at the spine [1.19 (95%CI 1.12-1.25) vs 1.25 (95%CI 1.21-1.28), p=0.03]. Weight was an effect modifier at the hip and total body. Among non-obese men (BMI<30; n=1165), BMD among SSRI users was 6.5% lower at the femoral neck [0.91 (0.85-0.96) vs 0.97 (0.94-0.99), p=0.006], 8.1% lower at the Ward's triangle [0.71 (0.66-0.77) vs 0.78 (0.74-0.81), p=0.02], 7.3% lower at the trochanter [0.83 (0.78-0.88) vs 0.89 (0.86-0.92), p=0.005] and 3.0% at the total body [1.19 (1.15-1.22) vs 1.22 (1.20-1.24), p=0.02]. These patterns were sustained after adjustment for SES, smoking, physical activity, depression, alcohol and dietary calcium intake, bone active medications, and other antidepressants. No differences in BMD were detected among the obese (all p>0.05).
Conclusion: Our data suggest that SSRI use is associated with reduced BMD among men. Considering the growing coalescence of basic and clinical evidence, it may be appropriate for safety monitoring guidelines to incorporate recommendations for prevention and treatment of bone disease in psychiatric patients.

P128

The association between AT_1 receptor antagonists and the risk of fragility fracture in women

Gould H[1,2], MacInnis RJ[3,4], Pasco JA[1,2], Jenkins M[3] and Kotowicz MA[1,2]
[1]*NorthWest Academic Centre, Department of Medicine, The University of Melbourne, St Albans, Australia*
[2]*School of Medicine, Deakin University, Geelong, Australia*
[3]*Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, School of Population Health, Melbourne, Australia*
[4]*Cancer Epidemiology Centre, The Cancer Council of Victoria, Melbourne, Australia*

Aim: A report that angiotensin II subtype 1 (AT_1) receptor antagonists reduce the risk of fracture in hypertensive men and women is not supported by some animal and *in vitro* studies and whether bone mineral density (BMD) affects the association is unknown. We aimed to examine the relationship between use of AT_1 receptor antagonists and the risk of fragility fracture in older hypertensive women accounting for femoral neck BMD.
Methods: Hypertensive women aged ≥50 years at baseline of the Geelong Osteoporosis Study (1993-97) were followed until the end of 2006 (n=434; 2268 person years). Use of AT_1 receptor antagonists (n=77) and incident fragility fractures (n=56) were ascertained prospectively. Associations were modelled using Cox proportional hazards regression. Analysis time was split when individuals commenced using AT_1 receptor antagonists.
Results: Use of AT_1 receptor antagonists was not associated with fracture after adjusting for age, femoral neck BMD T-score, body mass index, other antihypertensive medications, glucocorticoids, hormone replacement therapy, alcohol consumption, falls, activity, smoking and prior fragility fracture (HR: 1.71; 95% CI: 0.63, 4.69). Subgroup analyses showed an increased risk of fragility fracture (HR: 3.37; 95% CI: 1.18, 9.62) for users who had not sustained a fragility fracture prior to their baseline assessment.
Conclusion: Given the wide confidence intervals, this study provides insufficient evidence to conclude that AT_1 receptor antagonists are protective or harmful in hypertensive women. However, the impact of previous fragility fracture on the relationship between use of these medications and subsequent fragility fracture needs to be investigated further.

P129

Timed Up and Go Test predicts falls related hospitalization in older community-dwelling women

Zhu K[1,2], Lewis J[1,2], Radavelli-Bagatini S[1,2] and Prince RL[1,2]
[1]*Department of Endocrinology and Diabetes, Sir Charles Gardiner Hospital.* [2]*School of Medicine and Pharmacology, University of Western Australia.*

Aims: To examine the relationship between Timed Up and Go (TUG) test performance and risk of falls related hospitalization.
Methods: A 10-year longitudinal study with 1500 community-dwelling older women aged 70-85 years. Data collected at baseline include age, anthropometry, hand grip strength, TUG, Barthel activity of daily living (ADL), physical activity, SF-36 Quality of Life and comorbidity. Falls related hospitalization in the 5 years prior to baseline and during the 10-year follow-up was extracted from the WA Hospital Morbidity Database.
Results: 326 participants had at least one falls related hospitalization during follow-up. Compared to non-fallers, fallers were older (76.2±2.9 vs 74.9±2.6 years, P<0.001), had significantly lower hand grip strength, TUG performance, Barthel ADL and SF-36 physical and mental component score at baseline, and significantly more baseline comorbidity and falls related hospitalization in the previous 5 years. In Cox proportional regression analysis, significant predictors of falls related hospitalization include age, height, grip strength, TUG, SF-36 mental component score, comorbidity and falls history. Women in the 3rd (9.5-10.8 seconds) and 4th (≥10.8 seconds) quartiles of TUG performance had 46% (Hazard ratio: 1.46, 95%CI 1.02-2.10) and 49% (Hazard ratio: 1.49, 95%CI 1.06-2.09) higher risk for falls related hospitalization compared to the 1st quartile (<8.2 seconds) after adjustment of covariates. Using a reference cut-off value of 10.2 seconds, the risk is 44% higher in those with TUG performance worse than average.
Conclusion: TUG test is a feasible inexpensive physical performance assessment for screening patients with increased risk of falls that could result in hospitalization.

P130

A histomorphometric survey of breast and prostate cancer induced bone lesions in SCID mice: Breast cancer cells induce osteolytic bone lesions through a reduction in osteoblast activity

Gregory LS, Choi W and Clements JA
[1]*Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology*

Bone metastases are severely debilitating and treatment is often only palliative, warranting improved understanding of the complex mechanisms that lead to bone lesion development. Interestingly, whilst breast-derived bone metastases are characterised by osteolytic lesions, most prostate-derived bone metastases are characterised by mixed or osteoblastic lesions suggesting unique regulatory patterns. This study aimed to measure the changes in bone formation and bone resorption activity at two timepoints during development of the bone lesion within Severely Combined Immuno-Deficient (SCID) mice following intratibial injection of MDA-MB-231 human breast cancer cells (18 and 36 day sacrifice) or LNCaP human prostate cancer cells (36 and 72 day sacrifice) into the left tibiae. Phosphate-buffered saline was injected into the right tibiae as control. Tibiae were extracted, methylmethacrylate embedded and cut into longitudinal sections for comparative analysis using bone histomorphometry. We have provided evidence that the bone loss observed following exposure to MDA-MB-231 cells was due to a significant reduction in mineral apposition rate, rather than increased levels of bone resorption. This suggests that osteoblast activity was impaired in the presence of breast cancer cells, contrary to previous reports of osteoclast-dependent bone loss. Furthermore significant preservation of trabecular bone tissue was observed with age in LNCaP (prostate-cancer) treated animals with an increase in trabecular width with time and higher trabecular number and lower trabecular separation at 72 days compared to controls. These novel insights into the mechanisms through which bone lesions develop may be important in the development of new treatment strategies for metastatic cancer patients.

P131

Chemotherapy with anti-metabolite methotrexate increases expression of neurotrophins in bones of young rats

Zhou FH, Georgiou KR, King TJ, Su Y, Zhou XF and Xian CJ
Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5001, Australia.

Neurotrophins including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neutrophin (NT-3) are produced not only by neurons but by many cell lineages including bone and cartilage, and have capabilities to signal cells to survive, differentiate or grow. In this study we investigated the temporal and spatial expression of neutrophins in tibias of rats treated with methotrexate, an anti-metabolite commonly used in treating cancers and rheumatoid arthritis and known to cause bone defects. Male 6-week-old Sprague Dawley rats received subcutaneous injections of MTX (0.75 mg/kg/day) for 5 consecutive days, and tibia samples were collected on days 6, 9 and 14 (n=6 rats) following the initial dosing for real-time RT-PCR and immuno-histological analysis. We found that while all three neurotrophins were expressed at low levels in bones of untreated rats, NGF and BDNF were more abundant than NT3. Moreover, MTX treatment resulted in gradual and transient up-regulation of NGF, BDNF and NT3 mRNA levels from day 6 to day 9, which coincided with the bone destruction process. By day 14 when tibial bones from the treated rats regained structural integrity, mRNA expression of NGF, BDNF and NT3 also returned to basal levels. In addition, immunoreactivity of NGF, BDNF and NT3 was found in bone forming osteoblasts lining the trabecular bone on day 9. Our findings indicate these neurotrophic factors are expressed by bone cells in metaphysis bone, and may potentially regulate the osteoblasts or other cells in response to MTX bone damage.

P132

DNA methylation does not contribute to Wif1 epigenetic control in osteosarcoma

Baker EK[1], Chalk AM[1], Bhattacharya S[1], Strbenac D[2], Green A[1], Ng AJM[1], Gupte A[1], Kocovski P[1], Martin TJ[3], Purton LE[1], Robinson MD[4] and Walkley CR[1]
[1]*Stem Cell Regulation Unit, St. Vincent's Institute.* [2]*Epigenetics Research Group, Cancer Research Program, Garvan Institute of Medical Research.* [3]*Bone Cell Biology and Disease Unit, St. Vincent's Institute.* [4]*Institute of Molecular Life Sciences, University of Zurich.*

Activation of the Wnt pathway is essential for normal bone development. Therapeutic targeting of the Wnt pathway is of high clinical interest. However inappropriate activation is a hallmark of many cancers. Wif1, an antagonist of Wnt signalling, was recently proposed as a tumour suppressor of osteosarcoma (OS) due to DNA methylation mediated silencing[1].

Here we challenge that Wif1 is a tumour suppressor of OS. Using a mouse model of OS[2], we confirm Wif1 expression is reduced compared to normal osteoblasts. However genome-wide methylation profiling did not identify Wif1 as being differentially methylated and Wif1 expression was not upregulated by the demethylating agent 5-aza-2'-deoxycytidine (5-AzadC). Interestingly, HDAC inhibition by TSA induced Wif1 expression up to 38-fold in OS cell lines, similar to effects seen in normal osteoblastic cell types. Significantly, increased Wif1 expression was observed in OS cells undergoing *in vitro* differentiation to osteoblastic maturation as defined by alizarin red staining and QPCR analyses, similar to that seen in normal pre-osteoblastic cells undergoing maturation. To exclude potential Wif1 regulatory differences between the mouse OS model and human OS, we also treated a panel of human OS cell lines with 5-AzadC and TSA. In contrast to previous findings, Wif1 was not upregulated in response to 5-AzadC. TSA treatment however increased the expression of Wif1 suggesting the mechanism of regulation is HDAC dependent.

Together these results suggest Wif1 is not targeted for epigenetic silencing in OS but instead its reduced expression is in context with the stage in developmental maturation.

1. Kansara et al (2009) J Clin Invest 119: 837-851.
2. Walkley et al (2008) Genes Dev 22: 1662-1676.

P133

Fish oil in comparison to folinic acid for protection against the adverse effects of methotrexate chemotherapy in bones

Raghu Nadhanan R [1], Fan CM [1], Su Y-W [1], Howe PR[2] and Xian CJ[1]
[1]*Sansom Institute for Health Research, School of Pharmacy and Medical Sciences;* [2]*Nutritional Physiology Research Centre, School of Health Sciences, University of South Australia, Adelaide 5001, Australia*

Methotrexate (MTX) is commonly used to treat cancers and rheumatoid arthritis; however it is known to cause bone loss for which there is no antidote. Using a rat model, this study investigated the damaging effects of MTX injections (0.75mg/kg/day) for 5 consecutive days and the potential protective benefits of omega-3 fatty acid-rich fish oil at different doses (0.25, 0.5 or 0.75 mL/100g/day, p.o.) in comparison to folinic acid (0.75mg/kg i.p. 6 hours post MTX). MTX significantly reduced primary spongiosa bone height and metaphyseal trabecular bone volume while reducing density of osteoblasts at the secondary spongiosa. *Ex vivo* differentiation assays with bone marrow stromal cells of MTX treated rats revealed a significant reduction in osteogenic differentiation but an increase in adipogenesis, consistent with gene expression analyses revealing lower expression of osteogenic transcription factors Runx2 and Osx and bone matrix protein osteocalcin but significantly upregulated adipogenesis-related genes FABP4 and PPARγ. MTX increased the density of osteoclasts within the metaphyseal bone and osteoclast precursor cell pool and increased expression of proinflammatory and osteoclastogenic cytokines IL-1, IL-6, TNF-α, and the RANKL/OPG ratio. Fish oil (0.5mL/100g) or folinic acid significantly preserved metaphyseal trabecular bone volume, osteoblast density and bone marrow stromal cell osteogenic differentiation and suppressed MTX-induced adipogenesis, inflammation and osteoclastogenesis. MTX chemotherapy creates an inflammatory condition leading to bone loss by increasing osteoclast and decreasing osteoblast formation. Supplementation with fish oil or folinic acid can counteract these effects, helping to conserve bone formation, suppress bone resorption, marrow adiposity and prevent bone loss during chemotherapy.

P134

Modelling distinct osteosarcoma subtypes *in vivo* using Cre:LoxP & lineage restricted transgenic shRNA

Ng AJM[1], Mutsaers AJ[1], Russell MR[1], Wall M[2], Baker EK[1], Ho P[1], Liddicoat B[1], Slavin J[3], Martin TJ[1], Goriada A[1], Purton LE[1], Dickins RA[4] and Walkley CR[1]
[1]*St. Vincent's Institute, Fitzroy, VIC, Australia;* [2]*Victorian Cancer Cytogenetics Service, St. Vincent's Hospital, Fitzroy, VIC, Australia;* [3]*Department of Pathology, St Vincent's Hospital, Fitzroy, VIC, Australia;* [4]*Molecular Medicine Division, Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia.*

Background: Genetically engineered murine models recapitulate many aspects of human osteosarcoma (OS). We have determined the efficacy of transgenic shRNA to *in vivo* tumour modeling with direct comparison to Cre:loxP approaches.
Methods: Mice transgenically expressing a p53 shRNA (*p53.1224*) were bred with *Osterix*-Cre transgenic mice. This allows the expression of Cre-GFP & *p53.1224* shRNA to be restricted to the osteoblastic-lineage. These mice were crossed with a conditional *Rb* allele ($pRb^{fl/fl}$) that cooperates in tumour development.
Results: *Osx*-Cre p53.1224 $pRb^{fl/fl\ or\ fl/+}$ mice developed OS with near 100% penetrance at a median age of 415 days (n=24). By comparison, *Osx*-Cre $p53^{fl/fl}Rb^{fl/fl\ or\ fl/+}$ mice developed tumours after a median of 177 days (n=49). Both models metastasized to the lungs and the liver. Histology & μCT imaging demonstrated increased mineralization in shRNA OS than the Cre:LoxP OS. Gene expression analysis by qRT-PCR & FACS-based cell surface marker expression were consistent with the shRNA OS being a relatively homogenous mature-osteoblastic phenotype, as compared to a pre-osteoblastic OS generated in the Cre:LoxP model.
Conclusion: The complete loss of *p53* from the Cre:LoxP model resulted in a pre-osteoblastic OS, while the gradual shRNA-directed suppression of *p53* produced a mature OS phenotype. Thus, the improved modeling of distinct OS subtypes allows for a better representation of the clinical spectrum of human OS for therapeutic development.

P136

Potential role of sFRP-1 in the attenuated Wnt/β-catenin signalling, bone loss and bone marrow adiposity following methotrexate chemotherapy in rats

Georgiou KR, King TJ and Xian CJ
School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, Adelaide, SA 5000

The antimetabolite methotrexate (MTX), typically used in the treatment of acute lymphoblastic leukaemia and osteosarcoma, is commonly observed to cause myelosuppression, bone loss and marrow adiposity, for which the underlying mechanisms remain largely unclear. We have recently observed attenuated activation of Wnt/β-catenin signalling in bone of MTX-treated rats, which is associated with decreased osteogenesis and increased adipogenesis of bone marrow stromal cells (Georgiou KR et al 2012 *Bone*). However, the pathobiology for Wnt/β-catenin signalling deregulation is unknown. Known to be secreted by osteoblasts, osteocytes and adipocytes, expression and potential regulatory function of the Wnt antagonist secreted frizzled related protein 1 (sFRP-1) was investigated in rats following 5 daily doses of 0.75mg/kg MTX. sFRP-1 protein expression was found increased in bone marrow supernatant 9 days after the initial MTX dose when compared to control, 6 and 14 days after treatment. Consistently, sFRP-1 mRNA expression in whole metaphyseal bone was significantly elevated on days 6 and 9 when compared to control and day 14. In an effort to define the cell types responsible for the increased sFRP-1, sFRP-1 immunohistochemistry found osteoblasts at the endosteum on days 6 and 9 to be strongly stained, when compared the control. Day 9 bone marrow adipocytes were also positively stained. These preliminary findings suggest increased sFRP-1 expression is associated with the attenuated Wnt/β-catenin signalling following MTX chemotherapy. However whether it is responsible for the bone-fat switch and the bone and bone marrow defects following MTX is currently being investigated.

P137

Trolox inhibits osteolytic bone metastasis of breast cancer due to an anti-osteoclastic activity

Lee J-H and Lee ZH
Department of Cell and Developmental Biology, School of Dentistry, Seoul National University, 28 Yeongon-dong, Jongno-gu, Seoul 110-749, Republic of Korea.

Breast cancer frequently metastasizes to bone, in which tumor cells initiate a vicious cycle of bone destruction. Tumor expressed PTHrP participates in vicious cycle acting on osteoblasts, leading to increased production of an indispensable osteoclast stimulator RANKL. Activation of osteoclasts is critical for the formation of osteolytic lesions by breast cancer metastasis. Therefore, the prevention of osteolytic bone metastasis is clinically important. We previously reported that Trolox, a water-soluble vitamin E analogue, had an antiosteoclastic activity in pathological bone destruction. In this study, we investigated the effect of Trolox against breast cancer-mediated osteolytic bone metastasis in a mouse model. We found that Trolox potently inhibited PTHrP-induced osteoclast formation in bone marrow cell-osteoblast coculture by suppressing RANKL induction in osteoblasts. Pretreatment with Trolox did not affect PTHrP-induced early signaling pathways, including ERK, P38, CREB and PKC, required for RANKL production. This RANKL reduction was attributed to the reduced production of prostaglandin E2. Trolox also suppressed breast cancer conditioned medium-induced RANKL expression in osteoblasts. When we examined its ability to prevent breast cancer-induced bone loss in animals, Trolox (15 mg/kg of body weight) administered through the intraperitoneal route significantly decreased osteolytic lesion and inhibited weight reduction in tumor bearing mice. Overall, our results indicate that Trolox is a potent inhibitor of osteoclastogenesis and osteolytic metastasis induced by breast cancer.

P138

Attenuated megalin expression in hyperfunctioning parathyroid glands – possible role of vitamin D signaling

Nagata Y[1], Imanishi Y[1], Yamagata M[1,2], Kobayashi I[1], Ishii A[1], Michigami T[3], Yukimura T[2], Kato S[5], Arnold A[4] and Inaba M[1]
[1]*Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine.*
[2]*Faculty of Pharmacy, Osaka Ohtani University.*
[3]*Department of Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health.*
[4]*Center for Molecular Medicine, University of Connecticut Health Center*
[5]*Institute of Molecular and Cellular Biosciences, University of Tokyo.*

Megalin is a multiligand endocytotic receptor involving in the reabsorption of 25-hydroxyvitamin D (25OHD) and vitamin D binding protein (DBP) in renal proximal tubules. Megalin expression decreased as well as vitamin D receptor (VDR) in hyperfunctioning parathyroid tumors observed in patients with primary hyperparathyroidism (PHPT) and secondary hyperparathyroidism of uremia (SHPT) (ANZBMS 2011). Attenuated expressions of megalin will reduce 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) production from 25OHD in parathyroid tumors, resulted in enhanced secretion of parathyroid hormone (PTH). The aim of our study is to determine the role of vitamin D signaling in parathyroid megalin expressions.
72 week-old PC mice, in which parathyroid-targeted overexpression of the cyclin D1 oncogene causes PHPT, and 12 week-old conventional VDR KO mice were used in this study.
The PC mice exhibited biochemical hyperparathyroidism such as hypercalcemia (PC 12.4 ± 2.3 vs WT 9.1 ± 0.2 mg/dl, $P< 0.05$) and elevated PTH levels (PC 1217 ± 1195 vs WT 102 ± 42 pg/ml, $P< 0.05$). The megalin expressions were reduced in PC mice parathyroid glands as well as VDR. The VDR KO mice exhibited hypocalcemia (5.8 mg/dl) and an elevated PTH level (3370 pg/ml). The parathyroid megalin expressions in VDR KO mice were also diminished.
In conclusion, the decreased megalin expression in hyperfunctioning parathyroid tumors were replicated *in vivo* as well as human PHPT and SHPT. Attenuated vitamin D signaling via VDR may cause hypo-expression of megalin in hyperfunctioning parathyroid tumors.

P139

Characterization of a Murine Model of Tamoxifen-Inducible Cartilage-Specific Glucocorticoid Receptor Knockout

Tu JW[1], Stoner S[1], Zhang YQ[1], Kelly J[1], Chen D[2], Tuckermann J[3], Seibel MJ[1,4] and Zhou H[1]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney,*
[2]*Tissue Department of Biochemistry, Rush University Medical Center, USA*
[3]*Tissue specific Hormone Action, Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany,*
[4]*Dept of Endocrinology & Metabolism, Concord Hospital, Sydney, Australia*

The mechanisms by which glucocorticoids (GCs) exert their receptor-mediated effects on cartilage and bone cells are poorly understood. We aimed to elucidate the role of the glucocorticoid receptor (GR) in chondrocytes, and its interaction with bone, through characterisation of a cartilage-specific GRKO mouse line.
$GR^{flox/flox}$ mice were crossed with a transgenic mouse model (Col2a1-$CreER^{T2}$) to generate tamoxifen-inducible Col2-GRKO mice, in which the cre recombinase is expressed under the control of type II collagen (Col2) promoter. GRKO was induced at 2, 4, and 10 weeks of age by daily i.p. injection of tamoxifen (1mg/10g BW/day) for 5 days. Mice were then monitored for 8 weeks for body weight and long bone growth. Bones were analysed by histology and micro-CT at end point.
Body weight and bone length were similar in GRKO and WT mice in all three age groups. Histology of knee joints, growth plates and intervertebral discs showed normal cartilage structure in both GRKO and WT mice. However, compared with WT littermates, male GRKO mice induced at 10 weeks of age displayed significantly increased BV/TV (19.6% vs. 13.9%) and Tb.N (3.2 1/U vs. 2.6 1/U) 8 weeks post induction. These differences had resolved at 24 weeks. Female mice induced at 10 weeks of age, or GRKO mice induced at 2 or 4 weeks displayed no changes in bone mass.
These results indicate that cartilage-specific GRKO has no apparent effects on cartilage in postnatal and adult mice. However, tamoxifen may affect bone mass in mature male mice.

P140

Fracture healing is delayed in mice with tamoxifen-induced chondrocytic glucocorticoid receptor knockout

Tu JW[1], Zhang YQ[1], Kelly J[1], Dunstan CR[2], Chen D[3], Tuckermann J[4], Seibel MJ[1,5] and Zhou H[1]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney,*
[2]*Department of Biomedical Engineering, University of Sydney*
[3]*Tissue Department of Biochemistry, Rush University Medical Center, USA*
[4]*Tissue specific Hormone Action, Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany,*
[5]*Dept of Endocrinology & Metabolism, Concord Hospital, Sydney, Australia*

Background: Although long-term use of glucocorticoids (GC) increases the risk of fracture and hinders fracture repair, the mechanisms by which endogenous GC exert their receptor-mediated effects on fracture healing are poorly understood. In this study, we used a tibial fracture model in tamoxifen-inducible, cartilage-specific glucocorticoid receptor knockout (GRKO) mice to elucidate the role of endogenous GC in fracture healing.
Methods: $GR^{flox/flox}$ mice were crossed with Col2a1-$CreER^{T2}$ mice to generate the tamoxifen-inducible Col2-GRKO mice, in which the cre recombinase is expressed under the control of the type II collagen (Col2) promoter. An open mid-diaphyseal tibial fracture was generated in 10-week-old male mice and fixed by needle insertion. GRKO was induced by tamoxifen (1mg/10g BW/day) on day 1, 3 and 5 post-fracture. Healing was monitored by weekly X-rays and by histology and micro-CT at end point.
Results: Compared to WT mice, cartilage callus volume (CV) was significantly increased in GRKO mice on day 7 post-fracture ($p=0.03$). After 14 days, CV declined in both WT and GRKO mice but remained higher in GRKO animals ($p=0.06$), suggesting a delay in bone remodelling. Micro-CT of the previous fracture area, obtained on day 28 post-fracture, revealed significantly increased BV/TV in WT compared to GRKO mice ($p=0.02$).
In summary, our results indicate that cartilage-specific GRKO results in impaired fracture healing, pointing to a role of GCs during endochondral bone formation and fracture repair.

P141

Glucocorticoids regulate mineralised nodule formation in a biphasic, dose-dependent manner

Zhang YQ[1], Stoner S[1], Tu JW[1], Dunstan CR[1,2], Tuckermann J[3], Seibel MJ[1,4] and Zhou H[1]
[1]*Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, Australia*
[2]*Department of Biomedical Engineering, University of Sydney, Sydney, Australia*
[3]*Tissue specific Hormone Action, Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, Germany*
[4]*Dept of Endocrinology & Metabolism, Concord Hospital, Sydney, Australia*

The mechanisms underlying the anabolic and catabolic effects of glucocorticoids on bone are poorly understood. We investigated the effects of endogenous glucocorticoids at different circulating levels on osteoblast differentiation using ex-vivo calvarial osteoblast cultures derived from mice with a deletion of the pituitary/ hypothalamic glucocorticoid receptor (GR).
GRflox/flox mice were crossed with Fabp4-Cre mice to generate $GR^{Fabp4Cre}$ mice, with expression of cre recombinase controlled by the mouse fatty acid binding protein 4 (Fabp4) promoter. Cre was detected in mature adipocytes, and at high levels in the pituitary and hypothalamus. GR deletion in the latter tissues causes progressively increasing corticosterone levels in postnatal mice through lack of negative feed-back. We took advantage of this feature to determine the capacity of calvarial cells to differentiate into functional osteoblasts by generating primary osteoblast cultures from 1, 3 and 10-day-old $GR^{Fabp4Cre}$ mice. Cre-negative GRflox/flox (WT) littermates served as controls.
Serum corticosterone levels increased in $GR^{Fabp4Cre}$ mice from 168nmol/L on d1 to 1,127nmol/L on d10. In WT mice, corticosterone levels plateaued at~30nmol/L. Cultures derived from 1-day-old $GR^{Fabp4Cre}$ mice demonstrated a 54% increase in mineralized nodule formation, compared to WT ($p<0.01$). Cultures derived from 3-day-old $GR^{Fabp4Cre}$ mice were similar to those of WT controls ($p=0.874$). In contrast, nodule formation was reduced by 42% in cultures derived from 10-day-old $GR^{Fabp4Cre}$ mice ($p< 0.01$ compared to WT).
We conclude that glucocorticoids have dose-dependent, biphasic effects on calvarial cell differentiation: Moderately increased glucocorticoid concentrations stimulate, while excessive levels suppress calvarial cell differentiation into functional osteoblasts.

P142

Histochemical examination on osteocytes and their lacunae after administration of parathyroid hormone in mice

Hongo H[1], Sasaki M[1], Yamada T[1], Hasegawa T[1], Yamamoto T[1], Nakano T[3], Shimoji S[2] and Amizuka N[1]
Departments of [1]Developmental Biology of Hard Tissue, [2]Periodontology and Endodontology, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Japan
[3]*Course of Materials Science & Engineering, Division of Materials & Manufacturing Science, Graduate School of Engineering, Osaka University, Suita, Japan*

The idea of “osteocytic osteolysis”, which is respondent to elevated concentration of parathyroid hormone (PTH) or low calcium diet, was proposed by Bélanger in 1960’s. Regularly-arranged osteocytic lacunae-canalicular system (OLCS) in cortical bone was shown to be functionally efficient, *e.g.,* for sensing mechanical stress, when compared to the irregularly-distributed OLCS in metaphysis. In order to clarify the occurrence of osteocytic osteolysis, we have examined osteocytes and their lacunae after the PTH administration in the tibial metaphysis and cortical bone of mice.
At six hours after the PTH administration, enlarged osteocytic lacunae were observed mainly in the cortical bone, but not in the metaphysis. Transmission electron microscopy and von Kossa staining demonstrated broadly demineralized bone matrix surrounding osteocytes in the cortical bone. Intense immunoreactivity of vacuolar type H-ATPase was found in many osteocytes in the PTH-treated cortical bone, indicating that proton secreted by osteocytes might erode the surrounding bone minerals. In addition, immunopositivity of fibroblast growth factor 23, a regulator for serum phosphate concentration by mediating kidney, was intense in osteocytes of the PTH-treated cortical bone. Unlikely, the control bone without PTH administration did not reveal such histological alteration of osteocytic lacunae.
Taken together, it seems likely that osteocytes in cortical bone, rather in metaphysis, would participate, at least, in part, in controlling serum concentration of calcium and phosphate by responding to PTH, *i.e.,* osteocytic osteolysis in bone.

P143

Over-expression of osteoblastic Vitamin D Receptor (VDR) levels in a mouse model mediates anabolic or anti-anabolic activity which is associated with dietary calcium-mediated changes to circulating 1,25D levels

Triliana R[1], Lam NN[2], OLoughlin PD[1], Morris HA[1,2,3] and Anderson PH[2,3]
[1]School of Medicine and [2]School of Medical Sciences, Faculty of Health Sciences, University of Adelaide, Adelaide, SA, Australia, 5000;[3]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia, 5000

The mechanisms by which osteoblastic vitamin D receptor (VDR) can mediate both positive and negative effects on bone structure are currently unclear. 1,25D can stimulate RANKL-mediated catabolic processes, and also can directly inhibit or stimulate bone formation under varying conditions. While the overexpression of VDR in osteoblasts *in vivo* (OSVDR Tg mouse) demonstrates increased bone volume, increased BFR and reduced osteoclast activity with adequate dietary calcium, whether these mice exhibit enhanced catabolic or anti-anabolic activities during calcium depletion is less clear. Female (6w) OSVDR and WT mice were fed either a 1% Ca diet or a low 0.1% Ca diet for 4 months. At time of death, mean serum 1,25D levels in OSVDR mice fed 0.1% Ca were 168 (+/-25) pmol/L, 1.8-fold higher levels than OSVDR mice fed 1% Ca diet. OSVDR mice fed 1% Ca exhibited increased tibial metaphyseal BV/TV (up 12%) and cortical bone volume (up 15%) compared to WT levels ($P<0.001$). However, the BV/TV and cortical bone volume phenotype in OSVDR mice fed 0.1% Ca, was absent and comparable to WT mice fed 0.1% Ca. The reduced bone volume in OSVDR mice fed 0.1% Ca occurred without an increase in osteoclastogenesis. However, BFR and mRNA for Runx2, ALP, Col1, and osteocalcin were markedly lower in OSVDR mice fed 0.1% Ca compared to all other groups ($P<0.05$). Thus, increased VDR levels in osteoblasts mediate anti-anabolic activities but not increased catabolic activity which is associated with a low dietary calcium induced increase in serum 1,25D levels.

P144

Phosphate sensing in osteocytes: Extracellular phosphate induces FGF23 expression in IDG-SW3 osteocyte like cells

Ito N[1], Findlay DM[1], Ormsby R[1], Anderson PH[2], Bonewald LF[3] and Atkins GJ[1]
[1]Discipline of Orthopaedics & Trauma, University of Adelaide, Adelaide, Australia. [2]School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia. [3]Dental School, University of Missouri Kansas City, MO, USA.

FGF23 is a osteoblast/osteocyte-derived hormone that acts on the kidney to regulate Pi homeostasis by down-regulating NaPi-IIa, IIc and CYP27b1. Several reports indicate that Pi and 1,25D stimulate the production of FGF23 *in vivo* and also *in vitro* (1). This indicates that osteoblasts and/or osteocytes can sense Pi, although the mechanism has not been elucidated. In this study, the mouse osteocyte-like cell line, IDG-SW3 was differentiated for 35d, giving rise to a mature osteocyte-like phenotype including FGF23 expression (2). The expression of several bone-related genes was then evaluated in response to extracellular Pi (1, 4, 10mM), in the presence or absence of 1,25D (10nM). Pi increased the expression of FGF23 mRNA up to 40-fold, as well as genes associated with bone formation and mineralisation, including OCN, SOST, PHEX, DMP1 and MEPE, and key genes related to bone resorption, OPG and RANKL. 1,25D up-regulated the expression of OCN and RANKL, and down-regulated OPG, SOST, PHEX, DMP1, MEPE. Additionally, 1,25D decreased CYP27b1 mRNA levels in the presence of Pi at 10nM. Interestingly, the prominent induction of CYP24 mRNA by 1,25D was attenuated by high levels of Pi. These findings provide strong evidence that osteocytes can sense the extracellular concentration of Pi. Our results indicate that a variety of osteoblast-osteocyte specific genes are regulated in response to Pi. They also demonstrate the interaction between 1,25D and Pi acting at the level of the osteocyte.

1. Yamamoto et al. 2010 J Endocrinol 206:279-286.
2. Woo et al. 2011 J Bone Miner Res 26:2634-2646.

P145

Regional up-regulation of 25-hydroxyvitamin D 1alpha-hydroxylase (CYP27B1) gene is associated with the pathogenesis of ectopic calcification in the alpha klotho mutant mice

Yamamoto H, Otani A, Yokoyama N, Onishi R, Takei Y, Taketani Y and Takeda E
Department of Clinical Nutrition, Institute of Health Biosciences, University of Tokushima.

Alpha klotho plays an essential role in the signaling pathway of fibroblast growth factor (FGF) 23, which is a critical regulator of the phosphate and vitamin D metabolism in kidney. Alpha klotho mutant (*kl/kl*) mice have hyperphosphatemia, hypercalcemia and hypervitaminosis D, and develop ectopic calcification, together with short lifespan. It has been reported that these disorders are caused by over-production of 1,25-dihydroxyvitamin D through the renal up-regulation of 25-hydroxyvitamin D 1a-hydroxylase (CYP27B1) gene. In the present study, we investigated the relationship between the expression of CYP27B1 and the pathogenesis of ectopic calcification in *kl/kl* mice. Real-time PCR analysis confirmed that renal cortex CYP27B1 mRNA expression was up-regulated in *kl/kl* mice compared with wild type mice. Surprisingly, immunohistochemical analysis and von Kossa staining revealed that the regional high-expressed CYP27B1 in kidney cortex from 3-week-old mice without ectopic calcification, in other hand, in 6-week-old mice, its localization merged with calcifying renal arterioles and tubular cells. Importantly, we found that the CYP27B1 was also co-localized with the calcified lesions of heart and aorta in *kl/kl* mice. In addition, its co-localization was also detected in the heart of renal failure rats. Furthermore, in vitro analysis using rat aortic vascular smooth muscle (A-10) cells, inorganic phosphate (Pi) loading did not increase the CYP27B1 mRNA levels, however, promoted its protein accumulation in mitochondria. These results suggest that the regional up-regulation of CYP27B1 expression by abnormality of FGF23-klotho signaling is implicated with the pathogenesis of ectopic calcification.

P146

An *in vivo* model mimicking human subchondral bone and osteocyte pathophysiological changes in osteoarthritis

Jaiprakash A[1], Wille M-L[1], Chakravorty N[1], Crawford R[1, 2], Feng JQ[3] and Xiao.Y[1]
[1]*Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia*
[2]*Prince Charles Hospital, Brisbane, Queensland, Australia*
[3]*Texas A&M Health Science Center, Baylor College of Dentistry, Texas, USA*

Subchondral bone sclerosis is recognized as a clinical sign of osteoarthritis (OA). Although osteocytes play a central role in regulating bone remodelling, their involvement in OA pathophysiology is poorly defined. Our recent studies suggest that dysregulated osteocytic proteins contribute to the pathological changes in subchondral bone of knee OA patients. The aim of this study was to establish a suitable animal model, which resembles the clinical situation and allows the investigation of progressive osteocyte changes in OA pathophysiology.
We induced OA in 12 week old Wistar Kyoto rats by: (I) removal of the medial meniscus (MSX, n=12), (II) injection of Mono-idoacetate (MIA, n=6) and (III) transecting the anterior cruciate ligament (ACLT, n=6). We investigated changes in subchondral bone and osteocytes 8 weeks post-surgery by microCT and histology and compared it to SHAM controls and human knee subchondral bone samples.
Based on microCT data we excluded the MIA and ACLT group, since they showed a decreased bone volume compared to the controls. MSX showed high subchondral bone volume like the human situation (Fig.1). Further histological analysis of the MSX group demonstrated higher numbers of osteocytes expressing DMP1, E11, apoptosis, and TRAP compared to SHAM controls, whereas a decreased number of SOST expressing osteocytes were found.
This indicates a potential regulatory role of osteocytes in subchondral bone remodeling and mineral metabolism during OA pathogenesis. OA MSX experimental rat model resembles the human situation more closely and is suitable to study OA subchondral bone pathogenesis.

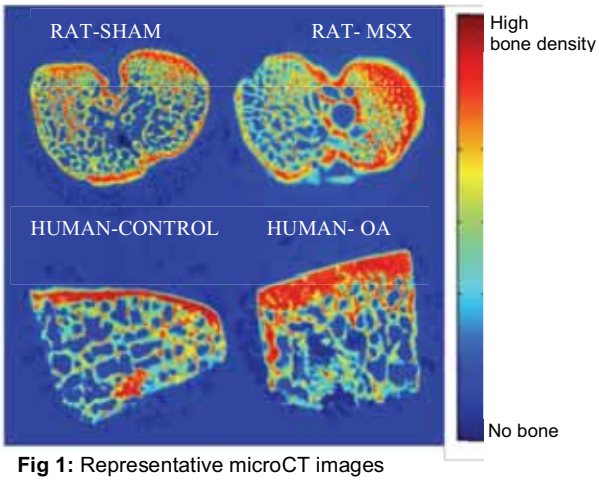


Fig 1: Representative microCT images

P147

Computational and experimental characterization of drug release kinetics in trabecular bone from titania nanotube implants

Kaiser J[1], Buenzli PR[1], Sinn AW M[2], Khalid KA[3,4], Gulati K[2], Atkins GJ[3], Pivonka P[1], Findlay DM[3] and Losic D[2]
[1]*Engineering Computational Biology Group, University of Western Australia, Perth, WA 6009, Australia.*
[2]*School of Chemical Engineering, University of Adelaide, Adelaide, SA 5005, Australia.*
[3]*Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA 5005, Australia.*
[4]*Department of Orthopaedics, Traumatology & Rehabilitation, Faculty of Medicine, International Islamic University Malaysia, Kuantan, Pahang 25200, Malaysia.*

Local drug delivery using drug-releasing implants located inside bone is recognised as a promising strategy to address the limitations of systemic drug administration to bone. However, the studies of drug-release kinetics are not possible to perform using existing *in-vitro* drug releasing systems and 2-D bone cell models. The aim of this work is to demonstrate the use of a 3-D bone bioreactor for studying the drug-release kinetics and distribution of drugs in the *ex-vivo* cancellous bone environment. Bovine trabecular bone was used as the bone substrate, in which drug-releasing implants were embedded in the form of nano-engineered titanium wires covered with a layer of titania nanotube (TNT) arrays. A hydrophilic fluorescent dye (rhodamine B) was used as a model drug, loaded inside the TNT/Ti implants to monitor drug release and transport in trabecular bone under *ex-vivo* conditions. In order to better understand how the bioreactor perfusion rate and the local drug release from the titanium wire affect overall drug distribution in the bone sample we utilize 3D finite element modelling. This model is based on porous media theory and takes into account advective-diffusive transport of the drug through the micropores of bone. The results showed a consistent, gradual release of model drug from the TNT/Ti implants, with a characteristic three-dimensional distribution into the surrounding bone over a period of 5 days. These results demonstrate the utility of this system for *ex-vivo* drug release studies in bone, which can be applied to optimise the administration therapeutics in bone for specific therapies and design of new drug delivery systems.

P148

Effects of systemic administration of phenylephrine on healing of rat rib fractures

McDonald SJ, Schuijers JA, Ward AR and Grills BL
Department of Human Biosciences, La Trobe University, Victoria, Australia.

Previous work from our laboratory has shown that early rib fracture callus contains a substantial population of cells with a myofibroblastic phenotype. This early callus displays smooth muscle-like properties *ex vivo* including the phenomenon of active contraction instigated by phenylephrine (PE). These findings led to the hypothesis that administration of contractile agonists such as PE may potentiate callus contraction and thereby accelerate fracture healing.
Thirteen week-old male rats had their sixth rib fractured under anaesthesia. Five days post-surgery, rats were implanted with a 200 µl mini-osmotic pump that delivered either saline or PE (200 µg/kg/h) for 7 days. Fractures from saline and PE-treated mice were analysed (42 days post-fracture) for their biomechanical properties using a 3-point bending apparatus. Expression of genes associated with osteogenesis and chondrogenesis were compared between saline and PE-treated fractures at 14 days post-fracture. Serum osteocalcin levels were compared between saline and PE-treated mice at 14 and 21 days post-fracture.
Systemic administration of PE did not induce significant changes to the mean values for callus peak force, stiffness, toughness, cross-sectional area, ultimate tensile stress and Young's modulus. Callus expression of most genes associated with osteogenesis and chondrogenesis were not altered by PE treatment, however mRNA expression of type II collagen was however significantly lower in PE-treated rats (2-fold decrease, $P < 0.05$). Serum concentrations of osteocalcin at 14 and 21 days post-fracture were not altered by PE treatment.
This study found that systemic treatment with PE had no dramatic effects on healing of rat rib fractures. It is possible however that either by topically administering PE or by delivering an alternate agonist that induces myofibroblastic contraction, could both be more likely to induce sustained, high tensile conditions that are known to promote osteogenesis. Future work will investigate such alternate treatment modalities.

P149

HydroxyColl as a multi-drug delivery system for bone tissue engineering

Murphy CM[1, 3], Yu NYC[1], Mikulec K[1], Peacock L[1], Aiken A[1], Schindeler A[1,2], O'Brien FJ[3] and Little DG[1,2]
[1] *Orthopaedic Research & Biotechnology, Westmead Children's Hospital.*
[2] *Paediatrics and Child Health, Faculty of Medicine, University of Sydney.*
[3] *Department of Anatomy, Royal College of Surgeons in Ireland.*

Recombinant human Bone Morphogenetic Proteins (rhBMPs) and bisphosphonates (BPs) have shown synergy in the induction of new bone. However, it is generally considered that delivery systems currently used for rhBMP-2 are sub-optimal. Combining orthopaedic concepts with emerging technologies in tissue engineering scaffolds, we utilised a recently developed novel collagen-hydroxyapatite scaffold (HydroxyColl) as a delivery system for rhBMP-2 and the bisphosphonate Zoledronic Acid (ZA). In this study we describe *in vivo* testing of HydroxyColl in an ectopic bone model.
6 x 3 mm scaffold blocks loaded with rhBMP-2 or ZA were implanted in rat hind limb muscle pouches. Six experimental groups were examined: (1) saline alone, (2) 5µg rhBMP-2, (3) 5µg rhBMP-2 + 10µg ZA, (4) 10µg rhBMP-2, (5) 10µg rhBMP-2 + 2µg ZA, (6) 10µg rhBMP-2 + 10µg ZA (N=4 rats per group). Rats were sacrificed at 4 weeks for microCT analysis.
Bone volume (BV, mm^3 Fig 1) measurements showed bone formation in all groups including saline alone, indicating *de novo* osteoconductivity of HydroxyColl. Consistent with bisphosphonates preserving rhBMP-2 induced bone, ZA increased BV by 5-6 fold in all treatment groups compared to rhBMP-2 alone.
This study supports further work using HydroxyColl as a method for rhBMP-2/ZA co-delivery and future experiments plan to test this combination in a rat critical defect model using 5 µg BMP-2 and 10 µg ZA.

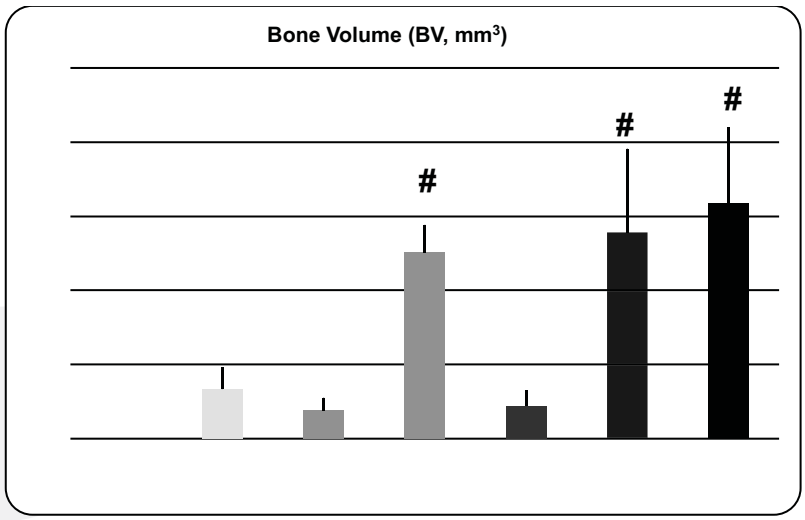


Figure 1: MicroCT quantification of BV showing significant increases with ZA co-delivery (#P <0.05).

P150

Local modulation of anabolic and catabolic responses in a rat critical-sized bone defect model

Yu NYC[1], Gdalevitch M[1], Schindeler A[1,2], Mikulec K[1], Peacock L[1], Fitzpatrick J[3], Cooper-White JC[3] and Little DG[1,2]
[1] *Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead.*
[2] *Paediatrics and Child Health, Faculty of Medicine, University of Sydney.*
[3] *Australian Institute for Nanotechnology and Bioengineering, University of Queensland.*

Bone tissue engineering approaches to heal critical defects typically focus on increasing bone anabolism, but we have hypothesized that concomitant suppression of resorption will yield superior outcomes. In this study we have built on prior work to test local co-delivery of recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) with the bisphosphonate zoledronic acid (ZA).

Our approach was to impregnate drugs in a highly porous poly(lactic-co-glycolic acid) (PGLA) scaffolds manufactured by thermally induced phase separation. All groups were loaded with 20μg rhBMP-2, test groups also contained 5μg ZA and 5μg ZA pre-adsorbed to hydroxyapatite (ZA+HAp). A control scaffold of Medronic porous collagen was also used. N=20 rats/scaffold group.

3mm diameter x 5 mm height cylindrical scaffolds were inserted into 6mm critical-sized femoral defects made in Wistar rats. Animals were sacrificed at 6 weeks. MicroCT analysis was used to quantify callus 3D bone volume (BV, mm^3, **Figure 1**) and generate 3D reconstructions. Addition of ZA+HAp increased BV by 73% ($p<0.01$) rhBMP-2 alone, and this was also superior to rhBMP-2 in the control collagen scaffold (30%, P=0.03). For all TIPS scaffolds, a suboptimal bone-scaffold interface was seen, suggesting an overlapping design for implants may yield superior results.

In summary, co-delivery of rhBMP-2 + ZA + HAp via a porous PLGA scaffold showed potential as an alternative bone graft material with superior bone healing over rhBMP-2 via commercial collagen, but further optimization may lead to improved repair strength in future experiments with mechanical outcomes.

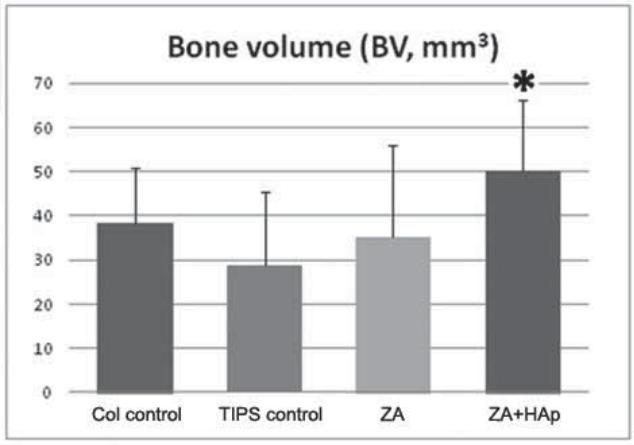


Figure 1. MicroCT quantification of bone volume showed significant increases with rhBMP2+ZA+HAp (BV, mm^3) (*=$p<0.01$ vs. TIPS PGLA carrier/rhBMP-2 control and p=0.03 vs. Collagen carrier/rhBMP-2 control)

P151

MEK inhibition modulates normal fracture healing

El-Hoss J[1,2], Kolind M[1], Mikulec K[1], McDonald M[1], Schindeler A[1,2] and Little DG[1,2]
[1] *Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead.*
[2] *Paediatrics and Child Health, Faculty of Medicine, University of Sydney.*

The Ras-MAPK pathway is an important mediator of cell proliferation and differentiation. Prior studies have highlighted an important role for Ras-MAPK signalling in osteoblast and chondrocyte cell biology, but its role in bone repair remains unclear.

To elucidate the role of Ras-MAPK in bone healing, closed tibial fractures were made in 10 week old C57/Bl6 mice. Mice were treated with PD0325901, a highly-specific third generation MEK inhibitor. Taking surgery as day 0, PD0325901 was dosed 10mg/kg/d during early stage repair (day -2 to day 10), late stage repair (day 11 to day 21), or throughout repair (days -2 to day 21). A no treatment group was also included.

PD0325901 was found to affect bone and cartilage both in the growth plate and the fracture callus. PD0325901 significantly increased the growth plate hypertrophic zone, but this was reversible after cessation of treatment. Histomorphometry showed treatment significantly increased fracture callus cartilage by 53% at day 10 post fracture (55% vs. 84% cart/TV), and continued dosing led to cartilage retention at day 21 (0% vs 10% cart/TV). When PD0325901 treatment ceased after day 10, cartilage was removed by day 21. Conversely, commencement of PD0325901 at day 11 prevented complete cartilage removal. PD0325901 treatment also led to a 25% increase in callus BV/TV versus vehicle at day 21.

These data support Ras-MAPK as a negative regulator of chondrogenesis and a positive regulator of endochondral ossification and cartilage removal. These experiments also demonstrate that modulation using PD0325901 can differentially impact each stage of fracture repair.

P152

Reduced radiation sterilization dose of bone allografts improves surgical outcomes while retaining sterility assurance levels

Nguyen[1,2], Morgan DAF[2,3], Gineyts E[4] and Forwood MR[1]
[1] *School of Medical Science and Griffith Health Institute, Griffith University, Gold Coast, Australia*
[2] *Queensland Bone Bank, Organ and Tissue Donation Service, Division of Chief Health Officer, Queensland Health, Brisbane, QLD, Australia*
[3.] *Brisbane Private Hospital, Brisbane, Australia*
4. INSERM U1033, Faculte de Medecine Laennec, University of Lyon, France

Our objective was to determine an optimal radiation sterilization dose that achieves sterility assurance while minimizing deleterious effects on allograft bone quality. For sterility testing, we inoculated allograft bone with *S. epidermidis* and *B. pumilus*, then exposed them to gamma irradiation at 0, 5, 10, 15, 20 and 25 kGy. Mechanical and biological properties of cortical allografts and morsellised bone were determined following irradiation at these doses. A dose of 20-25 kGy eliminated both inoculated organisms at concentrations from 10^1-10^3, while 10-15 kGy sterilized bone samples to a bioburden concentration of 10^2. Irradiation did not generate pro-inflammatory bone surfaces, as evidenced by macrophage activation, nor did it affect attachment or proliferation of osteoblasts. At a dose of 15 kGy or greater, there was a significant decline in the energy absorption capacity of cortical and morsellised bone ($p < 0.05$); and the attachment and fusion of osteoclastic cells onto irradiated bone ($p < 0.05$). We observed no significant change in the content of bone collagen cross links, but there was a dose-response increase in denatured collagen in irradiated bones ($p < 0.05$). We conclude that 15 kGy is a threshold for radiation sterilization of bone allografts that provides an acceptable sterility assurance level, and above which allograft strength and biocompatibility declines significantly.

P153

Upregulation of MCP-1 gene expression following stress fracture initiation, *in vivo*, is blocked by the dominant negative mutant, 7ND

Wu AC, Kelly WL, Morrison NA and Forwood MR
School of Medical Science and Griffith Health Institute, Griffith University, Gold Coast, Australia

Monocyte Chemotactic Protein 1 (MCP-1) belongs to the CC chemokine superfamily and plays a critical role in the recruitment and activation of immune cell precursors. We observed that within 4 hours of stress fracture (SFx) initiation, MCP-1 gene expression was significantly elevated, followed by increased serum levels within 24 h. Specific inhibition of MCP-1 would determine the significance of MCP-1 expression to bone cell recruitment for remodelling. We hypothesise that treatment of rats with a plasmid DNA encoding a dominant negative mutant of MCP-1 (7ND) will inhibit the increase in gene expression associated with SFx repair. SFx was created in the right ulna of mature wistar rats using cyclic end-loading. Unloaded animals were used as a control. 24 h prior to loading, 7ND plasmid vector was injected in the thigh muscle to overexpress 7ND protein, which was then secreted into systemic circulation. Rats were euthanized 4h after loading (n=5/group) and RNA extracted and converted to cDNA for quantitative real time PCR analysis using TaqMan gene expression assays. In untreated rats, there was ~33 fold increase ($P<0.001$) in MCP-1 expression 4h after loading. Treatment with 7ND abolished the loading related increase in MCP-1, with gene expression levels lower than un-loaded control rats. We hypothesise that activation of the remodelling phase of SFx repair will be inhibited following this suppression of MCP-1. Because MCP-1 is markedly upregulated by SFx and by PTH, we propose that it provides important regulation of chemotaxis and osteoclast differentiation during initiation events of bone remodelling.

P154

Bone and muscle interaction in young individuals with Cystic Fibrosis (CF)

Brookes DSK[1], Briody JN[2], Hill RJ[1], Munns C[2] and Davies PSW[1]
[1] *The University of Queensland, Children's Nutrition Research Centre, Australia;*
[2] *The Children's Hospital at Westmead, Sydney, Australia.*

Aims: (1) Apply a 4-step algorithm to DXA data, and (2) assess bone health using pQCT, in individuals with CF.
Methods: Fifty-three individuals with CF were compared against 53 controls following stratification by sex and Tanner stage (TS), (TS1: CF=16(9F), controls=18(11F); TS2-5: CF=37(25F), controls=35(23F)). DXA (Prodigy) acquired total BMC and LTM. Age and height Z-scores were calculated using a paediatric control database [(1)] and applied in the algorithm to determine the origin of low BMC for age. pQCT (XCT3000) acquired BMC, vBMD and bCSA at distal (4%) and shaft (66%) sites of the tibia and radius. Additionally, estimated bone strength indices and mCSA were measured.
Results: DXA data showed pubertal females with CF had: less BMC for age (p=0.02), were shorter (p=0.03), and less BMC for LTM (p=0.01). pQCT data showed, pre-puberty bone structural parameters were not different between groups. Pubertal females with CF had less BMC at 4% (p=0.01) and 66% (p=0.05) tibia, and smaller bCSA at 4% (p=0.00) and 66% (p=0.01) tibia. Pubertal males (p=0.02) and females (p=0.01) with CF had smaller mCSA at tibial shaft. Bone strength and mCSA were progressively lower with age, and smaller mCSA predicted lowered bone strength in the CF cohort, even when size adjusted.
Conclusion: Pubertal females with CF appeared to have a primary bone defect. The bone phenotype, via pQCT data, changed during puberty for CF. In this CF cohort, altered bone structural parameters, and their implications for bone strength, are potentially due to reduced bone strain, secondary to reduced muscle force.

1. Hogler W et al. J Pediatr. 2003;143(1):81-8.

P155

Effects of hypoxia during pregnancy on offspring bone development

Lee AMC[1], Morrison JL[1], Botting KJ[1,2], Shandala T[1] and Xian CJ[1]
[1] *Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA 5001, Australia*
[2] *Discipline of Physiology, School of Medical Sciences, University of Adelaide, Adelaide, SA 5005, Australia*

Studies suggested that weight at birth is associated with bone mass in adult life. Maternal hypoxia reduces birth weight; yet its effects on offspring bone development are unknown. The current study investigated effects of fetal growth restriction induced by maternal hypoxia during pregnancy on bone of the offspring. At 35d gestation day, mated guinea pigs were provided *ad libitum* feed at 21 or 12% O_2. Femur and tibia were collected at 62d gestation and 129d guinea pigs for bone histomorphometric measurements, and bone marrow cells were isolated for assessing osteoprogenitor cell contents (CFU-f assays). CFU-f alkaline phosphatase assays showed no changes in osteogenic potential pools in the fetal bone with maternal hypoxia in comparison to controls. Whilst histological analysis showed that maternal hypoxia resulted in thicker growth plate thickness in postnatal offspring, there were no significant changes in levels of gene expression of matrix degrading enzymes and hypoxia-induced factor in the growth plate. Histological analysis showed significantly higher bone marrow adipocyte counts despite no changes in bone volume in postnatal offspring from hypoxic mothers. Moreover, levels of expression of adipogenic genes were elevated in the metaphysis of these animals. These results suggest that maternal hypoxia during gestation did not change the pool of osteogenic cells in early life and bone volume in the early adulthood of offspring, but it induced bone marrow adiposity in early adulthood of offspring. Further studies are required to examine potential effects for maternal hypoxia during pregnancy on offspring's bone development.

P156

Evaluation of bone mineral density and bone/muscle geometry using pQCT in children after spinal cord injury

Biggin A[1,2], Middleton A[2], Ramjan KA[1,2], Briody JN[3], Waugh MCA[2,4] and Munns CF[1,2]
[1]*Institute of Endocrinology & Diabetes, The Sydney Children's Hospitals Network.* [2]*Discipline of Paediatrics & Child Health, University of Sydney.* [3]*Department of Nuclear Medicine, The Sydney Children's Hospitals Network.* [4]*Department of Rehabilitation Medicine, The Sydney Children's Hospitals Network*

Spinal cord injury (SCI) is associated with a significant reduction in bone mineral density (BMD) and increased bone fragility. The aims of this study were to quantify regional changes in bone mineral density and bone/muscle geometry in children following SCI using Peripheral Quantitative Computer Tomography (pQCT).
A retrospective cohort study of 19 patients (10 males and 9 females) with SCI was undertaken. The group comprised 9 paraplegics (6 complete, 3 incomplete) and 10 tetraplegics (5 complete, 5 incomplete). The mean age at SCI was 6.6 years. pQCT assessment was performed at a mean of 5.7 years post SCI. A total of 7 patients also had serial scans performed: The first at a mean of 7.6 years and the second at a mean of 10.7 years post SCI.
Reduced bone mass following SCI was regional. Lower limb involvement was universal and upper limb involvement was only seen in association with tetraplegia (Table). There was a significant loss of muscle cross sectional area in the calves. Analysis of serial pQCT data revealed a further reduction in trabecular volumetric bone mineral density (vBMD) Z-scores between 7.6 and 10.7 years post SCI, while cortical vBMD did not change. Incomplete paraplegics and tetraplegics, who were able to stand, had greater trabecular vBMD, cortical bone mineral content and cortical thickness tibial Z-scores than those with a complete SCI.
pQCT provides a valuable insight into the regional changes in bone and muscle development in children following SCI. Residual muscle function with the ability to weight bear, even if only in a frame, provides a significant benefit to bone development.

	4% site	66% site
Tibial Z-score:		
vBMD trabecular	-2.9 +/- 1.3*	
vBMD cortical		0.5 +/- 1.5
Total CSA		-1.8 +/- 2.6*
BMC		-3.9 +/- 3.4*
pSSI		-2.7 +/- 3.5*
Radial Z-score:		
vBMD trabecular	-3.2 +/- 3.6*	
vBMD cortical		-0.2 +/- 3.1
Total CSA	-1.4 +/- 1.9*	-1.1 +/- 0.8*
BMC	-2.5 +/- 1.5*	-3.9 +/- 3.0*
pSSI		-1.7 +/- 1.5*

Table: pQCT data of tibial and radial Z-scores at 4% and 66% sites
Values represent mean Z-scores +/- SD, asterisk represents p<0.05 compared to controls.
Tibial data for paraplegics and tetraplegics (n=19), radial data for tetraplegics only (n=10)
vBMD - volumetric bone mineral density, CSA - cross sectional area, BMC - bone mineral content,
pSSI – polar strength-strain index

P157

Six monthly intravenous zoledronic acid in childhood osteoporosis

Biggin A[1,2], Ooi HL[1], Briody JN[3], Cowell CT[1,2] and Munns CF[1,2]
[1]Institute of Endocrinology & Diabetes, The Sydney Children's Hospitals Network. [2]Discipline of Paediatrics & Child Health, University of Sydney. [3]Department of Nuclear Medicine, The Sydney Children's Hospitals Network. [4]Department of Rehabilitation Medicine, The Sydney Children's Hospitals Network

Childhood osteoporosis can be treated with intravenous bisphosphonates in order to improve bone mass and density. The aims of this study were to evaluate the safety and efficacy of six-monthly zoledronic acid (ZA) in children with osteoporosis.

A retrospective cohort study of 27 patients (16 males and 11 females) were treated with six-monthly ZA (0.05mg/kg/dose) for a minimum of one year. 17 patients were immobile, 4 had steroid-induced osteoporosis, 2 had osteogenesis imperfecta and 4 had other diagnoses. 16/27 (59%) had long bone fractures and 12/27 (44.4%) had vertebral wedging at baseline. Mineral homeostasis, bone mineral density by DXA and vertebral morphometry were evaluated at baseline and 1 year.

The median age at commencement of treatment was 12.3 years (range 8-15.8). Following the first infusion, 2/27 (7%) and 1/27 (4%) developed asymptomatic hypocalcemia at 48 hours and 72 hours, respectively. A fever above 38˚C developed in 14/27 (52%), generalised aches/pains in 13/27 (48%) and nausea in 6/27 (22%). At 1 year there was a significant reduction in bone turnover and improvement in bone mineral density (BMD) (see Table). Patients with vertebral wedging at baseline showed significant improvement in anterior, middle and posterior vertebral height ratios at 1 year. Only one patient fractured after starting ZA. There was normal growth.

Six monthly ZA was associated with an acute phase reaction to the first dose and improvement in BMD, reduction in bone turnover and improved vertebral shape at 1 year.

	Baseline	1 year
Calcium (mmol/L)	2.38 (2.35-2.44)	2.36 (2.28-2.42)
Alkaline phosphatase (U/L)	188 (143-271)	148.5 (127.25-205.5)*
Osteocalcin (nmol/L)	7.9 (4.35-11.35)	2.5 (1.1-3.95)*
25-OH-VitD (nmol/L)	75 (67-94)	76 (57.5-86)
Parathyroid hormone (pmol/L)	3.5 (2.3-4.1)	3.7 (2.9-5.4)
Total body aerial BMD Z-score	-0.56 (-1.7 to 0.35)	-0.03 (-1.13 to 0.86)*
L2-4 aerial BMD Z-score	-1.73 (-2.43 to -0.96)	-0.37 (-1.44 to 0.09)*
Bone mineral content for lean tissue mass Z-score	-1.68 (-2.51 to -0.60)	-0.10 (-0.9 to 1.35)*

Table: Mineral homeostasis and DXA data at baseline and 1 year
Values represent median (interquartile range), asterisk represents $p<0.05$ compared to baseline

Abboud, M.	OR19	Berk, M.	P118	Byun, D.W.	P88
Abe, M.	OR38	Berk, M.	P61	Byun, D.W.	P122
Abe, M.	OR7	Berk, M.	P127	Callon, K.E.	P27
Abel, T.	OR15	Bernhardt, J.	OR12	Callon, K.E.	P29
Abel, T.	P32	Bhattacharya, S.	P132	Callon, K.E.	P52
Abel, T.	P60	Biggin, A.	P156	Cantley, M.	P24
Abrahamsen, B.	P12	Biggin, A.	P157	Carpentier, V.T.	OR26
Adams, L.	P46	Bilezikian, J.P.	OR24	Center, J.R.	OR11
Adams, L.	P58	Bilezikian, J.P.	OR1	Center, J.R.	OR6
Ahn, K.J.	P59	Binkley, N.	OR20	Center, J.R.	P117
Aiken, A.	P149	Bjørnerem, Å.	P15	Center, J.R.	P17
Aiken, A.	P25	Bjørnerem, Å.	P38	Center, J.R.	P86
Aizawa, R.	OR8	Bleasel, J.	P17	Center, J.R.	OR27
Aizawa, S-I.	P96	Bliuc, D.	OR27	Chakravorty, N.	P146
Akel, N.S.	OR3	Boadle, R.	P70	Chakravorty, N.	P50
Akita, S.	P82	Bobyn, J.D.	OR2	Chakravorty, N.	P99
Akiyama, H.	OR30	Bodeen, G.R.	P106	Chalk, A.M.	P132
Alexander, K.A.	OR14	Bolland, M.J.	IS7	Chan, A.	OR10
Alexander, K.A.	P68	Bolognese, M.A.	P12	Chan, K.	P121
Amachi, R.	OR7	Bone, H.	OR20	Chan, M.Y.	P86
Amin, S.	OR5	Bone, H.G.	P12	Chang, J.S.	P122
Amizuka, N.	P142	Bone, H.G.	OR21	Chang, J.S.	P57
Anderson, P.H.	OR34	Bonewald, L.F.	P144	Chang, J.S.	P59
Anderson, P.H.	P105	Borgstrom, F.	P16	Chang, J.S.	P88
Anderson, P.H.	P144	Borromeo, G.L.	P124	Chang, M.K.	P68
Anderson, P.H.	P75	Borschmann, K.	OR12	Chapurlat, R.	OR1
Anderson, P.H.	P143	Botting, K.J.	P155	Chapurlat, R.	OR21
Anderson, P.J.	P104	Bowden, D.	P83	Chapurlat, R.	P12
Arai, M.	P53	Boyd, S.K.	OR1	Chauchard, M.A.	P120
Arakaki, R.	OR38	Bracken, A.	P23	Chauhan, A.	P119
Arnold, A.	P138	Brady, R.D.	P5	Chen, D.	P139
Arns, C.H.	P41	Brandi, M.L.	OR21	Chen, D.	P140
Asaoka, K.	OR38	Brennan, H.J.	OR13	Chen, D.Y.	P63
Askmyr, M.	P22	Brennan, H.J.	OR31	Chen, J.S.	P17
Atkins, G.J.	OR34	Brennan, S.L.	P109	Cheng, T.	IS11
Atkins, G.J.	P147	Brennan, S.L.	P111	Cheng, T.L.	P35
Atkins, G.J.	P63	Brennan, S.L.	P123	Cheng, T.S.	OR33
Atkins, G.J.	P75	Brennan, S.L.	P125	Chhana, A.	P27
Atkins, G.J.	OR16	Brennan, S.L.	P126	Chia, L.Y.	P102
Atkins, G.J.	OR9	Brett, A.D.	P106	Chim, S.M.	OR15
Atkins, G.J.	P101	Briggs, A.M.	P108	Chim, S.M.	P91
Atkins, G.J.	P144	Briggs, A.M.	P18	Choi, A.	P29
Atkins, G.J.	P76	Briody, J.N.	P154	Choi, W.	P130
Austin, M.	OR1	Briody, J.N.	P156	Chow, S.	P1
Austin, M.	OR24	Briody, J.N.	P157	Chow, S.T.	P91
Avlani, V.A.	P23	Brookes, D.S.K.	P154	Christopoulos, A.	P23
Baddock, H.T.	P26	Browett, P.	P81	Christopoulos, A.	P94
Bai, J.	P52	Brown, A.P.	P26	Chung, H.Y.	P57
Bai, J-Z.	P77	Brown, J.K.	P106	Chung, H.Y.	P59
Baker, E.K.	OR13	Brown, J.P.	OR21	Chung, M.H.	P57
Baker, E.K.	P132	Brown, K.	P107	Chung, R.	OR36
Baker, E.K.	P134	Brown, M.A.	P34	Chung, R.	P93
Baker, E.K.	P22	Buchbinder, R.	IS9	Chung, R.	P95
Bala, Y.	OR5	Bucknill, A.	P120	Cicolini, J.	OR18
Baldock, P.A.	P25	Buenzli, P.R.	P147	Clarke, M.V.	OR39
Baldock, P.A.	OR17	Buenzli, P.R.	P85	Clement, J.G.	P14
Baldock, P.A.	P10	Bui, M.	P15	Clement, J.G.	P85
Baron, K.	JOINT SYM	Bui, M.	P38	Clements, J.A.	P130
Bartold, P.M.	P24	Buttgereit, F.	P33	Codrington, J.D.	P18
Bava, U	P52	Byun, D.W.	P112	Cole, L.	OR19
Bennett, A.J.	P78	Byun, D.W.	P59	Coleman, B.	P27

Collier, F.M.	P56	Driessler, F.	OR17	Flicker, L.	IS30
Collier, F.M.	P61	Duan, R.	P34	Foo, B.	P119
Conigrave, A.D.	P23	Ducher, G.	OR22	Foo, B.	P87
Conigrave, A.D.	P26	Duncan, E.	IS13	Forwood, M.R.	P152
Conigrave, A.D.	P31	Dunstan, C.R.	P1	Forwood, M.R.	P153
Conigrave, A.D.	P71	Dunstan, C.R.	P100	Foster, B.K	P93
Conigrave, A.D.	P94	Dunstan, C.R.	P140	Foster, B.K.	OR36
Cooper, M.S.	P48	Dunstan, C.R.	P141	Fowler, T.W.	OR3
Cooper, M.S.	P54	Duque, G.	P13	Fowler, T.W.	P8
Cooper-White, J.C.	P150	Duque, G.	P16	Franchimont, N.	OR21
Copple, I.M.	P78	Duque, G.	P70	Fraser, D.R.	OR19
Cornish, J.	P27	Dwivedi, P.P.	P104	Fujihara, Y.	P64
Cornish, J.	P29	Ebeling, P.R.	P126	Fujii, S.	OR7
Cornish, J.	P52	Ebeling, P.R.	P13	Fujimoto, M.	P6
Cornish, J.	OR32	Ebeling, P.R.	P16	Fukuda, T.	P6
Cornish, J.	P77	Ebeling, P.R.	P56	Fukuta, O.	P55
Costa, J.L.	OR32	Ebeling, P.R.	P11	Fuller, P.J.	P83
Costa, J.L.	P29	Ebeling, P.R.	P124	Gaber, T.	P33
Coudrier, E.	P69	Ebina, K.	P82	Gaddy, D.	OR3
Cowell, C.T.	P157	Eeles, D.G.	P97	Gaddy, D.	P8
Cox, K.	JOINT SYM	Eisman, J.A.	OR11	Gagel, R.F.	P102
Crawford, R.	P146	Eisman, J.A.	OR17	Gamble, G.	OR29
Crawford, R.	P30	Eisman, J.A.	OR20	Gamble, G.	P27
Crawford, R.	P50	Eisman, J.A.	OR27	Gamble, G.	P81
Crawford, R.	P92	Eisman, J.A.	OR6	Ganda, K.	P17
Crawford, R.	P99	Eisman, J.A.	P17	Garcia, M.	P28
Crimeen-Irwin, B.	OR13	Eisman, J.A.	P86	Gdalevitch, M.	P150
Cross, M.	P16	Eisman, J.A.	P117	Geller, M.L.	OR21
Crotti, T.N.	P51	El-Hoss, J.	P151	Georgiou, K.	P93
Croucher, P.	IS18	El-Hoss, J.	P42	Georgiou, K.R.	P131
Cummings, S.R.	OR21	Endo, I.	OR7	Georgiou, K.R.	P136
Cundawan, W.	OR15	Endo, I.	P96	Ghasem-Zadeh, A.	OR12
Cunningham, R.B.	OR22	English, D.	P11	Ghasem-Zadeh, A.	OR5
Czerwinski, E.	OR21	Enomoto-Iwamoto, M.	P74	Ghasem-Zadeh, A.	P110
Czerwinski, E.	P12	Enriquez, R.E.	P10	Ghasem-Zadeh, A.	P15
Daizadeh, N.S.	OR21	Enriquez, R.F.	OR17	Ghasem-Zadeh, A.	P38
Dalbeth, N.	P27	Eser, P.	OR22	Ghasem-Zadeh, A.	P39
Daly, R.	P11	Ezura, Y.	P21	Ghasem-Zadeh, A.	P40
Daly, R.M.	OR22	Ezura, Y.	P98	Ghasem-Zadeh, A.	P43
Darby, I.B.	P124	Fairlie, D.P.	P24	Ghosh, P.	IS23
DaSilva, C.	OR20	Fan, C.M.	P133	Gilchrist, N.	OR20
Davey, R.A.	OR39	Fan, C.M.	P28	Gillespie, M.T.	P83
Davey, R.A.	OR34	Farrugia, R.	P120	Gillespie, M.T.	P97
Davies, B.N.	OR19	Fazzalari, N.L.	P14	Gineyts, E.	P152
Davies, P.S.W.	P154	Fazzalari, N.L.	P18	Glant, T.T.	P34
Dehghani, F.	P35	Fazzalari, N.L.	OR26	Goldring, C.	P78
Del Frate, D.	OR33	Feng, J.Q.	P146	Goldring, S.R.	P51
Delbridge, L.	P23	Feng, J.Q.	P50	Goode, A.	P78
Delbridge, L.	P71	Fernandes, T.J.	P56	Goolam, M.A.	P26
Denker, A.	OR20	Filmus, J.	P104	Goolam, M.A.	P31
Deo, N.	P42	Findlay, D.M.	OR16	Goradia, A.	P4
Dhaliwal, S.S.	OR23	Findlay, D.M.	OR9	Gordon-Thomson, C.	OR19
Dhaliwal, S.S.	P115	Findlay, D.M.	P101	Goriada, A.	P134
Dhaliwal, S.S.	P20	Findlay, D.M.	P144	Goto, S.	P53
Dharmapatni, A.	P101	Findlay, D.M.	P147	Goto, S.	P62
Dharmapatni, A.A.S.S.K.	P63	Findlay, D.M.	P63	Gould, H.	P123
Dickins, R.A.	P134	Findlay, D.M.	JOINT SYM	Gould, H.	P125
Dobbins, A.G.	P111	Fisher, T.	P119	Gould, H.	P128
Dodd, S.	P127	Fitzpatrick, J.	P150	Grauer, A.	OR1
Dong, B.	P96	Flannery, M.R.	P51	Grauer, A.	P12
Dore, D.	OR18	Flegg, M.B.	P44	Gravallese, E.M.	P68

Green, A.	P132	Hool, H.C.	P60	Kale, A.	P108
Gregory, L.	OR14	Hopper, J.L.	P15	Kamijo, R.	OR8
Gregory, L.S.	P130	Hopper, J.L.	P38	Kamioka, H.	P3
Grey, A.	IS7	Horne, A.	P81	Kanazawa, I.	OR25
Grey, A.	P29	Hosaka, Y.	OR30	Kaneshiro, S.	P82
Grey, A.	P81	Howe, P.R.	P133	Kantor, S.	P108
Grey, A.B.	OR32	Hui,J.	IS5	Kantor, S.	P18
Grills, B.L.	P97	Hülso, C.	P48	Kantor, S.	P80
Grills, B.L.	P148	Hülso, C.	P54	Kartsogiannis, V.	P83
Grills, B.L.	P5	Hunter, D.	IS19	Katagiri, T.	P6
Grønborg, M.	OR33	Huscher, D.	P33	Kato, S.	OR35
Gronthos S.	IS22	Hwang, Y.C.	P59	Kato, S.	P138
Grose, R.H.	P104	Hyde, Z.	IS30	Kaur, S.	OR14
Gruntmanis, U.	P12	Iimura, T.	OR8	Kawaguchi, H.	OR30
Gulati, K.	P147	Ikegawa,S.	IS4	Kawamura, I.	P103
Gunaratnam, K.	P70	Imaizum, K.	OR37	Kawamura, I.	P2
Guo, X.E.	P14	Imamura, K.	P103	Kawamura, I.	P49
Gupte, A.	P132	Imamura, K.	P2	Kawanabe, N.	P3
Ha, Y-C.	P112	Imamura, K.	P49	Ke, H.Z.	IS17
Ha, Y-C.	P122	Imanishi, Y.	P138	Kelly, J.	P1
Hall, J.W.	P12	Inaba, M.	P138	Kelly, J.	P139
Hamdorf, G.	P58	Inderjeeth, C.A.	P119	Kelly, J.	P140
Han, P.	P45	Inderjeeth, C.A.	IS3	Kelly, W.L.	P153
Hankey, G.J.	IS30	Inderjeeth, C.A.	P121	Kendler, D.L.	P12
Hanley, D.A.	OR1	Inderjeeth, C.A.	P87	Kersh, M.E.	P37
Hanrath, M.	P105	Intini, G.	JOINT SYM	Kersh, M.E.	P41
Harada, T.	OR7	Iseki, S.	P9	Khalid, K.A.	OR9
Hardy, R.S.	P48	Ishidou, Y.	P103	Khalid, K.A.	P147
Hardy, R.S.	P54	Ishidou, Y.	P2	Khan, B.K.	P11
Hardy, R.S.	P65	Ishidou, Y.	P49	Khoo, B.C.C.	P107
Hasegawa, T.	P142	Ishihara, Y.	P3	Khor, E.C.	OR17
Hashimoto, J.	P82	Ishii, A.	P138	Khosla, S	OR5
Hata, K.	P9	Ishimaru, N.	OR38	Kim, J.H.	P57
Hayashi, Y.	OR38	Ito, N.	P144	Kim, J.W.	P57
Hayata, T.	P21	Iuliano, S.	P116	Kim, J.W.	P88
Hayata, T.	P98	Iuliano, S.	P16	Kim, S.	P1
Haynes, D.R.	P101	Iuliano-Burns, S.	OR12	Kim, S.H.	P113
Haynes, D.R.	P24	Iuliano-Burns, S.	P43	Kim, Y.S.	P113
Haynes, D.R.	P63	Ivanovski, S.	P99	Kimura, H.	P36
Haynes, K.H.	P34	Izumi, S.	OR37	Kimura-Suda, H.	P36
Herrmann, M.	P13	Jacka, F.N.	P127	King, H.	P22
Herzog, H.	OR17	Jackson, C.J.	P73	King, T.	P93
Herzog, H.	P10	Jaiprakash, A.	P146	King, T.J.	P131
Hiasa, M.	OR38	Jaiprakash, A.	P50	King, T.J.	P136
Hiasa, M.	OR7	Jaiprakash, A.	P99	Kivitz, A.	P12
Hii, C.S.T.	P104	Javaid, A.	OR22	Knackstedt, M.A.	P41
Hill, B.	OR22	Jenkins, M.	P128	Kniessel, M.	P34
Hill, R.J.	P154	Jeon, Y.J.	P88	Knölker, H.J.	P69
Hirao, M.	P82	Jeong, I.K.	P59	Kobayashi, I.	P138
Ho, P.	P134	Jeong, J.K.	P72	Kobayashi, Y.	OR35
Ho, P.W.M.	OR13	Jeong, J.K.	P73	Kobayashi, Y.	P66
Ho, P.W.M.	OR4	Johnson, R.W.	OR31	Kocovski, P.	P132
Ho, P.W.M.	P4	Jones, A.C.	P41	Kocovski, P.	P4
Hodge, J.A.	P127	Jones, G.	IS20	Kogawa, M.	OR16
Hodge, J.M.	P56	Jones, G.	OR18	Kogawa, M.	OR9
Hodge, J.M.	P61	Josh, L.	OR23	Kogawa, M.	P101
Hogue, W.R.	OR3	Josh, L.	P115	Koh, T.	OR31
Holding, C.A.	P63	Jovic, T.	P22	Kokabu, S.	P6
Hong, S.	P113	Kagawa, K.	OR7	Kolind, M.	OR2
Hong, W.J.	OR10	Kaiser, J.	P147	Kolind, M.	P151
Hongo, H.	P142	Kakoi, H.	P2	Komiya, S.	P103

Komiya, S.	P2	Lim, B.	P60	Matthews, B.G.	P77
Komiya, S.	P49	Lim, E.M.	P121	McClung, M.R.	P12
Komoto, S.	P55	Lim, Y-T.	P122	McCorquodale, T.	P13
Kondo, H.	P47	Lim, Y-T.	P84	McDonald, M.	P151
Kondo, H.	P55	Lin, J.M.	OR32	McDonald, M.M.	P25
Kondo, H.	P62	Lin, J.M.	P29	McDonald, S.J.	P148
Kondo, H.	P64	Lin, J-M.	P77	McDonald, S.J.	P5
Kondo, M.	P62	Lin, S.	P10	McDonald, S.J.	P97
Kondo, T.	P96	Lin, Z.	P32	McGregor, N.E.	OR31
Koo, K-K.	P112	Little, D.G.	P35	McGregor, N.E.	OR4
Kotowicz, M.A.	P109	Little, D.G.	P42	McHugh, K.P.	P51
Kotowicz, M.A.	P123	Little, D.G.	OR2	McKelvey, K.D.	OR3
Kotowicz, M.A.	P126	Little, D.G.	P149	McQueen, F.M.	P27
Kotowicz, M.A.	P127	Little, D.G.	P150	Mellström, D.	OR21
Kotowicz, M.A.	P114	Little, D.G.	P151	Melton III, L.J.	OR5
Kotowicz, M.A.	P125	Little, D.G.	P25	Michigami, T.	P138
Kotowicz, M.A.	P128	Liu, R.	OR2	Middleton, A.	P156
Krieg, M-A.	OR21	Liu, X.S.	P14	Miki, H.	OR7
Kubo, K.	P36	Liu, Y.	P48	Mikulec, K.	OR2
Kular, J.	P60	Liu, Y.	P54	Mikulec, K.	P149
Kuliwaba, J.S.	OR26	Ljunggren, Ö.	P12	Mikulec, K.	P150
Kumarasinghe, D.D.	P76	Lloyd, D.	IS21	Mikulec, K.	P151
Kurten, R.C.	P8	Lombardi, A.	OR20	Milat, F.	P83
Kurusumuthu, P	P89	Long, J.E.	P78	Miller, P.D.	P12
Lam, N.N.	P143	Loo, L.S.	OR10	Min, K.H.	P59
Landao, E.	OR10	Losic, D.	P147	Min, Y.K.	P59
Landao, E.	P69	Lotinum, S.	JOINT SYM	Min, Y-K.	P112
Landao, E.	P79	Lowery, J.W.	JOINT SYM	Min, Y-K.	P122
Lane, N.	IS8	Lu, Z.F.	P100	Minami, Y.	OR35
Lane, N.E.	JOINT SYM	Ma, W.	P23	Mishima, K.	P74
Lane, S.E.	P114	MacInnis, R.J.	P128	Miyamoto, A.	P6
Langdahl, B.	OR20	Mackie, E.	OR4	Miyazawa, K.	P62
Langdahl, B.L.	P12	Macsai, C.E.	P95	Mizoguchi, T.	P66
Langdon, W.	P32	Maeda, K.	OR35	Moon, K.H.	P113
Langton, C.M.	P44	Maeda, S.	P103	Moon, M.H.	P72
Layfield, R.	P78	Maeda, S.	P2	Moon, M.H.	P73
Le Bailly De Tilleghem, C.	OR20	Maeda, S.	P49	Morgan, D.A.F.	P152
Lee, A.M.C.	P155	Man, Z.	OR21	Mori, H.	OR7
Lee, J-H.	P137	Manning, L.	P108	Moriyama, K.	P21
Lee, Y.J.	P72	Manolagas, S	IS16	Morris, H.A.	OR34
Lee, Y.J.	P73	Manolagas, S.	IS1	Morris, H.A.	P143
Lee, Y-K.	P112	Manolagas, S.	IS27	Morris, H.A.	P105
Lee, Y-K.	P122	March, L.	P16	Morris, H.A.	P75
Lee, Z.H.	P137	March, L.	P17	Morrison, J.L.	P155
Lee, Z-H.	IS6	March, L.	IS2	Morrison, N.A.	P153
Leem, Y.H.	P57	March, L.	OR28	Morse, A.	OR2
Leslie, W.D.	P126	Martin, T.J.	OR13	Morse, A.	P25
Leung, A.	OR20	Martin, T.J.	OR31	Mun, H-C.	P71
Lewiecki, E.M.	P12	Martin, T.J.	OR35	Mun, H-C.	P94
Lewis, J.	P129	Martin, T.J.	OR4	Munns, C.	P154
Lewis, J.R.	P20	Martin, T.J.	P102	Munns, C.F.	P156
Lewis, J.R.	P46	Martin, T.J.	P132	Munns, C.F.	P157
Lewis, J.R.	P58	Martin, T.J.	P134	Muratovic, D.	OR26
Li, A.	P33	Martin, T.J.	P4	Murphy, C.M.	P149
Li, A.	P65	Marumo, K.	OR35	Musson, D.	P29
Li, G.	IS24	Mason, R.S.	P23	Musson, D.S.	P77
Libanati, C.	OR1	Mason, R.S.	OR19	Mutsaers, A.J.	P134
Libanati, C.	OR24	Matsuda, T.	P67	Mylankal, J.K.	IS30
Liddicoat, B.	P134	Matsumoto, T.	OR38	Nagata, Y.	P138
Liew, D.	P120	Matsumoto, T.	OR7	Nair, P.	P121
Liew, J.L.	P46	Matsumoto, T.	P96	Nakamoto, T.	P21

Nakamura, S.	OR7	Otani, A.	P145	Purton, L.E.	P22
Nakano, T.	P142	Pandy, M.G.	P41	Qin, A.	P32
Nakashima, T.	IS10	Pandy, M.G.	P37	Qin, J.	IS29
Nakayama, T.	P66	Pang, M.Y.C.	OR12	Quach, J.	P22
Nam, M.S.	P113	Papapoulos, S.	OR21	Quinn, J.M.W.	P56
Nampei, A.	P82	Park, B.K.	P78	Quinn, J.M.W.	P97
Naot, D.	OR32	Park, H.M.	P59	Radavelli-Bagatini, S.	P129
Naot, D.	P27	Park, H-M.	P112	Radavelli-Bagatini, S.	P20
Naot, D.	P29	Park, H-M.	P84	Radominski, S.C.	OR21
Naot, D.	P52	Park, S.Y.	P72	Raggatt, L..	OR14
Naruse, K.	P3	Park, S.Y.	P73	Raggatt, L.J.	P68
Naughton, G.	OR22	Parkinson, I.H.	OR26	Raghu Nadhanan, R.	P133
Ng, A.J.M.	P132	Parkinson, I.H.	P14	Ramjan, K.A.	P156
Ng, A.J.M.	P134	Parkinson, I.H.	P18	Ratajczak, T.	P78
Ng, P.Y.	OR33	Pasco, J.A.	P114	Rea, S.	P78
Ng, P.Y.	P69	Pasco, J.A.	P118	Reginster, J-Y.	OR21
Nguyen, N.D.	OR11	Pasco, J.A.	P125	Reginster, J-Y.	P12
Nguyen, N.D.	OR27	Pasco, J.A.	P127	Reid, I.R.	IS7
Nguyen, N.D.	OR6	Pasco, J.A.	P128	Reid, I.R.	P52
Nguyen, N.D.	P117	Pasco, J.A.	P109	Resch, H.	OR20
Nguyen, N.D.	P86	Pasco, J.A.	P111	Resch, H.	OR21
Nguyen, S.C.	OR6	Pasco, J.A.	P123	Reynolds, E.C.	P124
Nguyen, T.	P152	Pasco, J.A.	P126	Reynolds, K.J.	P18
Nguyen, T.V.	OR27	Pavlos, N.	OR15	Robbins, J.	P116
Nguyen, T.V.	P117	Pavlos, N.	P51	Robinson, M.D.	P132
Nguyen, T.V.	OR11	Pavlos, N.	P60	Rogers, J.	P119
Nguyen, T.V.	OR6	Pavlos, N.J.	OR10	Román, J.A.	OR21
Nguyen, T.V.	P86	Pavlos, N.J.	OR33	Romas, E.	P7
Nicholson, G.C.	P16	Pavlos, N.J.	P69	Rosen,V.	JOINT SYM
Nicholson, G.C.	P56	Peacock, L.	OR2	Rosenberg, E.	OR20
Nicholson, G.C.	P13	Peacock, L.	P149	Roux ,C.	OR21
Nicholson, G.C.	P61	Peacock, L.	P150	Russell, M.	P4
Nishimura, R.	P9	Peacock, L.	P25	Russell, M.R.	P134
Nishita, M.	OR35	Perilli, E.	P18	Russell, P.K.	OR39
Niwa, Y.	P67	Peterson, J.	OR5	Ruygrok, P.	OR29
Noda, M.	P21	Petta, A.	P121	Saeki, Y.	P82
Noda, M.	P98	Pettit, A.P.	P34	Sainsbury, A.	OR17
Noguchi, T.	P64	Pettit, A.R.	OR14	Saito, A.	OR37
Nombela-Arrieta, C.	P22	Pettit, A.R.	P68	Saito, S.	OR30
Norman, P.E.	IS30	Pivonka, P.	P147	Saleh, H.	P97
Notomi, T.	P21	Pivonka, P.	P85	Sanders, K.M.	P114
Nowson, C.	P11	Pool, B.	P27	Sanders, K.M.	P13
O'Brien, F.J.	P149	Portales, J.R.	OR20	Sanders, K.M.	P16
O'Brien-Simpson, N.M.	P124	Poulton, I.J.	OR31	Sarah, B.	P126
Ohnishi, Y.	P96	Poulton, I.J.	OR4	Sasaki, M.	P142
Ohshima, S.	P82	Powell, B.C.	P104	Sasanuma, H.	P6
Ohte, S.	P6	Prasadam, I.	P92	Sato, T.	P53
OLoughlin, P.D.	P143	Price R.I.	P107	Scherer, M.	P28
Oloyede, A.	P99	Prince, R.	P16	Schindeler, A.	OR2
Omatsu, T.	P96	Prince, R.L.	OR23	Schindeler, A.	P149
Onishi, R.	P145	Prince, R.L.	P107	Schindeler, A.	P150
Ooi, H.L.	P157	Prince, R.L.	P115	Schindeler, A.	P151
Ormsby, R.	OR16	Prince, R.L.	P129	Schindeler, A.	P35
Ormsby, R.	P144	Prince, R.L.	P20	Schindeler, A.	P42
Ormsby, R.T.	P76	Prince, R.L.	P46	Schuijers, J.A.	P148
Orwoll, E.	P12	Prince, R.L.	P58	Schuijers, J.A.	P5
O'Sullivan, R.P.	P51	Puech, M.	P17	Schuijers, J.A.	P97
O'Sullivan, S.	OR29	Puglisi, D.A.	OR19	Searle, M.S.	P78
O'Sullivan, S.	P81	Purdue, P.E.	P51	Seeman, E.	OR1
O'Sullivan, S.	IS14	Purton, L.E.	P132	Seeman, E.	P16
Otahal, P.	OR18	Purton, L.E.	P134	Seeman, E.	OR24

Seeman, E.	OR5	Sutherland, R.L.	P1	Tuckermann, J.	P139
Seeman, E.	P110	Suva, L.J.	P8	Tuckermann, J.	P140
Seeman, E.	P15	Suva, L.J.	OR3	Tuckermann, J.	P141
Seeman, E.	P38	Suzuki, D.	OR8	Tuckermann, J.	P65
Seeman, E.	P39	Swain, F.L.	OR3	Turner, A.G.	P105
Seeman, E.	P40	Takada, I.	OR35	Turner, A.G.	P75
Seeman, E.	P41	Takahashi, N.	OR35	Udagawa, N.	OR35
Seeman, E.	P43	Takahashi, N.	P66	Udagawa, N.	P66
Seibel, M.J.	OR22	Takano-Yamamoto, T.	P9	Uehara, S.	OR35
Seibel, M.J.	P139	Takashima, R.	P9	Uehara, S.	P66
Seibel, M.J.	P140	Takayanagi, H.	IS15	Ueno, H.	P36
Seibel, M.J.	P141	Takeda ,E.	P145	Ung-il, C.	OR30
Seibel, M.J.	P33	Takei, Y.	P145	Upton, A.R.	P63
Seibel, M.J.	P48	Taketani, Y.	P145	Valchev, P.	P35
Seibel, M.J.	P54	Takeuchi, S.	P53	van Doornum, S.	P108
Seibel, M.J.	P1	Takyar, F.	OR4	Van, V.	P119
Seibel, M.J.	P17	Takyar, F.M.	OR13	Vander Schilden, J.	OR3
Seibel, M.J.	P19	Tam, P.P.L.	P25	Viola, H.M.	P60
Seibel, M.J.	P65	Tan, J.	P46	Wagman, R.B.	OR21
Seol, J.W.	P72	Tan, J.	P58	Wagman, R.B.	P12
Seol, J.W.	P73	Tanaka, E.	OR7	Walker, E.C.	OR31
Shahmoradi, N.	P43	Tatarczuch, L.	OR4	Walkley, C.R.	P132
Shandala, T.	P155	Teglbjaerg, C.S.	P12	Walkley, C.R.	P134
Shee, S.	P32	Telford, R.D.	OR22	Walkley, C.R.	P4
Shi, K.	P82	Telford, R.M.	OR22	Wall, M.	P134
Shimoji, S.	P142	Teo, J.	P39	Walsh, K.A.	P124
Shin, M.	P6	Teo, J.W.	P40	Walsh, N.	P22
Shore-Lorenti, C.	P13	Thekkedam, C.	P70	Walsh, N.C.	P102
Siddhanti, S.	P12	Thien, C.	P32	Walsh, N.C.	P68
Silberstein, L.E.	P22	Thomas, C.D.L.	P14	Walsh, N.C.	P7
Sims, N.A.	P7	Thomas, C.D.L.	P85	Wang, G.C.	P100
Sims, N.A.	OR13	Thomas, G.P.	P34	Wang, J.	P14
Sims, N.A.	OR31	Thompson, M.J.W.	OR18	Wang, J.Z.	P46
Sims, N.A.	OR4	Tickner, J.	OR15	Wang, J.Z.	P58
Sims, N.A.	P102	Tickner, J.	P60	Wang, Q.	P39
Singh, P.	P97	Togari, A.	P67	Wang, Q.	P40
Singh, P.S.	P56	Togari, A.	P47	Wang, Q.	P43
Sinn, A.W.M.	P147	Togari, A.	P53	Wang, T.	OR29
Skinner, R.A.	OR3	Togari, A.	P55	Wang, T.	P79
Slavin, J.	P134	Togari, A.	P62	Wang, X.	P40
Smith, M.D.	P63	Togari, A.	P64	Wang, X.F.	P15
Smith, S.	OR21	Tomoda, K.	P36	Wang, X.F.	P38
Song, J-Y.	P84	Tonkin, B.A.	P7	Wang, X.F.	P39
Speerin, R.	P17	Tonna, S.	OR13	Wang, X.F.	P43
Spies, C.M.	P33	Tonna, S.	OR4	Ward, A.R.	P148
Steck, R.	OR14	Torpy, A.M.J.	P109	Ward, A.R.	P5
Stoner, S.	P139	Tran, C.	P28	Ward, D.T.	P71
Stoner, S.	P141	Triliana, R.	P143	Ward, J.H.	P31
Stoner, S.	P48	Tsao, C.E.	P124	Wark, J.D.	P108
Stoner, S.	P54	Tseng, H.	P34	Wark, J.D.	P120
Stoner, S.	P65	Tsimos, S.	P80	Wark, J.D.	P18
Strauss, B.	P83	Tsuboi, H.	P82	Wark, J.D.	P80
Strbenac, D.	P132	Tsuji, K.	JOINT SYM	Watanabe, C.	P21
Stuart, A.L.	P118	Tsukamoto, S.	P6	Watanabe, K.	OR7
Su, Y.	P131	Tsunashima, Y.	P67	Watson, M.	OR32
Su, Y.W.	P93	Tu, J.	P33	Watson, M.	P29
Su, Y-W.	P133	Tu, J.	P48	Watson, M.	P77
Sugawara, Y.	P3	Tu, J.	P54	Wattie, D.	P81
Sugimoto, T.	OR25	Tu, J.W.	P139	Waugh, M.C.A.	P156
Sugita, S.	OR30	Tu, J.W.	P140	Wenger, G.R.	OR3
Sullivan, K.	P42	Tu, J.W.	P141	White, K.E.	P22

Wiebe, E.	P33	Xu, J.	P60	Zebaze, R.	P110
Wijenayaka, A.R.	OR16	Xu, J.	P91	Zebaze, R.	P15
Wijenayaka, A.R.	OR9	Xue, M.	P73	Zebaze, R.	P38
Wijenayaka, A.R.	P101	Yamada, A.	OR8	Zebaze, R.	P39
Will, R.	P119	Yamada, T.	P142	Zebaze, R.	P41
Wille, M-L.	P146	Yamagata, M.	P138	Zebaze, R.	P43
Wille, M-L.	P44	Yamaguchi, A.	OR8	Zebaze, R.M.	OR24
Wille, M-L.	P50	Yamaguchi, T.	OR25	Zhang, J.T.W.	P19
Williams, L.J.	P111	Yamamoto, A.	OR38	Zhang, X.	P92
Williams, L.J.	P127	Yamamoto, G.	OR8	Zhang, Y.	P114
Williams, L.J.	P61	Yamamoto, H.	P145	Zhang, Y.Q.	P139
Williams, L.J.	P118	Yamamoto, M.	OR25	Zhang, Y.Q.	P140
Wilson, S.	IS28	Yamamoto, M.	OR8	Zhang, Y.Q.	P141
Winzenberg, T.	OR18	Yamamoto, T.	P142	Zhen, Lin.	P79
Winzenburg, T.	P16	Yamashiro, T.	P3	Zheng, M.H.	OR10
Wiren, K.M.	OR39	Yamashita, T.	P66	Zheng, M.H.	OR15
Wolfram, U.	P37	Yamato, H.	P36	Zheng, M.H.	OR33
Wong, D.S.K.	P95	Yamauchim, M.	OR25	Zheng, M.H.	P60
Wong, P.	P83	Yang, D.	P105	Zheng, M.H.	P69
Woods, J.	P116	Yang, D.	P75	Zheng, M.H.	P32
Wraight, P.R.	P80	Yang, S.	OR11	Zheng, M.H.	P79
Wright, T.	P78	Yang, Y-C.	P12	Zheng, T.S.	P63
Wu, A.	P68	Yano, S.	OR25	Zheng, Y.	P1
Wu, A.C.	OR14	Yasuhara, R.	P74	Zheng, Y.	IS12
Wu, A.C.	P153	Yates, C.J.	P120	Zhou H.	P1
Wu, C.	P45	Yokouchi, M.	P103	Zhou, B.	P14
Wullschleger, M.E.	OR14	Yokouchi, M.	P2	Zhou, F.	P93
Xian, C.J.	OR36	Yokouchi, M.	P49	Zhou, F.H.	P131
Xian, C.J.	P131	Yokoyama, N.	P145	Zhou, H.	P65
Xian, C.J.	P133	Yoneda, T.	P9	Zhou, H.	P139
Xian, C.J.	P136	Yoneyama, K.	P6	Zhou, H.	P140
Xian, C.J.	P155	Yoshida, M.	P9	Zhou, H.	P141
Xian, C.J.	P28	Yoshikawa, H.	P82	Zhou, H.	P33
Xian, C.J.	P93	Yu, M.	OR23	Zhou, H.	P48
Xian, C.J.	P95	Yu, M.	P115	Zhou, H.	P54
Xiao, Y.	P146	Yu, N.Y.C.	P149	Zhou, X.F.	P131
Xiao, Y.	P30	Yu, N.Y.C.	P150	Zhou, X.F.	P93
Xiao, Y.	P45	Yukimura, T.	P138	Zhou, Y.H	P30
Xiao, Y.	P50	Yulyaningsih, E.	OR17	Zhu, K	P107
Xiao, Y.	P92	Zainuddin, M.Z.	OR31	Zhu, K.	OR23
Xiao, Y.	P99	Zajac, J.D.	OR39	Zhu, K.	P115
Xu, J.	OR17	Zanchetta, J.	OR1	Zhu, K.	P129
Xu, J.	P32	Zawawi, M.	P51	Zhu, K.	P20
Xu, J.	P69	Zebaze, R.	OR1	Zreiqat, H.	P100
Xu, J.	OR15	Zebaze, R.	OR5	Zysset, P.	P37

YOU CAN HELP HALVE MY RISK OF HIP FRACTURE*

* The relative risk of hip fracture was reduced by 63% in women with prior vertebral fracture ($p<0.05$) following 18 months of treatment with FOSAMAX® (alendronate sodium) 5/10mg.[1,2]

FOSAMAX PLUS D-CAL®

alendronate sodium/colecalciferol + calcium carbonate

Where vitamin D and calcium supplementation is required.

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

The alendronate in the FOSAMAX PLUS tablet was shown to be bioequivalent to the alendronate in the 70mg tablet. The therapeutic equivalence of alendronate 70mg weekly and 10mg daily was established.[2]

BEFORE PRESCRIBING, PLEASE REVIEW PRODUCT INFORMATION.
APPROVED PRODUCT INFORMATION IS AVAILABLE FROM MSD.

Minimum Product Information: FOSAMAX® (alendronate sodium) / FOSAMAX PLUS™ (alendronate sodium/colecalciferol) / FOSAMAX PLUS D-Cal® (alendronate sodium/colecalciferol and BoneCal® (calcium carbonate)). **Indications:** FOSAMAX: Treatment of: osteoporosis, glucocorticoid-induced osteoporosis or Paget's disease of bone. Prevention of: glucocorticoid-induced osteoporosis or osteoporosis in postmenopausal women with low bone mass. FOSAMAX PLUS: Treatment of osteoporosis in select patients where vitamin D supplementation is recommended. FOSAMAX PLUS D-CAL: Treatment of osteoporosis in select patients where vitamin D and calcium supplementation is recommended. Dosage: FOSAMAX tablets contain 40mg or 70mg alendronic acid. FOSAMAX PLUS tablets contain a fixed-dose combination of alendronic acid/colecalciferol 70mg/70µg (2800IU vitamin D3) or 70mg/140µg (5,600IU vitamin D3). FOSAMAX PLUS D-Cal contains FOSAMAX PLUS 70mg/140µg tablets plus BoneCal (calcium carbonate 1250mg) tablets. FOSAMAX is given as: 70mg once weekly for osteoporosis, 40mg daily up to 6 months for Paget's disease of bone, or 5mg (not available) daily for prevention of osteoporosis in postmenopausal women or treatment and prevention of glucocorticoid-induced osteoporosis (except in postmenopausal women not on oestrogen, 10mg (not available) daily). FOSAMAX PLUS is given once weekly for osteoporosis in patients requiring vitamin D supplementation. FOSAMAX PLUS D-Cal is given for osteoporosis in patients requiring vitamin D and calcium supplementation as a weekly tablet of FOSAMAX PLUS and 1-2 tablets BoneCal (calcium carbonate 1250mg) daily for 6 days of the week but never taken on the same day as FOSAMAX PLUS. FOSAMAX-containing tablets must be swallowed whole with a full glass of water at least 30 minutes before the first food, beverage or medication of the day. Patient must remain upright for at least 30 minutes and until after their first food of the day. **Contraindications:** Abnormalities which delay oesophageal emptying, inability to remain upright for at least 30 minutes, hypocalcaemia or hypersensitivity to any component of the products. In addition, BoneCal component of FOSAMAX PLUS D-Cal: hypercalcaemia or severe hypercalciurea. **Precautions:** Severe oesophageal ulceration has been reported with alendronate (follow full dosing instructions). Discontinue use if patient develops dysphagia, odynophagia or retrosternal pain. Use caution in patients with active upper gastrointestinal disorders (see full PI). Localised osteonecrosis of the jaw (ONJ) generally associated with tooth extraction and/or local infection has been reported with oral bisphosphonates, including FOSAMAX (for management of patients at risk of ONJ or who develop ONJ and/or require invasive dental surgery while on bisphosphonate therapy, see full PI). Atypical stress fractures of the proximal femoral shaft have developed in patients on long-term (usually >3 years) alendronate treatment (for information relating to management of atypical stress fractures see full PI). Not recommended in patients with creatinine clearance <35mL/min. Not to be used in pregnant or breast-feeding women or in children. In patients with disturbances in mineral absorption or metabolism (vitamin D deficiency, hypocalcaemia, hypercalcaemia, osteomalacia, Paget's disease of bone, history of nephrolthiasis and/or hypercalciuria, overproduction of calcitriol or those receiving glucocorticoids), appropriate diagnostic tests and/or treatment should be used (see full PI). The colecalciferol doses in FOSAMAX PLUS preparations are not suitable for correction of vitamin D deficiency. Interactions: Alendronate must be taken at least 30 minutes before taking any other oral medication. Caution during concomitant use of NSAIDs. Colecalciferol and calcium carbonate may alter the absorption or metabolism of concomitantly administered medications or vice versa (see full PI). **Adverse effects:** Oesophagitis, oesophageal erosions/ulcers, gastric/duodenal ulcers, melena, stomatitis, oropharyngeal ulceration, dysgeusia, ONJ, abdominal pain/distension, gastritis/ulceration, dyspepsia, acid regurgitation, nausea, vomiting, diarrhoea, constipation, flatulence, musculoskeletal pain, joint swelling, atypical stress fracture, muscular cramps, headache, dizziness, vertigo, pruritus, severe skin reactions, hypersensitivity reactions, acute phase response, hypocalcaemia, rash, erythema, alopecia, ocular disorders and peripheral oedema. On calcium carbonate; nausea, abdominal pain, constipation, flatulence, hypercalcaemia, hypercalciuria and nephrolithiasis. Based on TGA approved PI, last amended 10 August 2010. **PBS Dispensed Price:** FOSAMAX PLUS D-CAL, $45.16, FOSAMAX PLUS 70mg/70µg, $45.16, FOSAMAX PLUS 70mg/140µg, $45.16, FOSAMAX 70mg, $40.38; 40mg, $104.21; (10mg not PBS listed). Updated 7 December 2011. •• **References: 1.** Black DM, Thompson DE, Bauer DC et al, J Clin Endocrinol Metab 2000;85(11):4118–4124. **2.** Product Information for FOSAMAX PLUS D-CAL. ••

MSD