ANZBMS 24th Annual Scientific Meeting

7th - 10th September 2014

Queenstown, New Zealand

Meeting Handbook

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Sunday 7 September 2014

12:00	Registration opens
16:30	**Official Opening of the 24th Annual Scientific Meeting**
16:40 – 18:30	**Session 1**
	Chairs: Markus Seibel, Jill Cornish
16:40	IS1 — OSTEOCYTE DYNAMICS AND DIFFERENTIATION **Sarah Dallas (USA)**
17:15	IS2 – LESSONS FROM RARE BONE DISEASES **Rory Clifton-Bligh (Sydney)**
17:40	OR1 — AMGEN – ANZBMS OUTSTANDING ABSTRACT — BIOMEDICAL PRESENTATION
17:52	OR2 — AMGEN – ANZBMS OUTSTANDING ABSTRACT — CLINICAL PRESENTATION
18:04	OR3 — MSD OUTSTANDING CLINICAL ABSTRACT AWARD
18:16	Professor Philip Sambrook Award Presentation
18:30 – 20:00	**Welcome Cocktail Party, Exhibition, and Plenary Posters (P1 – P18, attended)**

Monday 8 September 2014

08:00 – 09:50	**Session 2A: Clinical Abstracts**
	Chairs: Nigel Gilchrist, Kerrie Sanders
	OR4 – OR12
08:00 – 09:50	**Session 2B: Biomedical Abstracts**
	Chairs: Nathan Pavlos, Ashika Chhana
	OR13 – OR21
08:30 – 09:50	**Meet the Professor Session**
	UPDATE ON BONE DENSITOMETRY **Nick Pocock**
09:50 – 10:10	**Morning Tea**
10:10 – 12:00	**Session 3A: Clinical Plenaries**
	Chairs: Michael Dray, Graeme Jones
	IS3 — BONE DENSITY TESTING: AN UNDERUTILISED HEALTH EDUCATION TOOL FOR OSTEOPOROSIS PREVENTION **Tania Winzenberg**
	IS4 — INDIVIDUALISED FRACTURE RISK ASSESSMENT: PROGRESS AND CHALLENGES **Tuan Nguyen**

IS5 – BONE IMAGING
Anthony Doyle

OR22 & OR23

10:10–12:00 **Session 3B: Orthopaedics**

Chairs: Matthew Gillespie, Jennifer Tickner

IS6 – BISPHOSPHONATES TO IMPROVE IMPLANT FIXATION
Per Aspenberg

IS6 – TENDON COMPLICATIONS: A CLINICAL PERSPECTIVE
Brendan Coleman

IS7 – TENDON REGENERATION: FROM BIOREACTOR TO CLINICAL TRIALS
Minghao Zheng

IS8 – LOAD TRANSFER IN THE PELVIS WITH PERIPROSTHETIC BONE LOSS
Jacob Munro

OR24

15:45–17:15 **Poster Session 1 (P21–P55)**

17:15–18:15 **Session 4: Tissue Engineering/Regenerative Medicine**

Chairs: David Musson, Hala Zreiqat

IS9 – BIOCERAMICS IN THE TISSUE ENGINEERING OF BONE: POTENTIAL AND CHALLENGES
Colin Dunstan

IS10 – RATIONAL USE OF SCAFFOLDS, BIOCHEMICAL AND BIOPHYSICAL FACTORS IN ACHIEVING BONE REPAIR
Nicole Yu

IS11 – STEM CELLS
Justin Cooper-White

18:15–19:30 **Roger Melick Young Investigator Award Finalists**

Chairs: Natalie Sims, Dorit Naot

OR25–OR30

20:00 **Young Scientists' and Students' Dinner**

Tuesday 9 September 2014

08:30–10:00 **Session 5A: Osteocytes**

Chairs: Jack Martin, Julian Quinn

IS12 – OSTEOCYTE MICROVESICLES AS A MECHANISM FOR CELL–CELL COMMUNICATION IN BONE
Sarah Dallas

IS13 – EPHRIN/EPH B INFLUENCES IN OSTEOCYTES
Natalie Sims

IS14 – OSTEOCYTES AND BONE REGENERATION
Gerald Atkins

OR31 & OR32

08:30–10:00 **Session 5B: Clinical Case Presentations**

Chairs: Fran Milat, Emma Duncan

08:30 IS15 – DRUGS FOR FRACTURE HEALING
Per Aspenberg

09:00 CLINICAL CASES

10:00–10:30 **Morning Tea**

10:30–12:00 **Session 6A: Clinical Abstracts**

Chairs: Elaine Dennison, Michael Hooper

OR33–OR39

10:30–12:00 **Session 6B: Biomedical Abstracts**

Chairs: Michelle McDonald,

OR40–OR46

12:00–13:30 **Lunch, Poster Session 2 (P56–P89)**

13:30–15:30 **Session 7: Matrix Biology and Joint Disease**

Chairs: Allison Pettit, Paul Baldock

IS16 – DYNAMICS OF ASSEMBLY OF BONE MATRIX
Sarah Dallas

IS17 – BASIC – CARTILAGE/BONE INTERFACE
David Findlay

IS18 – ARE CHONDROCYTES THE TARGET CELLS OF GLUCOCORTICOID THERAPY IN AUTOIMMUNE ARTHRITIS?
Hong Zhou

IS19 – MECHANISMS OF BONE EROSION IN GOUT: WHAT WE HAVE LEARNED FROM IMAGING AND LABORATORY STUDIES
Nicola Dalbeth

OR47 & OR48

15:30–15:40 **Afternoon Tea**

15:40–17:00 **ANZBMS AGM**

17:00–18:00 **Meet the Professor Sessions**

CAREER DEVELOPMENT
Eleanor Mackie, Paul Baldock

MTP1 – ADVANCED APPLICATIONS FOR MICRO CT IN BONE AND SOFT TISSUE
Egon Pirelli, Justin Fernandez

BONE CELL DYNAMICS
Nathan Pavlos, Sarah Dallas

SECONDARY FRACTURE PREVENTION
Paul Mitchell

19:00 for 19:30 **Conference Dinner**

Wednesday 10 September 2014

08:30–10:00 **Session 8: Paediatric Bone Disease**

Chairs: Tim Cundy, Craig Munns

08:30 IS20 — RARE MENDELIAN DISORDERS OF SKELETOGENESIS; WINDOWS INTO BONE DEVELOPMENT
Stephen Robertson

08:55 IS21 – A NEWLY IDENTIFIED DISORDER WITH ELEVATED BONE MINERAL DENSITY
Kieran Bunn

09:15 IS22 – MULTICENTRIC CARPOTARSAL OSTEOLYSIS
Syndia Lazarus

09:30 IS23 – HEREDITARY HYPOPHOSPHATAEMIC RICKETS WITH HYPERCALCIURIA
Komal Vora

09:45 IS24 – A MUTATION IN THE CLEAVAGE SITE OF C–PROPEPTIDE OF COL1A1 CAUSES IRREGULAR HIGH BONE MASS, BONE FRAGILITY AND JAW LESIONS: A NEW CAUSE OF GNATHODIAPHYSEAL DYSPLASIA?
Aideen McInerney-Leo

10:00–10:30 **Morning Tea**

10:30–12:30 **Session 9: Osteoporosis Management**

Chairs: Ian Reid, John Delahunty

10:30 IS25 – ROLE OF CALCIUM AND VITAMIN D IN OSTEOPOROSIS MANAGEMENT
Arthur Conigrave

10:55 IS26 – HISTOPATHOLOGY OF STRESS FRACTURE HEALING: AN IN VIVO MODEL FOR ACTIVATED REMODELLING AND ATYPICAL FRACTURES
Mark Forwood

11:15 IS27 – ATYPICAL FRACTURES
Per Aspenberg

11:40 IS28 – SELECTING AN OSTEOPOROSIS THERAPY – THE PROS AND CONS OF EXISTING AGENTS
Jeffrey Zajac

12:05 IS29 – NEW AGENTS FOR OSTEOPOROSIS MANAGEMENT
John Eisman

12:30 **CONCLUSION**

Citation: IBMS BoneKEy **11,** Article number: 576 (2014) | doi:10.1038/bonekey.2014.71

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Invited Speaker

IS3
Bone Density Testing: An Underutilised Health Education Tool for Osteoporosis Prevention

Tania Winzenberg
Menzies Research Institute Tasmania and School of Medicine, University of Tasmania, Hobart, TAS, Australia

Bone density testing is common with a reach of hundreds of thousands of Australian women annually, yet feedback of fracture risk based on bone mineral density (BMD) is underexplored as a potential osteoporosis education intervention. Our recent systematic review identified only a few, short-term studies but these suggest that feedback can lead to short-term improvements in behaviour and BMD. This includes data from our own 2-year trial of individualised fracture risk feedback in 470 premenopausal women. Women receiving feedback of high risk had a greater increase in femoral neck, but not lumbar spine BMD compared to the low risk group (1.6% p.a. vs. 0.7% p.a., p=0.0001). A greater proportion also commenced calcium supplements and reported changes in physical activity and mother's report of increasing their children's calcium intake was also increased (OR 2.0, 95% CI 1.2, 3.3). Given the nature of osteoporosis, these effects are only likely to be beneficial for fracture prevention if they are ongoing. We reassessed these women 10 years later. Compared to the normal risk group, women who received feedback of high risk had a smaller annual decrease in FN BMD (β=0.0023, 95% CI =0.0006 to 0.0041) and were more likely to quit smoking (7.9% vs. 4.2%, p=0.053) and commence calcium supplements (23.4% vs. 14.2%, p<0.001). Lumbar spine BMD change was similar in both groups. These new data strengthen the case for considering bone density feedback in young women as a strategy to improve long-term bone health and prevent osteoporosis in later life.

IS4
Individualised Fracture Risk Assessment: Progresses and Challenges

Tuan V Nguyen
Osteoporosis and Bone Biology Program, Garvan Institute of Medical Research; UNSW School of Public Health and Community Medicine. UNSW Australia; University of Technology, Sydney, NSW, Australia

Fragility fracture imposes a significant health burden to an individual as well as to the society, because fracture is associated with a substantial morbidity and increased risk of premature mortality. In previous years, fracture risk assessment and hence treatment decision were often based on bone mineral density (BMD) and a personal history of fracture. However, the two factors accounted for a modest proportion of fracture susceptibility, and models based on multiple risk factors are required. Risk prediction models, including the FRAX® and Garvan Fracture Risk Calculator (GFRC), have been developed to provide a useful clinical framework for communicating the risk of fracture. These models estimate an individual's risk of fracture based on the individual's unique risk profile, including clinical and non-clinical risk factors.

Recent validation studies suggested that the are under the ROC curve in fracture discrimination ranged from 0.61 to 0.83 for FRAX®, and from 0.63 to 0.88 for GFRC, with hip fracture having a better discrimination than fragility fractures as a group. FRAX® substantially under-estimated the risk of fracture, whereas the predicted risk by GFRC was close to or slightly higher than the actual risk. Results of post-hoc analyses of clinical trials indicated the anti-fracture efficacy of alendronate, coronate, bazedoxifene, and denosumab was greater in patients with higher predicted risk of fracture. However, there was no correlation between anti-fracture efficacy and predicted fracture risk among patients on raloxifene and strontium ranelate.

The prognostic performance of FRAX and GFRC for fracture prediction is not perfect, but there are rooms for further improvements. Genetic profiling and bone turnover markers have been shown to independently predictor fracture risk, and can help raise the accuracy of prediction. Nevertheless, these predictive models can aid patients and doctors communicate about fracture risk in the medium-term and to make rational decision.

IS9
Bioceramics in the Tissue Engineering of Bone: Potential and Challenges

Colin R Dunstan[1]*, Yongjuan Chen*[1]*, Iman Roohani-Esfahani*[2]*, ZuFu Lu*[2]*, Hala Zreiqat*[2]
[1]Biomedical Engineering, University of Sydney, Sydney, NSW, Australia; [2]Tissue Engineering & Biomaterials Research Unit, Sydney, NSW, Australia

The reconstruction of extensive bone defects, resulting from trauma, tumour resection surgery, or infections, remains one of the major challenges in orthopaedic surgery. Current treatment options are limited, and are associated with a high incidence of complications, often leading to non-unions or re-fractures. The filling of the defect with autologous bone grafts harvested from the patient's iliac crest—the current 'gold standard', leads to donor site morbidity in 20–30% of cases.

Particularly promising alternatives are synthetic bone substitutes. To successfully repair and regenerate bone using a synthetic-based tissue engineering approach, a scaffold/implant must meet these essential requirements: (i) mechanical

stability; (ii) bioactivity; (iii) biocompatibility; (iv) bio-degradability; and (v) high porosity and (vi) high interconnectivity to enable efficient migration of bone cell precursors and the vascularization necessary for bone formation. The synthetic materials currently available fall short of meeting the combined requirements for high porosity and interconnectivity while maintaining mechanical properties and bioactivity. These current ceramics tend to be brittle, lacking the toughness of bone itself. They tend to be monophasic in nature and allow rapid and catastrophic failure to occur when under repetitive load.
We have made major advances in developing strong and tough scaffold by producing ceramic composite materials with multiple crystal or glass phases which more closely reflect the hierarchical composite complexity of bone. To increase bioactivity we have investigate adding bioactive ions such as strontium to our ceramics. We have developed a new multi-component ceramics containing zirconium (which we have shown to have osteogenic properties) or strontium. These materials have increased mechanical properties and enhanced bioactivity relative to ceramics based on hydroxyapatite and calcium triphosphate. Further increases in toughness and bioactivity and the development of controlled fabrication methods to optimize strut architecture remain challenges.
Disclosure: Some bioceramics we have developed are the subject of licensing arrangements.

IS10
Exogenous and Endogenous Optimisation of In Situ Osteogenesis Using Biochemical and/or Mechanical Factors

Nicole YC Yu[1,2], *Aaron Schindeler*[2], *Jane Fitzpatrick*[3], *Marie Gdalevitch*[2], *Kathy Mikulec*[2], *Lauren Peacock*[2], *Ciara M Murphy*[2], *Laurence C. Cantrill*[4], *Andrew J Ruys*[5], *Justin J Cooper-White*[3], *David G Little*[3], *Melissa L Knothe Tate*[1]
[1]Graduate School of Biomedical Engineering, University of New South Wales, Sydney, NSW, Australia; [2]Department of Orthopaedic Research & Biotechnology, Kids Research Institute, Children's Hospital at Westmead, Sydney, NSW, Australia; [3]Tissue Engineering and Microfluidics Laboratory, Australian Institute for Nanotechnology and Bioengineering, University of Queensland, QLD, Australia; [4]Microscopy Services, Kids Research Institute at Westmead Children's Hospital, Sydney, NSW, Australia; [5]School of Aerospace, Mechanical and Mechatronic Engineering, University of Sydney, Sydney, NSW, Australia

Critical sized defects often result from trauma, tumour resection, debridement after infection, and congenital defects. Defects beyond this "critical size" exceed the body's natural bone healing capacity, leading to non-union. Despite current advances in surgery and implementation of bone grafts, the treatment of such defects remains a major challenge in orthopaedic medicine. Endogenous and exogenous tissue engineering approaches with biochemical factors and/or mechanical factors offer viable alternative methods for treatment of critical size bone defects.
In orthopaedics, focus is often placed on increasing bone anabolism with biochemical growth factors, such as recombinant human bone morphogenetic protein (rhBMP-2). However, rhBMP has also been associated with osteoclastic catabolism, leading to premature or excessive bone catabolism. Furthermore, implant stress shielding and instability can often lead to premature catabolism of newly engineered bone. Bone regeneration and maintenance processes are intrinsically linked to the mechanical environment which modulates the chemical environment directly and indirectly. The periosteum, which bounds every nonarticular bone surface of the body, provides a niche for mechanosensitive osteoprogrenitor cells and exhibits great regenerative capacity. This talk describes two approaches to modulate bone tissue engineering: (1) combination biochemical approach, specifically, local co-delivery of anabolic rhBMP-2 and anti-catabolic bisphosphonate; and a (2) mechanochemical approach, which modulates mechanosensitive periosteal-derived stem cells and transport in the defect zone.

IS13
The Role of Ephrins in the Osteocyte Network

Natalie A Sims
St. Vincent's Institute, Fitzroy, VIC, Australia

Ephrins are membrane-bound tyrosine kinases that signal through cell-contact dependent pathways both through their intracellular domain, and through the membrane-bound receptors (Ephs) to which they bind. A subset of ephrins and Eph receptors are expressed in the osteocyte network, which is characterised by a high level of cell-cell contact, both within the network itself, by contact with cells on the bone surface, including osteoblasts.
Within the osteoblast/osteocyte lineage, the EphrinB2:EphB4 interaction is unique since (a) both receptor and ligand are expressed by osteoblasts and osteocytes (b) EphrinB2 expression in osteoblasts is rapidly upregulated by parathyroid hormone and insulin-like growth factor, and (c) specific inhibition of the interaction of ephrinB2 with EphB4 (but not with other receptors) inhibits osteoblast differentiation in vivo and in vitro.
Cell specific knockouts of EphrinB2 either in the entire osteoblast lineage, or specifically in committed osteocytes, using Osx1.Cre- and DMP1.Cre-directed recombinase expression, respectively exhibit profoundly different phenotypes. Osteoblast-lineage deletion of ephrinB2 causes to an osteomalacia-like phenotype, with bones that had delayed mineralization and increased bone softness, a high level of osteocyte and osteoblast apoptosis, and reduced expression of osteocyte-specific marker genes. This confirms the role of late stage osteoblasts and osteocytes in stimulating osteoid mineralization. Surprisingly, osteocyte-specific deletion of EphrinB2 resulted in a brittle bone phenotype in the presence of normal osteocytic gene expression.
In conclusion, cell-contact dependent ephrinB2:EphB4 signalling plays unique roles in the osteoblast and osteocyte; both are required for normal bone strength, osteoblast activity and osteoclast generation.

IS21

A Newly Identified Disorder with Elevated Bone Mineral Density

Kieran J Bunn,[1] *Phillip B Daniel*[1]*, Tim Morgan*[1]*, Heleen Rösken*[1]*, Angeline Lai*[2]*, Azza Al-Ani*[3]*, Mauro Farella*[3]*, Susan Craw*[4]*, Stephen P Robertson*[1]

[1]Department of Women's and Children's Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand; [2]Genetics Service, Department of Paediatrics, KK Women's and Children's Hospital, Singapore; [3]Department of Oral Sciences, School of Dentistry, University of Otago, Dunedin, New Zealand; [4]Department of Radiology, Dunedin Hospital, Dunedin, New Zealand

Mendelian syndromes can often offer new insights into genetic contributors of key developmental processes. In this study, a novel constellation of features has been identified in three unrelated individuals. The three probands were all clinically diagnosed with a heterogeneous form of dwarfism known as Robinow Syndrome. Additionally, and somewhat anomalously for this disorder, all three also had dramatic and progressive osteosclerosis of both the axial and appendicular skeleton. Undertubulation of the long bones, hypoplasia of the distal phalanges, and bifurcation of the distal phalanx of the halluces and thumb were all demonstrable.

Mutations in genes encoding components of the WNT signalling pathway have previously been implicated in disorders featuring abnormal bone mineral density as well as in Robinow Syndrome. Defects in the non-canonical WNT mediators ROR2 and WNT5A that reduce their activity have been linked with Robinow Syndrome, while multiple components of canonical/beta-catenin dependent WNT signalling lead to osteosclerosis, examples including SOST mutations in Van Buchem disease and LRP5 mutations in osteopetrosis type I.

To address the cause of this newly identified disorder we employed next generation sequencing to identify mutations in a gene encoding a protein that is central to both canonical and non-canonical WNT signalling. We hypothesise that these mutations confer a gain-of-function, and that the new mutant product up-regulates canonical, while down-regulating non-canonical, WNT signalling. These findings offer new insights into how canonical and non-canonical signalling is interconnected and partitioned in different tissues during vertebrate development.

IS29

New Agents for Osteoporosis Management

John A Eisman

Clinical Translation and Advanced Education, Osteoporosis-Bone Biology, Garvan Institute of Medical Research; Endocrinologist, St Vincent's Hospital; SOMS, University of Notre Dame Australia; and UNSW University, Sydney, NSW, Australia

Treatment to reduce risk of initial and subsequent fragility fractures and of premature mortality is largely neglected in women and men, despite benefits of effective treatment. Uncertainty about adverse events with long-term treatment and failure to completely abolish fracture risk has led to continuing interest in new agents.

The anti-RANKL antibody, denosumab, produces marked osteoclast suppression with year-on-year increases in bone density. This is associated with halving of fracture risk similar to the other potent agents, the oral and IV bisphosphonates. Importantly its effect rapidly wears off in the 7th month after each SC injection. Compared with bisphosphonates, this potential disadvantage with respect to long-term adherence is an advantage regarding concern about long-term adverse effects.

Another new anti-resorptive, odanacatib, a cathepsin K inhibitor has potent anti-osteoclast action, also with rapid offset of action. It may allow release of osteoblast stimulating factors from bone. Initial data show year-on-year increases in bone density. The Phase III anti-fracture efficacy trial is due to be reported this year.

Use of the first anabolic agent, teriparatide, was restricted by rat osteosarcoma data and its high cost.

Another anabolic approach using anti-sclerostin antibodies has generated considerable interest. The optimal timing of treatment and benefit are being evaluated and Phase III anti-fracture efficacy trials are underway.

The new agents being developed have the potential to safely improve and maintain bone mass with potential for more effective prevention of fracture risk.

Disclosure: Professor Eisman provides consulting advice to and/or has received research support from Amgen, Lilly, Merck, Sharp and Dohme, sanofi-Aventis, Servier and Novartis.

Meet the Professor

MTP1

Advanced Applications of Micro-CT with Focus on Soft Tissue: Beyond Bone

Egon Perilli
Medical Device Research Institute, School of Computer Science, Engineering and Mathematics, Flinders University, Adelaide, SA, Australia

Micro-CT allows the non-destructive quantification and visualization of bone changes at the micro-meter level in 3D in-vivo and in-vitro in small animals and in-vitro in humans. It has been extensively used in diseases affecting bone and joints, such as osteoporosis, osteoarthritis and rheumatoid arthritis. In other studies performed in animal models, micro-CT has been used to visualize and quantify soft tissue such as lungs, lung tumors and body fat, in 3D. However, typically, bone and soft tissue are considered separate aspects and hence studied separately, with different techniques. This a priori separation is not always justified.

Over the years, there have been improvements in micro-CT systems including scanning, image processing and quantification protocols. By using contrast agents, when micro-CT scanning a rat in-vivo or in-vitro, in osteoarthritis studies the articular cartilage can be visualized and quantified. Similarly, the vasculature in fracture healing and soft tissue trauma models can be quantified, in 3D. Moreover, depending on the study, the readily available micro-CT images used for quantifying the bone, can also be used for quantifying soft tissue, hence examining more than the "just" typically-looked at bone information from these scans. For example, the paw swelling can be measured both in-vivo and ex-vivo in a murine inflammatory arthritis model, without the need of contrast agents, as demonstrated by a current study of ours. In this case, soft tissue volume changes and joint bone erosion can be visualized and quantified, within the same micro-CT scans, without altering the scanning protocol.

In muscoloskeletal research performed by using micro-CT on small animal models, there exist possibilities to combine the soft tissue evaluation with the typically-examined bone changes, within the same scan. This is an exciting feature and has the potential of turning micro-CT in an increasingly integrated 3D analysis tool in future.

Oral Presentations

OR1
AMGEN-ANZBMS OUTSTANDING ABSTRACT-BIOMEDICAL PRESENTATION

Macrophages are a Viable Therapeutic Target to Enhance Fracture Repair via Endochondral Callus Formation

Allison R Pettit[1], *Andy C Wu*[1], *Kylie A Alexander*[2], *Simranpreet Kaur*[1], *Susan M Millard*[1], *Michelle M Maugham*[1], *Roland Steck*[3], *Laura s Gregory*[3], ***Martin E Wullschleger***[4] *and Liza J Raggatt* [1]

[1]Mater Research Institute-UQ, Translational Research Institute, Woolloongabba, QLD, Australia; [2]Queensland Institute for Medical Research, Herston, QLD, Australia. [3]Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, QLD, Australia; [4]Queensland Health and The University of Queensland, Herston, QLD, Australia

Macrophages (macs) have been indirectly implicated in the inflammatory phase of fracture repair, but their roles in later phases are unknown. It is likely that both, resident tissue and inflammatory macs, participate in fracture healing. Resident osteal macrophages (osteomacs) promote osteoblast function. Recruited inflammatory macs are polarized toward an appropriate activation pathway depending on the local environmental cues. M2 polarized macs have been shown to promote wound healing in soft tissue models.

Mac distribution and phenotype through the multiple stages of fracture repair were examined using an internally plated (MouseFix) femoral fracture model that heals via periosteal endochondral callus formation. An in vivo inducible mac depletion model (Mafia) was used to examine mac requirement during different healing phases. Callus formation was assessed via quantitative histology.

Inflammatory M2-like macs ($F4/80^+Mac\text{-}2^+Arginase\text{-}1^+$) were distributed throughout the fracture granulation tissue during the late inflammatory phase. Inflammatory macs persist in granulation tissue at the expanding callus fronts and were associated with soft callus vascular invasions. In the late anabolic and remodeling phases osteomacs ($F4/80^+Mac\text{-}2^{neg}$) predominated in the maturing hard callus. Local depletion of macs, initiated at the time of surgery, resulted in catastrophic failure of fracture healing, clinching the importance of mac contributions to the initiation of fracture healing. Delayed systemic mac depletion commencing at the transition to early anabolism resulted in a significant 3.3 fold reduction in callus associated macs and 50% reduction in soft callus area ($p=0.006$). Local administration of the mac-trophic cytokine CSF-1 (therapy initiated at transition to early anabolism) resulted in a 2 fold increased in soft callus formation ($p=0.03$), supporting that macs make substantive contributions to early anabolic process during callus formation.

Overall, M2-like inflammatory macs are required for initiation and optimal early anabolic progression of fracture healing and are viable targets for development of novel anabolic fracture therapies.

Disclosure: The authors declare no competing interests.

OR2
AMGEN-ANZBMS OUTSTANDING ABSTRACT-CLINICAL PRESENTATION

Antiresorptive Therapy is Sometimes 'Too Late'

Zebaze R[1], *Chiang C*[1], *Bala Y*[1], *Iuliano S*[1], *Shahmoradi N*[1], *Zendeli A*[1], *Wang X*[1], *Peng Y*[2], *Ghasem-Zadeh A*[1], *Seeman E*[1]

[1]Department of Medicine and Endocrinology, Austin Health, The University of Melbourne, Melbourne, VIC, Australia; [2]Straxcorp Pty Ltd, Melbourne, VIC, Australia

The aim of treatment of bone fragility is to prevent fractures. Current antiresorptive therapies reduce non-vertebral fracture (NVF) risk by only 20%. Even in the setting of randomized trials NVF risk reduction is only ~ 20% despite compliance with therapy. We propose that if structural decay is too advanced (i.e., severe porosity), fragility will remain despite therapy.

About 80% of the skeleton is cortical, 70% of all bone loss is cortical and occurs mainly by intracortical remodeling which produces porosity a 'footprint' of bone loss that is an independent predictor of fractures. We therefore hypothesized that there is porosity value above which treatment does not reduce fracture risk.

We studied 69 postmenopausal women; 37 sustained a NVF despite being compliant with therapy; 32 remain fracture free during therapy, matched by age (71.2±1.2 vs. 73.6±1.64 years; NS) type and duration of therapy (5.3±0.56 vs. 4.7 ± 0.8; NS). Images of the distal radius using HRpQCT was quantified using StrAx1.0. The groups were stratified on percent porosity of the compact-appearing cortex (CC) of >25%; >30%; >35%; >40%; 45%; >50%; >55%, and >60%.

In groups with porosity <45%, fracture prevalence was no different and independent of porosity. In groups with porosity >45%, fracture prevalence increased exponentially with increasing porosity ($R^2=0.97$; $p<0.0001$) and was 1.6 fold higher than in those with porosity <45% (66.7 vs 41.6%; $p=0.05$). The OR increased exponentially to reach 2.8 in patients with porosity>45% (similar to that reported in a similar population of untreated postmenopausal women) [1].

We infer that a porosity >45% signals a level above which antiresorptive therapy is ineffective. This calls into question the use of antiresorptives as first line therapy in all subjects.

Reference

1. Bala Y *et al. JBMR* 2014.

OR3
MSD OUTSTANDING CLINICAL ABSTRACT AWARD

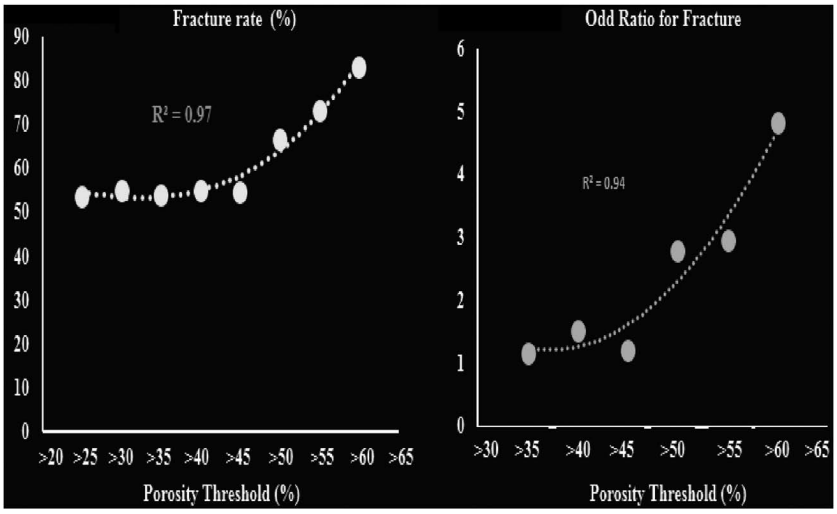
The Response of Bone Cells to Infection Following Total Hip Replacement

Masakazu Kogawa, *L Bogdan Solomon, Caroline Moran, Renee T Ormsby, David M Findlay, Gerald J Atkins*

Centre for Orthopaedic and Trauma Research, University of Adelaide, Adelaide, SA, Australia

Total hip replacement (THR) is a principal surgical procedure for advanced osteoarthritis (OA). However, bacterial infection

[OR2]



following surgery can result in prosthesis loosening and the need for revision surgery. Bacterial infections activate immune responses, resulting in the release of pro-inflammatory cytokines, which are known to stimulate osteoclast formation and activity. However, the effect of these infections on the osteocyte, which plays key roles in the regulation of physiological bone remodelling by controlling other cell types in bone, remains unclear. In this study, we have begun to address this. Bone samples were collected from age-matched patients undergoing either revision THR for infection-related prosthesis loosening (n = 7) or THR for primary OA (n = 18). Small samples of bone were collected from the acetabulum prior to reaming and from the iliac wing as a control. Bone samples were rinsed in PBS and examined for the analysis of mRNA expression by real-time RT-PCR. Our preliminary analysis of these samples has revealed the acetabular bone of the infected group has significantly increased mRNA expression of the osteocyte-related gene SOST, in comparison to the Primary OA group. This is consistent with our previous finding that pro-inflammatory cytokines TNF and TWEAK induced the expression of SOST/sclerostin in human osteoblasts, osteocyte-like cells and human bone samples cultured ex vivo [1]. The expression of sclerostin in bone is also known to reflect local strain perceived by osteocytes. Consistent with this, in the primary OA group, the expression of SOST mRNA from the acetublum, a load bearing site, was significantly lower than that in the iliac samples, a relatively unloaded site. Interestingly, this relationship did not exist in the infected group. These preliminary results suggest that in the situation of infection, sclerostin may play a role in implant loosening.

Reference

1. Vincent *et al*. *J Bone Miner Res* 2009;**24**(8):1434–1449.

OR4

Intergenerational Difference in Presentation of Paget's Disease in People with SQSTM1 Mutations

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Background: The cause of Paget's disease of bone (PDB) is unknown, but genetic factors, particularly SQSTM1 mutations, and environmental factors are important. We investigated the development of PDB in relatives with SQSTM1 mutations to determine if a secular trend toward a less severe phenotype is evident, and to estimate prospectively the rate at which PDB emerged in this genetically-susceptible population.
Methods: We recruited first degree relatives of patients with PDB (33 adult offspring [mean age 45] and 1 sibling) with a familial SQSTM1 mutation. Initial skeletal scintigraphy demonstrated PDB in 6; 26 of the remaining 28 unaffected subjects had a second scintiscan, a mean 5.1 years later.
Results: We identified 2 new cases of monostotic PDB in 134 patient-years of follow up. In the total 8 adult offspring diagnosed with PDB, the age of diagnosis was significantly greater, by at least 10 years, than that in the 21 probands with clinically-identified PDB ($p = 0.005$). In adult offspring who were older at the time of skeletal scintigraphy than their affected parents were at the time of clinical diagnosis, the difference was even more marked ($p<0.001$), and their disease was significantly less extensive than in their affected parent, as judged by alkaline phosphatase and disease extent ($p<0.003$).
Conclusions: These findings suggest a substantial gene-environment interaction: the emergence of PDB in offspring inheriting SQSTM1 mutations is delayed by at least a decade, has a substantially attenuated phenotype, and occurs at a low rate between the (mean) ages of 45 to 50 years.

OR5

Cortical Porosity in Women Over 80 Years of Age

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About 85-90% of the skeleton in over 80 year-olds is cortical because most trabecular bone has been resorbed leaving mainly cortical fragments in the medullary canal. High remodeling due to sex hormone deficiency is exacerbated by secondary hyperparathyroidism variously due to vitamin D deficiency, a low calcium intake and malabsorption. As intracortical remodeling is the major source of cortical bone loss, we hypothesized that cortical porosity will be elevated in these women and more severely in those with elevated circulating PTH.

We imaged distal tibial microstructure in 36 women (mean age 89±4 years) and 73 post-menopausal women (mean age 60±5 years) using HR-pQCT and quantified porosity using StrAx1.0.

Despite similar total bone area, in older compared with younger women respectively, the compact-appearing cortical area was ~12% smaller (101±24 v 114±17 mm^2), ~21% less dense (586±90 v 745±75 mgHA/cc) and porosity ~25% higher (60.9±8.7 v 45.4±7.4%) (all $p<0.01$). In the older women, PTH was elevated (9.0 ± 4.4 pmol/L) and dietary calcium intake low (636±175 mg/day) with 2/3 of women consuming <600 mg/day.

In all women, porosity was related to age ($r=0.72$) and PTH ($r=0.46$) (both $p<0.001$). In the older women, age accounted for 10% of the variance in porosity. PTH levels in the elderly women were systemically elevated, and the small sample size may have limited the ability to detect a relationship with porosity. Dietary calcium in the elderly was related to porosity in those with intakes below 600mg/day ($r=0.47$, $p=0.05$).

We infer that a reduced calcium intake independently contributes to deficits in cortical mineralized bone matrix volume by influencing intracortical remodelling. Studies are needed to examine the effects of calcium intakes below 600 mg/day on bone loss and the effects on repletion in this high-risk elderly population.

OR6

Marked Interlaboratory Variation of Biochemical Markers of Bone Turnover – Results of a Quality Assessment Trial Across Australia

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Biochemical markers of bone turnover are increasingly used in the management of patients with metabolic bone diseases, however, their practical utility is challenged by significant variability. To determine the interlaboratory reproducibility of bone markers we conducted a quality assessment trial across 19 Australian laboratories.

Urine and serum was collected from 5 subjects, then pooled, aliquoted and frozen. Identical serum and urine aliquots were sent on dry ice to 19 laboratories across Australia for analysis.

Sixty-two results were received from 19 participating laboratories located in 8 Australian metropolitan cities for the following markers: serum aminoterminal propeptide of type I collagen (sPINP; n=17); serum osteocalcin (sOC; n=11); serum carboxyterminal telopeptide of type I collagen (sCTx; n=12); urine aminoterminal telopeptide of type I collagen to creatinine ratio (uNTx:Cr; n=8); and urine deoxypyridinoline to creatinine ratio (uDPD:Cr; n=14). As no single laboratory performed all five bone turnover markers in-house, samples were shipped extensively between laboratories, both locally (n= 16) and interstate (n=16). Some samples routinely travelled from Sydney to Perth, or from Perth to Melbourne and there were 6 accounts of samples being sent interstate when the test was available from a laboratory within the same city. The inter-laboratory coefficients of variation (CV_{IL}) were as follows: sPINP, 5% (n=16 results across 9 laboratories); sOC 6–26% (n=8 results across 4 laboratories); sCTx, 11% (n=12 results across 7 laboratories); uNTx, 9% (n=7 results from 1 laboratory); uNTx:Cr, 5% (n=7 results from 1 laboratory); uDPD, 14% (n=12 results across 6 laboratories); uDPD:Cr, 13% (n=14 results across 7 laboratories).

We conclude that inter-laboratory variability is high for certain bone markers, possibly due to extensive shipping of samples across the Australian continent. Implementation of routine quality control programs for bone markers is required.

OR7

Withdrawn

OR8

Impact of Subchondral Bone Plate Integrity on the Homeostasis of the Underlying Subchondral Trabecular Bone in Late-stage Osteoarthritis

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Subchondral bone plate (SBP), a thin dense mineralized lamella lying immediately underneath cartilage, has a significant mechanical and biochemical function in the joint homeostasis. In the present study, we aimed to investigate the impact of SBP integrity on the homeostasis of the underlying subchondral trabecular bone (STB) in late-stage osteoarthritis (OA) when articular cartilage is eroded in full-thickness.

Peri-articular bone cylinders were extracted in the load-bearing region of femoral heads from 110 patients with late-stage OA. After micro-CT scanning, the bone samples were categorized into two groups: (1) samples with partial-thickness abrasion of SBP, without exposure of STB; and (2) samples with full-thickness abrasion of SBP, with exposure of STB. Micro-CT and histology were performed to analyze the

microarchitecture, bone remodeling and pathological changes of STB in all samples.
In samples with full-thickness abrasion of SBP, STB was detected with a more sclerotic microarchitecture and more active bone remodeling, compared to the counterparts with partial-thickness abrasion of SBP. In samples with partially abrased SBP, most microarchitecture and bone remodeling parameters of STB did not correlate with thickness and porosity of SBP. However, in samples with full-thickness abrasion of SBP, the larger SBP defect area became, the more sclerotic in microarchitecture and more active in bone remodeling STB displayed. In samples with full-thickness abrasion of SBP, there were also higher occurrence of pathological lesions in STB, including bone cysts, bone marrow edema, fibrosis, and blood vessels with fibrinoid deposition and hyperplastic walls.
Our results demonstrate that SBP plays a pivotal role in the maintenance of the underlying STB homeostasis, especially in late-stage OA when cartilage is completely eroded in thickness.

OR9

Is the Bone Response to Physical Activity Related to Skeletal Maturity? A Cross-Sectional pQCT Examination of Children and Young Adults Aged 5-29 Years

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Growth is the opportune time to modify bone accrual. The relative responsiveness of the skeleton to loading according to timing in relation to puberty is unclear. The differences in strain that are experienced around the cross-section of a long bone under load are reflected in location-specific adaptative responses. We therefore speculated that examining differences in bone strength parameters at different locations around tibial cross sections between individuals who vary in terms of physical activity exposure and pubertal status may enlighten the question of optimal timing of loading during growth. Data from 304 individuals aged 5–29 years (172 male, 132 female) were examined. Peripheral quantitative computed tomography (pQCT) was applied at 4%, 14%, 38%, and 66% of tibial length. Maturity was established by estimating age at peak height velocity (APHV). Loading history was quantified with the bone-specific physical activity questionnaire (BPAQ). Comparisons, adjusted for height, weight and age were made between sex, maturity, and BPAQ tertile groups. Few to no differences were observed between sexes or BPAQ tertiles prior to APHV, whereas marked sexual dimorphism and differences between BPAQ tertiles were observed after APHV. Cross-sectional location-specific differences between BPAQ tertiles were not evident prior to APHV, whereas clear location-specificity was observed after APHV. In conclusion, the skeletal benefits of physical activity are location-specific in the tibia. The present results indicate that the peri- or post-pubertal period is likely a more favourable window of opportunity for enhancing cross-sectional bone geometry than pre puberty. Further, increased loading during the peri-pubertal period may be expected to effectively enhance the bone of both sexes.

OR10

Serial Femoral Neck Bone Mineral Density Predicts Fracture Risk Better than Baseline Bone Density

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Existing fracture risk calculators have been developed using single measurements of femoral neck BMD made at baseline. However, femoral neck BMD (FNBMD) changes over time; hence models built using single measurements may not accurately reflect the relationship between FNBMD and fracture. This study examined the association between FNBMD and risk of fracture using models derived from serial FNBMD measurements.
This study used data from the Dubbo Osteoporosis Epidemiology Study collected between 1989 and 2014, and included 2500 women and 1484 men (age range: 50–94; mean: 69), with a median follow-up of 11 years. FNBMD was measured by DXA (GE-Lunar) at baseline and approximately every 2 years, with an average of 4 measurements per participant (range: 1–12). Low trauma and non-pathological fractures were ascertained from X-ray reports. Models were developed using time-variant Cox's regression to allow for use of serial measurements.
During the study period, a significantly higher proportion of women sustained fragility fractures (770 (30.8%)) compared to men (239 (16.1%)). In the model built using serial FNBMD measurements, each SD decrease in FNBMD (0.15 g/cm^2) was associated with 83% (HR: 1.83; 95% CI: 1.69–1.99), and 96% (HR: 1.96; 95% CI: 1.70–2.26) increased risk of fracture in women and men, respectively. All associations remained significant following adjustment for age and prior fracture (fractures prior to entry but after age 50). These models which used serial measurements yielded higher concordance indices than models built using baseline measurements only. In men, concordance index improved from 0.649 in the model with baseline to 0.666 in the model with serial FNBMD measurements. In women, the corresponding improvement was from 0.645 to 0.651.

OR11

Gout is associated with an excess risk of osteoporotic fracture

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Although metabolic syndrome is common in gout patients, recent reports that bone mineral density may actually be reduced (and falls common) in this group have led researchers to hypothesise that osteoporotic fracture may be more common in subjects with gout than in healthy controls. We tested this hypothesis in a national Danish registry.

We identified subjects as new users of allopurinol, a proxy for gout, for the years 1996–2010. Each incident user was assigned up to 10 age- and gender matched controls. We used propensity score matching to identify a highly matched control population. Patients with a diagnosis of malignancy in the year prior to the first allopurinol prescription were excluded. A final propensity score model included hospital diagnoses since 1994; Charlson index components; and prior osteoporotic fractures; use of drugs (including osteoporosis medication, prednisolone and HRT) in the last year. Conditional Cox regression modelling was undertaken.

We studied 86,129 patients and the same number of controls (58,129 men and 28,000 women). Thirteen thousand and ninety one cases and 12,188 controls sustained any osteoporotic fracture; the number of major osteoporotic fractures was 5,574 in the cases and 4,893 in the control group. We found a modest adjusted effect of allopurinol prescription on major osteoporotic fractures; an association with hip fractures just failed to attain statistical significance (see Table). Among patients who were incident allopurinol users and who also had at least one hospital contact with a gout diagnosis (about 20% of allopurinol users, median number of allopurinol prescriptions 12 versus 6 in non-hospital group), we found stronger associations.

These data suggest that gout requiring allopurinol prescription is a risk factor for osteoporotic fracture.

OR12

The Effect of High Intensity Exercise on Undercarboxylated Osteocalcin and Insulin Sensitivity in Obese Men

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Acute exercise improves insulin sensitivity for hours after the exercise is ceased. The skeleton contributes to glucose metabolism and insulin sensitivity via osteocalcin (OC) in its undercarboxylated (ucOC) form in mice. We tested the hypothesis that improvement in insulin sensitivity following exercise is partly related to ucOC in human subjects.

Eleven middle-aged (58.1±2.2year mean±SEM), obese (BMI=33.1±1.4 $kg \cdot m^{-2}$) non-diabetic men completed a euglycaemic-hyperinsulinaemic clamp at rest (Rest-Control) and at 60 min post-exercise (4×4 min of cycling at 95% of HRpeak). Insulin sensitivity was determined by glucose infusion rate relative to body mass (GIR, $ml \cdot kg^{-1} \cdot min^{-1}$) as well as GIR per unit of insulin (M-value). Blood samples and 5 muscle biopsies were obtained; two at the Resting-control session, one before and one after clamping, and three in the Exercise session, at rest, 60min post-exercise and after the clamp.

Exercise increased serum ucOC (6.4±2.1%, p= 0.013) but not total OC (p>0.05). Blood glucose was ~6% lower and insulin sensitivity ~35% higher post-exercise compared with control (both p<0.05). P-AKT was higher after exercise and insulin compared to exercise alone (no insulin, ~2 fold, p=0.006,) and insulin alone (no exercise, ~1.8 fold, p=0.029). In a multiple-linear regression model that included BMI, age and aerobic fitness, ucOC was an independent predictor for whole body insulin sensitivity at rest and post-exercise (β=0.59, p=0.023 and β= 0.66, p=0.005, respectively) as was BMI.

Insulin sensitivity at rest and post-acute exercise is related to circulating levels of ucOC in obese men. Whether ucOC has a direct effect on skeletal muscle insulin sensitivity after exercise is yet to be determined.

[**OR11**] Table

	Major osteoporotic fracture	*Hip fracture*
Allopurinol	1.075 (1.031–1.121) p<0.001	1.060 (0.990–1.134) p=0.093
Allopurinol + gout diagnosis	1.211 (1.093–1.343) p<0.001	1.188 (1.007–1.403) p=0.04

OR13

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

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Glucocorticoids (GCs) are widely used in the treatment of rheumatoid arthritis (RA), however, the function of endogenous GCs in the pathogenesis and maintenance of RA is less clear. The current study aimed to determine whether and how disruption of endogenous GC signaling in chondrocytes modulates the course and activity of K/BxN serum-induced arthritis in mice.

Chondrocyte-targeted GR knockout (chGRKO) mice were generated by breeding $GR^{flox/flox}$ mice with tamoxifen-inducible Cre mice (Col2a1-$CreER^{T2}$). chGRKO mice and their Cre-negative $GR^{flox/flox}$ littermates (WT) were injected with tamoxifen at 4-weeks of age. Arthritis was induced at 8 weeks of age via injection of K/BxN serum ("chGRKO-K/BxN" and "WT-K/BxN", n=10). Arthritis was monitored daily using an established scoring system. RNA was isolated from inflamed ankle joints at days 7 (D7) and 14 (D14) for gene expression analysis.

Both chGRKO and WT mice developed acute arthritis following injection of K/BxN serum. From D6 onwards, arthritis scores were significantly higher in chGRKO than in WT mice, suggesting that disruption of endogenous GC signaling in chondrocytes aggravates K/BxN serum-induced arthritis. This increased inflammatory activity in chGRKO mice was associated with significantly higher expression of IL-1β (4.4-fold) and neutrophil-recruiting chemokines: CXCL1 (4.6-fold), CXCL2 (2.8-fold), CXCL5 (3.6-fold), CCL7 (2.3-fold) and their receptors: CXCR1 (2.1-fold) and CXCR2 (4.3-fold) on D7 (compared to WT mice). In addition, flow cytometry analysis of spleens harvested on D7 confirmed a significant expansion of CXCR2 positive neutrophils in chGRKO mice (compared to WT littermates). While the above cytokine and chemokine gene expression changes were less pronounced on D14, the expression of MMP-9, an enzyme involved in cartilage degradation, was significantly up-regulated in chGRKO mice.

We conclude that chondrocytes modulate autoimmune arthritis via a GR-dependent pathway through the regulation of neutrophil activity and cartilage degradation.

OR14

Comparison of the Effect of Calcilytics and Teriparatide in Activating Mutations of Calcium-Sensing Receptor (CaSR) Knock-in mice Model of Autosomal Dominant Hypocalcemia (ADH)

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Activating mutations of calcium sensing receptor (CaSR) cause autosomal dominant hypocalcemia (ADH). ADH patients exhibit reduced PTH secretion, hypocalcaemia, hyperphosphatemia, and hypercalciuria. The current treatment of choice for ADH is active vitamin D_3, but PTH replacement has also been investigated. However, neither of them can reverse all the abnormalities of ADH. Because calcilytics stimulate endogenous PTH secretion and reduce urinary Ca excretion, they can become a specific therapeutic agent for ADH. Previously, we demonstrated that a calcilytic, JTT305, suppressed the exaggerated response to extracellular Ca^{2+} in HEK cells transfected with CaSR genes with activating mutations in vitro.

In order to clarify the effect of calcilytics on ADH in vivo, we generated two strains of ADH model knock-in (KI) mice with activating mutations of human CaSR (C129S, A843E). These KI mice exhibited hypocalcemia, hyperphosphatemia, hypercalciurea, renal calcification, reduced serum $1,25(OH)_2D$, urinary cAMP excretion and renal CYP27b1 mRNA encoding 1α-hydroxylase, mimicking almost all the features of ADH patients. A843E KI mice exhibited more prominent phenotypes as in human. Similar to hypoparathyroidism patients or PTH null mice, both KI mice showed increased bone mineral density with low bone turnover. Treatment with JTT305 rapidly increased serum PTH, improved serum and urine Ca/Pi levels, increased urinary cAMP and renal CYP27b1 mRNA expression in a dose-dependent manner. After long-term treatment with JTT305, serum Ca recovered to normal range, and urinary Ca excretion decreased with no renal calcification. JTT305 also increased BMD in both wild type and KI mice with higher bone remodeling. In contrast, teriparatide replacement improved serum Ca/Pi in KI mice, but urinary Ca remained elevated with development of renal calcification.

In conclusion, JTT305 but not teriparatide reversed all the abnormalities in bone and Ca/Pi metabolism in mice models of ADH. Calcilytics can become a promising therapeutic agent for ADH.

OR15

Disruption of Glucocorticoid Signaling in Osteoblasts Attenuates Surgically Induced Osteoarthritis

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Transgenic (tg) overexpression of the glucocorticoid (GC) inactivating enzyme, 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), under the control of a 2.3 Kb collagen type I promoter (Col2.3-HSD2), abrogates intracellular GC signalling in mature osteoblasts and osteocytes. Since cells of the osteoblast lineage play a role in the development of osteoarthritis (OA), we investigated the impact of osteoblast/osteocyte-targeted disruption of GC signalling in a rodent model of surgically induced OA.

We induced OA in 22-week-old male Col2.3-HSD2 tg mice (tg-OA, n=7) and their wildtype littermates (WT-OA, n=7) by surgical destabilization of the medial meniscus (DMM). Six tg and 6 WT control mice were sham operated (tg-CTR & WT-CTR). Knee joints were harvested 8 weeks after surgery.

At endpoint, WT-CTR and tg-CTR mice displayed no signs of arthritis as assessed by micro-CT and histology, whereas WT-OA and tg-OA animals developed features of significant cartilage degeneration and osteophytes formation. Tibial subchondral bone volume (BV/TV, by micro-CT) in the lateral 1/3 region of the medial subchondral compartment was significantly increased in WT-OA but not in tg-OA mice ($p<0.01$ and $p<0.001$, compared to respective controls). Similar changes were observed in the lateral 1/2 region of the medial subchondral compartment. Histology showed significantly less cartilage erosion in the medial femoral condyle and tibial plateau ($p<0.01$) and medial tibial plateau (MTP) ($p<0.001$) of tg-OA mice compared to their WT-OA littermates. Moreover, osteophyte formation (as assessed by histology) was less pronounced in the MTP of tg-OA compared to WT-OA mice ($p<0.01$).

We conclude that GC signalling in mature osteoblasts and osteocytes promotes the development of subchondral-bone hyperplasia, cartilage erosion and osteophyte formation in a mouse model of surgically induced osteoarthritis.

OR16

Noncanonical Wnt5a Enhances Wnt/b-catenin Signaling Through the Up-regulation of Lrp5/6 During Osteoblastogenesis

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Wnt ligands regulate bone formation through beta-catenin-dependent canonical and –independent noncanonical Wnt signaling pathways. The two signaling pathways cooperate with each other during osteoblastogenesis, although non-canonical Wnt5a antagonizes the canonical Wnt pathway in various types of cells. Here, we show that Wnt5a up-regulates the expression of Lrp5/6, co-receptors for the canonical Wnt pathway, thereby enhances osteoblastogenesis.

(1) Pretreatment of ST2 cells with Wnt5a enhanced canonical Wnt3a-induced Tcf/Lef transcription activity. (2) The expression of Wnt5a, Wnt10b and Lrp5/6 but not Ror2, a co-receptor of Wnt5a, was increased in osteoblast-lineage cells from calvariae (calvarial cells) under osteogenic culture conditions. (3) Short hairpin RNA-mediated knockdown of Wnt5a, but not treatment with Dkk1, which disrupts Lrp5/Wnt interactions, reduced the expression of Lrp5/6 in calvarial cells under osteogenic culture conditions. (4) Calvarial cells from Wnt5a-deficient mice exhibited impaired mineralization in culture. The expression level of Lrp5/6 in Wnt5a-deficient calvarial cells was lower than that in wild-type cells. This finding was further confirmed by reduced expression of Axin2, a target gene of the canonical Wnt pathway, in Wnt5a-deficient calvarial cells. (5) Treatment with recombinant Wnt5a up-regulated Lrp5/6 expression, and rescued the impaired mineralization in Wnt5a-deficient calvarial cells. (6) Adenovirus-mediated gene transfer of Lrp5 into Wnt5a-deficient calvarial cells rescued their phenotypic features. (7) Treatment with recombinant Wnt5a and BMP-2 up-regulated Sp7 expression, and then, overexpression of Sp7 up-regulated Lrp5 expression in wild-type calvarial cells. (8) microCT analysis showed that osteoblast-lineage-specific Wnt5a-deficient mice (Osterix-Cre: Wnt5a-floxed mice) exhibited low bone mass, but osteoblast-lineage-specific Ror2-deficient mice (Osterix-Cre: Ror2-floxed mice) did not.

These results suggest that Wnt5a-induced noncanonical signals enhance the canonical Wnt pathway through up-regulation of Lrp5/6 to achieve proper bone formation, and that the stimulatory effect of Wnt5a on the canonical Wnt pathway is independent of Ror2-mediated signals.

OR17

Molecular Mechanism of Glucocorticoid (GC)-induced Autophagy in Osteocytes

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Mechanosensory osteocytes (OYs) are the most abundant and long-lived (up to decades) cells entombed in bone. The longevity of osteocytes is achieved, in part, by their unique ability to adapt to enviromental and chemical stresses and elicit cell survial response mechanisms, e.g., autophagy; among which mTORC (mTORC1 and mTORC2) and FoxO signaling complexes are rapidly emerging as key regulators.
Here we investigate the role of mTORC and FoxO signalling in glucocorticoid (GC)-induced OY autophagy. For this, we established an in vitro system to induce autophagy in OYs (MLO-Y4 cells) using dexamethasone (dex) as stimulus. Autophagic flux was monitored using CytoID and mCherry-EGFP double-tagged LC3 construct which enables the rapid visualization and quantification of acidified vs neutral autophagic vesicles. Additionaly, autophagic signaling pathways induced by dex-treatment were monitored by immunoblotting. Our data indicate that dex dose-dependently inhibits AKT/mTORC1 signaling as well as activating the FoxO3a via inhibition of mTORC2/AKT/ERK signaling pathways to induce autophagy. In the mTORC1 pathway, Akt phosphorylation at both Ser473 (phosphorylated by mTORC2) and Thr308 (phosphorylated by PI3K-PDK1) were significantly inhibited (from 14 hrs). This coincided with inhibition of mTORC1 activity demonstrated by the inactivation of S6K1, a direct substrate of mTORC1, and the initiation of autophagy. By comparison, inhibition of the mTORC2/AKT signaling cascade also blocked the phosphorylation of ERK resulting in nuclear translocalisation and transcriptional activation of FoxO3a induced autophagy related genes. In addition, we identified two novel GC-responsive genes (i.e., redd1 & mkp1) that were upregulated at both gene and protein levels in OYs following dex-treatment.
Taken together, our data suggest that GC-induced autophagy in MLO-Y4 cells is governed by the intricate crosstalk that exists between mTORC1, mTORC2, and FoxO3a signaling cascades.

OR18

Central Regulation of Bone Mass by SNORD116, a Non-Translated, Imprinted Gene: Potential Role in Skeletal Abnormalities in Prader-Willi Syndrome.

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Here we describe the first non-translated, imprinted pathway from the brain to bone, and its potential role in disease.
SNORD116 exists as a 29 copy cluster of unknown function; it is not translated but produces small nucleolar RNA's (snoRNA). SNORD116 is widely expressed in the brain in humans and is exclusively expressed in the brain of mice.
SNORD116 resides within the Prader-Willi Critical Region, and is therefore imprinted, with the maternal copy silenced. Prader-Willi Syndrome (PWS) individuals display additional dysfunction and/or deletion of the non-silenced paternal allele. Loss of SNORD116 has recently been associated with the PWS phenotype; obesity, insatiable appetite, cognitive impairment, hormonal imbalance, short stature and osteoporosis.
Consistent with reduced stature in PWS, Snord116 KO mice showed delayed skeletal development, with reduced bone size and mass in femurs and vertebrae of both sexes. The osteopenia in Snord116 KO was due to reduced cortical bone volume and cortical mineral apposition rate, with no change in cancellous bone. Importantly, the reduced cortical bone formation was evident in skeletally mature mice, indicating ongoing suppression of osteoblast activity by loss of Snord116 expression in the brain, beyond developmental processes.
These skeletal changes were consistent with reduced somatotropic axis activity; with reduced serum IGF-1 levels in Snord116 KO mice.
The site of Snord116 activity in bone was isolated to the hypothalamus, with viral-mediated over supply of Snord116 enhancing the skeletal phenotype of Snord116 KO mice. Moreover, this response indicates that normal skeletal homeostasis requires a physiological range of Snord116 expression.
In conclusion, SNORD116 deletion in mice recapitulates the short stature, low BMD and somatotropic imbalance of PWS. Moreover, it demonstrates for the first time, that non-translated RNA, expressed solely within the brain can regulate bone mass in health and disease; highlighting the potential complexity of central regulation of bone mass.

OR19

Thymosin β_4 Enhances Mechanical and Morphological Properties of Healing Murine Fractures

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Thymosin β_4 ($T\beta_4$) is a peptide with multi-functional regenerative properties. Clinical trials are investigating the potential use of $T\beta_4$ in accelerating corneal, dermal and cardiac wound repair. We hypothesised that treatment with $T\beta_4$ may also promote healing of fractured bone.
In a previous pilot study we showed that $T\beta_4$ enhanced the integrity of healing murine fibular fractures. Here we extend these studies to further characterize mechanical properties of fractures and analyse morphological changes of fracture callus from mice treated with $T\beta_4$.
Mice received bilateral fibular fractures and were given i.p. injections of either $T\beta_4$ (6 mg/kg) or saline at 0-, 3-, 6-, 9- and 12-days post-fracture. Calluses from saline- and $T\beta_4$-treated mice were analysed for: (i) mechanical properties at 42 day post-fracture using three-point bending and (ii) composition

at 14- and 21-days post-fracture using micro-computed tomography (μCT), histology and histomorphometry.
Mechanical testing revealed that $T\beta_4$ treatment enhanced peak force to failure (41% increase, P<0.01) and stiffness (25% increase, P<0.05) of healing fractures compared with controls.
μCT analysis of 14 day calluses showed no differences in any measured parameter between treated and control animals. By 21 days post-fracture, the fractional volume of mineralised tissue and highly mineralised tissue were increased (18%, P<0.01 and 26%, P<0.05 respectively) in calluses from $T\beta_4$-treated mice compared with controls. Histomorphometry complemented the μCT data; at 21 days post-fracture, $T\beta_4$-treated calluses were 23% smaller (P<0.05), had almost half the amount of old cortical bone (P<0.05) and had 31% more new trabecular bone area/total callus area fraction compared with controls (P<0.05).
Overall, results from this study demonstrate that $T\beta_4$ enhances bone formation and mechanical integrity in healing murine fractures. As such, $T\beta_4$ may prove to be a suitable therapeutic agent for promoting bone fracture healing.

OR20
Osteoblast Lineage Specific Knockout of Chemokine MCP1 Blocks the Anabolic Effect of PTH on Bone

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Gene array studies showed that the chemokine MCP1 was the highest induced gene during anabolic PTH treatment in rats. Immunochemistry showed MCP1 expression in bone lining cells, presumably osteoblasts, after PTH treatment. A PTH dependent increase in MCP1 in osteoblasts was also observed using in vitro cell lines. Therefore the osteoblast seems to be a reasonable source for the MCP1 increase in the anabolic effect of PTH, rather than osteoclast. MCP1 gene knockout (KO) mice completely lost the anabolic effect of PTH on bone volume (BV/TV). Although bone formation rate was unaffected by MCP1 KO the increased osteoid failed to mineralize, possibly due to a lack of increase in osteoclast numbers in PTH treated animals. The blunting of the anabolic effect of PTH in global MCP1 KO suggests that the chemokine signalling is necessary for PTH actions. A cell specific knockout approach was used to test the hypothesis that osteoblast-lineage (osteoblast/osteocyte) specific expression of MCP1 is necessary for the anabolic effect. The type1 collagen promoter (COL-CRE) was used to drive CRE recombinase in conjunction with a floxed (flanking LoxP sites) MCP1f/f allele. Of all microCT parameters tested, the most sensitive to the anabolic PTH treatment is connectivity density (ConnD) which measures the interconnectedness of trabecular bone. In wildtype animals, PTH resulted in a 65% increase in vertebral ConnD (p=0.0001) while global MCP1 KO showed a 3% change (p=0.63). In the appropriate control animal (CRE-negative MCP1f/f) PTH increased ConnD by 68% (p=0.0001) as expected. In contrast, in the animal where CRE deletes MCP1 in osteoblast/osteocyte (CRE+ MCP1f/f) the PTH mediated increase in ConnD was severely blunted and not significant (18%, p=0.22).
These data support the hypothesis that osteoblast-lineage expression of MCP1, resulting from PTH stimulation, is an essential component of the PTH anabolic effect in mice.

OR21
The Anabolic Actions of CYP27B1 Expression in Mature Osteoblasts

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The hormone 1,25-dihydroxyvitamin D (1,25D) has been shown to regulate bone formation and bone resorption. A conclusion made by some, is that the overall activity of 1,25D plays a negative role on bone mineral levels. However, we have demonstrated that cells of the osteoblast lineage convert 25-hydroxyvitamin D (25D) to 1,25D by virtue of the activity of the CYP27B1 enzyme, which appears to be necessary for the regulation of osteoblastic activity. To test these findings, we have created two genetically modified mouse models in which Cyp27b1 expression is either ablated or enhanced within osteoblasts. $Cyp27b1^{loxP/loxP}$ and $Osteocalcin^{Cre+}$ mouse lines were used to generate Osteoblast-specific Cyp27b1-KO mice ($ObCYP27B1^{-/-}$ mice). Transgenic mice were also generated in which Cyp27b1 gene transcription is increased specifically in osteoblast cells by virtue of a human Cyp27b1 transgene driven by the 3.6 kb human osteocalcin promoter (ObCYP27B1-Tg). For each line, altered CYP27B1 activity within osteoblasts does not affect serum calcium or 1,25D levels. In female $ObCYP27B1^{-/-}$ mice at 6 weeks of age, vertebral BV/TV% was decreased (-18%, P<0.01) compared to $Cyp27b1^{loxP/loxP}$ littermates, largely as a result of reduced TbTh (-12%, P<0.01). Conversely, in 7 week old ObCYP27B1-Tg transgenic mice BV/TV% was increased within the vertebra (+14%, P<0.01), associated with an increase in TbTh (+9%, P<0.01). Serum levels of alkaline phosphatase were increased in ObCYP27B1-Tg mice (+14%, P<0.05), with no apparent change in bone resorption as measured by Oc.N/BS and serum cross-laps. These data are supported by in vitro models that demonstrate that osteoblast cells convert 25D to 1,25D with subsequent changes in gene expression. Our data support a physiological role for CYP27B1 activity in osteoblasts to promote bone formation, consistent with our previous studies that demonstrate the necessity of adequate serum 25D levels to optimise bone formation.

OR22

MRI Sequence-Specific Bone Marrow Lesions in Human Knee Osteoarthritis are Characterised by Different Degrees of Degenerative Change to Cartilage and Subchondral bone

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MRI-identified bone marrow lesions (BMLs) are discrete subchondral bone abnormalities that have potential prognostic value in osteoarthritis (OA). BMLs can be present/absent depending on the MR sequence used. There is limited knowledge of what a BML represents at the cartilage-subchondral bone tissue-level, particularly for BMLs detected by different MR sequences. The present study addressed this question for human knee OA.

Tibial plateaus were obtained from 46 knee OA arthroplasty patients (24 men, 22 women; mean age 68±8 years). To identify BMLs, ex vivo MRI scans were performed using specific T1- and PDFS-weighted sequences. Cartilage volume was quantitated from MR images. Whole tibial plateaus were micro-CT imaged prior to sampling cartilage-subchondral bone containing BML/No-BML for OARSI grading and histomorphometric assessment of bone remodelling and microdamage.

No MRI pathology was detected for 20% tibial plateaus (No-BML). BMLs were detected in 78% specimens; 2% had subchondral cyst. Of all BMLs, 64% were seen only by PDFS sequences (PDFS-BML) with 36% seen by both sequences (T1/PDFS-BML). A high OARSI score accompanied by reduced cartilage volume was observed for T1/PDFS-BML compared to No-BML ($p<0.02$, $p<0.007$) or PDFS-BML ($p<0.03$). T1/PDFS-BML had increased subchondral BV/TV ($p<0.04$), Tb.Th ($p<0.02$), and decreased TB.Pf ($p<0.02$), SMI ($p<0.0005$) compared to No-BML. Osteoid volume and thickness were higher for T1/PDFS-BML compared to No-BML ($p<0.005$, $p<0.0008$) or PDFS-BML ($p<0.03$, $p<0.02$). Osteoid and erosion surfaces, microcrack and diffuse microdamage parameters were not different between BML/No-BML.

PDFS and T1 sequences appear to detect BMLs representative of different stages of OA disease progression. The characteristic features of subchondral bone in the 'late stage' T1/PDFS-BMLs may be an adaptive modelling response to minimise the accumulation of microdamage. Further tissue-level characterisation of MRI sequence-specific BMLs will inform on their use as potential biomarkers for monitoring OA progression/therapy, as well as providing novel insights into the pathophysiology of OA.

OR23

Closing the Gap in Osteoporosis Management: Implementation and Outcome Analysis of Secondary Fracture Prevention Programs

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We present three analyses relating to Secondary Fracture Prevention programs (SFPP) that have been instituted to address the gap in osteoporosis management:

(1) Systematic review and meta-analysis of 42 publications on SFPPs published between 1996 and 2011: Outcome measures extracted included bone mineral density (BMD) testing and osteoporosis treatment initiation rates. Studies were grouped into 4 models from Type A (assessment & treatment) through to type D (patient education only). Meta-analyses demonstrated increased BMD testing ($p=0.06$) and treatment initiation rates ($p=0.03$) with increasing intervention intensity.

(2) A 2-year RCT of 102 patients initiated on oral bisphosphonate therapy at the Concord SFPP, randomised to 6-monthly follow-up with the SFPP (Group A) or primary care physician follow-up (Group B). Compliance & persistence were measured using claims data and their predictors analysed. At 24-months, medication possession ratio (MPR) and persistence were high and similar in both groups. In the adjusted analysis, patients in group A were not more likely to be compliant or persistent than those in group B, indicating that initiation of therapy within an SFPP is associated with high long-term therapeutic adherence. (3) In a 7-year prospective study, we determined predictors of refracture amongst 234 subjects managed by the Concord SFPP. In multivariate analysis, comorbidity (HR 2.04 if >3, 95%CI 1.10–3.79), corticosteroid use (HR 1.75, 1.12–2.73), total hip BMD (HR 1.36 per 0.1g/cm^2 decrease, 1.08–1.70) and a MPR of <50% (HR 3.36, 1.32–8.53) were significantly associated with refracture, indicating patients with these criteria are at high refracture risk, requiring intensive management.

Our results demonstrate that (a) intensive SFPPs (Type A) are effective in raising treatment rates; (b) following treatment initiation by the program, patients are likely to adhere to therapy outside the SFPP; and (c) therapeutic compliance remains the major determinant of refracture.

OR24

Evidence that Metal Wear Particles Exert a Pro-osteoclastogenic Effect via Osteocytes.

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Aseptic loosening of orthopaedic implants following total hip replacement (THR) has been associated with the induction of osteoclastogenesis. Osteocytes have been shown to have a central role in mediating osteoclastogenesis [1, 2] and we have

reported that polyethylene wear particles induce a pro-catabolic phenotype in osteocytes [3]. It is therefore possible that osteocytes play a role in the response to metal particle wear. Our aim was to analyse the short-term response of mouse and human osteocyte-like cells exposed to metal wear particles in vitro. Human trabecular bone derived osteoblasts were differentiated for 28 days under mineralising conditions prior to treatment, which we have shown induces a mature osteocyte-like phenotype. The mouse osteoblastic cell line IDG-SW3, which differentiates to a mature osteocyte stage cells was also cultured for 28 days under mineralising conditions. The resulting osteocyte-like cells were treated with varying concentrations of micron-sized titanium/aluminium and cobalt chrome wear particles for 48 hours. RNA was extracted and used for the analysis of gene expression using real time RT-PCR. Similar to our previous findings with polyethylene wear particles [3], metal particles showed evidence of promoting a pro-osteoclastogenic phenotype in osteocytes, evidenced by the induction of a high RANKL:OPG mRNA expression ratio in human primary osteocyte-like cells and differentiated mouse IDG-SW3 cells. Furthermore, the mRNA expression of genes associated with osteocytic osteolysis (CA2, CTSK, MMP13) [4] increased in both cell types. SOST mRNA expression was also increased. We have reported that the protein product of SOST, sclerostin promotes osteoclastogenesis by induces the expression of RANKL in mature human osteocyte-like cells in vitro[2]. In summary, osteocytes exposed to metal wear particles showed increased expression of genes associated with osteoclastogenesis and osteocytic osteolysis, identifying osteocytes as potentially important regulators of bone loss in peri-prosthetic loosening.

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OR25

A role for V-ATPase V0 domain subunit e1 in bone homeostasis

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Vacuolar-type H^+-ATPases (V-ATPases) proton pumps function to acidify a diverse range of intracellular organelles required to sustain cellular homeostasis. In osteoclasts (OCs), V-ATPases are uniquely enriched on the surface of the ruffled border membrane where they serve to acidify the underlying extracellular milieu, a prerequisite for bone resorption. The mammalian V-ATPase complex is composed of at least 14 subunits (organized into two functionally and structurally distinct domains, the cytoplasmic V1 and the membrane-embedded V0), of which the hydrophobic V0 domain e subunit(s) (i.e. e1 and e2 paralogs) remain poorly characterized. Here, using the Cre-LoxP system, we demonstrate that e1 is the major functional isoform expressed in OCs. Whereas mice with conditional knockout (cKO) of the e2 isoform in either mature OCs (Cathepsin K ($CtsK^{Cre}$) or OC precursors ($RANK^{Cre}$) have normal bone mass, conditional deletion of the e1 counterpart results in severe OC-rich osteopetrosis ($CtsK^{Cre}$-e1 cKO mice; designated $e1^{\Delta OC}$ herein) and/or embryonic lethality ($RANK^{Cre}$-e1 cKO), respectively. Histomorphometric assessment revealed that the marrow spaces of femurs from $e1^{\Delta OC}$ mice were completely occluded by unresorbed bone. Consistently, deletion of e1 significantly impaired the bone resorptive function of OCs derived from $e1^{\Delta OC}$ spleen cells, but did not alter overall OC differentiation. Mechanistically, this impairment is due to a disruption in extracellular and intracellular acidification as evidenced by live cell confocal microscopy and in vitro acidification assays. Interestingly, loss of e1 resulted in destabilization of the V-ATPase complex with reduced expression of V0 a3 subunit and uncoupling of V1 and V0 domain assembly. This disruption was further confirmed by Bioluminescence Resonance Energy Transfer (BRET) proximity assays which found that the integrity of the V0 domain was compromised due to reduced association between critical V0 subunits. Collectively, our data indicate that the V-ATPase V0 domain e1 subunit is an integral component of the OC acidification machinery.

OR26

Neurological Heterotopic Ossification Requires Both Spinal Cord Injury and Macrophage-Dependent Soft Tissue Inflammation

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Neurological heterotopic ossification (NHO) is a frequent complication of spinal cord and traumatic brain injuries and manifests as abnormal ossification of soft tissues near joints. NHO is debilitating, causing pain, joint deformation, ankylosis and vascular and nerve compression. The mechanisms leading to NHO are unknown with complicated and expensive surgical resection, the only effective treatment approach. To elucidate NHO pathophysiology we have developed the first animal model of NHO in genetically unmodified mice. Mice underwent a spinal cord transection (SCI); muscular inflammation was induced by cardiotoxin injection in limbs (IM-CTX). SCI alone or muscular inflammation alone did not induce NHO. The combination of SCI with muscular inflammation was necessary to induce NHO which is consistent with clinical observations (NHO incidence is higher in patients with severe trauma or concomitant infection). Abundant $F4/80^+$ macrophages, which can provide pro-anabolic support in bone formation, were detected within the inflamed muscle and associated with areas of intramuscular bone formation (confirmed by von

Kossa and collagen type 1 staining). In vivo depletion of macrophages with clodronate-loaded liposomes prevented NHO formation. Zoledronate treatment exacerbated NHO. This supports that macrophage-mediated inflammation is a key activator of NHO following SCI.
SCI was necessary for NHO development suggesting that it causes release of systemic factors priming NHO. Indeed, NHO developed in non-paralysed inflamed front limbs with SCI. Also, blood plasma from mice with SCI and IM-CTX induced osteogenic differentiation of cultured muscle mesenchymal progenitor cells (mMPC) sorted from naïve mice.
In conclusion, our model suggests that NHO is a 2-insult process with (1) SCI inducing the release of factors that sensitize mMPC to abnormal osteogenic differentiation in muscles and (2) macrophages accumulating in inflamed muscles then triggering abnormal osteogenic differentiation of mMPC. This study represents a significant advance in the understanding of NHO, revealing two targetable pathophysiologic mechanisms.

OR27

Chondrocytes Mediate the Anti-inflammatory Effects of Therapeutic Glucocorticoids in K/BxN Serum-induced Autoimmune Arthritis

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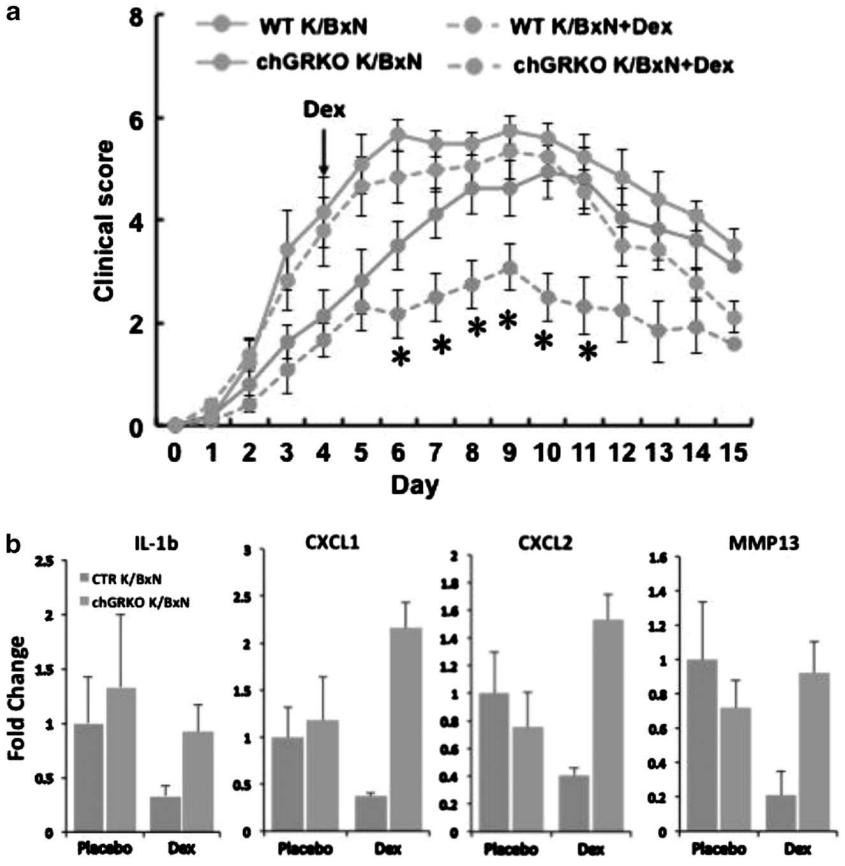
Therapeutic glucocorticoids (GC) are widely used in the treatment of autoimmune arthritis. This study aims to investigate whether chondrocytes have a role in mediating the anti-inflammatory effects of therapeutic GCs in K/BxN serum-induced autoimmune arthritis using a chondrocyte-specific glucocorticoid receptor knock-out (chGRKO) mouse line.
Tamoxifen-inducible chGRKO mice were generated by crossing $GR^{flox/flox}$ mice with Col2a1-$CreER^{T2}$ mice. Four-week-old male chGRKO mice and their Cre-negative $GR^{flox/flox}$ littermates (WT) were injected with tamoxifen (0.1 mg/g/day).

[OR27]



Arthritis was inducted at 8 weeks of age by injection of K/BxN serum (WT-K/BxN and chGRKO-K/BxN) followed by treatment with either placebo or 1 mg/kg/day i.p. dexamethasone (Dex) from day 4 through to day 15. Arthritis was assessed daily (ankle size, clinical score) and inflammation, cartilage degradation and bone erosion were measured by histology at endpoint (day 15). RNA was isolated from inflamed ankle joints for gene expression analysis.
Both chGRKO-K/BxN and WT-K/BxN developed acute arthritis as assessed by clinical score and measurement of ankle size. Dex significantly suppressed joint inflammation in WT-K/BxN mice ($p<0.05$) while chGRKO-K/BxN mice were completely resistant to the anti-inflammatory actions of Dex (Fig. a). Histological analysis revealed that Dex significantly reduced inflammatory activity, cartilage degradation and bone erosion in WT-K/BxN mice ($p< 0.05$ for all parameters, compared to WT-K/BxN placebo). In contrast, Dex treatment had no effect on any of these measures in chGRKO-KBxN animals. Gene expression analysis revealed that treatment with Dex suppressed the expression of pro-inflammatory cytokines (IL-1β), neutrophil recruiting chemokines (CXCL1, CXCL2) and the cartilage-degrading enzyme, MMP13, in WT-K/BxN mice (Fig. b) with no effects on gene expression in chGRKO-K/BxN mice.
We conclude that in KBxN serum-induced arthritis, the therapeutic effects of glucocorticoids are mediated via a GR-dependent signaling pathway in chondrocytes.

OR28

Prolonging Osteocytic JAK/STAT Signalling by Deletion of SOCS3 Results in a Profound Sex-divergent Change in Trabecular Bone Mass

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The SOCS (suppressor of cytokine signalling) family of proteins limit JAK/STAT cytokine signalling. SOCS3 binds directly to receptors that play key roles in bone metabolism, including glycoprotein 130 (gp130), leptin receptor and G-CSF receptor. SOCS3 expression in the osteoblast lineage is stimulated by parathyroid hormone (PTH), mechanical loading and IL-6/gp130 family cytokines, all of which increase bone formation, at least in part, by acting on the osteocyte to inhibit sclerostin expression.
To investigate the role of osteocytic SOCS3 in bone remodelling, we examined the skeletal phenotype of mice with SOCS3 conditionally deleted in osteocytes (DMP1Cre.SOCS3$^{fl/fl}$). Femora were analysed by microCT, and tibiae by histomorphometry, at five different ages.
DMP1Cre.SOCS3$^{fl/fl}$ mice demonstrated a sex-divergent trabecular bone phenotype, after sexual maturation. At 2 and 6 weeks of age, trabecular bone volume (BV/TV) was significantly greater (1.5 to 4.5-fold, $p<0.01$) in both male and female DMP1Cre.SOCS3$^{fl/fl}$ mice compared to age- and sex-matched controls (DMP1Cre.SOCS3$^{w/w}$). However, at 12 weeks of age, while both male and female DMP1Cre.SOCS3$^{fl/fl}$ mice demonstrated a high level of bone remodelling, including significantly greater osteoid, osteoblast and osteoclast surfaces (all $p<0.05$), the resulting effect on trabecular bone mass was profoundly different between sexes. A 7-fold higher BV/TV was observed in female DMP1Cre.SOCS3$^{fl/fl}$ mice ($p<0.0001$), whereas in males, BV/TV was half that of control ($p<0.01$). Females lost 50% of their high bone mass phenotype by 16 weeks, and completely resolved to control levels by 26 weeks. Male DMP1Cre.SOCS3$^{fl/fl}$ mice retained their low BV/TV to 26 weeks.
These data indicate that SOCS3-mediated inhibition of osteocytic JAK/STAT signalling restrains both bone formation and resorption, with the balance being sex- and age-specific. The striking sexually divergent phenotype at 12 weeks indicates that osteocytic JAK/STAT signalling is a key influence that determines sexual dimorphism in adult trabecular bone remodelling and bone mass.

OR29

Effects of Calcium Citrate, Fortified Juice and Dairy Products on Serum Calcium in Postmenopausal Women

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Recent evidence suggests calcium supplementation increases cardiovascular risk. Although the mechanism is presently unclear, it may involve the elevation in serum calcium that occurs following the ingestion of a calcium supplement. Most evidence suggests dietary calcium is not associated with cardiovascular risk, which could be related to its smaller calcaemic effect. The effects of different calcium sources on serum calcium are therefore of interest. We recruited 10 postmenopausal women into a cross-over trial. Participants received each of the following four treatments in random order: 500 mg of calcium citrate fasting, 500 mg of calcium citrate after a meal, 500 mg of calcium as fortified juice, or 500 mg of calcium from dairy products. Blood was sampled before, and 1, 2, 4 and 6 hours after the treatment was ingested. Serum calcium increased significantly from baseline, within the normal range, after each intervention, and remained elevated at 6 hours after calcium citrate and fortified juice. The elevation in serum calcium was identical after calcium citrate fasting and fortified juice. In comparison, the elevation in serum calcium was similar but delayed when calcium citrate was taken with a meal, and was smaller when calcium was taken from dairy products. These findings indicate serum calcium is elevated for at least 6 hours following the ingestion of a calcium supplement, and that this elevation is not diminished when calcium is taken with a meal or as a fortified juice. The smaller increase in calcium after dairy products might explain the difference in cardiovascular risk between supplemental and dietary calcium.

OR30

Explaining Gender Difference in Fracture Risk: The Role of Volumetric Bone Mineral Density

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Men and women with the same age and areal bone mineral density (aBMD) are assumed to have the same risk of a fragility fracture. However, it is not clear whether this assumption holds true for different fracture sites and when volumetric bone mineral density (vBMD), instead of aBMD, is taken into account. The present study aimed to determine the gender difference in any and site-specific fracture risk using volumetric and areal bone mineral density.

This population-based prospective study involved 2279 women and 1379 men aged 57+ years (median: 68), who had been followed for a median of 9 years (range 1–25 years). Femoral neck aBMD (g/cm^2), was measured by dual energy X-ray absorptiometry (GE-Lunar) at baseline. Femoral neck vBMD (g/cm^3), was estimated from aBMD and bone mineral content (BMC) using this published algorithm: vBMD=π/6(BMC/aBMD)2. Low-trauma fracture was ascertained from X-ray reports.

During the follow-up period, 728 (32%) women and 227 (17%) men sustained at least one fracture. On average, aBMD was lower in women than in men (0.81 ± 0.14 vs. 0.93 ± 0.15, P<0.0001). Volumetric BMD, however, was not different between women and men (0.32 ± 0.07 vs. 0.31 ± 0.06, P<0.117). For a given aBMD and age, women had a higher risk of any fracture compared to men (HR: 1.4; 95%CI: 1.18-1.62), but not for hip or vertebral fracture. However, for a given vBMD and age, the risk of fracture was consistently greater in women than men for any fracture (HR 2.04; 95%CI: 1.76-2.36), for hip fracture (HR: 2.02; 95%CI: 1.37-2.96), and for vertebral fracture (HR: 1.93; 95%CI: 1.51–2.45).

These results indicate that women have higher risk of fracture than men for a given age and vBMD. The data also suggest that vBMD migh be a better predictor of fracture risk than aBMD, and that the diagnosis of osteoporosis should be based on vBMD rather than aBMD.

OR31

Quantifying the Extent of the Osteocyte Network: How do the Numbers Stack Up?

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Osteocytes form an extensive cellular network throughout the hard tissue matrix of the skeleton. However due to limitations in imaging techniques, the magnitude and complexity of this network remains undefined.

We have used data from recent papers obtained by new imaging techniques to estimate absolute and relative quantities of the human osteocyte network to form a more complete understanding of the extent of this communication system.

We estimate that the total number of osteocytes within the average adult human skeleton is ~42 billion (roughly 50% of the number of neurons in the brain). We calculate a total number of osteocyte dendritic processes projecting from these cells of ~1.67 trillion, forming a total of 21.5 trillion connections that extend through the skeleton (20% of the number of neural connections in brain). If all the osteocytic processes were connected end-to-end this would extend 175,000 km (>4x the earth's circumference). We calculate the total surface area of the lacuno-canalicular system to be 156 m^2, which is greater than the skin (2 m^2) and equivalent to the floor space prescribed for 1092 free-range chickens. However, the residing osteocytes leave only enough space for 10 mL extracellular fluid, thus microscopic changes in pressure can be readily detected by the osteocyte. Calculations based on measurements in lactation-induced murine osteocytic osteolysis indicate a potential total loss of 14 mm^3 bone in this process. Finally, based on the average speed of remodelling in the adult, we calculate that 8.7 million osteocytes are replenished throughout the skeleton on a daily basis (101/second), indicating the dynamic nature of the osteocyte network.

We conclude that the osteocyte network is breath-taking in its complexity and has great information processing power. Furthermore, continual replenishment of large numbers of osteocytes allows therapeutic changes to the continually renewed osteoblast population to be rapidly incorporated into the skeleton.

OR32

Isolation and Characterisation of Osteocytes from Human Trabecular Bone

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The importance of osteocytes in maintaining bone homeostasis is becoming ever more apparent [1,2]. However, studying primary osteocytes is difficult due to their location within the mineralised bone matrix. Techniques for isolating osteocytes from mouse bone have been described [3] but as yet no such techniques have been documented for human osteocytes. We have developed a method for isolating osteocytes from trabecular bone from patients undergoing knee arthroplasty. Trabecular bone pieces were subjected to sequential digestions in collagenase/EDTA. Cells were harvested after each digest and cultured over a 5 day time course. Osteocyte marker gene expression was analysed by RT-PCR and cell morphology was demonstrated by phalloidin staining and confocal microscopy. Cells from digests 1 and 2 expressed low levels of the osteocyte markers SOST and DMP1, with increased levels in digests 3 and 4. The highest levels were observed in digests 5 and 6, with a 20-fold relative increase in DMP1 and a 9-fold increase

in SOST mRNA compared to digest 1. FGF23 mRNA expression was observed from digest 3 onwards, increasing up to 150-fold in expression in digest 6. The osteocyte markers PHEX and MEPE were also increased in the later digests. The cells isolated in digests 1 and 2 displayed a mixed morphology, with osteoblast-like cells and some dendritic osteocyte-like cells. Digests 3-6 contained many stellate, highly-dendritic cells. Treatment of isolated cells from digests 3-6 with 50 nM PTH or 10 nM $1,25(OH)_2D_3$ for 24 hours resulted in the downregulation of SOST and upregulation of FGF23 mRNA levels, respectively. In conclusion, we have developed for the first time a reproducible method of isolating osteocytes from human bone. The isolated cells express osteocyte markers, display a dendritic morphology and respond to anabolic hormones similar to osteocytes in vivo. Such cells will be invaluable for furthering human osteocyte research.

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OR33

Denosumab Reduces Hip Cortical Porosity in Women with Osteoporosis

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Cortical thickness, area, mass, and porosity are determinants of bone strength and so, contribute to nonvertebral fracture risk. Cortical porosity is a marker of structural decay associated with an exponential worsening in bone fragility and results from unbalanced and accelerated intracortical remodeling upon Haversian canal surfaces. Canals enlarge, coalesce, and fragment the cortex. Reducing remodeling will limit worsening of porosity but for individuals already at increased risk for fracture, reducing porosity is preferred. Using MDCT hip images from the FREEDOM study[1], we previously reported that hip cortical mass and thickness improved over 3 years of denosumab administration. We postulated that this could be explained by infilling of porosity in the inner cortical region adjacent to the medullary canal. Here, we used a subset of these images from FREEDOM to evaluate changes in hip porosity. Percentage porosity in both the compact and the trabecularized (outer and inner transitional zones) cortical volumes of the subtrochanter region were measured using StrAx1.0 software [2] from MDCT hip images obtained at baseline and year 3 (placebo, n=22; denosumab, n=28).

The percentage volume occupied by porosity at baseline was 72% in the inner transitional zone adjacent to the medullary compartment, 37% in the outer transitional zone, and 29% in the compact-appearing cortex. Cortical porosity correlated positively with serum CTX (p=0.017) and negatively with hip strength estimated using finite element analysis (p=0.027). Denosumab reduced porosity compared with baseline and placebo at year 3 across the entire cortex (Figure) and in each compartment, reaching treatment effect (denosumab–placebo) improvements of –1.8% (inner transitional zone), –5.6% (outer transitional zone), and –7.9% (compact-appearing cortex) (all p<0.001).

Since reductions in cortical porosity equate to increased mineralised bone matrix mass and both are relevant to strength, these improvements are expected to contribute to the observed reductions in nonvertebral fractures associated with denosumab administration.

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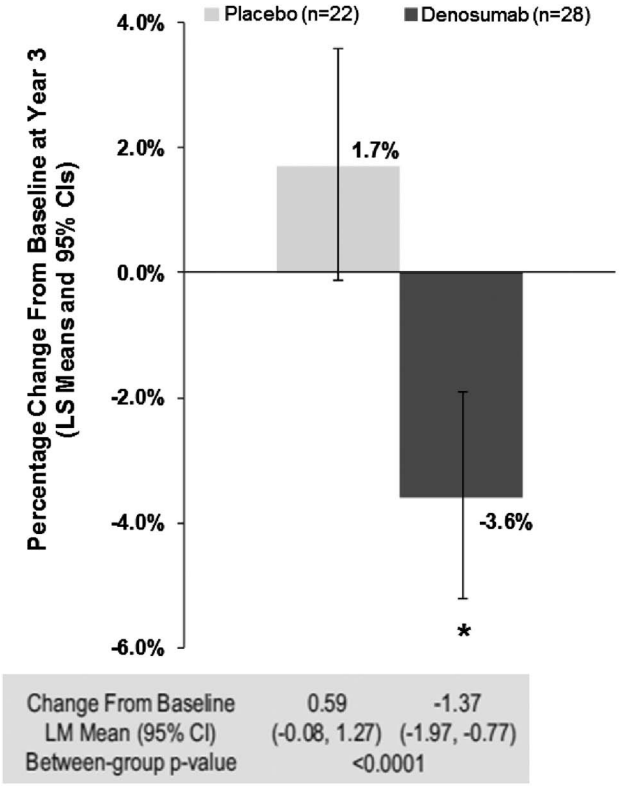

n = number of subjects with available data at baseline and year 3.
*p<0.001 compared with baseline and placebo.

OR34

Effects of Calcium Supplements on Blood Pressure and Blood Coagulation: a Randomised Controlled Trial

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Calcium supplements appear to be associated with increased cardiovascular risk; however the mechanism underlying the increase in risk is presently unclear. We studied the acute and 3-month effects of calcium supplements on blood pressure and their acute effects on blood coagulation. We recruited 100 postmenopausal women into a randomised, placebo-controlled trial. Participants were allocated to calcium (1 g/day as citrate, carbonate or microcrystalline hydroxyapatite) or a placebo containing no calcium. Blood was sampled and blood pressure measured 2 hourly for up to 8 hours after the first dose, and after 3 months of supplementation. Blood pressure was measured on participants from all groups. Systolic and diastolic blood pressure declined from baseline in the control group and all calcium groups over 8 hours. When the calcium groups were pooled, the reduction in blood pressure was less at some time-points in the calcium group compared with the control group. After 3 months of supplementation blood pressure was not different from baseline in the control or calcium groups. Blood coagulation was measured by thromboelastography on participants allocated to calcium citrate or to placebo (n = 36). The coagulability of blood increased over 8 hours in the control and calcium citrate groups. The time to clot initiation was significantly shorter in the calcium citrate group compared with the control group at 4 hours, and the coagulation index (an overall assessment of coagulation status) was significantly increased in the calcium citrate group compared with the control group at 4 hours. We found adverse trends in blood pressure and blood coagulation acutely following the ingestion of a calcium supplement. However, because of the borderline significance of these findings and small size of our trial, they should be investigated in larger studies.

OR35

Secular Decline in Fracture Incidence is Not Associated with Better Post-fracture Outcomes

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During the last decade, hip fracture incidence has declined and life expectancy improved. However, it is unclear whether the outcomes following osteoporotic fracture have changed accordingly. The aim of this study was to compare re-fracture risk and excess mortality following osteoporotic fracture between two cohorts with similar ages but separated in time.
Study participants comprised women and men 60+ participating into DOES1 (recruited from 1989 and born prior to 1930) and DOES2 (recruited from 2000 and born between 1930 and 1945). All fractures excluding head, fingers and toes were recorded. Mortality data was obtained from Dubbo media records. Age-standardised fracture incidence and mortality rates were calculated. The difference in excess mortality between the 2 cohorts was assessed using standardised mortality ratios (SMR) calculated for each study cohort using time-specific population mortality rates.
The prevalence of osteoporosis declined between the 2 cohorts in both women and men by approximately 40%. Correspondingly, the fracture incidence also declined by ~1/3 in both genders. Interestingly, re-fracture risk was similar for DOES2 and DOES1 [women age-adjusted RR 2.0 (95% CI, 1.6-2.5) in DOES1 and 1.9 (95% CI, 1.7–2.3) in DOES2 and men, 3.5 (95% CI, 2.7–4.8) in DOES1 and 3.4 (95% CI, 2.7–4.5) in DOES2]. Crude mortality rates decreased during study follow-up. However, after taking into account the difference in general population life expectancy during the study periods, the excess mortality post-fracture was similar [women, SMR 2.1 (95% CI, 1.7–2.6) in DOES1 and 1.7 (95% CI, 1.2–2.4) in DOES2, and men, 1.9 (95% CI, 1.5–2.5) in DOES1 and 1.9 (95% CI, 1.3–2.7) in DOES2].
Thus despite a reduction in the prevalence of osteoporosis and fracture incidence over the last 2 decades, re-fracture risk and fracture-associated excess mortality was similar. The reasons for this deserve urgent exploration.

OR36

Vascular Calcification Does Not Progress Following Kidney Transplantation

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Vascular calcification (VC) is associated with increased cardiovascular (CV) and all-cause mortality in patients with chronic kidney disease (CKD). Kidney transplantation improves CV mortality, but whether transplantation affects VC progression is unclear. The abdominal aortic calcification score (AACS) assessed from lateral abdominal X-ray correlates to coronary artery calcification and identifies patients at increased CV risk. We examined progression of VC after kidney and simultaneous pancreas kidney (SPK) transplantation using the AACS.
Patients transplanted at Westmead Hospital are assessed using a validated 24-point AACS scale at 4 weeks, 1 year and 5 years post-transplant, with demographic and laboratory data also collected. Changes in VC were assessed by paired T-test and ANOVA. Results are presented as mean and standard deviation, median and interquartile range and mean difference (MD) with 95% confidence intervals (CI).
Baseline to 12 month data was available for 161 patients (51 SPK), with age at transplant 43 years (35–53) and dialysis vintage 18 months (5-54). Baseline AACS was 2.8 ± 5.3 and 2.8 ± 4.8 at 12 months (correlation 0.91; p<0.0001). On paired sample T-test, MD 0.04 ± 2.3 (95% CI -0.31 to 0.39; p=0.84) with no differences in progression by gender, presence of diabetes, kidney or SPK, or use of bisphosphonates or calcitriol in the 1st post transplant year. For 28 patients, 5-year data was available. Age at transplant was 40 years (33–47), 59% male and dialysis vintage 10 months (2–20). Baseline AACS was 3.1 ± 6.1 and 3 ± 5.2 at 5 years, and did not progress; MD 0.04 (95% CI—1.4 to 1.4; p=0.96).

VC progresses rapidly in patients on dialysis and changes are often reported during 12 months observation. However, no differences in VC scores were detected over 1–5 years following transplant, and this is likely to contribute to reduced post-transplant mortality.

OR37

Social Disadvantage and Incident Fractures of the Major Osteoporotic Sites: Data from the Geelong Osteoporosis Study Fracture Grid

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Few data exist that investigate associations between socioeconomic status (SES) and fracture sites other than the hip. We investigated age-specific associations between SES and incident major osteoporotic fractures of the hip, humerus, (clinical) spine and forearm.

Incident fractures that occurred 2006–2007 for men and women aged 50 years and over were identified using a computerized keyword search of all radiological reports from the radiological centers servicing the Barwon Statistical Division (BSD), south eastern Australia. SES was determined by cross-referencing residential addresses with Australian Bureau of Statistics census data and categorized in quintiles based upon the BSD reference range. We tested the frequencies of observed fractures for each quintile of SES using chi square comparison, and calculated age-specific fracture incidence across SES quintiles.

During 2006–2007 we identified 1,869 incident fractures (495 hip; 295 humerus; 950 spine; 129 forearm) in 543 men and 1,326 women. Differences existed between observed vs. expected fractures across SES quintiles ($p \leq 0.001$). SES showed a linear association with fracture incidence, whereby the greatest proportion of fractures was observed in the most disadvantaged SES quintile and the lowest proportion observed in the least disadvantaged quintile. When stratified by 10 year age groups, the pattern was consistent, with the exception of 50–59 years where no association with SES was observed.

Socially disadvantaged individuals have higher incidence of fracture at the major osteoporotic sites compared to less disadvantaged individuals; this reflects the well-documented social gradient of many other chronic diseases. The differences between observed and expected fracture incidence across SES quintiles appear large enough to warrant public health concern.

OR38

An International Comparison of Quality of Life Utility Changes During the First 4 Months Following Osteoporotic Fracture in Australian Adults (AusICUROS)

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In 2009 the International Costs and Utilities Related to Osteoporotic Fractures Study (ICUROS) was initiated in eight centres across Australia (AusICUROS). Insufficient Australian data were available for hip and vertebral fractures to contribute to the first ICUROS publication documenting the protocol and early quality of life (QoL) changes worldwide [1]. Following the same methodology [2] the current work adds Australian data to these international results.

QoL utility scores using the EQ-5D from 2,758 fractured patients from 10 countries were compared with those from 624 Australians (hip: 224; vertebral: 92; wrist: 308). The Australian mean QoL before fracture was amongst the highest internationally for all fracture groups (Table 1a–c). At 4-months post-hip fracture Australian and Swedish patients rated QoL similarly with significant improvements post-fracture but still 15–20% lower than pre-fracture.

Changes in QoL immediately following wrist fracture were similar internationally except for France with a greater decline and the USA where there was a longer lag-time between fracture and assessment. Although the immediate decline in QoL is less dramatic for vertebral fractures, at 4-months the decline is similar to hip fracture patients in Australia, Austria and Russia with other countries demonstrating even less improvement. Higher pre-fracture QoL in Australians regardless of fracture type is associated with larger subsequent declines in QoL (regression analysis; $p<0.05$) consistent with international data. QoL loss was not different between gender or those with and without previous fracture ($p>0.05$).

These results suggest QoL decline following fracture is significant but not greater in Australia than elsewhere. At 4-months post-fracture vertebral fracture patients report similar declines in QoL to hip fracture patients.

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Table 1a to c: Utility scores derived from UK weights[2]
a: Hip fracture participants

OR39

Risk of Fracture in Men with Prostate Cancer on Androgen Deprivation Therapy: a Population-Based Cohort Study in New Zealand

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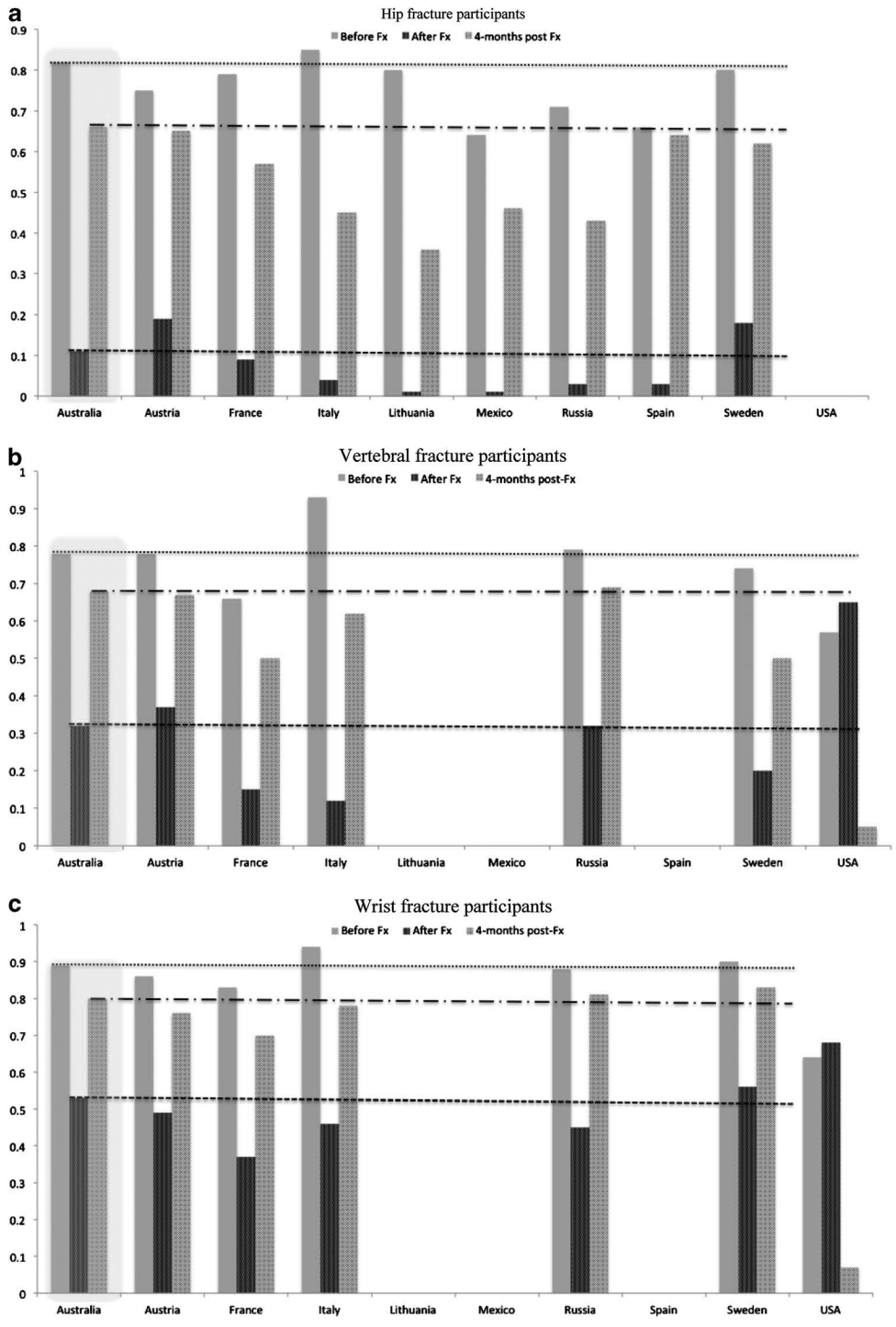
The use of androgen deprivation therapy (ADT) has been reported to accelerate bone loss and increase the risk of

[OR38]



fracture. We aimed to examine the association between different types of ADT and fracture risk in the New Zealand prostate cancer (PCa) population, and to evaluate the subsequent risk of mortality after a fracture.

Using datasets created through linking records from the New Zealand Cancer Registry to the National Minimal Dataset, Pharmaceutical Collection and Mortality Collection, we studied 25544 men (aged ≥40) diagnosed with PCa between 2004 and 2012. ADT was categorized into the following groups: gonadotropin-releasing hormone (GnRH) agonists, anti-androgens, combined androgen blockade (GnRH agonists plus anti-androgens), bilateral orchiectomy, and bilateral orchiectomy plus pharmacologic ADT (anti-androgens and/or GnRH agonists).

Among patients receiving ADT, 10.8% had developed a fracture as compared to 3.2% of those not receiving ADT ($p<0.0001$). After controlling for age and ethnicity, the use of ADT was associated with a significantly increased risk of any fracture (OR = 2.83; 95% CI 2.52 to 3.17; $p<0.0001$) and of hip fracture requiring hospitalization (OR = 1.82; 95% CI 1.44 to 2.30; $p<0.0001$). With respect to different types of ADT, those who received combined androgen blockade consisting of GnRH agonists plus anti-androgens (OR = 3.48; 95% CI 3.07–3.96) and bilateral orchiectomy with pharmacologic ADT (OR = 4.32; 95% CI 3.34–5.58) had the greatest risk of fracture. Men experiencing a fracture had a 1.91-fold higher mortality risk than those who did not (95% CI 1.78 to 2.06).

ADT was significantly associated with an increased risk of any clinical fracture and hip fracture requiring hospitalisation in men with PCa. Men who experienced a fracture after the diagnosis of PCa was associated with decreased survival. Identification of those at higher risk, and monitoring and managing their bone health are critical.

OR40

Loss of vitamin D Receptor in Breast Cancer Cells Enhances Bone Metastasis in a Murine Model of Breast Cancer

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Breast cancer is one of the most prevalent malignancies and although diagnostic and therapeutic strategies have consistently improved over the past decades, up to 40% of patients will eventually develop bone metastases. We have previously demonstrated that vitamin D deficiency promotes the growth of human breast cancer cells implanted into the tibiae of nude mice. In the current study we aimed to define the role of the vitamin D receptor (VDR) in systemic breast cancer cell metastasis.

VDR expression was knocked down in the human breast cancer cell line, MDA-MB-231 ($MDA^{VDR-/-}$). Knock-down efficiency was ~80% compared to non-target (NT) controls. $MDA^{VDR-/-}$ and NT cells were transfected with a luciferase gene, and cells were injected into the left ventricle of female nude mice (n=11 for each, $MDA^{VDR-/-}$ and NT). Systemic spread and growth of tumour cells were monitored by sequential in vivo bioluminescent imaging (BLI), high resolution X-ray and µ-CT imaging for a period of 30 days.

Compared to animals injected with NT-cells, mice receiving $MDA^{VDR-/-}$ cells developed more metastases at 5 ($p=0.009$) and 10 days ($p=0.03$) following intra-cardiac injection (Fig.1), with light emission measurements generating significantly higher values at all time points in $MDA^{VDR-/-}$ mice ($p<0.01$). After 20 days, multifocal metastases were observed in all animals and tumours were visible on X-ray. However, mice injected with $MDA^{VDR-/-}$ cells had significantly larger bone lesions compared to control mice ($p<0.01$).

We conclude that knockdown of the VDR in human breast cancer cells increases their systemic metastatic potential and promotes intra-skeletal growth, resulting in significantly greater tumour burden in mice injected with $MDA^{VDR-/-}$ cells. Our results indicate that the VDR itself impacts breast cancer cell invasiveness and growth.

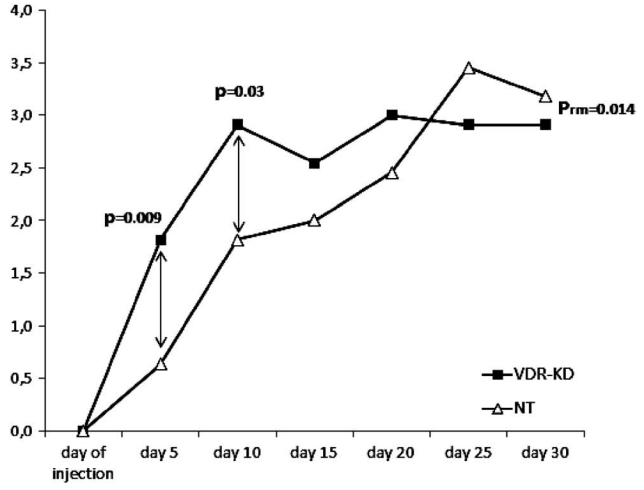

Fig.1. Graphs demonstrate the average number of metastatic bones for each group ($MDA^{VDR-/-}$ and NT) on different time points. There was a significant difference between the two groups at five ($p=0.009$) and ten days ($p=0.03$) following intra-cardiac tumor cell injection. Moreover, a significant difference in the run of both curves could be shown (p-value for repeated measures [$p_{rm}=0.014$]).

OR41

Deletion of Furin in Osteoclasts Leads to a High Bone Mass Phenotype: A Role of Furin in Osteoclastic Bone Resorption

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Using comparative microarray analysis on osteoclasts and macrophage-progenitors, we investigated the expression profile of nine members of the proprotein convertase family that regulate proteolytic maturation of proteins. Among these, furin was identified as the most abundantly expressed

member in osteoclasts, but the role of osteoclastic furin in bone remodeling remains unknown. The global deletion of furin leads to embryonic lethality precluding investigations of its role in postnatal bone. To address the physiological role of furin in osteoclast biology, we generated osteoclast-specific furin conditional knockout mice under the control of Cathepsin K-Cre recombinase (Furin$^{\Delta OC}$) and the bone phenotype was assessed. MicroCT analysis of 12 week old female mice demonstrated a significant increase in trabecular bone volume in the femora of Furin$^{\Delta OC}$ mice compared to floxed littermate controls (by 62.0%, p=0.003). Histomorphometric analysis revealed a trend of decrease in the number and surface area of osteoclasts in Furin$^{\Delta OC}$ mice in vivo. However, osteoclast formation and fusion was not significantly affected by furin deletion in vitro. In comparison, osteoclast bone resorptive function was significantly impaired from the lack of furin by reduced CTX (by 32%, p=0.037) and reduced bone resorption pit depth (by 51%, p=0.002). Consistent with the role of furin as the primary processing enzyme of the proton pump v-ATPase accessory subunit Ac45, proteolytic maturation of Ac45 was significantly reduced in osteoclasts lacking furin. In line with the altered Ac45 processing and disrupted function of v-ATPase, intracellular acidification was also impaired in furin deficient osteoclasts. Taken together; these data imply that furin functions as a positive regulator of osteoclastic bone resorption during bone homeostasis, in part through the proteolytic processing of Ac45.

OR42

Monocyte Chemotactic Protein-1 (MCP-1) is a Key Regulator of Remodeling Activation

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Monocyte chemotactic protein-1 (MCP-1, or CCL2) plays a critical role in recruitment and activation of leukocytes. It also has the highest level of gene induction in bone following anabolic PTH treatment [1]. We reported that MCP-1 is specifically regulated during bone remodeling that is activated to repair stress fracture (SFx) [2]. We hypothesized that MCP-1 is a necessary regulator of recruitment and activation of osteoclasts required for skeletal repair and remodeling. We used the ulnar stress fracture model, allowing scrutiny of focal remodeling with a known time course and precise anatomical location. Within 4 hours of SFx initiation, we observed significant increases in MCP-1 gene expression (P<0.01), followed by increased serum levels within 24h (P<0.05). To test our hypothesis, we used a plasmid DNA encoding a dominant negative mutant of MCP-1 (7ND) to specifically inhibit MCP-1 in vivo. SFx was created in the right ulna of Wistar rats using cyclic end-loading. Unloaded animals were used as a control. 24 h prior to loading, 7ND plasmid vector, saline or empty vector control (pcDNA3.1), were injected in the thigh muscle to overexpress 7ND protein, effecting its secretion into systemic circulation. Rats were euthanized 4h (n=5/group) or 2 weeks (n=10/group) after loading for gene expression or histomorphometry. Using qPCR analysis, there was 33-fold increase in MCP-1 expression 4h after loading (P<0.001), which was abolished by 7ND treatment. At 2 weeks, there was a profound suppression of osteoclast number (61%), resorption area (50%) and new bone formation (60%) in basic multicellular units that initiate remodelling of the SFx (P<0.05). Conversely, 7ND treatment had no effect on formation of periosteal woven bone. MCP-1 is markedly upregulated by SFx, but also by intermittent PTH treatment. We therefore conclude that MCP-1 is a critical regulator of chemotaxis and osteoclast differentiation during initiation events of bone remodelling.

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OR43

Role of MCP1 in Human Osteoclast Formation.

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The chemokine MCP1 is a small signalling molecule that is usually thought of as a mediator of monocyte trafficking and inflammatory reactions by macrophages. We previously showed that human osteoclasts become larger and have greater bone resorption activity when exposed to MCP1. In addition, MCP1 is involved in determining the cell fate of osteoclast precursors isolated from blood. We now examined the effect of blockade MCP1 action on human osteoclast precursors from blood and from umbilical cord. The amount of cytokines produced in peripheral blood CD14+ monocytes cultured over a 7 day period of osteoclast differentiation was measured using a Bioplex assay system. Of 27 cytokines examined, by far the most abundant in the culture medium was MCP1 which reached concentrations of 50 ng/ml. In comparison, other chemokines such as CCL3, 4 and 5 were in pg/ml concentrations. Using gene expression analysis, chemokine receptors CCR1, CCR2 and CCR5 were found in these osteoclast progenitor cultures. CCR2 is considered the prime MCP1 receptor, although chemokines become receptor-promiscuous at high concentration. Gene expression profiles were consistent with protein assays indicating that MCP1 is the dominant chemokine produced in human osteoclast cultures. Treatment of osteoclast precursors with GM-CSF, which inhibits osteoclast formation, resulted in suppression of MCP1 mRNA and protein production. A truncated form of MCP1, known as 7ND, forms inactive dimers with normal MCP1 and therefore acts as a dominant negative. 7ND protein added to cultures of human osteoclasts at the same time as RANKL resulted in complete inhibition of osteoclast differentiation. This inhibition coincided with failure to induce calmodulin with subsequent flow on failure to induce NFATc1 and NFATc2 and other characteristic osteoclast genes.

These data support the hypothesis that early induction of MCP1 is a necessary component of osteoclast differentiation.

OR44

Characterisation of the Osteoclast-Specific Vitamin D Receptor knockout mice

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We have previously demonstrated that osteoclasts express the enzyme 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) and the vitamin D receptor (VDR) and thus can synthesise 1,25D and respond directly to vitamin D. Our in vitro studies demonstrate that while the absence of vitamin D activity results in osteoclasts that are smaller, these cells resorb deeper pits than normal osteoclasts, which is possibly due to reduced motility.
To assess the role for VDR-mediated activity in osteoclasts in vivo, we have generated an osteoclast-specific vitamin D receptor knockout mice (OclVDR$^{-/-}$) by mating Cathepsin K^{Cre} with floxed VDR mice (VDR$^{fl/fl}$). Successful deletion of VDR gene and marked reduction in VDR mRNA in bone was determined in OclVDR$^{-/-}$ mice. Female OclVDR$^{-/-}$ mice and VDR$^{fl/fl}$ controls were fed a chow diet ad libitum and were killed at 6 weeks age for biochemical, histomorphometric and molecular analyses.
In OclVDR$^{-/-}$ mice, numerous osteoclastic genes were reduced when compared to VDR$^{fl/fl}$ mice, including 60% reduction in calcitonin receptor (CTR) mRNA ($P<0.01$) as well as non-significant reductions in TRAP and Cathepsin K mRNA levels. Femoral metaphyseal Oc.Pm/B.Pm was modestly increased (+4%, $P=0.08$) in OclVDR$^{-/-}$ mice due to an increase in Oc.N/B.Pm (Females: +17% $P=0.09$) and despite a non-significant reduction in mean osteoclast size (-11%). While serum X-laps were not significantly different between OclVDR$^{-/-}$ and VDR$^{fl/fl}$ mice, femoral metaphysis BV/TV% was marginally reduced, due to a decrease in trabecular number (Tb.N). Thus, our in vivo findings generally support our in vitro findings that vitamin D activity in cells of the osteoclast lineage regulates osteoclastogenesis or osteoclast survival and overall osteoclast size. Our data suggest that the absence of vitamin D activity in osteoclasts results in more numerous, smaller osteoclasts which are capable of greater focal resorptive activity.

OR45

Diet-induced Obesity Inhibits Bone Accrual through a Neuropeptide Y-mediated Mechanism

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Studies in murine models demonstrate that diet-induced obesity (DIO) is detrimental to bone mass, despite the positive contribution of load-bearing to bone. The mechanisms involved have not been fully elucidated.
Neuropeptide Y (NPY) is a powerful modulator of energy homeostasis and bone metabolism. Central overexpression of NPY reduces bone formation and results in bone loss. We have previously shown that NPY expression is upregulated in DIO models. In this study, we aimed to determine the contribution of NPY to the skeletal response during DIO.
6 week old male WT and NPYKO mice were fed either standard chow diet (6% fat) or HFD (23% fat) for 10 weeks. We monitored feeding, energy metabolism and whole body bone mass, prior to commencement and at 2, 6 and 10 weeks on diet. Post-cull, femurs were analysed using microCT and histomorphometry.
In WT, HFD increased body weight compared to chow, attributed to a body-wide increase in fat mass. NPYKO animals were less susceptible to DIO with no difference in body weight; however, a significant increase in fat mass was also observed.
WTs on HFD had a 40% reduction in BV/TV compared to chow. NPY deficiency attenuated this DIO-induced cancellous bone loss. NPYKO on HFD had a 20% reduction in BV/TV in comparison to their chow counterparts. A similar pattern of cortical bone loss was observed in WTs on HFD; however this effect was lessened/absent in NPY-deficient mice.
Together our results confirm the detrimental action of HFD to bone metabolism and demonstrated that NPY contributes significantly to this effect. In addition, we have shown that blocking NPY signalling can rescue bone accrual and inhibit bone loss due to obesity.

OR46

EGFL7 is Expressed in Bone Microenvironment and Promotes Angiogenesis via ERK, STAT3 and Integrin Signaling Cascades

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Angiogenesis plays a pivotal role in bone formation, remodeling and fracture healing. The regulation of angiogenesis in the bone microenvironment is highly complex and orchestrated by intercellular communication between bone cells and endothelial cells. Here, we report that EGF-like domain 7 (EGFL7), a member of the epidermal growth factor (EGF) repeat protein superfamily is expressed in both the osteoclast and osteoblast lineages, and promotes endothelial cell activities. Addition of exogenous recombinant EGFL7 potentiates

SVEC (simian virus 40-transformed mouse microvascular endothelial cell line) cell migration and tube-like structure formation in vitro. Moreover, recombinant EGFL7 promotes angiogenesis featuring web-like structures in ex vivo fetal mouse metatarsal angiogenesis assay. We show that recombinant EGFL7 induces phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), signal transducer and activator of transcription 3 (STAT3), and focal adhesion kinase (FAK) in SVEC cells. Inhibition of ERK1/2 and STAT3 signaling impairs EGFL7-induced endothelial cell migration, and angiogenesis in fetal mouse metatarsal explants. Bioinformatic analyses indicate that EGFL7 contains a conserved RGD/QGD motif and EGFL7-induced endothelial cell migration is significantly reduced in the presence of RGD peptides. Moreover, EGFL7 gene expression is significantly upregulated during growth plate injury repair. Together, these results demonstrate that EGFL7 expressed by bone cells regulates endothelial cell activities through integrin-mediated signaling. This study highlights the important role that EGFL7 plays in the regulation of endothelial cell activities in bone microenvironment and might help to develop novel therapeutic approaches for bone fracture and bone disorders.

OR47

Anti-angiogenic Factors are Essential Regulators in Cartilage Homeostasis and Osteoarthritis

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Articular cartilage is an avascular tissue by nature. It is well known that physiological response to hypoxia will result in angiogenesis. However, cartilage under hypoxia can maintain avascular and the abrogation of avascularity is related to joint diseases including osteoarthritis (OA). The anti-angiogenic factors in cartilage homeostasis are far from understanding. This study aim to clarify whether anti-angiogenic factor is crucial in chondrocyte maturation and stabilizing chondrocyte phenotype, and whether maintenance of anti-angiogenic property (via chondromodulin-1 (ChM-1) overexpression) could protect chondrocyte from inflammatory cytokine-induced hypertrophy.

Gene profiling of angiogenic-related cytokines in human OA cartilage were evaluated by angiogenesis PCR arrays. Lentivirus vectors carrying ChM-1 cDNA (LV-ChM-1) and ChM-1 siRNA was constructed to study the role of ChM-1 in chondrogenesis and hypertrophy. The MAPK pathways were studied to unveil the mechanisms involved.

Imbalance of angiogenic and anti-angiogenic factors was found in OA cartilage (Figure A). ChM-1 expression was strongly correlated with the chondrogenesis in cells both from cartilage and bone marrow. ACCs with ChM-1 overexpression could significantly increase chondrogenic genes (ChM-1, COL2 and ACAN) expression and decrease hypertrophic markers (RUNX2, COL10, MMP13, ALP and VEGF); while decreasing ChM-1 by siRNA led to reduced chondrogenesis and increased hypertrophic marker expression. The angiogenesis PCR arrays revealed that in OA cartilage, majority of anti-angiogenic factors showed a decreased trend; while angiogenic-related cytokines were increased (Figure B). ChM-1 overexpression protected chondrocytes from TNFα-induced hypertrophy (Figure C); whereas decreasing ChM-1 by siRNA resulted in loss of chondrogenic markers leading to hypertreophy and became more sensitive to inflammatory cytokines (Figure D). It was noted that ChM-1 suppressed TNFα-induced phosphorylation of ERK-1/2 and p38 in ACCs. In conclusion, the potent anti-angiogenic factors ChM-1 is essential in maintaining chondrocyte phenotype. ChM-1 can protect chondrocytes from cytokine-induced hypertrophy, indicating anti-angiogenesis could be a potential target for novel therapies of OA.

OR48

Changes of Subchondral Bone and Osteocyte Phenotype in a Surgically Induced Osteoarthritis Rat Model

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Subchondral bone sclerosis is recognized as a clinical sign in osteoarthritis (OA). Although osteocytes play a central role in regulating bone remodelling, their involvement in OA subchondral bone pathophysiology is poorly defined. Our recent studies suggest that dysregulated osteocytic proteins contribute to the pathological changes in subchondral bone of OA patients. The aim of this study was to investigate whether surgically induced rat OA model (medial minesectomy) could induce similar subchondral bone changes identified at the clinical situation.

OA was induced in twelve week-old Wistar Kyoto rats by the removal of the medial meniscus (MSX). For bone remodelling study, fluorochrome calcein was injected 6 weeks post-surgery intraperitoneally and fluorochrome alizarin was injected 10 days after calcein injection. Rats were sacrificed at 8 weeks post-surgery and tissue samples were collected and processed by both decalcified and undecalcified methods for the study of subchondral bone and osteocyte phenotype by micro-CT, scanning electron microscope (SEM), histology and immunohistochemistry.

Increased progression of subchondral bone remodelling was detected in rat MSX model with significant accumulation of calcein and alizarin, indicating increased net bone formation at both time points of fluorochrome injection in MSX surgery compared to the SHAM. Micro-CT and SEM showed high subchondral bone volume with altered osteocyte morphology in the MSX group. 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. WNT signalling pathway proteins (β-catenin, AXIN2) were significantly increased in MSX osteocytes. Conversely, DKK1 was significantly decreased in MSX osteocytes compared to SHAM controls.

These results demonstrated the changes of subchondral bone and osteocytes in a rat MSX model, which is similar to human OA, indicating a potential regulatory role of osteocytes in subchondral bone remodelling and mineral metabolism.

Plenary Poster Presentations

Plenary Poster P1

Continuous Modeling-based Bone Formation: A Novel Mechanism That Could Explain the Sustained Increases in Hip Bone Mineral Density (Bmd) with Denosumab Treatment

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In clinical studies, denosumab is associated with continued increases in BMD and low fracture incidence through 8 years despite persistently low bone turnover markers and limited iliac crest tetracycline labeling [1]. We hypothesised that, with persistently low bone remodeling, these BMD increases may result from a non-remodeling dependent mechanism to accrue bone matrix. Therefore we examined the fluorochrome labeling pattern in proximal femur sections from ovariectomised (OVX) cynomolgus monkeys (cynos) treated with denosumab.

Mature 9+ year old OVX cynos were treated with vehicle (n=20) or 25 mg/kg denosumab (n=14) QM for 16 months. Fluorochrome labels were administered at months 6, 12 and 16. Consistent with the potent anti-remodeling effect of this regimen (25x clinical dose), very low bone resorption and formation indices were observed histologically and by serum markers [2]. Despite these reductions, BMD continued to rise at the femur neck in the denosumab group, from 5.9% (month 6) to 11.3% above baseline (month 16). Examination of proximal femur sections confirmed the low surface extent of label within the trabecular compartment of the denosumab group. In contrast, consistent and prominent labeling was observed in the cortex, primarily on both the superior endocortex (12/14 cynos) and the inferior periosteal surface (11/14 cynos), consistent with increasing cortical thickness. These regions typically contained multiple superimposed labels over smooth cement lines, often spanning months 6–16 (Figure), suggesting that modeling-based bone formation was continuous during denosumab administration.

Importantly, this augmentation of bone mass occurred at biomechanically relevant sites on the superior and inferior aspects of the femur neck, and corresponded to significant increases in bone strength [3]. In conclusion, there is evidence that continual modeling-based bone formation occurs during denosumab therapy, providing the first histological evidence of a potential mechanism for the clinical observations of continued BMD increases and low fracture rates with long-term denosumab in the FREEDOM Extension [1].

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Plenary Poster P2

Novel Insights into the Renal-Bone Axis in Thalassemia Major

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Thalassemia major is a disorder of red blood cell production requiring chronic transfusion and iron chelation to prevent iron toxicity and multi-organ disease. Bone disease is common with marrow expansion, iron toxicity and endocrinopathies contributing to low bone mineral density (BMD) and fractures. Recently, reports of renal tubulopathy and hypercalciuria have raised concerns of a renal role in accelerated bone loss in this cohort. In a retrospective study, we reported that 18.1% of patients with β-thalassemia major had symptomatic renal tract calculi and identified an association between urolithiasis and low BMD. To determine the true prevalence and chemical composition of urolithiasis and its association to BMD in patients with β-thalassemia major, we investigated 27 subjects presently asymptomatic for stone disease. All subjects underwent an initial single energy CT of the renal tract, and if a calculus was detected, dual-energy data was acquired, enabling derivation of its chemical composition from a standardised atomic number plot. Synchronous serum and urine biochemistry were measured and BMD determined. Urolithiasis was present in 16/27 (59%). Affected patients generally had multiple stones, often of varying composition, with struvite (33.3%), calcium oxalate (31.3%) and cysteine (21.6%) stones being the most prevalent. Hypercalciuria was present in 77.8% of subjects, and those with calcium-containing urolithiasis had dramatically reduced femoral neck BMD compared to non-calcium stone formers (z score -2.40 vs -0.2, P=0.04). The unusually high prevalence of cysteine stones and its association with renal tubulopathy warrants further investigation. This study confirms the prevalence of urolithiasis in β-thalassemia major to be 10 fold that of the general population. It raises questions about the pathophysiology of

urolithiasis and highlights the importance of the renal-bone axis in the management of bone disease in this condition.

Plenary Poster P3
Identification and Management of Bisphosphonate-Related Atypical Femoral Fractures

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Atypical fractures comprise a significant proportion of femoral shaft fractures but may go unrecognized at initial presentation. Management may be complicated by delayed union. The aims of this study were to estimate the prevalence of atypical femoral fractures, the proportion related to bisphosphonates, and to assess outcomes following surgical fixation in a level 1 trauma centre.
The Radiological database was searched for all femoral shaft fractures occurring in patients aged ≥50 presenting between 2010 to mid 2013. These radiographs were then independently assessed and characterized as atypical femoral fracture by 3 specialists, (DK, CC and WI). Where there was discrepancy in the initial assessment, consensus was reached after consultation. For those fractures identified as atypical, further data regarding patient characteristics, nature of bisphosphonate use, surgical management and the union rate at 12 months were obtained from the medical records.
Out of 226 radiographs, there were 24 atypical fractures (11%) occurring in 23 patients (mean age 71). Eighty-eight percent of these were associated with bisphosphonate use, mean duration 8.9 years (range 1-25 years). Forty-eight percent of atypical fractures were not recognised as atypical at presentation and the patients continued using bisphosphonates. Six patients (30%) had bilateral atypical femoral fractures. Two out of 17 patients (12%) followed up at our institution had a non-union at 12 months.
Atypical femoral fractures account for a significant proportion of femoral shaft fractures in the elderly population, with the majority associated with bisphosphonate use. The high rate of non-union of these fractures should be considered when planning surgical fixation. Only half of the patients were recognised during their admission as having an atypical fracture, which resulted in the erroneous continuation of bisphosphonates. We have subsequently introduced institutional protocols which have improved our identification and management of these patients, including specialist Bone clinic review.
Disclosure: Warrick Inder has received speaker fees and has served on an Advisory Board for Amgen.

Plenary Poster P4
Prediction of Fracture Risk by Trabecular Bone Score (TBS)

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TBS is bone texture index that reflects bone microarchitecture. The present study sought to determine the association between TBS and fracture, and to define its prognostic value in terms of fracture prediction in the elderly.
The study was designed as a population based prospective cohort which included 1688 women aged 60 years and above. The women were recruited between 1989 and 1993. Baseline anthropometric and demographic characteristics were ascertained at baseline by a structured questionnaire. BMD at the femoral neck and lumbar spine was measured by DXA (GE-LUNAR Corp, Madison, WI). TBS was analysed blindly from clinical outcome by TBS iNsight® software v1.9(Medimaps SASU, Pessac, France). Low trauma and non-pathological fractures were ascertained from X-ray reports during the follow-up (from study entry till 31/12/2013).
During the follow-up period, 418 women had sustained a fragility fracture. Women with fracture were significantly older, had lower femoral BMD and lower TBS than those without a fracture. Each standard deviation lower TBS (0.12) was associated with a 67% increase in the odds of fracture (odds ratio [OR] 1.67; 95%CI 1.43–1.96). However, TBS was correlated with femoral neck BMD (r=0.38, P<0.001) and age (r=–0.18; P<0.001). After adjusting for FNBMD and age, the association between TBS and fracture risk was weaker but remained statistically significant (OR 1.37; 95%CI 1.15–1.64). Further adjustment for fall, quadriceps strength and prior fracture did not significantly alter the result. The same result was also observed in a subsample of women without osteoporosis (T-scores>2.5). The proportion of variance in fracture risk that could be attributed to variation in TBS was ~4%.
These results suggest that lower TBS is associated with an increase in fracture risk, and that TBS could improve the accuracy of fracture prediction over and above that of femoral neck BMD and other recognised risk factors.

Plenary Poster P5
Concern and Risk Perception: Effects on Osteo-Protective Behavior

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Purpose: To determine the effect level of concern for osteoporosis as well as self-perceived risk of osteoporosis and fracture on, seeking medical advice, bone mineral testing (BMD) and anti-osteoporosis medication (AOM).
Methods: Study subjects were female Australian participants of the Global Longitudinal study of Osteoporosis in Women

(GLOW) who were not on osteoporosis treatment at the time of the earlier assessment. Self-administered questionnaires were collected annually from 2007–2010. Study outcomes included self-reported seeking of medical advice regarding osteoporosis, BMD testing and use of anti-osteoporosis medications (i.e., estrogen, selective estrogen receptor modulators, bisphosphonates, calcitonin, parathyroid hormone, and strontium) in the last 12months at the late assessment. Logistic regression was used in the analysis. The lack of independence in the study outcomes for multiple assessments in the same woman was accounted for using generalised estimating equations (GEE).

Results: There were 2,874 assessments from 1,095 women (mean age 66Â±9.4, range 55–97 years) for the study. Of women with osteoporosis or with a higher perceived risk to osteoporosis, only 22.2% and 40.4% respectively noted a higher perceived risk to fracture. Concern significantly increased the likelihood of seeking medical advice however, had no significant impact on screening or treatment. Heightened self-perceived risks of osteoporosis and fracture both significantly increased the likelihood of seeking medical advice and BMD testing while an elevated self-perceived fracture risk increased the likelihood of taking AOM.

Conclusion: Concern and risk perceptions to osteoporosis and fracture were found to be significantly associated with certain bone-protective behaviours in this prospective study. However, the apparent lack of association between perceived risk of osteoporosis and fracture illustrates the need for future studies to explore this disconnect further and also challenges future education programs to emphasize the connection between osteoporosis and fracture as only elevated self-perceived fracture risk was associated with higher intake of AOM.

Plenary Poster P6
Appendicular Lean Mass, BMD and Fracture Risk

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Low appendicular lean mass (ALM) might contribute to fracture risk through interrelationships with low bone mineral density (BMD), frailty and falls. We aimed to evaluate the independent contribution of ALM to fracture risk in older women enrolled in the Geelong Osteoporosis Study.

During the period 1993 to 1997, baseline ALM was determined from whole body scans and BMD from femoral neck scans (Lunar DPX-L) for 616 women aged 50 years and older (median age 74 years, IQR 68–82). All cause incident fractures were identified from radiological reports. Prior fractures (low trauma since age 50 years) were documented by self-report. Subjects were followed longitudinally until the end of 2010, or until sustaining a fracture, death, or migration from the study region. Fifty-eight women had invalid DXA for ALM assessment; missing data were imputed using multiple imputation. Multivariable Cox proportional hazards regression was used to determine the risk for first incident fracture associated with ALM (expressed in SD).

ALM was positively associated with BMD, weight and height, and negatively with age. In total, 217 fractures (67 clinical vertebral, 38 hip, 30 wrist, 16 pelvis, 15 rib, 14 humerus, 11 ankle, 8 tarsal/metatarsal, 6 forearm, 5 femur, 4 tibia/fibula, 3 carpal/metacarpal) were sustained during 4,871 person-years of follow-up. Increasing ALM decreased the hazard of fracture (HR 0.81, 95%CI 0.70, 0.94) but this was attenuated by age (age-adjusted HR 0.94, 95%CI 0.80, 1.10). In a model adjusted for age, BMD and prior fracture, there was no independent contribution by ALM to fracture risk. No effect modifiers were identified.

Although low ALM was associated with increased fracture risk, the age-related interrelationship with low BMD may act as a surrogate for ALM.

Disclosure: The authors declare no competing interests.

Plenary Poster P7
The Neglected Health Burden of Non-hip/Wrist/Vertebral Fractures (AusICUROS)

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There is little information concerning the health burden imposed by non-hip/vertebral/wrist fractures even though these fractures account for over half of all fractures [1]. We assessed this burden by comparing hip/vertebral/wrist fractures with the cost and quality of life (QoL) associated with all ‘other’ fractures including specific estimates of ankle and humerus fracture.

As part of the Australian arm of the International Cost and Utility Related to Osteoporosis Study (AusICUROS) [2], 65 humeral, 89 ankle and 137 ‘other’ fracture-participants were followed for 18-months. Costs associated with fracture-treatment were collected and EQ-5D used to assess QoL pre-; immediately after and 4, 12, and 18-months post- fracture.

Like wrist fractures, ‘other’ fractures took 18-months to regain pre-fracture QoL while hip and vertebral fractures did not recover pre-fracture levels. Wrist fractures had the highest pre-fracture and the least decline in QoL (Table). The immediate post-fracture decline in QoL for ‘other’ fractures was more

Table: Costs and Quality of Life (QoL) in Australians (50+ years)

	Ankle (n=89)	*Humerus (n=65)*	*'Other' [c] (n=137+89+65)*	*Hip (n=224)*	*Vertebrae (n=92)*	*Wrist (n=308)*
Age (years; SD)	64 (9)	70 (11)	67 (11)	78 (10)	71 (11)	67 (10)
Total cost[a]	$6,860	$4,890	$10,011[d]	$31,820	$7,650	$6,080
QoL[b]						
Pre-fracture	0.86 (0.19)	0.82 (0.17)	0.85 (0.19)	0.84 (0.20)	0.80 (0.21)	0.90 (0.17)
~Fracture	0.34 (0.24)	0.34 (0.26)	0.36 (0.27)	0.26 (0.25)	0.43 (0.28)	0.58 (0.22)
4-months	0.70 (0.18)	0.72 (0.18)	0.71 (0.21)	0.69 (0.22)	0.68 (0.27)	0.82 (0.19)
12-months	0.79 (0.18)	0.78 (0.16)	0.77 (0.21)	0.74 (0.22)	0.73 (0.25)	0.88 (0.18)
18-months	0.87 (0.13)	0.83 (0.13)	0.84 (0.18)	0.73 (0.25)	0.71 (0.28)	0.90 (0.17)

[a]Total direct costs $AUD over 12 months per patient; [b]EQ-5D utilities using Australian TTO weights [3] (SD=standard deviation); [c]Includes ankle & humeral fractures; [d]High cost influenced by pelvic fractures (n=11).

than vertebral and wrist fractures. Costs were similar for ankle, vertebral and wrist fractures.

The significant costs and QoL decline from 'other' fractures including ankle and humerus warrants more focus on improving their prevention and treatment.

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Plenary Poster P8

Progressive Vicious Cycle between Acid Sensing and Survival Signaling in Myeloma Cells

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Multiple myeloma (MM) develops systemic devastating bone destruction. In the osteolytic lesions, MM cells and osteoclasts (OCs) interact with each other to enhance their growth and activity, while creating an acidic milieu by protons produced by OCs and lactate by MM cells. Although tumor acidity is known to confer drug resistance, acid sensing and its relation to tumor function are largely unknown in MM. In the present study, we therefore explored the mechanisms of acid sensing and its role in survival signaling and gene expression in MM cells in acidic conditions. The acidic conditions induced the expression of the anti-apoptotic mediator Pim-2 and phosphorylated Akt in MM cells. MM cell lines as well as primary MM cells variably expressed extracelluar pH sensors, including TDAG8, OGR1, and TRPV1, at pH 7.4; the expression of these pH sensors was up-regulated at mRNA levels in MM cells cultured at pH 6.8 or cocultured with acid-producing OCs. The addition of the PI3K inhibitor LY294002 abolished the up-regulation of these pH sensors along with the suppression of Akt phosphorylation at pH 6.8. The Pim inhibitor SMI16a also reduced the expression of these pH sensors in MM cells at pH 6.8. LY294002 and SMI16a in combination cooperatively induced MM cell death more markedly in acidic conditions than at pH 7.4. These results suggest that MM cells activate the PI3K-Akt and Pim-2-mediated survival pathways in response to acids, which in turn up-regulates their expression of pH sensors to form a progressive positive feedback loop between pH sensor editing and survival signaling in MM cells. Besides the skewed cellular microenvironment surrounding MM cells, acids may act as a niche factor for MM cells to elicit their survival potential while adapting to a harsh acidic environment in bone lesions.

Plenary Poster P9

Healing of the Tendon-Bone Interface: An In Vivo Study Using A Lactoferrin Seeded ECM Scaffold

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Healing of hard-soft tissue interfaces are a significant clinical problem. 50% of people over 50 yrs of age experience rotator cuff tears, which predominantly occur at the tendon-bone interface. Due to the complex nature of surgical repair, re-tear rates are high (>70%). A potential approach for improving surgical outcomes is scaffold augmentation with/without growth factors that improve healing. Decellularized extracellular matrix (ECM) scaffolds can improve soft tissue regeneration and our preliminary studies demonstrated that a novel ovine forestomach ECM is cytocompatable and has low immunogenicity. Furthermore, we established that lactoferrin (LF) is a potent bone anabolic factor. The aim of this study was to assess the potential use of the decellularized ECM, in combination with LF, for augmenting tendon-bone repair.

Complete unilateral supraspinatus tears were surgically created in 46 Sprague-Dawley rats. The tendons were surgically repaired via a single row technique and the rats randomised into 3 groups: 1 (control) received surgical repair only; 2 received an augmented repair using the ECM scaffold; and 3 received the ECM scaffold with LF. The repairs were assessed 6 and 12 weeks postoperatively. Biomechanical testing and blinded histological scoring of tendon-bone healing were assessed.
At 6 weeks the ECM scaffolds had completely degraded in all animals. Both groups 2 and 3 scored significantly higher than group 1 in the histological evaluation of healing, with improved collagen orientation and tendon-bone interdigitation at 6 and 12 weeks. Stiffness and modulus were increased in group 2 compared to other groups, although this did not reach significance (11.8 N/mm stiffness in group 2 vs. 9.1 N/mm and 9.5 N/mm in groups 1 and 3, respectively, at 12 weeks).
This study demonstrated the positive effects of LF and a decellularized ovine forestomach ECM for augmenting rotator cuff tendon repair in a rat model, and thus has the potential to improve clinical outcomes.

Plenary Poster P10 - Withdrawn

Plenary Poster P11
Generation of a T Lymphocyte-specific Protease-activated Receptor-2 Null Mouse for Investigation of Periodontal Disease-induced Bone Loss

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Periodontal diseases are characterised by gingival inflammation and alveolar bone loss. A major etiological agent is Porphyromonas gingivalis, which secretes proteases capable of activating protease-activated receptor-2 (PAR_2). Our laboratory has previously demonstrated that when subjected to an established P. gingivalis-induced periodontitis model PAR_2 global knockout mice display an impaired immune response and reduced bone loss. Hence, we hypothesised that PAR_2 expressed on T-cells is required for the establishment and progression of periodontal disease. To test this hypothesis, $CD4^+$ T-cells were adoptively transferred from $PAR_2^{+/+}$ or global $PAR_2^{-/-}$ mice into αβ T-cell receptor knockout mice. Following the adoptive transfer, the mice were subjected to an established P.gingivalis-induced periodontitis model. Significant alveolar bone loss was observed in αβ T-cell receptor knockout mice that received T-cells from $PAR_2^{+/+}$ mice, but not in αβ T-cell receptor knockout mice that received T-cells from $PAR_2^{-/-}$ mice, suggesting that PAR_2 expression by T-cells is pivotal in P. gingivalis induced bone loss. In order to further test the role of PAR_2 on T-cells and other cell types we have generated mice harbouring floxed PAR_2 alleles. These mice have been bred with lymphocyte protein tyrosine kinase (Lck)-Cre transgenic mice to produce mice in which PAR_2 is deleted in T-cells during the DN3 stage of differentiation. The efficiency of PAR_2 deletion was determined in T-cells isolated from various lymphoid tissues including spleen, thymus and lymph nodes. We observed a 94% and 40% deletion of the PAR_2 gene in the T-cells isolated from thymus and spleen respectively, suggesting a previously unidentified role for PAR_2 during β-selection in the early stages of T-cell maturation. The findings of this project will help us better understand the specific role of PAR_2 and T-cells in mediating the bone loss associated with periodontitis and help in designing novel therapies with which to treat the disease.

Plenary Poster P12
Transgenic Over-expression of Vitamin D Receptor in Mature Osteoblasts Enhances Anabolic and Catabolic Activities Depending on Dietary Calcium and Phosphate Levels

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The osteoblast-specific over-expression of vitamin D receptor (VDR) in a transgenic mouse model demonstrated reduction of osteoclastic bone resorption and enhanced bone formation resulting in increased bone mineral volume [1]. This observation appears to contradict the dogma that 1,25-dihydroxyvitamin D (1,25D) activity in osteoblasts enhances VDR-mediated RANKL gene expression and osteoclastic bone resorption. Whether over-expression of VDR in osteoblasts is capable of reducing bone resorption under limited dietary calcium and phosphorus is unclear.
To address this, 3w female $ObVDR^{+/-}$ transgenic and WT mice (C57black6 background) were fed diet containing either 1% Ca/0.625% P (NormCa/P) or 0.03% Ca/0.08% P (LowCa/P) for 17 weeks and culled for analyses.
WT mice fed LowCa/P diet exhibited decreased femoral cortical bone volume (24%, $P<0.001$) and metaphyseal trabecular bone volume (70%, $P<0.001$) consistent with osteopenia due to enhanced bone resorption. While $ObVDR^{+/-}$ Tg mice fed NormCa/P diet demonstrated increased cortical (8%, $P= 0.056$) and trabecular (64% $P<0.01$) bone volumes when compared to WT mice. Conversely, LowCa/P fed $ObVDR^{+/-}$ Tg resulted in marked reduction of trabecular BV/TV and cortical bone volume with marked intra-cortical porosity and 18% decline in cortical osteocyte density. In addition, these mice exhibited markedly disturbed growth plate, cortical splaying and reduced femur length and yet serum calcium and phosphorus levels were normal suggesting that these bone defects were not due to nutritional rickets. Serum FGF23 levels in LowCa/P fed $ObVDR^{+/-}$ Tg mice were profoundly lower (83.5 pmol/L vs 154.6 pmol/L in LowCa/P WT) and serum 1,25D levels were very high (1345.5 pmol/L vs 872.8 pmol/L in LowCa/P WT mice) which was associated with enhanced RANKL mRNA levels and RANKL:OPG ratio. Thus, while over-expression of VDR in osteoblasts can mediate anabolic activities, under limited dietary calcium and phosphorus, profound

bone catabolism prevails possibly due to a lack of appropriate FGF-23 feed-back on renal 1,25D synthesis.

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Plenary Poster P13

Impact of Extracellular Matrix Derived from Osteoarthritis Subchondral Bone Osteoblasts on Osteocytes: Role of Integrinβ1 and Focal Adhesion Kinase Signaling Cues

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Subchondral bone sclerosis is a well-recognised manifestation of osteoarthritis (OA). Our recent study indicated that subchondral bone pathogenesis in osteoarthritis (OA) is associated with osteocyte morphology and phenotypic abnormalities. However, the mechanism underlying this abnormality needs to be identified. In this study we investigated the effect of extracellular matrix (ECM) produced from normal and OA bone on osteocytic cells function.

De-cellularized matrices, resembling the bone provisional ECM secreted from primary human subchondral bone osteoblasts (SBOs) of normal and OA patients were used as a model to study the effect on osteocytic cells. Osteocytic cells (MLOY4 osteocyte cell line) cultured on normal and OA derived ECMs were analyzed by confocal microscopy, scanning electron microscopy (SEM), cell attachment assays, zymography, apoptosis assays, qRT-PCR and western blotting. The role of integrinβ1 and focal adhesion kinase (FAK) signaling pathways during these interactions were monitored using appropriate blocking antibodies. Our results showed that the ECM produced by OA SBOs contained less mineral content, showed altered organization of matrix proteins and matrix structure compared with the matrices produced by normal SBOs. Culture of osteocytic cells on these defective OA ECM resulted in a decrease of integrinβ1 expression and the de-activation of FAK cell signaling pathway, which subsequently affected the initial osteocytic cell's attachment and functions including morphological abnormalities of cytoskeletal structures, focal adhesions, increased apoptosis, altered osteocyte specific gene expression and increased Matrix metalloproteinases (MMP-2) and -9 expression.

This study provides new insights in understanding how altered OA bone matrix can lead to the abnormal osteocyte phenotypic changes, which is typical in OA pathogenesis.

Disclosure: The authors declare no competing interests.

Plenary Poster P14

Choline Kinase Beta is an Important Regulator of Bone Homeostasis

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The maintenance of bone homeostasis requires tight coupling between the bone-forming osteoblasts and bone-resorbing osteoclasts. However, the precise molecular mechanism(s) underlying the activities of these specialized cells are still largely unknown. In search of novel molecules involved in bone homeostasis, we screened ENU-induced mutant mouse lines and identified choline kinase beta (CHKB), a kinase involved in the biosynthesis of phosphatidylcholine, as a novel regulator of bone homeostasis. Choline kinase beta mutant mice (flp/flp) exhibit a systemic low bone mass phenotype comparable to osteoporosis. Consistently, osteoclast numbers are elevated in flp/flp mice both in vivo and in vitro. Bone resorption assays revealed a significant increase in the depth of resorption pits formed by flp/flp osteoclasts, accompanied with increased CTX release. Interestingly, osteoclasts derived from flp/flp mice exhibit altered responses to excessive levels of extracellular calcium coupled with reduced levels of the membrane phospholipid phosphatidylcholine. In contrast, calcein labelling revealed a significant reduction in the bone formation rate in the flp/flp mice, whilst osteoblast numbers were not noticeably affected, indicating a defect in osteoblast function in vivo. Consistently, osteoblasts derived from flp/flp bone marrow stromal cells generated less mineralised bone nodules in vitro than their wild type counterparts. ^{1}H-NMR assessment of choline metabolites in flp/flp osteoblasts showed levels of phosphocholine were reduced; furthermore, phosphate production was significantly reduced in matrix vesicles derived from flp/flp osteoblasts. This result is consistent with existing evidence showing phosphocholine is the substrate for Phospho1, an important source of phosphate during bone mineralization. Supplementation of CDP-choline (Cytidine 5´-diphosphocholine) in vivo and in vitro, a regimen which bypasses CHKB deficiency but does not rescue phosphocholine levels, restores osteoclast numbers to physiological levels, but has no impact on osteoblast number or function. Taken together, these data posit CHKB as a new modulator of bone homeostasis.

Plenary Poster P15
Disruption of Glucocorticoid Signaling in Osteoblasts Prevents Stress-induced Bone Loss in Mice
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Chronic stress and depression are risk factors for low bone mineral density and fractures. However, the mechanisms underlying stress-induced bone loss are ill defined. The present study aimed to define the effects of chronic mild stress (CMS) on the skeletal phenotype of young adult mice.
We used a transgenic (tg) mouse model in which glucocorticoid signaling had been selectively disrupted in osteoblasts and osteocytes via targeted overexpression of the glucocorticoid-inactivating enzyme, 11ß-HSD2. Eight-week-old tg mice and their wild type (WT) littermates were exposed to CMS for the duration of 4 weeks. Stressors included restraint stress, exposure to hot and cold, tilted cages and overnight illuminations. At endpoint, corticosterone serum concentrations were determined by LCMS and both the L3-vertebra and tibia were analyzed by micro-CT and dynamic histomorphometry.
Compared to control mice, serum corticosterone levels increased ~3-fold in stressed WT and tg mice. Exposure to CMS resulted in loss of vertebral trabecular bone mass in WT but not tg mice when compared to their respective controls (BV/TV WT: –16%; tg: +3%, $p<0.01$). This was mainly due to a decrease in trabecular number (WT: –14%; tg: +1%; $p<0.05$) and a corresponding increase in trabecular separation (WT: +12%; tg: +1%; $p<0.04$) in WT mice only. While trabecular bone in the tibia was unaffected in both WT and tg mice exposed to CMS, tibial cortical volume (WT: –9%; tg: +1%; $p<0.05$) as well as cortical thickness (WT: –6%; tg: +2%; $p<0.05$) were reduced in stress-exposed WT but not in stress-exposed tg mice. Dynamic histomorphometry of the tibia revealed reduced endocortical (WT: –14%; tg: +42%; $p=0.055$) and pericortical bone formation rates (WT: –86%; tg: –3%; $p=0.062$) in stressed WT mice only.
We conclude that in mice, CMS induces loss of both trabecular and cortical bone via increased glucocorticoid signaling in osteoblasts and osteocytes.

Plenary Poster P16
Manufacture of Scaffold-free Engineered Tendon in Ex Vivo Bioreactor System
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Background: Tendon injuries are common in both workplace and sport activities. The poor self-healing capacity of tendon and lack of efficient treatment spur a demand to develop engineering tissue for tendon repair and regeneration. Tendon progenitor cell (TPC) is multipotent stem cell, its application in tendon injury and tissue engineering seems to be promising. However guided differentiation of TPC into target cell type is the key to the success of tendon engineering. Biomechanical signal and bioscaffold have been reported as an essential factor to regulate cell differentiation, whereas most of exogenous biomaterial has poor biocompatibility and low capacity for inducing cell differentiation.
The objective is to construct a scaffold-free engineered tendon (SFET) under mechanical stimulation in a bioreactor system using TPCs.
TPCs were isolated from mice tendon, and characterized by colony forming, flow cytometry and differentiation assay. We produced a SFET via TPCs treated by connective tissue growth factor (CTGF) and ascorbic acid in vitro. The SFETs were then cultured either in loading-free environment or under mechanical stimulation (6% tensile strain at 0.25 Hz, 8 h/day) in a bioreactor system for 7 days. Histology, immunohistochemistry, qRT-PCR and mechanical test were performed to characterize the engineered tendon.
After the validation of various conditions, we have manufactured the SFET that exhibited well organized collagen structure with elongated cell morphology similar to native tendon tissue. Furthermore, mechanical stimulation is able to induce tenogenic differentiation of TPCs evidenced by increased expression of tenogenic marker and decreased expression of adipogenic, osteogenic and chondrogenic markers at both gene level and protein level. Lastly, subjected to cyclic tensile loading, SFETs showed improved mechanical properties compared to the other groups.
We conclude that TPCs in bioreactor system could be a potentially promising method for tendon engineering.

Plenary Poster P17
The Vacuolar Atpase Subunit D_2 is Associated with Chondrocyte Hypertrophy and Supports Chondrocyte Differentiation
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Osteochondrosis is a developmental orthopaedic disease in horses, which involves focal retention of growth cartilage in subchondral bone. We have previously identified the gene encoding subunit d_2 of the vacuolar H^+ ATPase (ATP6V0D2) as a novel chondrocyte-expressed gene associated with the cartilage component of equine osteochondrosis lesions. The current study was undertaken to investigate the role of ATP6V0D2 in growth cartilage. In quantitative PCR studies, expression of ATP6V0D2 mRNA was found to be significantly more highly expressed in the zone of hypertrophic chondrocytes than in the reserve (>3-fold; $P<0.001$) or proliferative (>2-fold; $P<0.01$) zones of growth plates from equine foetuses. Similarly, immunohistochemical analysis of ATP6V0D2 expression in sections of equine foetal growth plates showed much stronger staining in the hypertrophic zone than in reserve or proliferative zones. In the ATDC5 mouse chondrocyte cell line, expression of Atp6v0d2 mRNA was up-regulated by conditions inducing

expression of hypertrophy-associated genes (>6-fold; P<0.05), and differential expression was confirmed by immunocytochemistry. Transfection of ATDC5 cells with Atp6v0d2-targetting siRNA resulted in significant knockdown (>50%; P<0.05) of Atp6v0d2 expression (relative to sham transfection) in cells in which hypertrophy was induced as well as in control cells. In control but not hypertrophic cells, the siRNA consistently suppressed expression of Col2a1 (P<0.05). Hypertrophy-associated genes were not differentially expressed between siRNA- and sham-transfected cultures. Knockdown of Atp6v0d2 expression in hypertrophic ATDC5 cells caused a decrease (P<0.0001) in nuclear area as well as increases in the number of cells (P<0.05) and of mitotic figures (P<0.0001). These observations suggest that ATP6V0D2 expression (and presumably vacuolar H^+ ATPase activity) supports differentiation and suppresses proliferation of chondrocytes.

Plenary Poster P18
Intracellular Trafficking Dynamics of Degraded Bone Matrix in Osteoclasts

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Osteoclasts are large multinucleated cells exquisitely adapted to degrade bone matrix. Upon contact with bone, osteoclasts segregate their surface membrane into four unique polarized domains: (1) the sealing zone; (2) the basolateral membrane; (3) functional secretory domain (FSD) and; (4) the bone-apposed ruffled border (RB). The RB functions as the 'resorptive apparatus', serving as a release site for protons and osteolytic enzymes (i.e., cathepsin K) required to digest the mineral and organic phases of bone. At the same time, the RB facilitates the uptake and transcytosis of bone matrix by-products (via membrane-delimited carrier vesicles) to the opposing FSD where they are expelled into the extracellular milieu. Although the crucial importance of vesicular trafficking between the RB and FSD during the functional bone resorption cycle is now well-established, the spatiotemporal dynamics of this process has not yet been appreciated in real-time. Here using confocal microscopy, we have monitored the intracellular trafficking dynamics of degraded bone matrix in osteoclasts actively engaged in bone resorption. Using fluorescently-labelled bone substrates together with a panel of intracellular compartment markers we show that osteoclasts utilize multiple endolysosomal trafficking pathways to ingest degraded bone matrix particles at the RB. Interestingly, following internalization, bone particles were observed to undergo continual digestion before converging with a common transcytotic pathway destined for the FSD. In addition, we demonstrate that the mechanism of uptake employed at the RB is dependent on the size of the bone particle(s) being engulfed. Furthermore, utilizing cytoskeletal disrupting agents, we show that both the uptake and release of degraded bone matrix is intimately linked to actin and the microtubule network. Together, these studies provide the first dynamic account of the trafficking itinerary traversed by degraded bone matrix en route to the FSD in osteoclasts and establish a platform on which to monitor osteoclast activity in real-time.

Poster Presentations

P20

Tbs in Lumbar Spine Osteoarthritis

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Lumbar spine osteoarthritis results in a spurious increase in dual-energy X-ray absorptiometry lumbar bone mineral density (BMD). Trabecular bone score (TBS) is a new textural parameter of grey scale variation providing micro-architectural information. A recent report found that TBS is not influenced by lumbar spine osteoarthritis1 [1]. We set out to test this hypothesis.

Lumbar spine DXA scans were retrospectively selected based on the presence of OA which was discordant within the scanned lumbar ROI. BMD and corresponding TBS were calculated in the vertebrae affected by OA and compared to the control non affected, or minimally affected, vertebrae in the same patient.

The study included 39 subjects (30 female and 9 male) mean age 65 ± 10 years. BMD and TBS were lower in the upper lumbar vertebrae (L1-L2) compared to the lower vertebrae (L3-L4). As expected BMD was higher in the lumbar vertebrae affected by OA compared to the control vertebrae. TBS was significantly higher ($p<0.001$) in affected vertebrae compared to vertebrae less affected by OA. OA was, however, more common in the lower vertebrae and when TBS was corrected for the vertebral region the independent effect of OA was significantly reduced.

We conclude that in postmenopausal women, lumbar osteoarthritis is associated with increased lumbar spine BMD and higher TBS. The higher TBS in osteoarthritic regions is partly due to the greater prevalence of OA in the lower lumbar vertebrae.

Reference

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P21

Quantification of the Heterogeneity of Cortical Porosity and the Effect on Strength

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Porosity is the proportion of cortex within the periosteal and endocortical envelopes that is void space. About 80% intracortical void volume is formed by Haversian and Volkmann canals which have diameters of 50–80 μm. Remodeling upon canal surfaces enlarges them focally or creates new canals increasing canal number. Accurate measurement of cortical porosity is important because its porosity accounts for ~ 70% of all bone loss.

To define whether a given porosity compromises strength due to more small pores or due to fewer large pores, we used scanning electron microscopy images in a region of interest (ROI) of the anterior subtrochanteric cortex from cadavers (14 Caucasian women age 67–87 years) and finite element analysis (FEA) to simulate loading. In the FEA model, total porosity was kept constant at 25% while pore size was varied from 53 to 300 μm. Strain distribution was quantified while applying a 250N bending force along one edge of the cortex.

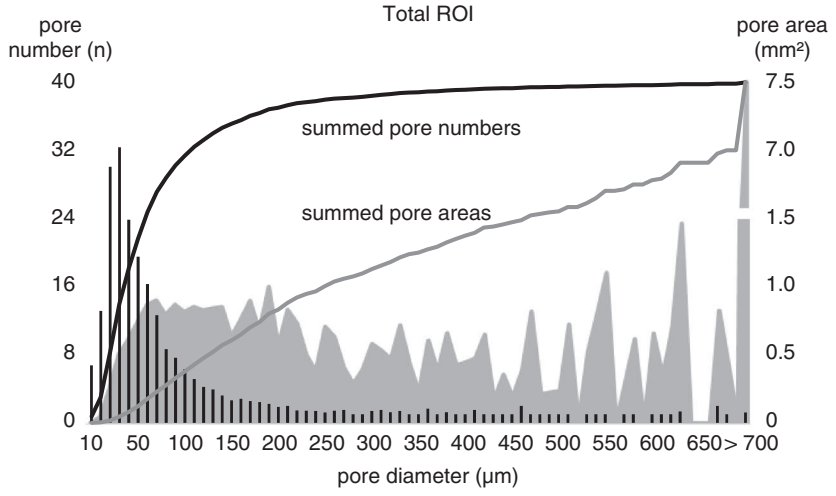

Figure 1. Frequency distribution curve for the region of interest (ROI) in total cortex. Each line shows the number of pores (left axis) for pore sizes at increments of 10 microns in diameter with the black line showing the exponential increase in total number of pores. The right axis, grey zones and grey line show the increase in pore area which is more linear.

In the region of interest, 82.87 ± 9.20% of pores in the total cortex; 89.98 ± 9.46% pores in the compact-appearing cortex, and 64.65 ± 15.03% in the transitional zone were ≤ 120 μm in diameter (Figure 1). These pores contributed 17.19 ± 6.45%, 41.66 ± 18.19% and 6.35± 2.56 % to porosity in the respective regions. However, for the simulated porosity of 25%, areas with highest strain increased with higher numbers of pores of smaller sizes (R^2= 0.72; p<0.001). When pore size was <125 μm, the areas subjected to >2600 μstrain increased by 135% compared to models with pores > 125 μm.
We infer that in vivo imaging using a voxel size of 82 μm (i.e., a resolution of ~125 μm) underestimates porosity and the fragility produced. Accurate account of all pores is important in identifying persons at risk for fracture.
Disclosures: Ali Ghasem-Zadeh is one of the inventors of the StrAx1.0 algorithm; Ego Seeman and Roger Zebaze are inventors of the StrAx1.0 algorithm and Directors of Straxcorp; All other authors state that they have no conflicts of interest.

P22
Puffing During Pregnancy; Are We Compromising Offspring Bone Development?

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Whilst the detrimental health outcomes associated with cigarette smoking are well-documented, including the increased risk of osteoporosis and subsequent fractures, much less is known regarding the potential of maternal smoking upon offspring bone development in utero. We investigated whether smoking during pregnancy impacted upon offspring bone measures.
We used data from the Vitamin D in Pregnancy study (VIP); a cohort of 475 pregnant women initially recruited from the Geelong Hospital antenatal clinic at 16 weeks gestation (2002–03). Current maternal smoking habits, anthropometric measures were obtained at recruitment; dietary information and blood samples were collected at recruitment and approximately 28 weeks gestation and analysed for serum 25-hydroxyvitamin D (25OHD). At birth, infant crown-heel length was measured using an Ellard newborn lengthboard (n=393) and knee-heel length was measured using with a handheld BK5 infant knemometer (n=391).
Of the 393 women, 71 were current smokers (18.07%). Babies of mothers who smoked had lower birth-weight [median IQR: 3.39 kg (3.11–3.77) vs. 3.55 kg (3.22–3.96), p=0.02], shorter crown-heel length [49.5 cm (47.5-50.5) vs. 50.9 cm (49.0–52.0), p<0.0001], shorter knee-heel length [84.41 mm (79.20–88.57) vs. 82.82 mm (80.96–91.51), p=0.01] and shorter gestation [39 wk (38–40) vs. 40 wk (39–41), p=0.09). After adjusting for maternal height, smoking was associated with shorter crown-heel length (β –1.11, SE 0.32, p=0.001), and a trend was observed for lighter birth-weight (β –0.130, SE 0.07) and shorter knee-heel length (β –2.1 SE 1.1) (both p≤0.08). Associations between maternal smoking and shorter infant crown-heel length persisted after adjusting for maternal height, dietary calcium and gestation length (β –0.86, SE 0.29, p=0.003); this was not confounded by maternal serum 25OHD measured at either visit.
Maternal smoking during pregnancy appears to have a detrimental effect on offspring bone development in utero. Further investigations are warranted within this cohort to investigate whether the effects of maternal smoking are transient or are maintained into childhood.

P23
Cut-off Points for Associations Between Vitamin D and Bone Mineral Density, Balance Measures and Lower Limb Muscle Strength Vary in Middle-aged Women

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To describe associations between serum 25-hydroxyvitamin D [25(OH)D] and musculoskeletal outcomes in middle-aged women.
In this cross-sectional analysis from a cohort of 348 women (mean age 50 yr), we used nonlinear least-squares estimation to determine flexion points for associations of 25(OH)D with lumbar spine (LS) bone mineral density (BMD), femoral neck (FN) BMD, lower limb muscle strength (LMS), timed up and go test (TUG), functional reach test (FRT), lateral reach test (LRT) and step test (ST) and piecewise regression to determine how associations differed by 25(OH)D level.
The prevalence of low 25(OH)D was 12% (<30 nmol/L) and 28% (<50 nmol/L) (mean 25(OH)D= 63 nmol/L). Flexion points were 25 (95%CI:16, 33), 42 (95%CI:16, 68), 26 (95%CI:20, 32), 41 (95%CI:23, 60), 18 (95%CI:12, 24), 48 (95%CI: –158, 254) and 24 (95%CI:12, 35) nmol/L for FN BMD, LS BMD, TUG, ST, FRT, LRT and LMS, respectively. Significant associations between 25(OH)D and musculoskeletal outcomes occurred only in participants with low serum 25(OH)D. Differences in slope between vitamin D groups became statistically significant at a level of 35 nmol/L for FN BMD (β (in low group)= 0.0044 (95%CI: 0.0009, 0.0078); between-group difference in β (Δβ) =0.0044, p=0.020) and TUG (β= –0.027 (95%CI: –0.052, –0.002); Δβ= –0.030, p=0.028); 30 nmol/L for LS BMD (β= 0.0059 (95%CI: 0.0002, 0.0117); Δβ= 0.0066, p=0.034), 25 nmol/L for FRT (β= 0.684 (95%CI: 0.075, 1.292); Δβ= 0.697, p=0.027) and LMS (β= 1.703 (95%CI: 0.241, 3.165); Δβ= 1.802, p=0.020), and 60 nmol/L for ST (β= 0.030 (95%CI: –0.001, 0.061); Δβ= 0.051, p=0.038).
In middle-aged women, the point at which associations between 25(OH)D and musculoskeletal outcomes change varies for different outcomes. The current cut-off of 50 nmol/L appears too high for some outcomes but reasonable overall to optimise bone and balance in this population.
Disclosure: The authors declare no competing interests.

P24

Linking Proximal Tibia Bone Microarchitecture to In Vivo Dynamic Joint Loads in End Stage Osteoarthritis: Preliminary Findings

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In human knee osteoarthritis (OA) the relationship between in vivo knee joint loading and subchondral bone microarchitecture remains unclear. The aim of this study is to examine, on end-stage OA patients undergoing total knee replacement, the relationships between knee joint loads measured using gait analysis prior to surgery, and variations in bone microarchitecture of their excised knees quantified with micro-computed tomography (micro-CT).

Six knee-OA patients (age 66±6 years, mean±SD) underwent pre-operative gait analysis. The following kinematic and kinetic data were collected with Vicon cameras and force platforms: peak external (ERM) and internal rotation moments (IRM), and peak knee adduction moment (KAM), all normalized by bodyweight; peak tibio-femoral joint contact force (JCF) was calculated. After surgery, tibial plateaus were retrieved and scanned with micro-CT. The following subchondral bone 3D microarchitectural parameters were analysed in four subregions of interest, in the antero-medial (AM), antero-lateral (AL), postero-medial and postero-lateral condyle: bone volume fraction (BV/TV), trabecular thickness, trabecular number and structure model index (SMI). Subregional differences were tested by repeated measures ANOVA followed by post-hoc analysis. Correlations between gait measurements and bone microarchitecture were examined.

Statistically significant differences ($p<0.05$) in subchondral bone microarchitecture were found among subregions: the AM subregion exhibited increased BV/TV (+119%), trabecular thickness (+32%), trabecular number (+68%), and decreased SMI (-81%) compared to AL. The BV/TV in the AM subregion was negatively correlated with peak ERM ($r=–0.88$, $p<0.05$). Positive trends were observed for "BV/TV AM vs. peak KAM" ($r=0.73$, $p=0.10$), and "BV/TV AM vs. peak JCF" ($r=0.75$, $p=0.09$).

Our preliminary results suggest that decreases in peak ERM during stance correlate significantly with increases in subchondral BV/TV in the AM tibial plateau. This bone volume increase could be linked to bone adaptation, due to increased stresses in this condyle. Further analysis is required to elucidate these relationships. Patients recruitment is ongoing.

P25

Automatic Exclusion Criteria for Dexa Lumbar Spine Scans do not Identify All Vertebrae that Require Further Morphometric Evaluation

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Fracture risk calculators preferentially use Hip DEXA data. Lumbar Spine DEXA artefacts include degenerative disease, scoliosis and compression fracture of the spine. Yet the presence of vertebral compression fracture increases future fracture risk prediction. Visual assessment is qualitative though some criteria for excluding vertebrae within a scan have been published. These include loss of expected BMD increases across Lumbar Vertebrae (%D-AV), T Score differences greater than 1SD between adjacent vertebrae (TSD-AV) or T Score differences greater than 0.9SD between the affected vertebrae and the L1-L4 T Score (TSD-LSV). T Score differences between the Lumbar Spine and Hip (TSD-LSH) have not been studied. The aim of this study was to evaluate the ability of these methods to direct further evaluation to a region within the Lumbar Spine thought to be demonstrating an artefact.

Qualitative assessment of DEXA at the Lumbar Spine has been recorded since 2005. 111 middle aged women from a study of bone loss following risk reducing salpingo-oophorectomy (RRSO) and not selected on the basis of osteoporosis were used as controls. 1704 women with osteoporosis (OP) based on Total Hip or Femoral Neck BMD were studied together with age, height, weight, BMI and major fracture history and age at menopause.

46% of Lumbar Spine DEXA scans had focal changes that were classified as suboptimal for diagnosis (OP-SFD). Women with scans optimal for diagnosis (OP-OFD) were younger, taller, lighter and had less fractures than OP-OFD.

Despite significant differences, %D-AV, TSD-AV, TSD-LSV or TSD-LSH did not accurately identify OP-SFD (ROC < 0.60). Conclusions are limited without radiological confirmation of confounders integral or adjacent to the Lumbar Spine. Automated DEXA exclusion criteria at the Lumbar Spine may not reliably exclude artefactual increases at this site. Quantitative Lumbar Spine DEXA cannot be used to direct morphometric evaluation of the spine.

[P25]

	n	*age*	*wt*	*BMI*	#	*LSBMD*	*LS TScore*	*THBMD*	*TH Tscore*
RRSO	111	49.8 (6.6)	71.4 (16.1)	27.1 (5.9)	13	1.16 (0.15)	-0.15 (1.30)	0.98 (0.12)	-0.16 (1.00)
OP	1704	74.9 (11.5)	56.5 (12.5)	24.9 (4.9)	717	0.91 (0.16	-2.26 (1.34)	0.64 (0.07)	-2.95 (0.57)

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%D-AV (n)	**(L2-L1)/L2 (**SD)	**(L3-L2)/L3** (SD)	**(L4-L3)/L4** (SD)*	
RRSO (111)	6.1 (5.7)	4.5 (5.3)	–3.7 (8.0)	
OP-OFD (901)	5.7 (10.2)	5.1 (9.6)	–0.1 (10.9)	
OP-SFD (791)	5.3 (11.7)	6.1 (10.5)	1.6 (11.9)	
TSD-AV	**Abs(L2-L1)***	**Abs(L3-L2)***	**Abs(L4-L3)***	**Abs(L3:4-L1:2)***
RRSO (110)	0.43 (0.36)	0.56 (0.40)	0.66 (0.48)	0.60 (0.43)
OP-OFD (901)	0.59 (0.54)	0.66 (0.54)	0.65 (0.58)	0.69 (0.60)
OP-SFD (791)	0.69 (0.63)	0.79 (0.65)	0.77 (0.67)	0.88 (0.76)
TSD-LSV	**L1:4-L1**	**L1:4-L2***	**L1:4-L3**	**L1:4-L4***
RRSO (109)	0.21 (0.52)	0.19 (0.41)	–0.25 (0.33)	0.05 (0.59)
OP-OFD (898)	0.18 (0.69)	0.31 (0.54)	–0.11 (0.47)	–0.16 (0.67)
OP-SFD (788)	0.27 (0.79)	0.40 (0.66)	–0.10 (0.54)	–0.31 (0.80)
TSD-LSH	**L1:4-TH***	**L1:4-FN***		
RRSO (109)	0 (1.09)	0.03 (1.17)		
OP-OFD (898)	0.57 (1.24)	0.34 (1.29)		
OP-SFD (788)	0.83 (1.37)	0.66 (1.44)		

*P<0.001 ANOVA

P26

Vitamin D and Metabolic Status in Young Women

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Vitamin D plays a pivotal role in bone and mineral homeostasis. Recent publications have suggested other critical roles for vitamin D in a variety of biological processes, including regulation of metabolism. Therefore, vitamin D, which is linked with metabolic profiles, may be associated with risk of many chronic diseases. Long term vitamin D deficiency may be particularly adverse. The aim of this study was to assess the relationship between serum 25-hydroxyvitamin D (25OHD) and metabolic profiles in young women.

The Young Female Health Initiative (YFHI) is a large cross-sectional study to evaluate physical and mental health in young females. To date, over 200 women aged 16-25 years old living in Victoria have been recruited through Facebook advertising. Participants completed consent forms, an extensive online health survey and attended the study site where metabolic profiles and serum 25OHD levels were measured.

Results are available in 93 participants to date. The mean (±SD) serum levels of 25OHD, total cholesterol, LDL, HDL and triglyceride were 63.51±26.48 nmol/L, 4.38±0.77 mmol/L, 2.39±0.72 mmol/L, 1.58±0.38 mmol/L and 0.91±0.43 mmol/L, respectively. Based on the linear regression analysis, serum 25OHD was negatively associated with total cholesterol and LDL (β= –0.218, 95%CI –0.012 to 0.00, p=0.036, and β= –0.238, 95% –0.012 to –0.001, p=0.022, respectively). There was no significant association between serum levels of 25OHD and triglyceride (β= –0.105, 95% CI –0.005 to 0.002, p=0.318) or HDL (β= 0.028, 95%CI –0.003 to 0.003, p=0.789).

Vitamin D status is associated with serum lipid profiles including total cholesterol and LDL in young women, providing a possible mechanism for adverse long term cardiovascular outcomes in vitamin D deficiency. A related YFHI study, Safe-D, will recruit up to 500 young women to further investigate vitamin D status, metabolic health and other health indices in this important demographic that is under-represented in health research.

Disclosures: The Safe-D study has received in-kind support from Swisse Wellness.

P27

Relationship Between Soluble α-klotho and Nutritional Intake in Postmenopausal Women

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The α-klotho gene has been identified as an anti-aging gene that encodes a single-pass transmembrane protein. There are two forms of α-Klotho, membrane and secreted, and each forms has different functions. Membrane α-Klotho acts as a co-receptor for fibroblast growth factor (FGF)-23. On the other hand, animal experiments suggest that a cleaved and secreted form α-Klotho found in serum, urine and other body fluids may also exert physiological effects by itself. We examined the relationship between secreted α-Klotho concentration and nutritional intake in postmenopausal women.

We enrolled 174 postmenopausal women who were undergoing examination for osteoporosis. Serum levels of FGF-23 and secreted α-Klotho were measured. Nutrient intakes were calculated using a food frequency questionnaire.

Mean values of age and BMI were 63.2±7.4 years and 22.9±3.1 kg/m^2, respectively. Mean serum levels of FGF-23

and secreted α-Klotho were 34.0±9.2 pg/mL and 596.7±171.9 pg/mL, respectively. Mean daily intake of vitamin A, vitamin D and vitamin K were 647±178 mg/RE, 9.9±4.0 mg and 272±82 mg. Secreted α-Klotho levels showed significantly negative correlation with serum levels of P and positive correlations with vitamin A and vitamin K intakes. Multiple regression analysis revealed that secreted α-Klotho showed significantly positive correlations with vitamin A intake [R=0.319, p<0.01] and vitamin K intake [R=0.266, p<0.05] after adjusting for age, BMI, Ca, P, Cr, PTH, 25(OH)D, FGF-23, Ca intake, P intake and vitamin D intake.
Membrane α-Klotho is cleaved by a disintegrin and metalloproteinase (ADAM) 10/17 into a soluble protein. Retinoic acid has been reported to stimulate expression of ADAM10. It is possible that vitamin A stimulates ADAM10 expression, thereby contributing to the release of secreted α-Klotho.
This is the first study to show that vitamin A and vitamin K intake may affect serum levels of secreted α-Klotho in postmenopausal women.
Disclosure: The authors declare no competing interests.

P28

Intensive Glycemic Control Improved the Excretion of Urine Mineral Ions in Type 2 Diabetes Mellitus

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Background: Patients with poorly controlled type 2 diabetes are known to show hypercalciuria. Although hypercalciuria is suggested to cause hyperparathyroidism and high bone turnover, the influence of changes in glycemic control on parathyroid hormone (PTH) and bone metabolism is still unclear. Aim: To examine the effects of intensive glycemic control on urinary levels of mineral ions and the association between urinary mineral ions versus PTH and bone turnover markers.
Methods: A total of 25 Japanese patients with poorly controlled type 2 diabetes (6 males and 19 females, 63 ± 13.3 years old, HbA1c 9.9 ± 1.9%) were recruited. Serum and urinary levels of calcium (Ca), phosphate (P) and magnesium (Mg), intact and whole PTH, 25-hydroxyvitamin D (25VD), 1,25-dihydroxyvitamin D (1,25VD), and bone turnover markers [bone specific alkaline phosphatase (BAP), total osteocalcin, undercarboxylated osteocalcin, urinary type I collagen cross-linked N-telopeptide] were measured at the start and end of glycemic control (mean period; 18.3 days).
Results: Fasting plasma glucose, HbA1c, glycosylated albumin, and urinary glucose were markedly reduced after glycemic control. Urinary levels of Ca, Ca/creatine (Cr) ratio, P, and Mg as well as BAP and 25VD were reduced significantly (p<0.05), while PTH, 1,25VD, or other bone turnover markers were not changed. By simple regression analyses, changes in Ca were significantly and negatively correlated with initial urinary Ca levels and urinary Ca/Cr ratio (r=–0.96, p<0.001 and r=–0.68, p<0.001, respectively). However, changes in Ca were not correlated with initial and changes in plasma and urinary glucose levels, PTH, vitamin D, or bone turnover markers. Discussion/Conclusion: The present study showed that urinary levels of Ca, P, and Mg were reduced after intensive glycemic control, and that changes in urinary Ca were independent of PTH, vitamin D, and bone metabolism. These findings suggest that intensive glycemic control improve the negative balance of Ca metabolism without disturbance of PTH secretion or bone metabolism.

P29

Management of Hypoparathyroidism in Pregnancy and Lactation— A Review of 11 Pregnancies

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Hypoparathyroidism is rare in pregnancy with limited case reports and no established management guidelines reported in the literature. However, hypoparathyroidism is important as it is associated with maternal morbidity and fetal loss. Optimal maintenance of calcium levels within lower normal range during pregnancy is required to minimise risk of related complications. Variable responses to calcitriol and calcium, and altered calcium homeostasis during pregnancy and lactation make the management of this condition challenging. Monash Health's maternity service is the largest maternity provider in Victoria, with an associated database that captures birthing outcomes in over 9,000 women each year. We audited the database between 2000–2014 to examine the clinical course, treatment, and maternal and fetal outcomes of pregnant women with hypoparathyroidism. We identified 11 pregnancies from 6 women with pre-existing hypoparathyroidism secondary to thyroid surgery for Graves disease (n=3) and thyroid cancer (n=1), DiGeorge syndrome (n=3), idiopathic hypoparathyroidism (n=3) and familial hypoparathyroidism (subsequently diagnosed with autosomal dominant hypocalcemia with hypercalciuria) (n=1). In all cases, maternal calcium levels were monitored through pregnancy, with calcitriol and calcium doses adjusted to maintain normocalcemia. One woman delivered by caesarean section at 34 weeks gestation in the setting of IUGR and oligohydramnios in two pregnancies. The perinatal course was otherwise uneventful in the remaining pregnancies. The postpartum period was complicated by severe hypercalcemia in one woman 9 days postpartum and by symptomatic, labile serum calcium levels during lactation in another woman which required close monitoring over a 6 month period. Although rare, hypoparathyroidism in pregnancy poses a management challenge for clinicians and co-ordinated care is required between obstetricians and endocrinologists to ensure optimal outcomes for mother and baby. Continued monitoring of maternal calcium levels during lactation and weaning is required to avoid the potential complications of hypercalcemia or hypocalcemia.
Disclosure: The authors declare no competing interests.

P30

Vitamin D and Bone Status in Young Women

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Young women are under-represented in health research despite transitioning a life stage critical for their future health outcomes. The Young Female Health Initiative (YFHI) is a comprehensive study of physical and mental health in young females. The aim of this analysis was to investigate the association between serum 25-hydroxyvitamin D (25OHD) concentrations and bone health in young women.

Study participants (aged 16-25 years living in Victoria, Australia) were recruited through the social media website Facebook. Consented participants completed an extensive online health survey and attended a study site visit. Dual-energy X-ray absorptiometry was used to measure bone mineral density (BMD) at the total hip, femoral neck and lumbar spine. The Kessler Psychological Distress Scale (K10) was used to measure anxiety and depressive symptoms. Serum 25OHD was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS).

To date, 161 participants have completed site visits and LC-MS/MS 25OHD results are available for 85. The mean serum (±SD) 25OHD concentration was 78.3±28.6 nmol/L with 10 (12%) 25-50 nmol/L, 35 (41%) 50–75 and 40 (47%) ≥75nmol/L. Fifty-three (62%) participants had normal BMD; 31 (37%) had osteopenia and 1 (1%) had osteoporosis at one or more sites. Using a regression analysis, serum 25OHD was positively associated with total hip and femoral neck BMD (β= 0.001, 95% CI 0.0004–0.002, p=0.003, and β= 0.001, 95%CI 0.0001–0.002, p=0.023 respectively) when adjusted for age, height and weight. No relationship between BMD and psychological distress was observed.

Approximately a third of young women in the YFHI study were found to have osteopenia or osteoporosis. Preliminary analysis revealed significant associations between vitamin D status and BMD at the total hip and femoral neck. Another YFHI study, Safe-D, will recruit up to 500 young women to further investigate vitamin D status, musculoskeletal health and other health measures in this underinvestigated demographic.

Disclosures: The Safe-D study has received in-kind support from Swisse Wellness.

P31

Hospitalisations and Direct Costs Related to Osteoporotic Fractures and Risk of Fracture-related Re-admissions in Western Australia: A 10-year Snapshot Using the Linked Wa Hospital Morbidity Data System

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Jurisdictional-level evidence relating to the health and financial impacts of osteoporosis is required to support implementation of models of care. The aims of this study were to quantify admission costs to Western Australia (WA) for osteoporosis-related fractures, and estimate risk of re-admission after incident fracture.

All hospitalisations due to osteoporotic fracture in WA residents aged ≥50 years between 2002–2011 were identified from the WA Hospital Morbidity Data System. Data linkage was used to identify first (index) fracture admission, to determine subsequent osteoporotic fracture-related readmissions to any WA hospital, and to quantify total admission costs and bed days. Cox proportional hazard models were used to assess factors influencing first re-admission including age, gender, bed days of index admission and clinically-relevant co-morbidities.

5,326 patients were admitted to WA hospitals for an index fracture. Of the 2,037 (38.2%) re-admitted patients, 1,223 (23.0%) sustained one, 453 (8.5%) sustained two, and 361 (6.8%) sustained three or more re-fractures requiring re-admission. 44.4% were readmitted within <6 months, 13.1% within 6 months to <1 year, 17.4% within 1 to <2 years, 10.5% within 2 to <3 years, and 14.6% within ≥3 years of the index admission. Cost of index admissions was $AU57,007,262 and $AU48,948,623 was associated with re-admissions (CPI-adjusted to 2011/12). Cumulative probability of re-admission within 6 months of the index admission was 20% and 17% for males and females, respectively. Probability increased to 25% for males and 24% for females within one year; 34% for both males and females within 2 years. The risk of first re-admission for males increased by 3% for every one year increase in age and 5% for each additional bed day during the index admission (2.7% for females).

Hospitalisations due to osteoporotic fractures impose a substantial financial impact to WA, exceeding $AU100M in a decade. Opportunities exists to initiate preventive interventions.

Disclosure: The authors declare no competing interests.

P32

Knowledge Change Regarding Osteoporosis Prevention: Translating Recommended Guidelines into User-friendly Messages for the Community

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Osteoporosis is a skeletal disorder characterised by low bone mineral density (BMD) and a subsequent increase in fracture risk. Nationally the total direct and indirect costs of this chronic disease are currently estimated at a significant $2.754 billion. Effective public health messages that provide clear recommendations and develop osteoporosis-related knowledge are vital in supporting efforts in osteoporosis prevention. This research aimed to investigate knowledge change associated with the translation of recommended preventive guidelines into accessible messages for the general community via community-based information session. The information session was designed to translated the recommended guidelines for osteoporosis prevention into lay terms; items focused on dietary calcium, vitamin D, physical activity, alcohol, smoking and general osteoporosis-related knowledge. We developed a 10-item questionnaire that reflected these key points and investigated knowledge change associated with the session. 47 participants (51% female), aged 21–94 years completed the pre- and post-test questionnaires. Pre-test knowledge was a combined score of 336 out of a possible 470 (71.5%). Relatively high pre-test scores were observed for the questions regarding sedentary activity and calcium intake. The lowest pre-test scores were observed for the item that questioned whether swimming and cycling strengthened bones, and the highest possible score post-test was achieved by participants for three of the items: 3–5 serves of calcium-rich food as a protective factor, and excessive alcohol and smoking as risk factors. The overall increase in knowledge change was a mean score of +2.08 (95%CI 1.58, 2.42). In conclusion, we found an increase in knowledge regarding osteoporosis prevention was demonstrated over the short term. Our findings suggest that the recommended guidelines concerning dietary calcium intake are generally well understood, however, the asymptomatic nature of osteoporosis and the types of physical activity that assist with bone strength are less well understood.

P33

Toxic Elements in the Blood and Hair Samples in Men From the North of Russia

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Minerals are key elements of the most important chemical processes in the human body. Bone belongs to the most mineralized tissues. Accumulation of toxic elements in the body can lead to the development-disruption of the bone mineral metabolism. Our goal is to assess the content of toxic and essential elements in hair samples and blood serum in the North.

A total of 99 people under the age of 50 years. There were included 63 people living in the North in the group I. Group II – 36 living in the Northwest. Mass spectrometry with inductively coupled plasma was measured content 10 of toxic elements (Al, Cd, Ni, Hg, Rb, Pb, Ag, Sr, Tl, Ce) and 13 of essential elements (Na, Mg, P, Ca, V , Cr, Mn, Co, Cu, Zn, As, Se, I) in serum, as well as 12 of toxic elements (Al, Cd, Ni, Hg, Rb, Pb, Ag, Sr, Tl, Ce, Ba, Be) and 19 of essential elements (Na, Mg, P, Ca, V, Cr , Mn, Co, Cu, Zn, As, Cd, Se, I, B, K, Ba, Li, Fe) in hair samples.

Group I samples revealed increasing hair: barium (95%), nickel (22%), aluminum (9%), cadmium (4%) and strontium (4%). Serum observed an increase of rubidium (18%), cadmium (9%) and mercury (4%). In Group II, we observed a high nickel content (3%) in hair samples. More pronounced changes are observed among the essential elements in a sample of hair, where deficiency of selenium was detected (100%), iodine (90%), cobalt (90%), identified as a significant reduction in magnesium (55%), copper (20%), zinc (35%). Serum also revealed deficiency of selenium (90%) and iodine (38%). Despite the fact that the median calcium content in hair samples from northern residents are within the boundaries of the reference range, a number of surveyed found their reduced levels compared with the control group.

Thus the inhabitants of the North, we are seeing a significant violation of the element status, characterized by a high content of barium, nickel, aluminum, cadmium, strontium, mercury, rubidium, lead. Against the background of the excess of toxic elements observed reduced levels of essential elements necessary for the formation and bone metabolism.

Disclosure: The authors declare no competing interests.

P34 – Withdrawn

P35

Discovery of Peroxidase Enzymes as Novel Regulators of Osteoblast Collagen Extracellular Matrix Biosynthesis and Mineralization

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Myeloperoxidase (MPO) and eosinophil peroxidase (EPO) are heme-containing enzymes whose functional involvement in human health has mainly been limited to providing a mechanism for oxidative defense against invading bacteria and other pathogenic microorganisms. Released by infiltrating neutrophils and eosinophils respectively, these endogenous peroxidases have often been associated with fibrotic tissue

in various organs without any direct involvement in extracellular matrix (ECM) biosynthesis attributed to their presence. We report here studies that characterize for the first time the ability of mammalian MPO and EPO as well as plant derived peroxidase proteins such as horseradish peroxidase (HRP) and soybean peroxidase (SBP) to directly stimulate the biosynthetic capacity of human osteoblasts to secrete collagenous proteins and generate a functional ECM while promoting mineral deposition. Mechanistic data that shows peroxidase enzymes regulate collagen biosynthesis at a post-translational level that is prolyl-4-hydroxylase (P4H)-dependent, but importantly does not require ascorbic acid. Our studies demonstrate that the catalytic activity of MPO and EPO is essential to support collagen biosynthesis, and superoxide has an active role in promoting P4H-dependant hydroxylation and subsequent release of pro-collagen from osteoblasts. Our novel findings that peroxidase enzymes like MPO and EPO can substitute for ascorbic acid to promote collagen biosynthesis strongly suggests these enzymes will play an important role not only in normal bone remodeling but also in bone pathology where infiltrating inflammatory cells are known to deposit peroxidases.
Disclosure: The authors declare no competing interests.

P36
Myocyte Enhancer Factor 2c, an Osteoblast Transcription Factor
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Controlling the differentiation potential and fate of cultured osteoblasts is a useful tool for stem cell-mediated therapies for fracture and other orthopaedic problems. Dimethyl sulfoxide (DMSO) is a small amphipathic solvent molecule capable of stimulating cell differentiation. In primary human osteoblasts, DMSO dose-dependently enhanced the expression of osteoblast differentiation markers alkaline phosphatase activity and extracellular matrix mineralization [1]. Similar DMSO-mediated mineralization enhancement is observed in primary osteoblast-like cells differentiated from mouse mesenchymal cells derived from fat. Using a mouse pre-osteoblast model cell line MC3T3-E1, we report that DMSO treatment is correlated with enhanced mineralization and the increased expression of osteoblast-expressed genes. Myocyte enhancer factor 2c (Mef2c) was the transcription factor most induced by DMSO, suggesting a role for Mef2c in osteoblast gene regulation. Immunohistochemistry confirmed expression of Mef2c in osteoblast-like cells in mouse mandible, cortical, and trabecular bone. shRNAi-mediated Mef2c gene silencing resulted in defective osteoblast differentiation, decreased alkaline phosphatase activity, matrix mineralization and knockdown of osteoblast specific gene expression. A flow on knockdown of bone-specific transcription factors, Runx2 and osterix by shRNAi knockdown of Mef2c, suggests that Mef2c lies upstream of these two important factors in the cascade of gene expression in osteoblasts. The co-transfection of Mef2c and Runx2 into MC3T3-E1 cells induced a significant increase in osteoblast differentiation markers alkaline phosphatase activity and extracellular matrix mineralization at 10-days following the induction of osteoblast mineralization ($p<0.05$). These data suggest that Mef2c is a transcription factor that acts in osteoblasts to regulate osteoblast specific genes.
Disclosure: The authors declare no competing interests.
Reference:
1. Stephens *et al*. *JBC* 2011;**286**:30071-30086.

P37
Vitamin D and Thyroid Hormone Enhance Sulphate Transporter mRNA Expression in Rat Osteoblasts.
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Sulphate is an important nutrient for bone growth and development. It is essential for numerous metabolic and cellular processes. Sulphate conjugation (sulphonation) of proteoglycans is required for maintaining the normal structure and physiological function of bone and cartilage. Several congenital chondrodysplasias are linked to reduced sulphonation of proteoglycans. Importantly, a sufficient supply of sulphate needs to be maintained for intracellular sulphonation of proteoglycans. Sulphate is transported into cells via sulphate transporters on the plasma membrane. The human and rodent genomes contain 10 sulphate transporter genes belonging to the solute linked carrier SLC13 and SLC26 gene families. However, there is limited knowledge regarding the expression profile and regulation of these genes in bone.
In the present study we used a rat osteoblast cell line (UMR-106) as a model to test several hormones on the transcriptional regulation of sulphate transporters. Cultured osteoblast cells were treated with Angiotensin II (10^{-7}M), Dexamethasone (10^{-7}M), Vitamin D_3 (10^{-6}M), T3 (10^{-6}M) and Estrogen (10^{-5}M), and all 10 sulphate transporter mRNAs were quantified using qPCR. While Angiotensin II, glucocorticoids and estrogen had no effect on the mRNA levels of all 10 sulphate transporters, Vitamin D_3 induced SLC13a1 (~10-fold), SLC26a1 (~5-fold) and SLC26a9 (~5-fold) mRNA expression, and T3 induced SLC13a1 (~5-fold), SLC26a1 (~10-fold), SLC26a2 (~10-fold) and SLC26a9 (~10-fold) mRNA levels, compared to untreated cells.
This is the first study to investigate the transcriptional profile of all 10 sulphate transporters in a rat osteoblast cell line. Our results indicate an important role of vitamin D_3 and T3 for up-regulating sulphate transporter expression in osteoblasts, which may be relevant to the link between perturbed vitamin D and T3 homeostasis and certain skeletal pathologies, including osteoporosis.
Disclosure: The authors declare no competing interests.

P38

Ephrinb2 Signalling in Late-stage Osteoblasts/osteocytes is Required for Good Quality Bone Matrix

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Osteoblastic ephrinB2 signalling is required for late stage osteoblast differentiation and bone mineralisation. Since ephrinB2 is also expressed in osteocytes, this study examined the role of ephrinB2 signalling in osteocytes in vivo.

Bones were collected from 12-week-old female mice with targeted ephrinB2 deletion in osteocytes (DMP1Cre.efnB2$^{f/f}$) or controls (Dmp1Cre.efnB2$^{w/w}$). Tibiae were analysed by histomorphometry, and femora by microCT, reference point indentation (RPI) and 3-point bending tests. mRNA levels were assessed in flushed femora and serum assayed for changes in bone formation/resorption markers.

Female DMP1Cre.efnB2$^{f/f}$ mice showed significantly greater tibial trabecular bone volume (by 40%, $p<0.01$) and trabecular thickness (by 10%, $p<0.05$), while femoral and tibial trabecular separation were significantly reduced (by 12% and 23% respectively, $p<0.01$, $p<0.05$). This was not caused by increased bone formation; neither osteoblast number nor bone formation rate was significantly altered, nor were there any changes in mRNA levels of osteoblast marker genes. In contrast, osteoclast size was ~12% greater ($p<0.05$) in DMP1Cre.efnB2$^{f/f}$ mice. Additionally, cartilage remnants within trabecular bone were significantly reduced in DMP1Cre.efnB2 f/f mice ($p<0.05$) but serum CTX1 levels were unchanged. This suggests that while the osteoclast's ability to remove cartilage template is enhanced its ability to resorb bone is impaired.

3-point bending tests demonstrated female DMP1Cre.efnB2$^{f/f}$ bones were more brittle than controls, with reduced toughness (33%, $p<0.001$) and energy absorbed to failure (35% less, $p<0.001$). A defect in matrix quality was confirmed by RPI, showing a significantly greater indentation distance increase (0.32 µm, $p<0.01$) in DMP1Cre.efnB2$^{f/f}$ femora compared to controls.

In summary, the defective bone matrix produced in mice lacking ephrinB2 in osteocytes appears to lead to poor bone resorption and increased trabecular bone mass. This suggests that ephrinB2 signalling in osteocytes in female mice is required to maintain bone matrix quality.

Disclosure: The authors declare no competing interests.

P39

Glucocorticoid Signaling in Osteoblasts Mediates Age-associated Metabolic Dysfunction in Mice

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The physiological ageing process is associated with changes in body composition and metabolism, including central obesity, diabetes and osteoporosis. The osteoblast has recently been identified as a mediator of GC-induced metabolic dysfunction in mice [1]. We therefore hypothesised that a mechanistic link exists between increased GC signaling in the osteoblast and changes in body composition and fuel metabolism during ageing.

To test this hypothesis, we investigated the ageing phenotype of transgenic (tg) mice in which glucocorticoid signaling had been selectively disrupted in osteoblasts/osteocytes via targeted overexpression of the glucocorticoid-inactivating enzyme, 11ßHSD2. Body weight and composition, insulin sensitivity and glucose tolerance, serum corticosterone (CS) and osteocalcin levels as well as hepatic gene expression patterns were assessed in female 11ßHSD2-tg mice and litter-matched wild-type (WT) controls at 2 and 18 months of age.

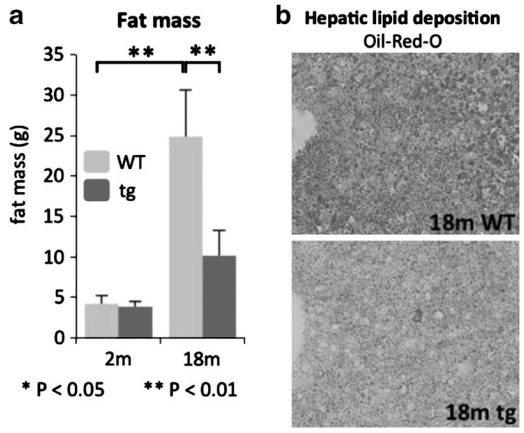
From 2 to 18 months of age, female WT mice gained more in body weight (WT: +32g vs tg: +16g, $p<0.01$) and overall fat mass (WT: +20.7g vs tg: +6.3g, $p<0.01$, Fig 1A) than their tg littermates. Eighteen-months-old WT mice exhibited reduced insulin sensitivity and hepatosteatosis, while insulin responsiveness and hepatic lipid deposition remained normal in their age-matched tg littermates (Fig. 1B). Hepatic mRNA expression of lipogenic and gluconeogenic genes was higher in aged WT compared to aged tg mice (acetyl-coA-carboxylase, WT: 12.8 vs tg: 5.6-fold increase on respective young controls, $p=0.051$; glucose-6-phosphatase, WT: 8.1 vs tg: 3.3-fold increase on respective young controls, $p=0.09$). Serum CS concentrations were similar in 18-month-old WT and tg mice and ~3-fold higher than in 2-month-old mice ($p<0.05$). Serum osteocalcin concentrations declined during ageing in both genotypes but remained significantly higher in tg mice at all time points ($p<0.05$).

Conclusion: Glucocorticoid signaling in osteoblasts is critically involved in the pathogenesis of age-related changes in glucose handling and body composition.

Reference

1. Brennan-Speranza *JCI* 2012;**122**(11):4172-4189

[P39]



P40

Utilising Vitamin D Receptor Knockout Mice Models to Understand the Role of Vitamin D Receptor Activity in Osteoblasts and Osteocytes

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Although the importance of vitamin D receptor (VDR)-mediated activity in normalising serum calcium levels is well established, some confusion remains as to the necessity of direct vitamin D activity in mediating osteoblastic activity. Previous reports in $VDR^{-/-}$ mice have demonstrated that supplying the growing animal with adequate dietary calcium and phosphorus to maintain serum homeostasis is sufficient to normalise bone mineralisation, suggesting that vitamin D activity in bone is redundant. In order to investigate further the role of VDR-mediated activity in the skeleton, we have utilised three mouse models–haploinsufficient VDRKO ($VDR^{+/-}$), osteoblast-specific VDRKO ($ObVDR^{-/-}$) and osteocyte-specific VDRKO ($OyVDR^{-/-}$) mice, to investigate effects of VDR on bone cell activities. We have shown that 12 week old chow-fed $VDR^{+/-}$ mice demonstrate a 27% increase ($P<0.01$) in metaphyseal BV/TV% when compared to WT animals, suggesting that reduced VDR activity in bone promotes bone accrual in the growing animal. Consistent with this finding, 6 week old $ObVDR^{-/-}$ mice display increases in metaphyseal BV/TV% (23%, $P<0.05$) and cortical width, due to decreased endosteal circumference (25%, $P<0.05$), associated with a marked reduction in RANKL mRNA-driven osteoclast number and serum X-laps when compared to $VDR^{fl/fl}$ control littermates. Decreases in mRNA levels of genes such as Runx2, Tnap and osteocalcin were also associated with a decline in osteoblast number and BFR, demonstrating that the absence of VDR in osteoblasts interrupts bone formation as well as bone resorption. In contrast, 6 week-old $OyVDR^{-/-}$ mice exhibited no change to trabecular or cortical bone parameters. These data demonstrate that in young, growing mice, VDR-mediated activity in osteoblasts or transitioning osteocytes, but not mature osteocytes, is a key regulator of bone accrual independent of changes to intestinal calcium supply, largely through the down-regulation of RANKL-mediated osteoclastic activities.

P41

Activation of Fgf/fgf Receptor Signaling in Hyp Osteocytes

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Osteocytes express multiple genes involved in mineral metabolism. In Hyp mice, a murine model for X-linked Hypophosphatemia (XLH), it has been established that Phex deficiency results in the overproduction of FGF23 in osteocytes, which leads to Hypophosphatemia. However, the role of osteocytes in the pathogenesis of Hyp mice is still not fully understood. In this study, to further clarify the abnormality in osteocytes of Hyp mice, we obtained detailed gene expression profiles in osteoblasts and osteocytes from Hyp mice and wild-type (WT) mice, using cells freshly isolated from the long bones based on the differentiation stage by sequential digestion and decalcification.

The expression of Fgf23, Dmp1, and Fam20c was higher in osteocytes than osteoblasts in both genotypes, and up-regulated in Hyp. Interestingly, the up-regulation of these genes in Hyp bones began before birth. Conversely, the expression of Slc20a1 encoding the sodium/phosphate (Na^+/Pi) co-transporter Pit-1 was increased in osteoblasts and osteocytes from adult Hyp, but not in Hyp fetal bones.

Previous studies suggest that induction of FGF23 in Hyp bone may be attributed to the activation of FGFR signaling. Therefore, we next examined the expression of the genes encoding Fgf1, Fgf2, Fgfr1–3, and a-Klotho in the osteocytes from WT and Hyp. The expression of Fgf1, Fgf2, Fgfr1, and Fgfr3 was significantly increased in Hyp, while that of a-Klotho was low in both genotypes. The expression of Egr-1, a target gene of FGF/FGFR signaling, was two-fold higher in Hyp osteocytes than in WT.

These results provide the evidence for the activation of FGF/FGFR signaling in Hyp osteocytes, which might play a role in the pathogenesis of Hyp mice through the dysregulation of multiple genes. Since the expression of Fgf1 and Fgf2 was increased in Hyp osteocytes, these FGF ligands rather than Fgf23 might be responsible for the activation of the signaling.

Disclosure: The authors declare no competing interests.

P42

Inhibition of Pdgfrb Reduces Osteoblast Mitogenesis and Enhances Osteoblast Differentiated Function.

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Imatinib is a tyrosine kinase inhibitor (TKI) used in the treatment of chronic myeloid leukemia (CML), gastrointestinal stromal cells tumor (GIST) and other malignant and proliferative disorders. Imatinib's receptors are present in bone and "bystander effects" on bone and calcium metabolism have been observed. In vitro imatinib inhibits osteoblast proliferation; at similar doses to those that inhibit proliferation, it increases differentiation of osteoblastic cells. It has been proposed that the mechanism of these osteoblastic effects is via inhibition of the PDGFRB. Recombinant PDGF enhances proliferation and reduces mineralization of osteoblastic cells. This effect is reversed by co-treatment with imatinib (and nilotinib with regards to the proliferative effect). When PDGFRB gene and PDGFRB protein expression is reduced using transient gene silencing techniques, there is a reduction in mitogenesis in ST2 cells. Using long-term gene silencing, achieved by lentiviral shRNA delivery, we have achieved stable knockdown of PDGFRB in MC3T3-E1 cells. These cells show reduced mitogenesis and enhanced mineralization, and have increased expression of genes associated with osteoblast differentiation. There is a reduced ability to respond to the mitogenic effects of recombinant PDGF, but not to other substances such as IGF-1 and lactoferrin. These results imply that PDGFRB is a potent regulator of osteoblast proliferation and differentiation, and that this may be a means by which imatinib inhibits osteoblast proliferation and enhance osteoblast differentiation.

P43

Exogenous and Endogenous Optimisation of In Situ Osteogenesis Using Biochemical and/or Mechanical Factors

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Critical sized defects often result from trauma, tumour resection, debridement after infection, and congenital defects. Defects beyond this "critical size" exceed the body's natural bone healing capacity, leading to non-union. Despite current advances in surgery and implementation of bone grafts, the treatment of such defects remains a major challenge in orthopaedic medicine. Endogenous and exogenous tissue engineering approaches with biochemical factors and/or mechanical factors offer viable alternative methods for treatment of critical size bone defects.

In orthopaedics, focus is often placed on increasing bone anabolism with biochemical growth factors, such as recombinant human bone morphogenetic protein (rhBMP-2). However, rhBMP has also been associated with osteoclastic catabolism, leading to premature or excessive bone catabolism. Furthermore, implant stress shielding and instability can often lead to premature catabolism of newly engineered bone. Bone regeneration and maintenance processes are intrinsically linked to the mechanical environment which modulates the chemical environment directly and indirectly. The periosteum, which bounds every nonarticular bone surface of the body, provides a niche for mechanosensitive osteoprogrenitor cells and exhibits great regenerative capacity. This talk describes two approaches to modulate bone tissue engineering: (1) combination biochemical approach, specifically, local co-delivery of anabolic rhBMP-2 and anti-catabolic bisphosphonate; and a (2) mechanochemical approach, which modulates mechanosensitive periosteal-derived stem cells and transport in the defect zone.

Disclosure: The author declares no competing interests.

P44

Assessing the Layered Distribution of Osteocytes in Secondary Osteons From High-resolution 3d Micro-ct Scans

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Osteocytes are commonly reported to form concentric layers around the Haversian canal of secondary osteons. However due to the irregular geometry of osteons and the high density of osteocytes, quantitative evidence for a layered distribution of osteocytes in three dimensions is lacking. Currently osteocyte distribution structure in 3D space is assessed from high-resolution μCT scans by low intensity projections, mean intercept lengths, image distortion realigning Haversian canals, or texture analysis quantities such as osteocyte distribution tensor and alignment tensor. These can provide quantitative indications of the collective grouping of osteocytes locally, but do not allow an understanding of this grouping in the whole cross-section of irregular osteons.

We have developed a method of analysis of osteocyte distribution in osteons of arbitrary shape based on the construction of a family of 3D mathematical surfaces. These surfaces gradually morph the osteon's cement line into the Haversian canal boundary and define pseudo-lamellae modelling the natural lamellar environment of the osteocytes. We applied this method to synchrotron-radiation μCT scans of human femoral cortical bone from which the Haversian canal, osteon boundary, and osteocyte lacunae were segmented.

We show that osteocyte lacuna density exhibits well-defined large-amplitude oscillations (20,000–45,000/mm^3) over sequential pseudo-lamellae. The period of 8–10 μm matches the width of two successive human bone lamellae. The plane of each lacuna aligns well with the local orientation of the pseudo-lamellae, showing that these surfaces are a valid model of the osteon's real lamellae. In contrast, lacunae outside the osteon's boundary do not align with pseudo-lamellae extrapolated there.
We conclude that the lamellar structure of secondary osteons is reflected in the 3D distribution of osteocytes, and likely composed of an alternate sequence of high-density and low-density lamellae. The orientation of osteocytes in bone is heterogeneous and strongly determined by the osteon geometry they belong to.

P45

Osteoblast Viability and Differentiation Following Treatment With A Novel Hydroxyapatite Based Product

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Despite advances in modern medicine, non-union rates following fracture and spinal fusion are as high as 20% and 35%, respectively. Furthermore, large areas of bone loss secondary to trauma or tumour resection may exceed the body's regenerative capabilities.
Clinically, autologous bone grafting remains the 'gold standard' treatment for non-unions. Although effective, high complication rates (>49%) have prompted steady growth in the use of Demineralised Bone Matrix (DBM). Current DBM products are based on human-derived bone matrix, which is associated with high costs.
Therefore, we aimed to determine whether a more readily available bovine bone product is a viable and sustainable solution. This product is manufactured using proprietary low temperature process to retain a 22% organic component. Previously we have shown that the organic component induced bone formation and decreased osteoclastogenesis, in vitro. Here, we evaluated the product in its entirety, incorporating it into a 3D collagen gel with human osteoblasts and compared it to collagen gel alone and collagen gel with the organic component denatured.
The viability and differentiation of human osteoblasts were assessed over a 14-day period using alamarBlue® and real-time PCR analysis of osteoblastic gene markers. Contrary to what we observed with the organic component alone, where osteoblast viability was decreased, in this study osteoblast viability increased over the 14-day period, but was unchanged between groups.
In the differentiation study, expression of ALP and OPG were increased 2-fold at the later time points. There was no difference in the other genes tested. This was similar to previous results with the organic component alone.
Overall, we demonstrated that this product is cytocompatible with human osteoblasts. Furthermore, the increase in ALP expression suggests that this product may increase bone formation, while the increase in the expression of OPG, but not RANKL, suggest that osteoclast formation would likely be inhibited.
Disclosure: The authors declare no competing interests.

P46

Native Sodium Currents in Murine Osteoblasts are Inhibited by Carbamazepine

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It is well-established that patients with epilepsy have an increase in fracture risk. However, the mechanism for this association is less well defined. We hypothesised that osteoblasts express ion channels which could be inhibited by anti-epileptic medication, potentially altering signalling during bone remodelling and repair. We aimed to investigate whether: (1) mouse primary calvarial osteoblasts express voltage-activated sodium currents, and (2) the anti-epileptic medication carbamazepine (CBZ) inhibits these currents.
Primary osteoblasts isolated from neonatal C57BL6 mice calvariae were used to examine the impact of CBZ on whole-cell current recordings produced using Patchliner, an automated planar patch-clamp system (Nanion, Germany). Currents were elicited using a voltage protocol stepping from –100 mV to +60 mV in 10 mV increments, for 20 ms, from a holding potential of –80 mV or –60 mV. CBZ (50 μM) was applied to the cells in the continued presence of external tetraethylammonium (10 mM) and internal Cs^+. Following washout of CBZ, 10 μM tetrodotoxin (TTX) a known voltage-gated sodium channel blocker was applied.
Robust voltage-activated inward currents were elicited and external application of CBZ (50 μM) resulted in a significant inhibition of current amplitude 31.6 ± 5.9 % ($n = 7$; $p<0.001$), which was partially reversed upon washout. Subsequent application of TTX (10 μM) produced almost complete inhibition of current amplitude 89.96 ± 2.14 % ($n = 6$; $p<0.0001$).
Our results demonstrate that mouse osteoblasts express native voltage-activated sodium channels, which are sensitive to CBZ. To our knowledge this is the first study to utilise a Patchliner to examine native primary osteoblast sodium currents, and to demonstrate an inhibitory effect of CBZ. Further study is required to determine whether the effects on ion channel activity observed here translate to clinically-relevant changes in bone signalling and bone quality.
Disclosure: The authors declare no competing interests.

P47

Endogenous Glucocorticoid Signalling in Osteoblasts is Required for Acquisition of Vertebral Trabecular Bone Mass in Mice

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We have previously shown that targeted enzymatic disruption of endogenous glucocorticoid (GC) signalling in osteoblasts at the pre-receptor level is associated with impaired bone acquisition in mice. The present study examined the skeletal phenotype of mice with osteoblast-specific deletion the glucocorticoid receptor (GR).
Osteoblast-specific GR knock-out mice (obGRKO) were generated by crossing $GR^{flox/flox}$ mice with a transgenic mouse line expressing cre recombinase under the control of a type I collagen (Col2.3) promoter. At 12 weeks of age, L3 vertebrae and tibiae from male and female obGRKO mice and their Cre-negative $GR^{flox/flox}$ littermates (WT) were harvested and analysed by micro-CT.
Body weight did not differ between obGRKO and WT mice across both genders. Micro-CT analysis revealed a significant difference of vertebral bone volume (p=0.025) across both male (BV/TV: 16.6% v. 21.3%) and female (BV/TV: 14% v. 18.7%) obGRKO mice compared to their WT littermates. This was largely due to a reduction in trabecular number ($3.2\,mm^{-1}$ v. $4.2\,mm^{-1}$, p=0.059 in males; $2.7\,mm^{-1}$ vs. $3.7\,mm^{-1}$, p=0.042 in females), and a corresponding increase in trabecular separation (0.19 mm vs. 0.17 mm, p=0.009 in males; 0.24 mm vs. 0.2, p=0.00 in females). Interestingly, vertebral trabecular thickness remained unaffected by osteoblast-targeted loss of the GR and trabecular bone parameters in the tibia were similar in WT and obGRKO mice.
Our results indicate that signalling of endogenous GCs in osteoblasts via the GR pathway is crucial for the accrual and maintenance of vertebral trabecular bone mass in young adult mice. In contrast, accretion of tibial trabecular bone mass appears to occur independent of endogenous GC signalling.

P48

Molecular Mechanisms Underlying Changes in Osteogenesis During Hyperhomocysteinemia

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Homocysteine is a sulphur containing non-protein amino acid which alters bone remodeling process. Though many clinical studies have shown that homocysteine induces bone loss, the underlying molecular mechanisms remain ambiguous. Herein we demonstrate how hyperhomocysteinemia affects osteogenesis during skeletal remodeling.
Two models systems were employed for this study: (i) Hyperhomocysteinemia induced in skeletally immature mice by administration of 5 mg/100 g body weight homocysteine i.p for 30 days and (ii) homocysteine (500 μM) induced MC3T3E1 osteoblast cultures.
Analysis of hyperhomocysteinemic bone by RT^2 Profiler mouse osteogenesis PCR array followed by qPCR revealed many pathophysiological changes like (i) altered chondrogenesis (increased Sox-9 and reduced type X collagen), (ii) augmented adipogenesis (increased PPAR-γ), (iii) loss of osteocyte integrity (reduced sclerostin), (iv) altered TGF-β/BMP signaling and (v) reduced osteovascularity (decreased VEGF). Interestingly we also observed high expressions of odontogenic proteins like ameloblastin, tuftelin and msx-1 in hyperhomocysteinemic bone. Experiments conducted in osteoblasts cultures in vitro demonstrated that homocysteine induced ameloblastin upregulation was a physiological process adopted by bone forming cells to counter the stress associated with alterations in osteogenesis during hyperhomocysteinemia.
The results indicate that the pathogenesis of bone loss during hyperhomocysteinemia is different from typical bone disorder prototypes like osteoporosis. This study unravels the molecular mechanisms that change bone formation during hyperhomocysteinemia and identifies candidate genes that alter bone formation during skeletal remodeling.
Disclosure: The authors declare no competing interests.

P49

Both Tnf and Sharpin are Required for Normal Osteoclastic Function

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SHARPIN is a component of the linear ubiquitin chain assembly complex (LUBAC), along with HOIL-1L and HOIP. LUBAC is involved in the NF-κB canonical signaling pathway activated by inflammatory cytokines such as TNF via TNF receptor 1 [1]. Loss-of-function SHARPIN mice (cpdm) develop a chronic proliferative dermatitis mutation and also develop an osteopenic phenotype [2]. The aim of this experiment was to determine whether this osteopenic phenotype was a result of disruptions to the TNF/SHARPIN pathway and the related effects on osteoclastic (OCl) differentiation and activity.
Bone marrow stromal cells were cultured from femora of control (CTRL), cpdm, $Tnf^{-/-}$ (TNF KO) and cpdm/TNF KO mice ($n \geq 4$). Cells were grown in OCl growth media (25 ng/ml m-CSF, 100 ng/ml RANKL) for 14 days prior to analyses, which included immunocytochemistry, tartrate-resistant acid phosphatase (TRAP) staining and qPCR.
Immunocytochemistry and qPCR identified the presence of sharpin (protein and gene expression) in treated bone marrow cell cultures of CTRL and TNF KO mice and an absence of SHARPIN in cpdm and cpdm/TNF KO cultures. TRAP staining identified a decrease in OCl number in all three experimental groups relative to CTRL ($P<0.01$). The apoptotic gene caspase-3 increased in cpdm mice compared with all other groups ($P<0.0001$). In the absence of SHARPIN, the osteoclastic

marker cathepsin K decreased compared with CTRL (P<0.05) and was not fully recovered in either TNF KO or cpdm/TNF KO cells. This may be due to the decrease in RANK expression in all experimental groups (P<0.0001).
Overall, our findings suggest that both TNF and SHARPIN are required for osteoclast formation and the regulation of apoptosis in osteoclastic progenitor cells.
Disclosure: The authors declare no competing interests.
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P50
Synthetic Analogues Display More Potent Activity in Inhibiting Osteoclastogenesis than the Natural Fatty Acid
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We previously found that saturated fatty acids of C14-18 are inhibitory to osteoclastogenesis. However, their action on osteoclasts is complicated by modest potency, rapid metabolism and low solubility. To optimise these fatty acids for potential therapeutic use, we have introduced two modifications for C16 fatty acids: insertion of ether or triazole units. The effects of these analogues on osteoclasts have been tested using mouse bone marrow and $RAW_{264.7}$ cell cultures. Among the eight ether-modified analogues, only three were found to be mildly inhibitory to osteoclasts, while all six triazole-modified analogues inhibited osteoclastogenesis by 60% to 80% at 10 μg/mL in mouse bone marrow cultures.
The two most potent analogues had their triazole group substituted within two carbons from either end of the chain. In the mid-range concentration (5 μg/mL), at which palmitate inhibited osteoclastogenesis by 19%, these two compounds exhibited 35.5% and 58.5% inhibition, indicating a much higher potency of the analogues than that of the natural fatty acid. In $RAW_{264.7}$ cell cultures in the absence of stromal cells, the two compounds reduced osteoclast numbers by 31.9% and 41.1%, while palmitate reduced the numbers by 20.8% (all at 10 μg/mL).
In conclusion, triazole-modified fatty acid analogues are more potent osteoclastogenesis inhibitors than either ether analogues or the natural fatty acids. They could act directly on osteoclasts, in a similar way to palmitic acid. The signalling pathways involved are still under investigation. This study has provided a novel approach to improve the efficacy of the natural product in targeting bone cells.

P51
Cell Stressors that Enhance Osteoclast Formation Cause Increased Microphthalmia Transcription Factor (Mitf) Protein but not mRNA Levels in Osteoclast Progenitors
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We have shown that a number of HSP90 inhibitors and cancer chemotherapeutic agents (such as doxorubicin) increase RANKL-dependent osteoclast formation. These actions depend upon the induction of heat shock factor 1 (HSF1)-mediated cell stress. However, how cell stress affects osteoclast formation is unclear. We therefore investigated the influence of these stressors on NFATc1 and MITF, key RANKL-induced transcription factors required for osteoclast differentiation.
HSP90 anti-cancer inhibitors 17-AAG and NVP-AUY922 dose-dependently enhanced osteoclast formation in RANKL-treated bone marrow and RAW264.7 cells, as did doxorubicin and cisplatin. We investigated NFATc1 and MITF induction by Western blotting, and NFAT activity by use of a stable NFAT-dependent luciferase reporter (RAW264.7) cell line. Since Mitf gene promoter regions have been reported to contain HSF1 binding domains we also investigated the effects of stressors on mRNA expression of MITF and known MITF splice variants by semi-quantitative RT-PCR methods.
None of the stimuli significantly induced NFATc1 activity. In contrast, all increased MITF protein levels. Consistent with a previous report (Lu *et al*., *Mol Biol Cell* 2010:**21**:1763), RANKL affected expression of only one variant of MITF, namely MITF-E, which it strongly induced in bone marrow macrophages and RAW264.7 cells. In contrast, 17-AAG failed to induce mRNA levels MITF-E (or any other variant) despite causing increased steady state MITF protein levels in these cells.
These data indicate that cell stressor-enhanced osteoclast formation does not principally involve increased NFATc1 (typically considered the master controller of osteoclast formation) but rather influences MITF protein levels. However, our results do not support a direct transcriptional control of MITF by HSF1 or another stress induced factor. This may suggest a post-translational control mechanism such as stabilization of MITF protein by stress-induced heat shock proteins.
Disclosure: The authors declare no competing interests.

P52
Prolonged Exposure to Nanomolar Concentrations of Zoledronic Acid Inhibits Rap1A Prenylation in Cultured Macrophages
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Bisphosphonate drugs (BPs) are the gold standard of treatment to inhibit bone resorption in patients with bone

diseases such as post-menopausal osteoporosis, Paget's disease and tumour-associated osteolysis. BPs target bone mineral, are selectively internalised by osteoclasts at high concentrations (at least micromolar) during bone resorption, and prevent the prenylation of small GTPases such as Rap1A by inhibiting the enzyme FPP synthase. Low (nanomolar), circulating concentrations of BPs could affect cells outside bone, particularly myeloid cells such as macrophages. However, effects of nanomolar concentrations of BPs on protein prenylation have never been described. Inhibition of prenylation can be monitored by western blotting to detect the accumulation of unpenylated Rap1A. We sought to determine whether nanomolar concentrations of the BP zoledronic acid (ZOL) could inhibit Rap1A prenylation in cultured macrophages.

J774 mouse macrophage cells were cultured acutely (1–2 days, mimicking effects of BP on osteoclasts) or chronically (3–31 days, mimicking effects of circulating BP on cells outside the skeleton) with 1 mM-50 mM or 1 nM-1000 nM ZOL. The accumulation of unprenylated Rap1A was measured using western blotting. Acute treatment with >2 mM ZOL inhibited Rap1A prenylation, but concentrations <1 mM had no detectable effect. In contrast, treatment for 3-7 days with 250 nM or 500 nM ZOL inhibited Rap1A prenylation; 1 mM ZOL dramatically inhibited Rap1A prenylation after 7 days' treatment. By day 31, prolonged exposure to as low as 10 nM ZOL inhibited the prenylation of Rap1A.

We provide the first evidence that nanomolar concentrations of the bisphosphonate drug ZOL can inhibit Rap1A prenylation in cultured macrophages, but that prolonged exposure to low concentrations of ZOL is required (as might occur in vivo in patients). Further studies are ongoing to examine short-term and long-term effects of ZOL treatment on Rap1A prenylation in vivo in cells such as macrophages outside the skeleton.

P53

Investigation of Pathways Involved in M-csf Augmentation of Rankl-induced Resorption

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Osteoporosis develops when bone homeostasis is disrupted in favour of osteoclastic bone resorption. RANKL is a cytokine which initiates osteoclast bone resorption and M-CSF greatly enhances RANKL-induced resorption activation in mature human osteoclasts. We have investigated the pathways involved in the M-CSF augmentation of resorption. We analysed the effect of various inhibitors targeting signalling pathways downstream of M-CSF. During investigation of the MEK/ERK pathway we found that PD98059 (MEK1 inhibitor) significantly reduced M-CSF-enhanced resorption. Since ERK1 is downstream of MEK1, we hypothesised that down-regulating Erk1 will further reduce resorption to RANKL-only levels. Using a mature osteoclast dentine resorption assay, we have been investigating the effect of supressing Erk1 on resorption. However, as yet our data showing the effect of down-regulation of Erk1 using siRNA on resorption is inconclusive. When osteoclasts are activated for resorption formation of F-Actin rings occur. Using this characteristic to gauge resorption activation, we are undertaking F-Actin ring immunofluorescence to complement our dentine resorption assays. To further investigate the M-CSF resorption phenomenon, we decided to look at IL-34 since, along with M-CSF, it is a ligand of the c-fms receptor. Surprisingly, we discovered that IL-34 does not enhance RANKL-induced resorption. Logically, therefore, it would be expected that M-CSF activates some pathway(s) separate to IL-34 which enables enhanced RANKL-induced resorption. Using Western-blot analysis, phospho-ERK1 was found to be in equal abundance in both M-CSF and IL-34 stimulated mature human osteoclasts. This suggests that ERK1 is not part of the unique pathway that results in M-CSF augmented resorption, contrary to the indication from our earlier results. We also examined the Akt/PI3K pathway, however, the results were similar to ERK1. Using the difference in resorption activation between M-CSF and IL-34 we are continuing to investigate potential candidates to target in order to minimise bone resorption by osteoclasts.

P54

Combined Effects of Soy Isoflavones and Docosahexaenoic Acid on Osteoclast Formation in Mouse Bone Marrow Cell Culture

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Soy isoflavones, such as daidzein and genistein, have a chemical structure similar to that of estrogen, and exhibit weak estrogenic activity. Many studies have shown that soy isoflavones inhibit bone resorption. Docosahexaenoic acid (DHA), one of the n-3 polyunsaturated fatty acids, also has an inhibitory effect on bone resorption. This study investigated the combined effects of soy isoflavones and DHA on osteoclast formation as well as nuclear factor of activated T-cells c1 (NFATc1) expression using mouse bone marrow cell culture.

Mouse bone marrow cells were obtained from 7- to 9-week-old male Balb/c mice, and pre-cultured in phenol red-free α-MEM containing 10% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin with 30 ng/ml macrophage colony-stimulating factor (M-CSF) for 3 days. For evaluation of osteoclast formation, cells were further cultured with 30 ng/ml M-CSF and 30 ng/ml receptor activator of nuclear factor κB ligand in the presence or absence of isoflavones and DHA. After 6 days, cells were stained for tartrate-resistant acid phosphatase (TRAP) and TRAP activity in the medium was measured. In addition, NFATc1 mRNA expression was also measured after 6 days of culture.

Ten μM daidzein and genistein, as well as 20 μM DHA significantly decreased the number of TRAP-positive multinucleated cells (TRAP(+)MNCs) and TRAP activity, and the combination of soy isoflavones and DHA further decreased the number of TRAP(+)MNCs. NFATc1 mRNA expression was decreased by each of daidzein, genistein, and DHA. However, the combination of soy isoflavones and DHA caused no further reduction in NFATc1 mRNA expression.
These results demonstrated that the combination of soy isoflavones and DHA enhanced the inhibition of osteoclast formation. Furthermore, this suppressive effect by these food ingredients alone and in combination on osteoclast formation might be explained in part by a decrease in NFATc1 mRNA expression.

P55

Alexidine Dihydrochloride Attenuates Osteoclasts Formation and Bone Resorption Via Induction of Mitochondrial-activated Apoptotic Program

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Excessive osteoclast (OC) formation and bone resorption are the main mechanisms that lead to severe osteolytic bone diseases, such as osteoporosis and bone metastatic tumours. Thus the induction of OC death (apoptosis) represents a potential therapeutic mechanism for the treatment of such osteolytic diseases.
Alexidine Dihydrochloride (AD), a positively charged dibiguanide compound found in contact lens cleaning solutions and oral mouthwashes, induces lipid-phase separation and domain formation of negatively charged cellular membranes and has been shown to exhibit apoptosis-inducing properties. The most negatively charged biological membranes known to nature and highly abundant in OC, the mitochondria is a potential candidate target for AD-induced apoptotic effects. In vitro RANKL-induced osteoclastogenesis was inhibited by AD in a dose-dependent manner and correlated with suppression of OC-specific marker genes (TRAP, CTSK, CTR and DC-STAMP). Additionally, AD attenuated the bone-resorbing activity of mature OCs. Mechanistically AD disrupted mitochondrial function and induced classical mitochondrial apoptotic program involving members of the Bcl-2 family, cytochrome c release, triggering activation of caspase-3 and -9 apoptotic cascades and ultimately leading to nuclear condensation and cell death.
Collectively, our findings demonstrate that AD inhibited OC formation and function via inducing mitochondrial-activated apoptotic program. Thus, our results prove that AD may represent an alternative therapeutic agent for the treatment of osteolytic bone diseases.

P56

Ca^{2+}-Dependent Control of 25-Hydroxyvitamin D-1α-Hydroxylase Expression Mediated by the Calcium-Sensing Receptor

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Elevated extracellular calcium (Ca^{2+}_o) represses 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) expression in the renal proximal tubule but activates it in the parathyroid, and thus appears to have opposing effects on 1,25-dihydroxyvitamin D synthesis in different tissues. Both these effects function to reduce elevations in Ca^{2+}_o concentration. However, the underlying mechanisms remain unknown. The present study aimed to elucidate the mechanisms of Ca^{2+}_o-dependent CYP27B1 expression using CYP27B1-luciferase constructs transfected into HEK-293 cells stably expressing the CaSR (HEK-CaSR cells), and control HEK-293 cells. At lower Ca^{2+}_o concentrations (0.5–3.0 mM), there was a Ca^{2+}_o-dependent increase in CYP27B1-luciferase expression in HEK-CaSR cells as Ca^{2+}_o was increased, which was absent in control HEK-293 cells. Interestingly, as Ca^{2+}_o concentrations increased further (5.0-6.5 mM), a reduction of expression was observed, exhibiting an overall biphasic Ca^{2+}_o-dependent response. Furthermore, addition of the CaSR selective positive modulator cinacalcet (1.0 μM) enhanced both these responses, shifting the peak response from 3.0 mM to 0.5 mM, whereas the negative modulator NPS 2143 (1.0 μM) abrogated the Ca^{2+}_o-dependent response, indicating both the stimulatory and inhibitory effects are CaSR-mediated. However, the peptide S-methylglutathione (30 μM) that binds in the Venus flytrap domain of the CaSR had no effect. To investigate the regulatory elements of the CYP27B1 promoter, we used a 305 bp truncation and observed that it remained sufficient for CYP27B1-luciferase expression in HEK-CaSR cells. Inactivating response elements within this region by site-directed mutagenesis, in particular AP1, CRE(p), CCAAT, and Sp1(p), reduced the Ca^{2+}_o-dependent increase in CYP27B1-luciferase expression at 3.0 mM Ca^{2+}_o. It is thus hypothesised that multiple response elements function in combination to control CYP27B1-luciferase expression in HEK-CaSR cells. This stimulatory response may provide a mechanistic basis for the activation of 1,25-dihydroxyvitamin D synthesis in the parathyroid, while elucidation of the inhibitory response may describe expression patterns observed in proximal tubule cells.

P57

An Inter-domain Disulfide Bridge Between the Calcium-binding Domain and the Heptahelical Signalling Domain that is Essential for Activation of the Calcium-sensing Receptor

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The calcium-sensing receptor (CaSR) is a class C GPCR that mediates extracellular Ca^{2+} (Ca^{2+}_o)-dependent feedback regulation of parathyroid hormone secretion and renal calcium excretion and is, thus, a critical component of the machinery

that supports whole body calcium homeostasis. Ligand binding in the CaSR's extracellular Venus Flytrap Domain (VFTD) induces intracellular signalling via a Cysteine-rich domain (CRD) coupled to a heptahelical transmembrane domain (HHD). However, the mechanism that supports this interdomain coupling has not been identified. We have now investigated the roles of two Cysteine residues that are highly conserved in Class C GPCRs and conclude that CaSR residues C236 in the VFTD and C561 in the CRD participate in a functionally critical interdomain disulfide that transmits Ca^{2+}_o-dependent turning moments from the activated VFTD to the HH signalling domains.

The key observations are as follows:

1. Mutant receptors including C236S, C561S and the double mutant C236S/C561S are non-functional in Ca^{2+}_i mobilization assays when expressed in HEK-293 cells and exposed to the VFTD ligands Ca^{2+}_o and L-Phe;
2. However, these mutants receptors retain signalling in the presence of the positive modulator Cinacalcet, which binds in the receptors' HHDs and directly activates them;
3. A FLAG epitope tagged CaSR construct in which a thrombin cleavage site was introduced between the VFTD and CRD was expressed and functionally active;
4. Following expression in HEK-293 cells, thrombin treatment, purification by immuno-precipitation based on the FLAG epitope, and western blotting for the presence of the FLAG tag, CaSR constructs of the expected sizes for intact monomers (160 kDa) and dimers (320 kDa) were observed in the absence of the disulfide reducing reagent β-mercaptoethanol, suggesting a second physical connection between the VFTD and the CRD.
5. In the presence of β-mercaptoethanol, however, there were significant reductions in the sizes of FLAG-tagged protein fragments corresponding to the expected sizes of monomeric VFT domains (i.e., 70 kDa). A similar fragmentation pattern was observed after expression of FLAG-tagged C236S/C561S mutant receptors in the presence of thrombin followed by immuno-precipitation and western blotting, regardless of whether β-mercaptoethanol was absent or present.

The study demonstrates that the formation of a disulfide between C236 and C561 is essential for functional coupling between the calcium-binding VFT domain and HH signalling domain.

P58

Roles of the Intraloops and Carboxy-terminus of the Calcium-sensing Receptor in Signalling Pathway Selection

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The calcium-sensing receptor (CaSR) is a Class C G-protein coupled receptor that is widely expressed. It contributes to the control of calcium metabolism and bone homeostasis via expression in the parathyroid, renal cortical thick ascending limb, calcitonin-secreting thyroid C cells, and in bone cells, including cells of the osteoblast lineage. The CaSR mediates diverse effects by selecting for signalling pathways in a ligand- and cell-type-specific manner. However, the mechanisms that underlie the selection of signalling pathways are not well understood.

In the present study, we have used alanine scanning site-directed mutagenesis to identify subdomains and residues in intraloops -2 and -3 that are critical for the coupling of the extracellular Ca^{2+} (Ca^{2+}_o) -activated receptor to distinct pathways including downstream of PI-PLC (IP_1 accumulation), phosphorylated $ERK_{1/2}$ (pERK), intracellular Ca^{2+} (Ca^{2+}_i) mobilization, and suppression of forskolin-stimulated adenylyl cyclase (intracellular cAMP levels).

The results demonstrate that distinct residues mediate coupling to distinct signalling pathways downstream of the receptor. Most strikingly, four mutants F706A (iL-2), L797A and E803A (iL-3), and a C-terminal truncation mutant R866X, which removes all but the proximal three residues of the C-terminus (863–865), none of which impaired cell surface expression, all markedly attenuated PI-PLC and pERK but had differential effects on Ca^{2+}_i mobilization and suppression of adenylyl cyclase. In particular, R866X exhibited complete loss of Ca^{2+}_i mobilization but retained intact suppression of adenylyl cyclase. In addition, E803A exhibited only partial impairment of Ca^{2+}_i mobilization but retained intact suppression of adenylyl cyclase.

The results demonstrate that pathway selection arises from distinct domains and sub-domains of the receptor's intraloops and C-terminus.

Disclosure: The authors declare no competing interests.

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Structural Studies of the Extracellular Domain of the Calcium Sensing Receptor

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The calcium-sensing receptor (CaSR) is a class C G-protein coupled receptor (GPCR), characterised by a large nutrient-sensing N-terminal extracellular region consisting of a bilobed Venus Fly Trap (VFT) module and a cysteine-rich (CR) domain. Two or more primary binding sites for the endogenous ligand Ca^{2+} and a binding pocket for positive allosteric modulator L-amino acids have been identified in the VFT. In addition, the VFT module is involved in receptor dimerisation. However, the locations of the ligand binding sites and the mechanisms that support co-operativity and receptor activation are unclear. Definitive solution of these problems requires determination of the structures of the CaSR's key domains in both unoccupied and ligand-bound forms. We have undertaken trials of expression and purification of the CaSR's extracellular domains with a view to solving these problems. Based on secondary structure predictions, disorder analysis and sequence alignments of CaSR across species and Class C GPCRs, six variants of CaSR extracellular domain (ECD) inserts were prepared and ligated into bacterial protein expression vectors pRSET, pGEX, pMal c2x and pMal p5x. Preliminary results from protein expression trials in *E. coli* Rosetta cells demonstrate overexpression of the His-tagged CaSR ECD constructs confirmed by western blotting using anti-His antibody. Overexpression of

GST tagged CaSR VFT proteins were also observed in Coomassie stained SDS-page gel and confirmed by a western blot using CaSR ADD polyclonal antibody. In both of these cases we have encountered problems with protein solubility and limited fragmentation indicating that further optimisation is required.

P60

Roquin is a Novel Regulator of Bone Homeostasis

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Many osteolytic diseases have highlighted the importance of osteoimmunology, a dialogue between the immune and skeletal systems. However, the underlying mechanism of osteoimmunology remains poorly understood. To gain insights into the molecular genetics and mechanisms of osteoimmunology, we have screened a chemically induced (ENU) mutagenesis library and identified a mouse line with M199R mutation in the Roquin (Rc3h1) gene. The mutation results in the dysregulation of follicular helper T cells (TFH) and the SanRoque mutant mouse line displays an autoimmune disease consistent with Systematic Lupus Erythematosus. X-ray analysis revealed that SanRoque mice possess a lower bone density in comparison to wildtype littermates. MicroCT and histological analyses confirmed a low bone mass phenotype in SanRoque mice with a significantly reduced bone volume (BV/TV) as compared to wildtype littermates. Real Time-PCR analysis revealed elevated RANKL expression in whole bone isolated from SanRoque mice. Flow cytometry analysis demonstrated that the increased RANKL expression is correlated with a significant expansion of putative osteoclast progenitor populations in the SanRoque mice relative to wildtype mice. Consistent with the increased in progenitors, we observed enhanced osteoclastogenesis and osteoclast activity from bone marrow macrophages derived from SanRoque mice, accompanied by enhanced RANKL-mediated MAPK signaling. In vivo bone calcein labeling showed a reduction in bone mineral apposition rate in the SanRoque mice indicating that osteoblast activity was also affected. Consistently, SanRoque calvarial-derived osteoblast culture showed a reduction in bone-nodule formation. Co-culture experiments further revealed that calvarial osteoblasts from SanRoque mice have a reduced ability to support osteoclastogenesis, suggesting an extrinsic contribution to the priming effect of osteoclast progenitors within the bone marrow niche in SanRoque mice. Taken together, these findings demonstrate that Roquin is an important regulator of bone homeostasis and SanRoque mice emerge as a useful model to gain osteoimmunological insight into systemic bone loss in autoimmune diseases.

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Enu Induced Chemical Mutagenesis Reveals that Morc3 is a Novel Regulator of Bone Homeostasis

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Bone homeostasis is tightly regulated by complementary activities of bone-resorbing osteoclasts and bone-forming osteoblasts, imbalance in which leads to clinical diseases like osteoporosis. Identification of novel molecules that regulate bone homeostasis may provide us with new therapeutic strategies. In the present study, a phenotype-driven N-ethyl-N-nitrosourea (ENU) mutant mouse screening approach was employed to identify an aberrant bone phenotype in a strain of (heterozygous) mutant mice with a mutation at splice donor site of intron 12 in microorchidia 3 gene (Morc3). Morc3 is an epigenetic regulator of transcription and DNA damage response with previously unknown function in bone homeostasis. MicroCT analysis of 3 month old mutant mice femurs revealed significant thinning of the cortical bone, with significantly reduced cortical area and cortical BMD (WT = 1.76 g/cm^3 vs Mutant = 1.71 g/cm^3, $p<0.05$). In vivo and in vitro analysis indicated significant reduction in osteoclast number, surface area and bone resorption in Morc3 mutant compared to WT. There was no detectable change in osteoblast bone nodule formation in vitro. Heterozygous mutant cells express Morc3 mRNA and 2 additional spliced variants which might lead to reduced levels of functional protein. Western Blot revealed reduced Morc3 protein expression during osteoclast differentiation in mutant osteoclasts. No difference in Morc3 protein levels were observed during osteoblast differentiation in osteoblasts from both WT and Morc3 mice. Gene expression of OPG and RANKL were differentially upregulated during osteoblast differentiation such that the ratio of RANKL/OPG was reduced in Morc3 mutant long bone compared to WT in accordance with observed reductions in osteoclast number. A remarkable increase in STAT1 protein levels and divergence of MAPK signalling was observed in mutant osteoclasts, during differentiation, which indicates a critical role of Morc3 in RANKL induced osteoclast regulation. Altogether our data suggest that Morc3 is a novel regulator of bone homeostasis.
Disclosure: The authors declare no competing interests.

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Sharpin is a Key Regulator of Skeletal Homeostasis in a Tnf-dependent Manner

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SHARPIN is a subunit of linear ubiquitin chain assembly complex (LUBAC) regulating the activation of NF-κB, a pivotal transcription factor in skeletal homeostasis. Mice with a mutation in the SHARPIN gene (cpdm) develop chronic proliferative dermatitis and systemic inflammation, whilst the hyper-inflammatory phenotype of cpdm mice is corrected by crossing with Tnf$^{-/-}$ (TNF KO) mice [1]. Cpdm mice have an osteopaenic phenotype which is characterised by decreased

cortical and trabecular bone volume [2]. As TNF has been shown to stimulate osteoclastic activity and inhibit osteoblastic activity, this current experiment examined the skeletal development of cpdm mice and compared them to both TNF KO and cpdm/TNF KO mice.

Both femora were collected from control (CTRL), cpdm, TNF KO and cpdm/TNF KO mice ($n \geq 4$). RNA was extracted from the proximal femur and expression of genes associated with osteoclastic, apoptotic and inflammatory markers were compared between groups. The other femur underwent μCT analysis of both trabecular and cortical bone.

Although a decreasing trend was identified in osteoclastic and osteoblastic gene expression (cathepsin K and collagen 1α1 respectively) between CTRL and cpdm groups, no significant differences were recorded. IL-1β, TNF and caspase-3 expression was elevated in cpdm mice ($P<0.05$); however expression was similar to control levels in both TNF KO and cpdm/TNF KO mice (TNF expression not present). There was a decrease in both trabecular and cortical bone measurements in cpdm mice compared to controls ($P<0.05$), while in the absence of TNF (TNF KO and cpdm/TNF KO), trabecular and cortical bone morphology was similar to CTRL.

Overall, the removal of TNF prevented the osteopaenic phenotype seen in cpdm mice, indicating that SHARPIN is responsible for mediating the role of TNF in bone remodelling. Further investigation utilising cell cultures of osteoblasts and osteoclasts of these four groups needs to be performed.

Disclosure: The authors declare no competing interests.

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P63

Vitamin K-dependent Gamma-glutamyl Carboxylase Regulates Bone Formation and Glucose Metabolism

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VK-dependent γ-glutamyl carboxylase (GGCX) is an enzyme that catalyzes a posttranslational modification, which changes glutamic acid to gamma-carboxyglutamic acid (Gla) residues in its target proteins. GGCX-dependent Gla-modified proteins such as osteocalcin (OC) and matrix Gla protein (MGP) are known to play modulatory roles in bone metabolism. Recently, OC and undercarboxylated OC (ucOC) has been paid much attention as they are supposed to be involved in glucose metabolism by regulating insulin sensitivity and secretion. The precise contribution of GGCX to bone formation and glucose metabolism, however, remains to be clarified. To address this question, we generated osteoblast-specific Ggcx-deficient (i.e., conditional knockout: cKO) mice using collagen type1 α-1 (Col1)-Cre mice. Ggcx cKO and control (Col1-Cre) mice were subjected to the analyses of bone mineral density (BMD), bone histomorphometry, bone strength, immunohistochemistry, transmission electron microscopy and oral glucose tolerance test (OGTT). We confirmed that immunoreactivites detected by an anti-GGCX antibody was absent in osteoblasts of Ggcx cKO mice. Intriguingly, BMD, bone strength, and osteoid formation were substantially increased in Ggcx cKO mice compared with controls. Electron microscopic examination revealed disassembly of mineralized nodules and aberrant calcification of collagen fibers in Ggcx cKO mice. OGTT showed that the rate of increase in serum insulin levels was lower in Ggcx cKO mice compared with controls. Interestingly, weights of white adipose tissue were decreased in Ggcx cKO mice. Taken together, our findings suggest that GGCX expressed in osteoblasts will play a multifaced role in various tissues, including bone homeostasis and glucose metabolism.

Disclosure: The authors declare no competing interests.

P64

Disruption of Glucocorticoid Signalling in Osteoblasts Prevents High-fat Diet-induced Bone Loss in Mice

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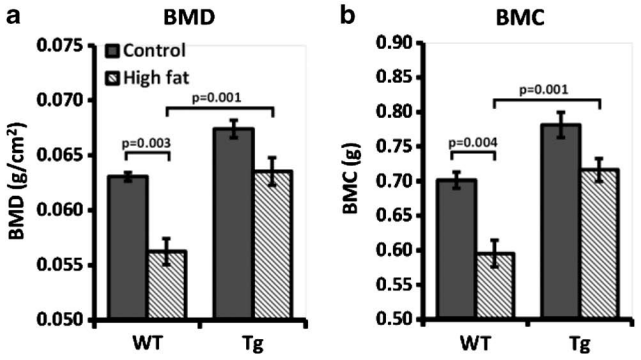
High-fat diets adversely affect bone strength in mice while simultaneously increasing systemic glucocorticoid levels. We hypothesise that a mechanistic link exists between high-fat intake, increased glucocorticoid signalling in osteoblasts and poor bone health. We tested this hypothesis in a transgenic (tg) mouse model in which glucocorticoid signalling has been selectively disrupted in osteoblasts/osteocytes via targeted overexpression of the glucocorticoid-inactivating enzyme, 11β-hydroxysteroid dehydrogenase type 2. Seven-week-old male tg mice and their wild type (WT) littermates (n=6–9/group) were fed ad libitum a control diet (14.3% energy from fat, 25.5% from protein) or an isoenergetic high-fat diet (HFD; 43.0% energy from fat, 25.5% from protein) for 16 weeks. Body weight and food intake were measured weekly. Serum corticosterone levels were quantified after 10 weeks of feeding, and body composition and bone parameters were assessed at endpoint.

As animals were fed an isoenergetic diet, changes in body weight and body composition did not differ between the four groups. Both WT and tg mice fed the HFD had higher serum corticosterone levels than WT or tg control mice (pooled measures: 386±35 nM for HFD vs. 285±36 nM for control diet; $p=0.054$). Corticosterone levels in WT and tg mice fed the same diets (either HFD or control) were similar. At endpoint, WT mice fed a HFD had significantly lower BMD than both WT mice receiving the control diet ($0.056\pm0.001 g/cm^2$ vs. $0.063\pm 0.0004 g/cm^2$; $p<0.005$) and tg animals fed the HFD ($0.056\pm0.001 g/cm^2$ vs. $0.064\pm0.001 g/cm^2$; $p<0.005$; Fig. 1A). Similarly, WT mice on HFD exhibited lower BMC than

WT control mice (0.60±0.02 g vs. 0.70±0.01 g; p<0.005) and tg mice receiving the HFD (0.60±0.02 g vs. 0.72±0.02 g; p<0.005; Fig. 1B). Neither BMD nor BMC differed significantly between tg control and tg HFD mice.

We conclude that high dietary fat intake negatively affects bone mass via glucocorticoid signalling in osteoblasts and osteocytes.



P65

Novel Technology to Develop Architecturally Controlled Ceramic Scaffolds with Outstanding Potential for Supporting Bone Regeneration under Load

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During the past two decades, research on ceramic scaffolds for bone regeneration has progressed rapidly; however, currently available porous scaffolds remain unsuitable for load-bearing applications. The key to success is to apply microstructural design strategies to develop ceramic scaffolds with mechanical properties approaching those of bone. Here we report on the development of a unique microstructurally designed ceramic scaffold, strontium–hardystonite–Gahnite (Sr–HT–Gahnite), with 85% porosity, 500 micro pore size, a competitive compressive strength of 4.1 ± 0.3 MPa and a compressive modulus of 170 ± 20 MPa. The in vitro biocompatibility of the scaffolds was studied using primary human bonederived cells. The ability of Sr–HT – Gahnite scaffolds to repair critical-sized bone defects was also investigated in a rabbit radius under normal load, with b-tricalcium phosphate/hydroxyapatite scaffolds used in the control group. Studies with primary human osteoblast cultures confirmed the bioactivity of these scaffolds, and the in vivo regeneration of segmental critical size bone defects in a rabbit model demonstrated that this material induces new bone defect bridging, with clear evidence of regeneration of original radial architecture and bone marrow environment.

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Effects for Osteosarcoma Cells by Carbon Nanotubes

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Sarcomas such as osteosarcoma are treated with surgery and chemotherapy by anticancer drugs. The anticancer drugs cause various severe side effects, and prospective enough effects may not be obtained. So-called nano-particles smaller than cells have a property to enter cells and they are expected as drug delivery system (DDS). We heretofore reported biocompatibility and safety of the carbon nanotubes (CNT). We report potential as DDS for chemotherapy to osteosarcoma cells with CNTs.

The 143B cells (human osteosarcoma cell line) were seeded at 5.0×10^5 cells/10 cm culture plate. After 24 hours, the culture medium was renewed to the medium contained 1 μg/ml or 10 μg/ml multi-walled CNT (MWCNT). Doxorubicin hydrochloride (DOX) (0.1 μM, 1.0 μM, 5.0 μM) was used as positive control. Each group was n=3. After more 24 hours, we observed the cells with light microscope and counted the number of 143B cells of each plate

In the light microscope images of the 143B cells that we added MWCNTs before 24 hours, the MWCNTs were taken in the cells. In the MWCNT 10 μg/ml group, much MWCNTs were taken in the 143B cells than the MWCNT 1 μg/ml group. The cell number after 24 hours culture was 23.3×10^5 cells/plate in control, 10.3×10^5 cells/plate in DOX 0.1 μM group, 5.6×10^5 cells/plate in 1.0 μM group, 2.8×10^5 cells/plate in 5.0 μM group, 21.3×10^5 cells/plate MWCNT 1 μg/ml group and 16.3×10^5 cells/plate MWCNT 10 μg/ml group.

When the MWCNTs are added to the osteosarcoma cell line; 143B cells, the MWCNTs are taken into the cells and inhibited a cellular proliferation in concentration-dependency. By adhering anticancer drugs to the MWCNTs, we expect to improve invasive efficiency to sarcoma cells of the anticancer drugs, to enhance the chemotherapeutic effect and to reduce the chemotherapeutic side effects.

Disclosure: The authors declare no competing interests.

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Time-elapsed Screw Insertion into Cancellous Bone

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"Time-elapsed" or "image-guided failure" assessment of bone is a relatively new technique that uses sequential image acquisition to analyse trabecular bone mechanics under a given loading regime. To date, this procedure has been employed to analyse trabecular mechanics during uniaxial compression [1, 2], screw pull-out [3], and screw push-in tests [4]. Nazarian *et al*. (2004) validated the use of this method for the assessment of microstructural trabecular mechanics, demonstrating

no difference in the macroscopic behaviour of cancellous bone specimens under continuous or step-wise loading conditions [2].

These methods have provided valuable insight into the failure mechanisms of bone under specific loading conditions. Work within our laboratory, however, has sought to better understand the interactions between bone and implant during screw placement. To understand the trabecular mechanics during screw tightening, a device has been developed that allows time-elapsed assessment of trabecular mechanics during the final tightening phase of screw insertion. Previous work by our group has established a strong relationship between the insertion torque measured just prior to screw head contact with the bone and the maximum tightening torque (T_{max}) [5]. This provides the ability to predict T_{max} based purely on the torque required to achieve head contact and allows us to stop and image the bone-screw interface at pre-defined points between head contact and T_{max}.

Here we present our novel device that has enabled, for the first time, the visualisation (using microCT) of trabecular mechanics during the final phase of screw tightening. Using this device, we can see the deformation of individual trabeculae in response to the turning of the screw threads. This information will prove useful in understanding the strains experienced by bone during screw insertion.

Disclosure: The authors declare no competing interests.

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Synergistic Effect of Nanomaterials and BMP-2 Signalling in Inducing Osteogenic Differentiation of Adipose Tissue-Derived Mesenchymal Stem Cells

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Mesenchymal stem cells (MSCs)-based therapeutic strategies hold great promise for large bone defects and non-union bone fractures, but their application is hindered by the lack of complete understanding in the signalling pathways which control the fate of MSCs being directed into osteogenic differentiation. The aim of this study is to gain insight into the interactions of different components within the bone tissue microenvironment in directing osteogenic differentiation of adipose tissue-derived mesenchymal stem cells (ASCs), and the underlying signalling pathway involved. We investigated how bone morphogenetic protein-2 (BMP-2) and the underlying substrata (in the form of three dimensional scaffold) direct osteogenic differentiation of ASCs and the underlying signalling pathways. We first demonstrated that 3 days of BMP-2 treatment increased osteogenic gene expression levels (Runx-2, collagen type I, osteopontin and bone sialop rotein) and *alkaline phosphatase (ALP)* activity in ASCs. Furthermore, we demonstrated that the underlying substrata; hydroxyapatite/β-tricalcium phosphate scaffolds (HA/TCP) coated with bioactive glass nanoparticles (nBG) can exert a synergistic effect with BMP-2 signalling to further promote osteogenic differentiation in ASCs. To elucidate these findings, we showed that ASCs grown on nBG-coated scaffolds had significantly higher Wnt-3a protein expression compared to those on uncoated scaffolds, and supplementation of Wnt signalling inhibitor in the culture medium inhibited the elevated expression of osteogenic markers in ASCs. Moreover, ASCs grown on nBG-coated scaffolds showed significantly higher expression of activated β1-integrin, and blocking the β1-integrin signalling pathway by neutralising antibody not only decreased Wnt-3a protein expression, but also inhibited the elevated expression of osteogenic markers in ASCs. In conclusion, we controlled in vitro culture conditions to mimic different components in the bone tissue microenvironment, and revealed their effects in directing the differentiation of MSCs into the osteogenic lineage through a coordinated signalling pathway network.

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In Vitro Evaluation of a Novel Silk Scaffold for use in Tendon Regeneration

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Tearing of the rotator cuff tendon in the shoulder is a significant clinical problem, with recurrent tear rates post-arthroscopic repair as high as 94%. Tissue engineered biomaterials, which induce host cell activity to promote tendon regeneration, are increasingly being investigated as a means to augment rotator cuff repairs. One of the challenges in tendon repair is that tenocytes tend to trans-differentiation down chondrocytic or osteoblastic lineages. Silk-derived materials have the mechanical strength and biocompatibility required for tendon repair, and in the current study, Spidrex®, a novel silk fibroin was evaluated in vitro for its potential to improve tendon regeneration.

Primary tenocytes cultured on Spidrex® were compared to tenocytes cultured in collagen gels. Primary human and rat tenocyte viability was assessed through fluorescent calcein-AM staining and alamarBlue fluorescence. Gene expression was determined by real-time PCR, and material immunogenicity

was assessed by the maturation and cytokine release of primary human dendritic cells.
Tenocytes proliferated rapidly on Spidrex® for 7 days, after which cell numbers remained stable for up to 14 days. Gene expression, analysed on days 1, 7 and 14, demonstrated an increase in the expression of tenocyte markers: scleraxis, tenomodulin and fibromodulin in cells cultured on either Spidrex® or collagen gels. However, in the collagen gels the expression of aggrecan and osterix also increased with time, indicating trans-differentiation into chondrocytic and osteocytic linages, whereas these genes remained mostly undetectable in tenocytes growing on Spidrex®. Immunogenicity tests showed that Spidrex® significantly enhanced dendritic cell maturation and cytokine release, suggesting it is likely to induce an early immune response post-implantation.
Overall, our in vitro data suggests that Spidrex® may have the cytocompatability and bioactivity required to support tendon regeneration. In vivo studies are currently underway to determine if these promising in vitro results transfer to clinically relevant animal models.

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Circulating Mediators of Bone Remodelling in Patients With Tophaceous Gout

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Disordered bone remodelling has been implicated in the development of bone erosion in tophaceous gout. The aim of this study was to determine the relationship between bone erosion and circulating mediators of bone remodelling in people with tophaceous gout.
One hundred patients with tophaceous gout were prospectively recruited from rheumatology outpatient clinics. Bone erosion at articular sites was assessed in conventional computed tomography (CT) scans of the feet and in plain radiographs (XR) of the hands and feet. Hip, spine and total body bone mineral density (BMD) was also measured. Circulating levels of OPG, sclerostin, dickkopf-1 (DKK-1) and fibroblast growth factor-23 (FGF-23) were measured in serum by ELISA. CT bone erosion scores positively correlated with circulating OPG concentrations (r=0.22, p=0.03), and negatively correlated with sclerostin concentrations (r=–0.29, p=0.003). Similar correlations were observed for XR erosion scores for OPG (r=0.30, p=0.002), and sclerostin (r=–0.21, p=0.04). Neck of femur BMD negatively correlated with OPG concentrations (r=–0.34, p=0.001), and positively correlated with sclerostin concentrations (r=0.24, p=0.02). Similar relationships were observed for total body BMD. No relationship was observed between bone erosion scores or BMD, and DKK-1 or FGF-23 concentrations. In linear regression analysis, OPG and sclerostin were independently associated with CT erosion score (p=0.005 for OPG and p=0.003 for sclerostin, R^2 for model 0.16, p<0.0001). Similarly, OPG and sclerostin were independently associated with neck of femur BMD (p=0.002 for OPG and p=0.04 for sclerostin, R^2 for model 0.13, p=0.001). These relationships persisted after adjusting for eGFR.
In people with tophaceous gout, circulating OPG and sclerostin levels are independently associated with both central and peripheral bone loss. The direction of the associations does not support a direct role for bone remodelling factors in pathogenesis of bone erosion, but may reflect compensatory or repair mechanisms to maintain bone homeostasis at both central and peripheral sites.
Disclosure: The authors declare no competing interests.

P71

Role of Micrornas in Regulation of the Acute Inflammatory Response to Monosodium Urate Crystals

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Acute gouty arthritis is an inflammatory arthritis induced by monosodium urate (MSU) crystals. In some patients with previous acute gouty arthritis MSU crystals are present within clinically uninflamed joints, suggesting that additional regulatory mechanisms provide a 'brake' on MSU crystal-induced acute inflammation in vivo. We hypothesized that miRNA play a role in regulating gene expression of pro-inflammatory cytokines in response to MSU crystals.
We stimulated human monocytic THP-1 cells with monosodium urate (MSU) crystals and/or interleukin (IL)-1β, and examined miRNA and pro-inflammatory cytokine gene expression using quantitative real-time PCR. The effects of miR-146a over-expression were examined by transfecting THP-1 cells with miR-146a precursor. The relative expression of miR-146a was also examined in tophus samples and in peripheral blood mononuclear cells (PBMC) from people with intercritical gout, and normouricaemic and hyperuricaemic control participants. In THP-1 cells, MSU crystals increased miR-146a expression, but not other miRNA implicated in IL-1 regulation. miR-146a and IL-1β expression were both maximal 20 hours after MSU crystal stimulation. Culture with IL-1β alone led to an increase in miR-146a, but addition of IL-1β did not further increase miR-146a expression following culture with MSU crystals. Inhibition of IL-1β using IL-1ra did not inhibit MSU crystal induced miR-146a expression. Transfection of THP-1 cells with miR-146a precursor led to high levels of miR-146a expression and reduced MSU crystal-induced IL-1β, TNFα, MCP-1 and IL-8 gene expression. In people with intercritical gout, PBMC expressed higher levels of miR-146a, compared with both healthy normouricaemic and hyperuricaemic control participants (ANOVA p<0.0001). Similar concentrations of IL-1 gene expression were observed in the three groups. PBMC expression of miR-146a correlated with IL-1β gene expression (r=0.35, p=0.028). Expression of miR-146a was also observed in tophus samples.
Together, these data suggest that miR-146a acts as a transcriptional brake in the acute inflammatory response to MSU crystals.

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The Transcription Factor Zinc Finger Homeobox 4 (Zfhx4) Plays a Novel Role in the Regulation of Endochondral Ossification

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Endochondral ossification is a unique and important biological process regulated by varieties of transcription factors including Sox9, Runx2, and Osterix. Despite that the study of these transcritption factors significantly has progressed our understanding of the transcriptional regulation of endochondral ossification, the precise regulatory mechanism remains elusive. We explored whether a yet-unidentified transcription factor contributes to the control of endochondral ossification.
To address this, we performed microarray analysis using mouse limb bud cells. Microarray analyses showed high expression levels of Zfhx4, a transcription factor putatively proposed to be responsible for 8q21.11 Microdeletion Syndrome. The syndrome is characterized by micrognathia, camptodactyly, and syndactyly. Consistently, RT-qPCR showed high expression of Zfhx4 in chondrocytes, long bones, and calvariae.
To investigate the role of Zfhx4 in endochondral ossification, we generated the Zfhx4 floxed mice and subsequently mated them with CAG-Cre transgenic mice. The Zfhx4 knockout (KO) mice died of respiratory failure within a day after birth. The skeletal preparation of the Zfhx4 KO mice at E15.5 exhibited trivialized thoracic cavity, malformation of skull, and dysplasia of femur, scapula, mandible and rib. Histological analyses indicated that endochondral ossification was arrested at hypertrophic zone in the femur of Zfhx4 KO mice. We next examined expression of chondrogenic markers by performing immunohistochemical staining of femur of Zfhx4 KO and wild-type (WT) littermates at E15.5. Col2 and Col10 expression levels were equivalent in Zfhx4 KO and WT. In contrast, MMP13 expression was dramatically down-regulated in Zfhx4 KO mice compared with WT mice. Of note, calcification of cartilage matrices was markedly suppressed in Zfhx4 KO at E16.5 as demonstrated by von Kossa staining. These results suggest that Zfhx4 is an important transcription factor, presumably regulating the degradation and calcification of cartilage matrices. In conclusion, our data suggest that Zfhx4 plays a novel role in the late stages of endochondral ossification.
Disclosure: The authors declare no competing interests.

P73

Nanostructural Analysis of Osteoarthritic Subchondral Bone Reveals Altered Crystal–collagen Bone Structure

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Increasing experimental and human studies demonstrate that the existence of remodelling abnormalities at subchondral bone specifically increased bone turnover and subsequent bone loss in early stages of osteoarthritis (OA). However, no studies have analyzed the structure of OA human bone at the nanoscale to understand how altered bone mineral density is affected by apatite crystal geometry and crystal organization. In this study we use transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FITR) to determine the nano-structural properties of OA bone. Our results indicate that in the normal human bone knee samples, apatite crystals showed a clear profile with collagen fibrils forming a typical cross-banding pattern and having their c-axis orientation parallel to the long axis of fibrils in trabecular and subchondral bone plate. However, OA subchondral bone samples showed a random, undulated arrangement, with certain localized areas demonstrating circular oriented patterns. Furthermore, collagen fibrils showed abnormal intra-fibrillar mineralization and higher ratio of calcium (Ca) to phosphorous (P) (Ca/P) in OA bone samples compared to normal bone. We further observed that the mineral content in OA bone lacks definite orientation with low crystallinity.
To our knowledge our investigation is one of the few studies focusing on crystal shape, size, and its arrangement with respect to collagen fibrils for normal compared with osteoarthritis bone. We conclude that there were significant structural differences between these two bone types at the crystal–collagen level.

P74

Chondrocyte-specific Deletion of Sod2 Exacerbates Age-related Cartilage Degeneration in Mice

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Superoxide dismutase 2 (SOD2) is a major antioxidant enzyme localized mitochondria to diminish mitochondrial superoxide. Human studies have reported that SOD2 protein and SOD2 gene expression were significantly decreased in osteoarthritic cartilage. The aim of this study is to conclude whether SOD2 loss in chondrocytes enhances osteoarthritis (OA) progression.
Chondrocyte-specific Sod2 cKO mice were newly generated using a Cre-loxP system. Surgical-induced OA model was created in knee joints by resecting the medial meniscotibial ligament at eight weeks of age and histologically evaluated at eight weeks after surgery. We also analyzed knee joints at 12 months of age without surgery as an age-associated OA model. Furthermore, cellular phenotypes were evaluated in primary articular chondrocytes isolated from knee joint of neonates.
Dehydroethidium, a specific dye for superoxide detection, staining revealed that Sod2 ablation significantly enhanced

superoxide generation by 400% in isolated chondrocytes from the cKO mice. Sod2 loss in chondrocytes of knee joint developed no obvious skeletal abnormalities during growth term, while the cKO mice significantly accelerated cartilage loss in the depth of tidemark at eight weeks after surgery. Moreover, Sod2 deficiency spontaneously exacerbated OA pathologies at 12 months of age. In vitro experiments also revealed that Sod2 depletion caused impaired mitochondrial membrane potential using JC-1 staining as well as swollen mitochondrial morphology associated with disrupted cristae using an electron microscope. Furthermore, gene expression analyses clarified that anabolic genes, including Col2a1 and Acan, were significantly down-regulated, while catabolic genes, including Mmp13 and Adamts5, were significantly up-regulated. Alcian blue staining confirmed a significant 80% decrease of proteoglycan in cKO chondrocytes.
Sod2 deficiency in chondrocytes induced mitochondrial superoxide generation and mitochondrial dysfunction associated with decreased membrane potential resulted in cartilage degeneration and loss via impaired proteoglycan metabolism. Our findings revealed that SOD2 plays a protective role in OA development and progression.
Disclosure: The authors declare no competing interests

P75
Influence of Experimental Traumatic Brain Injury on Bone
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Few studies have investigated the influence of traumatic brain injury (TBI) on bone homeostasis. TBI has been associated with accelerated bone fracture healing and an increased prevalence of heterotopic ossification. However, it is possible that mechanisms underlying brain injury; which include heightened sympathetic outflow, activation of inflammatory processes and free radical formation, may be detrimental to bone. Therefore, the current study aimed to determine the effect of TBI in a rat model on the quantity and quality of two different weight-bearing bones.
Adult rats were randomly assigned into either sham or severe lateral fluid percussion injury (LFPI) groups and killed at 1 or 12 weeks post-injury. Bones from sham and LFPI rats were compared using (i) Peripheral quantitative computed tomography (pQCT) of diaphysis, distal metaphysis and distal epiphysis of the femur, (ii) three-point bending of mid-points of diaphyses of both femora and humeri at 12 weeks post-injury and (iii) qPCR to determine expression of genes associated with bone remodelling in proximal tibiae.
pQCT analysis revealed decreased cortical thickness (10% decrease; $P<0.05$) at 1 week post-injury in the distal metaphyseal region of femora from LFPI animals when compared to controls. In the same location at 12 weeks post-injury, femora from LFPI animals had a 6% decrease in total density, an 8% decrease in cortical content, a 10% decrease in cortical thickness, and a 7% increase in endosteal circumference ($P<0.05$ for each). Three-point bending revealed no differences in mechanical properties of diaphyses of femora or humeri between sham and TBI animals. At 1 week post-injury, mRNA expression of cathepsin K, collagen type I and osteoprotegerin was reduced in LFPI animals ($P<0.05$ for each).
These preliminary data indicate that LFPI causes region-specific bone loss in rats and may substantiate a link between brain injury and bone remodelling. Additional studies are necessary to further characterize the effect of TBI on bone and the likely mechanisms involved.

P76
Cdc42 is Essential for Cartilage Development During Endochondral Ossification.
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Cdc42 is a universally expressed protein that belongs to the family of Rho GTPases that control a wide variety of signal transduction pathways including cytoskeletal organization, cell migration, proliferation, and apoptosis in a kind of cell type. However, its tissue-specific roles, especially in cartilage development, remain unclear. In the present study, to investigate the physiological functions of Cdc42 during cartilage development, we generated chondrocyte-specific inactivated Cdc42 mutant mice ($Cdc42^{fl/fl}$; Col2-Cre). The gross morphology of mutant neonates showed shorter limbs and body as compared to the control mice ($Cdc42^{fl/fl}$). Skeletal preparations stained with alcian blue and alizarin red also revealed that the body and the long bone length of the mutants were shorter. Furthermore, severe defects were found in growth plate chondrocytes of the femur sections of mutant mice, characterized by a reduced proliferating zone height, wider hypertrophic zone, and loss of columnar organization in proliferating chondrocytes. The expression levels of chondrocyte marker genes, such as Col II, Col X, and Mmp13 in mutant mice were decreased as compared to the control mice. Mineralization of trabecular bones in femur sections showed to be decreased in the mutants as compared to control mice, whereas osteoid volume was increased. The number of chondrocytes and ALP activity of costal cartilage were decreased in mutant mice. Together, these results suggested that chondrocyte proliferation and differentiation in growth plates of the present mutant mice were not normally organized, which contributed to abnormal bone formation. We concluded that Cdc42 is essential for cartilage development during endochondral bone formation.

P77

Bone-bound Bisphosphonates Inhibit Proliferation of Breast Cancer Cells

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Clinical studies have demonstrated that bisphosphonate treatment can prolong disease-free survival in breast cancer, suggesting its influences on tumour biology other than just activation of osteoclasts. We hypothesised that bisphosphonate on a bone surface reduces survival of metastatic breast cancer in the bone marrow environment. If this occurred, it could reduce seeding of tumour from these sites to other tissues. Therefore, we have quantified the growth of a breast cancer cell line, MCF7, on control bone slices and those previously treated with bisphosphonate.
Sterile bovine bone slices that were pre-incubated with either PBS or bisphosphonate solutions were washed and seeded with MCF7 cells. Cell proliferation was assessed by cell counts and thymidine incorporation for up to 72 hours. Cell lysates were used to determine levels of unprenylated Rap1A by Western blotting.
MCF7 cells cultured on zoledronate-coated bone had significantly decreased proliferation (by both cell number and thymidine incorporation) compared to cells on control bone, as measured at different time points during the 72 hours incubation time. The action of the drug was dose dependent. Experiments using five different bisphosphonates demonstrated that nitrogen-containing bisphosphonates were more potent inhibitors compared to non-nitrogen containing bisphosphonate, with zoledronate showing the greatest potency followed by alendronate. Thus, the potency of the different bisphosphonates in inhibiting MCF7 cell proliferation appeared to be related to their clinical potency. Western blot analysis showed the accumulation of unprenylated Rap1A in MCF7 cells treated with zoledronate, suggesting that the inhibition of cell proliferation was through the inhibition of FPP synthase downstream of the mevalonate pathway.
Our findings demonstrate inhibition of proliferation of MCF7 cells cultured on bone pre-incubated with bisphosphonate, suggesting a mechanism for the direct effect of the drugs on breast cancer cells in the bone marrow environment.

P78

Visualisation of Chemo-resistant Dormant Tumour Cells in the Skeleton by Two-photon, Intra-vital Imaging

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Cancers that develop in the skeleton can be treated with chemotherapies; however, despite the success of new, targeted, therapies tumors typically return and patients relapse. The mechanisms responsible are unknown. We hypothesize that dormant cancers cells, engage in the osteoblast niche, are resistant to chemotherapeutic agents and responsible for relapse. Using myeloma as a model, we developed intra-vital imaging to visualize dormant cells in live mice and examine the ability of dormant cells to resist chemotherapy.
5TGM1eGFP murine myeloma cells were labeled with a membrane dye (DiD), retained by dormant cells (DiD^{High}/GFP^{+ve}), but lost as cells divide (DiD^{Neg}/GFP^{+ve}). C57BLKalwRij mice were injected with labelled cells and treated with melphalan (3 times/week, 5mg/kg) or vehicle, from days 14–28. Cells were visualized after 7, 14, 21, or 28 days in intact tibia by two-photon, intra-vital, microscopy, and DiD^{High}/GFP^{+ve} and DiD^{Neg}/GFP^{+ve} cells isolated for flow cytometry and whole genome array analysis at day 28.
Individual, dormant, DiD^{+ve}/GFP^{+ve} cells were localized opposed to bone surfaces at all time points, including day 28. DiD^{Neg}/GFP^{+ve}, proliferating cells were identified from day 14, increasing in number to day 28. At days 14 and 21, limited numbers of individual DiD^{Neg}/GFP^{+ve} colonies were visualized. Longitudinal imaging of mice revealed persistent dormant DiD^{+ve}/GFP^{+ve} cells adjacent to actively growing tumours up to day 28. Dormant DiD^{+ve}/GFP^{+ve} tumour cells persisted after melphalan treatment, whereas actively growing tumour cells were reduced by >97%. Microarray analysis of DiD^{High}/GFP^{+ve} cells demonstrated a transcript profile consistent with cell cycle arrest and chemoresistance.
This study shows that intra-vital microscopy can identify individual dormant cancer cells and follow the activation of a limited number of dormant cells in live mice. Furthermore, we show that dormant cells are retained following chemotherapy and available to repopulate the tumour. Our transcript profiling has also identified potential new targets to overcome drug resistance.

P79

Novel Dairy Phospholipid for Bone Health in Aged Rats

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Phospholipids, particularly phosphatidyl serine (PS), are naturally occurring calcium-binding molecules found in milk which can be involved in skeletal mineralisation. Some phospholipids have direct effects on bone cells in vitro, inhibiting osteoclast formation. We have performed two independent studies on bone health in an aged animal model. Both dairy and soybean phosphatidyl serine were compared in the study 1 and dairy significantly increased bone volume compared to soybean. Consequently a further study, study 2, was performed using a mixture of milk phospholipids to confirm and expand the findings in study 1. In both studies, animals were fed phospholipid-rich diets (study 1 for 8 weeks and in study 2, 27 weeks) and effects on tibial bone microstructure were examined by micro-computed-tomography (SkyScan/Bruker).
The findings in study 1 demonstrate significant differences in bones of dairy versus soy PS diet-fed animals; with increases in weight-adjusted measurements of trabecular bone parameters in the dairy PS-fed rats compared to the soy PS-fed rats, including: bone surface ($p=0.045$) and parameters of trabecular connectivity (connectivity $p=0.030$; connectivity density $p=0.025$ and Euler number $p=0.034$). In the mixed

phospholipid-rich diet-fed animals, there were significant increases in the cortical bone parameters compared to control diet-fed animals. Bone surface (p=0.020), bone volume (p=0.049), mean total cross-sectional bone area (p=0.049) and mean total cross-sectional bone perimeter (p=0.022) were greater in cortical bone from the rats fed phospholipid-rich diets compared to control.

The findings in study 2 suggest improvements in the bones of the animals fed a mixed dairy phospholipid-rich diet versus beta serum or soy diet-fed animals. The rats fed the dairy phospholipid-rich diet compared to control animals exhibited significant increases in weight-adjusted measurements of trabecular bone number (p=0.031) and trabecular separation (p=0.022).

These findings demonstrate that diets enriched with various dairy phospholipids have positive effects on bone health in aged animals.

P80

The Short Term Effect of a Single Parathyroid Hormone (Pth) Injection on the Healing of Stress Fractures.

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Stress, or fatigue, fractures (Sfx), occur as a result of repetitive non-traumatic cyclic loading [1]. They are common in professional athletes, soldiers and dancers, and repair via a process of direct remodelling. Anti-inflammatory drugs (NSAIDs), commonly used in SFx patients, retard SFx healing, as do bisphosphonates (BPs)[1, 2]. Parathyroid hormone (PTH) has an anabolic effect that can accelerate bone remodelling and counteract effects of BP. Therefore, our aim was to investigate the short-term effect of a single PTH injection on the healing of SFx.

Sixteen female wistar rats 300 g were allocated to PTH and vehicle (VEH) groups. 24 hours after Sfx, PTH received a single dose of hPTH-(1-34) peptide (Sigma-Aldrich) (8 µg/100 g) dissolved in 0.9% saline with 1% rat heat-inactivated serum. SFx was created in the right ulna of both groups using cyclic end-loading. We used the ulnar SFx model, allowing scrutiny of focal remodeling with a known time course and precise anatomical location. Both groups had an ulnar stress fracture induced in a single session. Ulnae were harvested two weeks after loading, dissected, processed for histology and stained with Toluidine blue and for TRAP. Histomorphometry was conducted using Osteomeasure™.

There were no differences between groups for cortical area, woven bone area or length of fracture. There was a trend for increased SFx porosity (resorption), erosion and area of new bone formation in PTH groups; but significantly increased osteoclast number when compared to VEH group (P<0.01).

These data provide evidence that a single PTH injection, 24 hours after SFx initiation, results in active changes in dynamics of bone remodelling that may accelerate healing. Additional data is now required to demonstrate the long-term effect on healing time, and potential for daily PTH injections on the healing of SFx.

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P81

Detecting Effects of Nanomolar Concentrations of Bisphosphonate on the Rab Prenylome and on Cells Outside the Skeleton in Vivo Using a Highly Sensitive In Vitro Prenylation Assay

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Bisphosphonate (BP) drugs such as zoledronate (ZOL) are selectively internalised by osteoclasts at high concentrations (perhaps millimolar) during bone resorption, and prevent the post-translational prenylation of small GTPases such as Ras, Rho and Rab proteins. However, there is accumulating evidence that low, circulating (nanomolar) concentrations of BPs can have pleiotropic effects, probably via myeloid cells such as monocytes and macrophages outside the skeleton. However, effects of nanomolar concentrations of BPs have never been described because of the difficulty in detecting subtle changes in protein prenylation. We have developed an in vitro prenylation assay using recombinant Rab GGTase to detect effects of low concentrations of BPs on the prenylation of Rab proteins in cultured cells. We have combined this assay with mass spectrometery (MS) using Stable Isotope Labelling with Amino Acids in Cell culture (SILAC) to identify and quantify the Rab proteins that are most affected by low concentrations of ZOL.

Using this assay, we now provide the first evidence that concentrations of ZOL as low as 10 nM can inhibit Rab prenylation in cultured J774 macrophages, but that prolonged exposure to low concentrations (>7 days) is required. Using SILAC-MS we also identified the Rab proteins that are most abundant in J774 macrophages and that accumulate to the greatest extent in the unprenylated form after treatment with nanomolar concentrations of ZOL, including Rab5, Rab6, Rab7 and Rab11. After subcutaneous injection of ZOL (equivalent to the clinical dose) in mice, we also detected an inhibitory effect on Rab prenylation in peritoneal macrophages.

These studies finally confirm that prolonged exposure to low concentrations of BPs such as ZOL, as might occur in vivo in patients, can inhibit protein prenylation in macrophages. Furthermore, systemic treatment of mice with a clinically relevant dose of ZOL can indeed inhibit protein prenylation in macrophages outside the skeleton.

P82

Disruption of Glucocorticoid Signaling in Osteoblasts Prevents Adverse Lipid Accumulation in Brown Fat

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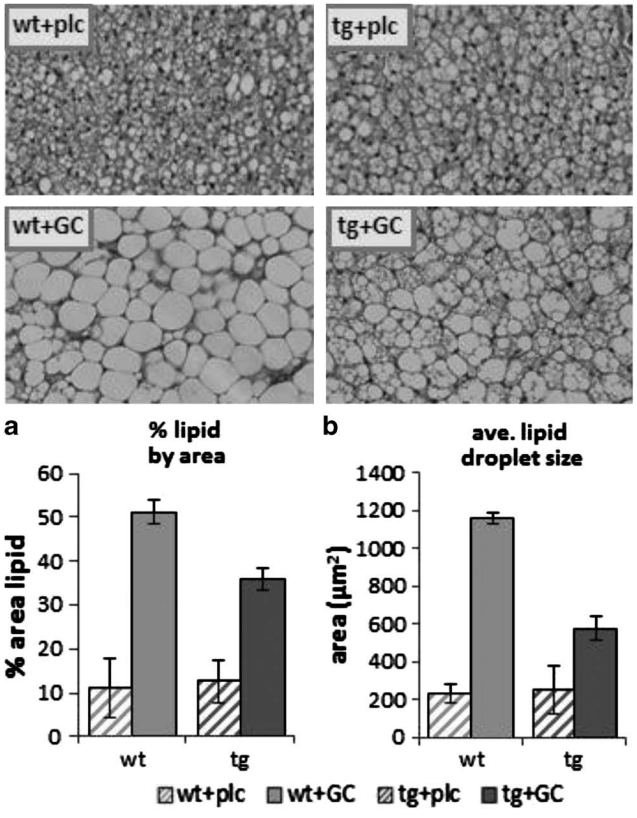
Glucocorticoids (GC) negatively affect brown adipose tissue function, which may contribute to GC-induced metabolic dysfunction. We have recently demonstrated that some of the adverse metabolic outcomes of GCs are mediated via the osteoblast [1]. The present study aimed to evaluate the contribution of osteoblastic GC signaling in GC-induced brown adipose tissue dysfunction.

We used a transgenic (tg) mouse model in which glucocorticoid signaling had been selectively disrupted in osteoblasts and osteocytes via targeted overexpression of the glucocorticoid-inactivating enzyme, 11ß-HSD2. Eight-week-old tg mice and their wild-type (wt) littermates were subcutaneously implanted with pellets containing either 1.5 mg corticosterone or placebo for the duration of 4 weeks. At endpoint, white intra-abdominal fat pads and the inter-scapular brown fat pad were excised, weighed and histologically assessed.

Compared to their respective placebo-treated controls (wt+plc/tg+plc), treatment of wt mice with corticosterone (wt+GC) resulted in a substantial increase in both white and brown adipose tissue mass, while in treated tg mice (tg+GC) only marginal changes in fat pad mass were observed (White fat: wt+GC +0.9 g vs. tg+GC +0.4 g, p<0.001; Brown fat: wt+GC +0.31g vs. tg+GC +0.18g, p<0.001). Histology revealed that treatment of WT mice with corticosterone increased lipid deposits in the brown fat pad, giving it the appearance of white adipose tissue with large, lipid filled adipocytes rather than the small and dense multilocular adipocytes typical of brown fat (Fig.1, upper panels). This effect was markedly attenuated in GC-treated tg mice, which displayed significantly lower fat accumulation and smaller lipid droplets than their wt counterparts (lipid area: wt+GC 51% vs. tg+GC 36%, p=0.005; lipid droplet size: wt+GC 1160 μm^2 vs. tg+GC 574 μm^2, p=0.006) (Fig.1, lower panels).



Our findings demonstrate that the actions of high-dose GCs on the osteoblast play a hitherto unknown role in the adverse effects of GC treatment on brown adipose tissue in mice.

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P83

Delivering Glucocorticoids Via the Drinking Water: An Effective Tool to Induce Dose-dependent Metabolic and Skeletal Pathologies.

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Examining the mechanisms underlying glucocorticoid (GC) induced disorders in animal models is often obscured by the wide variety of treatment delivery methods and dosages employed. We investigated the use and benefits of administering corticosterone (CS) to mice through the drinking water.

Eight-week-old mice received either vehicle (1%EtOH) or CS at a dose of 25 μg/ml, 50 μg/ml or 100 μg/ml dissolved in 1% EtOH (equivalent to ~100, 200 and 400 μg/day) in the drinking water for 5 weeks. Body weight and body composition were monitored, with insulin tolerance tests (ITT) being performed fortnightly. Serum levels of cholesterol, triglycerides, corticosterone and osteocalcin were measured at endpoint.

Compared to vehicle treated controls, mice exposed to 100μg/ml of CS for 5 weeks had gained significantly in both body mass (vehicle:+3.32 g vs 100 μg/ml:+7.3 g, p=0.012) and body fat mass (vehicle:+0.7 g vs 100 μg/ml:+8.2 g, p<0.0001). Similarly, serum cholesterol and triglyceride levels were ~2-fold higher in animals treated with 100 μg/ml CS (p=0.004 and p=0.06, respectively, compared to controls), although lower CS concentrations did not induce a significant rise in blood lipids. Insulin resistance increased in a dose-dependent manner (Fig. 1). Serum osteocalcin levels were profoundly suppressed in mice treated with 50 or 100μg/ml CS (p<0.001 each). Serum CS levels were elevated only in the group receiving 100 μg/ml CS of drinking water (3.5-fold higher than control, p=0.008). At higher CS doses, mice lost lean body mass (controls: +10% vs. 50 μg/ml: -3%, p=0.001 and 100 μg/ml: –1%, p=0.008) and failed to gain in bone area as measured by DXA (controls: +8% vs. 50 μg/ml: +3%, p=0.035 and 100 μg/ml: –0.5%, p=0.001). We conclude that delivery of CS through the drinking water is an inexpensive, easily adjustable and non-invasive method

of altering CS levels in mice, which maintains the characteristic diurnal rhythm, avoids animal stress and reliably induces metabolic as well as skeletal pathologies in a dose-dependent fashion.

P84

Exploring the Structural and Biological Difference in Early Haematoma Between Natural Healing and Delayed Bone Healing Defects

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The repair of critical-sized bone defects (CSDs) resulting from severe traumatic injury represents a major challenge in the field of orthopaedics. Despite bone's natural capacity to heal, severe morbidity often ensues and the clinical treatment is not always satisfactory. In the event of bone fracture, the natural healing process starts with bleeding within the injured bone and surrounding tissues, forming a fracture hematoma situated between the damaged fragments. A number of recently published studies have described the importance of hematomas in this healing process; however, a detailed study on the properties of haematoma, specifically comparing natural bone healing with a delayed healing fracture, has not previously been undertaken. In this study, a comparison of haematomas from natural and delayed healing defects have been investigated using scanning electron microscopy (SEM) and atomic force microscope (AFM) to determine differences of their structural properties at various time points. Furthermore, differences in the release of growth factors during clot formation and lysis have been determined by quantitative PCR and histology at days 1, 4, 7, and 28. Finally, the formation of mineralized bone tissue has been assessed at days 7 and 28 using micro-tomography and histology. Preliminary results from SEM and AFM analysis showed that a more loosely-woven fibrin network composed with larger diameters of fibrin fibres in haematomas has been found in natural healing defects. A potential outcome from this research is to develop a scaffold similar to haematomas properties found in bone healing samples that mimic the micro-environment, thereby optimizing the healing process. This study will provide an innovative solution to enhance large bone defects healing.

P85

Frax (Aus) As A Predictor of Falls Risk in Men and Women

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The World Health Organisation fracture risk prediction tool (FRAX®) utilises clinical risk factors to estimate the probability of sustaining a fracture over a 10-year period. Falls increase the risk for fracture, but are not incorporated into the FRAX tool. Recent data, suggests that inclusion of falls is not necessary because in elderly men, the FRAX score is a surrogate for falls risk[1]. The aim of our study was to investigate the association of falls risk and Australian-specific FRAX 10-year probability of hip fracture in an Australian cohort study.
Clinical risk factors were documented for 469 men and 522 women (age 40–90 years) assessed at follow-up (2006-2010 and 2000–2003, respectively) of the Geelong Osteoporosis Study. Probability of hip fracture was calculated using FRAX (Aus) including femoral neck BMD. Self-reported incident falls were documented from questionnaires returned approximately one year post-BMD measurement. Two multivariable analyses were performed: (i) the cross-sectional association between the FRAX scores and a falls risk score (Elderly Falls Screening Test, EFST[2]) using linear regression model and (ii) the prospective.

P86

Mutations Linked to Paget's Disease of Bone and Dementia Lead to Dysfunctional Autophagy and Cell Signalling

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Mutations in the SQSTM1/p62 (p62) gene are associated with Paget's disease of bone (PDB) and are a rare cause of Frontotemporal Dementia (FTD) and the phenotypically similar amyotrophic lateral sclerosis (ALS). p62 is an autophagy receptor, one of several such receptors that are reported to be mutated or implicated in FTD/ALS, PDB or the rare syndrome of "Inclusion body myopathy with FTD and PDB". In both dementia and PDB, protein aggregates are typically observed and may be involved in disease pathogenesis, possibly through effects on cell signalling.
We used luciferase assays to determine the effect of various UBA and non-UBA mutant p62 proteins on NF-kB activation in non-stimulated cells. We also conducted co-immunoprecipitation

experiments, where HEK293 cells were transfected with expression plasmids for FLAG-p62 (wild type or mutant) or empty vector. p62 proteins were immunoprecipitated with FLAG antibody and the bound LC3 detected by Western blot analysis. We transfected a cell line stably expressing mCherry-GFP-LC3 with p62 expression constructs and used confocal microscopy to determine the ratio of immature and mature autophagolysosomes that formed in cells expressing either mutant or wild type p62.

Our data shows that mutant p62 expression increases NF-kB activity and also impeded autophagosome maturation. By contrast, expression of wild type p62 promotes maturation. Together, our data suggests that p62 mutant proteins are defective mediators of the final stages of autophagy and this may be important for NF-kB regulation via perturbed degradation of specific protein substrates, including p62. Importantly, both dysfunctional autophagy and NF-kB signalling are implicated in the pathogenesis of both PDB and neurodegenerative disorders. Our study provides insight into the mechanisms underlying both PDB and dementia, and provides direction for research in the dementias caused by mutations to p62 and other autophagy receptors.

Disclosure: The authors declare no competing interests.

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How to Develop a Mobile Health Application to Support Optimisation of Vitamin D Status in Young Women

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In Australia, where skin cancer risk is extreme, 31% of 18–24 year olds are vitamin D deficient. Therefore, there is a need to provide balanced information and guidance to young people to enable them to safely achieve adequate vitamin D status. Our aim is to develop and evaluate a smartphone application (the Safe-D app) to safely improve users' vitamin D status. The Safe-D app will provide individualised UV-exposure recommendations and messages to young women to (1) improve vitamin D status, (2) follow SunSmart guidelines, and (3) achieve sustained appropriate behaviour change to safely maintain improved vitamin D status. A 3-arm randomised control trial (RCT) will evaluate the Safe-D app compared with vitamin D supplementation and education using brochure-based information.

A web survey was conducted to understand the smartphone landscape of females aged 16–25 years and to guide development of the Safe-D app. The survey was advertised via various media to target young women (a media release, youth forums, e-newsletters, emails to previous study participants). Researchers, expert clinicians and external developers jointly developed the app.

Of the eligible respondents (n = 57), 98% owned a Smartphone (95% Apple or Android). 77% wanted information regarding required sun exposure and 68% believed an app could help improve their vitamin D status. Sun exposure reminders (59%) and calculated sun exposure recommendations (34%) were among features that respondents recommended for inclusion in the Safe-D app. The Safe-D app was developed to accommodate the multifaceted determinants of skin safety and vitamin D status, providing simple interactive input of information, recommendations and education aiming to optimise sun exposure in young women. A key component was an algorithm that built in all variables that contribute to support safe sun exposure. The app was extensively beta tested (24 evaluators, 14 weeks) and modified, and is now under RCT evaluation.

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Comparison of Bone Health Status Among Vegans, Lacto-ovo-vegetarians and Non-vegetarians Among Malaysian

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In Malaysia, there are now appreciable numbers of individual practicing vegetarianism, attributed to contentment, animal welfare, environmental and health benefits. The aim of this cross-sectional study was to compare the Bone Health Status among vegans, lacto-ovo-vegetarians and non-vegetarians, involving 168, 187 and 198 respondents from the respective vegetarianism practices. The study subjects were members of the various Buddish Associations and the Malaysian Confucian Association, which has a large network of membership throughout the country. Information on socioeconomic status was obtained using structured questionnaire. Dietary assessment was conducted by using multiple days of food record while anthropometric measurements (body weight, height, body fat percentage) were measured according to standardized techniques. Bone health status of the calcaneus was assessed using Quantitative Ultrasound (QUS) which measured the Broadband Ultrasound Attenuation (BUA; in units of dB/MHz) of the bone. While the mean body weight was comparable between the lacto-ovo-vegetarians and non-vegetarians subjects, vegans were found to have a significant lower mean body weight ($p<0.05$). Mean intakes of dietary protein and dietary calcium were significantly higher among the lacto-ovo-vegetarians than their non-vegetarian or vegan counterparts ($p<0.05$). Using Kruskal-Wallis test followed by Mann-Whitney test, vegan subjects had the lowest mean BUA, followed by non-vegetarians and lacto-ovo-vegetarians, with significant differences found between the three groups (vegans v.s. non-vegetarians, non-vegetarians v.s. lacto-ovo-vegetarians, and lacto-ovo-vegetarians v.s. vegans) ($p<0.05$). Using multivariate linear regression analysis, age, body weight and dietary calcium intake were found to contribute significantly to BUA. Our study indicates that there were significant differences in bone health status between vegetarians and non-vegetarians. Specifically, there is a need to distinguish between lacto-ovo-vegetarians and

vegans in terms of bone health status as the later omit all dairy products which are good sources of dietary calcium, hence may attribute to lower bone health status among the vegans.

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Preptin Peptidomimetics for the Treatment of Osteoporosis

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Osteoporosis is a skeletal disorder of aging leading to bone fragility and an increase in bone fractures. The majority of current therapies for osteoporosis are anti-resorptive which do not have the ability to re-build lost bone. Anabolic agents however, have the ability to increase bone mass and strength. Therefore, the discovery of low cost, bone anabolic agents is a priority to treat osteoporosis and improve the quality of life of osteoporotic patients.

The 34-amino acid pancreatic peptide preptin has been shown to be anabolic to bone in vitro and in vivo possibly via phosphorylation of p42/44 MAP kinases. We have identified the smaller peptide fragment, preptin (1–16) and due to its smaller size and retained anabolic activity to bone formation, is a promising candidate for further therapeutic development.

Alanine scanning has been commonly used as a useful and valuable tool to probe the activity of peptides. Substitution of a residue side chain by a methyl group provides a convenient means to assess which side chains are responsible for the biological activity. An alanine scan study on preptin (1–16) was therefore undertaken and the results showed that substituting Ser-3, Asp-12, or Pro-14 with Ala retained the proliferation activity of the native preptin in vitro.

Preptin's peptidic nature suggests a moderately rapid systemic clearance and susceptibility to proteolytic degradation. Therefore, additional modifications using natural and non-natural amino acids were introduced on the native sequence in order to improve enzymatic resistance and enhance dosage profile.

In addition to that, we aim to explore the therapeutic potential of cyclic preptin (1–16) analogues. Because of their reduced conformational freedom relative to their linear precursors, cyclic peptides display improved metabolic stability and/or biological activity. We will therefore attempt different synthetic approaches for the chemical synthesis of three cyclic analogues of preptin (1-16), to evaluate for osteoblast proliferation activity and metabolic stability (see Figure 1).

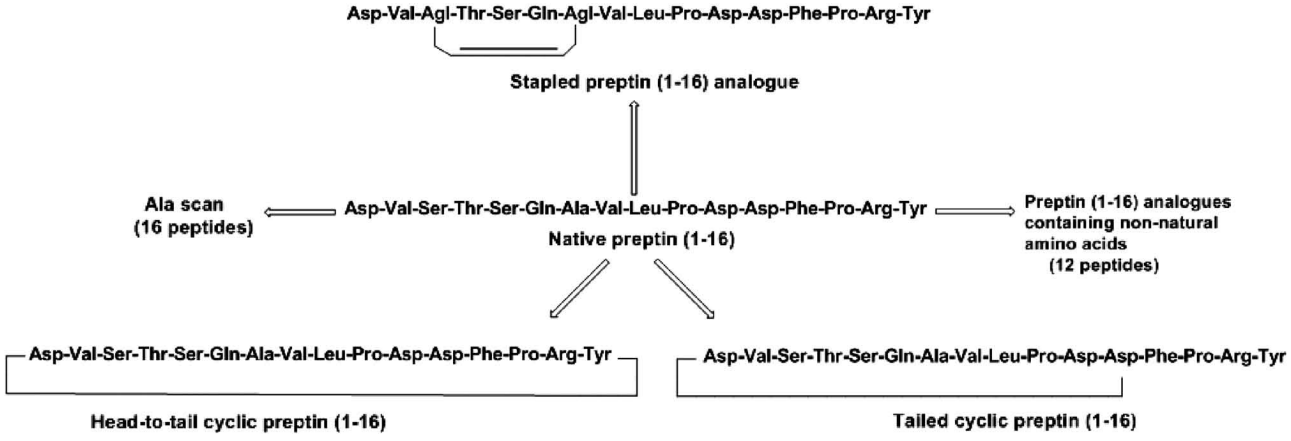

Figure 1: Primary structure of preptin (1-16) and the attempted modifications used.