ABSTRACTS

PTH resistance syndrome: genetics and epigenetics

Harald Jueppner[1]

1. Endocrine Unit, Department of Medicine and Pediatric Nephrology Unit, Department of Pediatrics, Massachusetts General Hospital and Harvard Medical School , Boston, MA

Hypocalcemia and hyperphosphatemia despite elevated levels of parathyroid hormone (PTH) are the typical laboratory abnormalities encountered in the different forms of pseudohypoparathyroidism (PHP). Most PHP variants caused by genetic mutations and/or epigenetic changes at the complex *GNAS* locus on chromosome 20q13.3 that undergoes parent-specific methylation changes at several sites, but in rare cases other molecular defects have been discovered.

GNAS encodes the alpha-subunit of the stimulatory G protein (Gsα) and several splice variants thereof. Inactivating mutations involving the maternal *GNAS* exons 1-13 cause PHP type Ia (PHP1A). These heterozygous defects cause disease because Gsα expression from the paternal allele is dramatically reduced and absent in certain tissues, such as the proximal renal tubules, thyroid, and pituitary. Consequently, there is little or no Gsα protein in the presence of maternal *GNAS* mutations thus leading to PTH-resistant hypocalcemia and hyperphosphatemia. When located on the paternal allele, the same or similar Gsα-specific mutations are the cause of pseudopseudohypoparathyroidism (PPHP). Besides the biochemical abnormalities, patients affected by PHP1A show developmental abnormalities, referred to as Albrights Hereditary Osteodystrophy (AHO). Some, but not all of these AHO features are encountered also in patients affected by PPHP, who typically show no laboratory abnormalities. If *GNAS* mutations cannot be found in such patients, defects in other genes, including *PTHLH, PDE4D, PDE3A,* and possibly others need to be considered.

PHP type Ib is another PHP variant. The autosomal dominant form of that disorder (AD-PHP1B) is caused by heterozygous maternal deletions within *GNAS* or *STX16*, which are associated with loss-of-methylation (LOM) at exon A/B alone or at all maternally methylated *GNAS* exons and their promoters. LOM of exon A/B and the resulting biallelic expression of A/B transcript reduces through as-yet unknown mechanisms Gsα expression thus leading to hormonal resistance. Epigenetic changes at all differentially methylated *GNAS* regions are also observed in sporadic PHP1B, the most frequent disease variant, which remains unresolved at the molecular level, with the exception of those cases with duplication of the paternal uniparental isodisomy or heterodisomy of chromosome 20q (patUPD20q). Some patients with clinical and laboratory abnormalities indistinguishable from those in PHP1B, but with normal *GNAS* methylation, can also be encountered because of PTH mutations thus expanding the PHP spectrum of related disorders.

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Dairy supplementation reduces fractures and falls in institutionalised older adults: a cluster-randomised controlled trial

Sandra Iuliano[1], Shirley Poon[1], Judy Robbins[1], Minh Bui[2], Xiao-Fang Wang[1], Lisette Dr Groot[3], Marta Van Loan[4], Tuan Nguyen[5, 6], Ego Seeman[7]

1. University of Melbourne / Austin Health, West Heidelberg, VIC, Australia

2. School of Population and Global Health, University of Melbourne, Parkville, Victoria, Australia

3. Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands

4. US Department of Agriculture-ARS, University of California, Davis, USA

5. Garvan Institute of Medical Research , Sydney, Australia

6. University of Technology Sydney, Sydney, Australia

7. University of Melbourne / Australian Catholic University, Melbourne, VIC, Australia

Publish consent withheld

3

Bone geometry is altered by follistatin-induced muscle growth in adult male mice

Audrey S Chan[1], Narelle E McGregor[2], Ingrid J Poulton[2], Justin P Hardee[1], Ellie HJ Cho[3], T. John Martin[2], Paul Gregorevic[1], Natalie A Sims[2], Gordon S Lynch[1]

1. Centre for Muscle Research, Dept Physiology, The University of Melbourne, Melbourne, VIC, Australia

2. Bone Cell Biology and Disease, St Vincent's Institute of Medical Research, Melbourne, VIC, Australia

3. Biological Optical Microscopy Platform, The University of Melbourne, Melbourne, VIC, Australia

Skeletal muscle size and the forces generated during muscle contraction shape bone structure during growth by providing mechanical stress to the developing skeleton. This is particularly evident in myostatin-*null* mice, where larger muscles increase bone mass and alter bone shape during development. However, whether muscle hypertrophy can influence the shape and strength of adult bones is unknown.

To answer this question, we assessed bone structure after inducing hypertrophy in the lower hindlimb muscles of 14-week-old adult male mice by intramuscular injections of recombinant adeno-associated virus vectors expressing follistatin (Fst), a potent antagonist of myostatin. To separate the local influence of Fst-induced muscle growth from any systemic action of Fst we used two Fst isoforms: the tissue-bound 288 amino acid isoform (Fst-288), and the longer circulating isoform (Fst-315).

Both Fst isoforms increased the mass of individual muscles, including the tibialis anterior (by 45%), extensor digitorum longus (by 45%), plantaris (by 46%), soleus (by 69%) and gastrocnemius (by 53%). In both Fst-treated cohorts, the anterior crest of the tibia, adjacent to the tibialis anterior muscle, was lengthened. Additionally, cortical bone adjacent to the hypertrophic muscles receded inward toward the central axis; an event driven by bone resorption on the periosteal surface abutting the gastrocnemius, and abundant osteoblast formation on the adjacent endocortical surface. In addition, expression of the circulating Fst isoform (Fst-315), but not tissue-bound Fst-288, increased trabecular bone volume. This was observed to a similar extent in both the tibia (increased by 53%) and the distal femur (increased by 40%), even though the latter is not adjacent to hypertrophied muscle.

These findings indicate that circulating Fst can increase trabecular bone volume, and that Fst-induced muscle hypertrophy in adult mice modifies bone shape. We conclude that muscle growth is capable of conferring local changes in bone shape, even in the adult skeleton.

4

Interaction between osteocyte SOCS3-dependent signaling and the bone marrow microenvironment maintains cortical bone integrity

Tsuyoshi Isojima[1], Emma C Walker[1], Blessing Crimeen-Irwin[1], Ingrid J Poulton[1], Narelle E McGregor[1], Jonathan H Gooi[1, 2, 3], T. John Martin[1, 2], Natalie A Sims[1, 2]

1. St Vincent's Institute of Medical Research, Fitzroy, VIC, Australia

2. Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Fitzroy, VIC, Australia

3. Bio21 Molecular Science and Biotechnology Institute, Parkville, VIC, Australia

Cortical structure is a crucial determinant of bone strength, yet the underlying mechanisms controlling its organization remain poorly understood. We recently reported that cortical bone develops through pore closure and accumulation of high-density bone by SOCS3-mediated suppression of gp130-STAT3 signaling in osteocytes. Since SOCS3 also suppresses G-CSFR signaling, we studied whether global G-CSFR (*Csf3r*) ablation could improve the structure of *Dmp1Cre.Socs3$^{f/f}$* cortical bone.

Dmp1Cre.Socs3$^{f/f}$.Csf3r$^{-/-}$ mice were generated by crossing *Dmp1Cre.Socs3$^{f/f}$*mice with *Csf3r* null mice on a C57BL/6 background. *Csf3r* null mice exhibited no change in bone structure compared to wild type. Surprisingly, *Dmp1Cre.Socs3$^{f/f}$* bone structure was worsened by *Csf3r* deletion. At 12 and 26 weeks of age *Dmp1Cre.Socs3$^{f/f}$.Csf3r$^{-/-}$* bone had a higher proportion of low-density bone than *Dmp1Cre.Socs3$^{f/f}$* (increased by 17%), and very little high-density bone (6%, compared to 18% in *Dmp1Cre.Socs3$^{f/f}$*). Despite this, femoral strength was not different. Histology revealed that *Dmp1Cre.Socs3$^{f/f}$.Csf3r$^{-/-}$* cortical bone contained a "double-shell" of lamellar bone separated by highly porous woven bone, containing 5x more osteoclasts and a 3-fold increase in blood vessel area compared to the already high levels in *Dmp1Cre.Socs3$^{f/f}$*. qPCR of flushed femora in *Dmp1Cre.Socs3$^{f/f}$.Csf3r$^{-/-}$* showed extremely high mRNA levels of osteoclast markers (*Dc-stamp, Acp5*), RANKL (*Tnfsf11*) and angiogenesis markers (*Endomucin, Tie-1*). A significant increase in phospho-STAT1 (~15%) and phospho-STAT3 (~20%) positive osteocytes, and elevations in STAT1 and STAT3 target gene mRNAs (*Socs1* and *Bcl3*), indicated that G-CSFR deletion further increased STAT signaling in *Dmp1Cre.Socs3$^{f/f}$* bone.

Since G-CSFR is not expressed in osteocytes, we suggest that G-CSFR deficiency promotes STAT signaling in osteocytes through its influence on the bone marrow microenvironment. In the absence of SOCS3 negative feedback, this increases cortical porosity by promoting local vascularization and bone resorption. This suggests cortical bone integrity requires communication between osteocytes and G-CSFR-mediated signals in the bone marrow microenvironment.

5

Subject characteristics and changes in bone mineral density after transitioning from denosumab (DMab) to alendronate (ALN) in the Denosumab Adherence Preference Satisfaction (DAPS) Study

Peter R Ebeling[1], David Kendler[2], Arkadi Chines[3], Patricia Clark[4], Michael McClung[5], Yumie Rhee[6], Shuang Huang[3], Robert K Stad[7], Nick Freemantle[8]

1. Monash University, Clayton, VIC, Australia

2. University of British Columbia, Vancouver, BC, Canada

3. Amgen Inc,, Thousand Oaks, CA, USA

4. Hospital Infantil de Mexico Federico Gómez, National University of Mexico, Mexico City, Mexico

5. Australian Catholic University, , Melbourne, VIC, Australia

6. Yonsei University College of Medicine, Seoul, Korea

7. Amgen Inc,, Zug, Switzerland

8. University College London, London, UK

Purpose: Investigate relationships between subject characteristics and BMD responses after transitioning from DMAb to ALN.

Methods: DAPS (NCT00518531) was a 24-month, open-label, randomised, cross-over study that compared adherence to 12 months (M) of DMAb (60 mg Q6M SC) with ALN (70 mg QW PO) in treatment-naïve postmenopausal women; T-score ≤−2.0 to ≥−4.0 at the lumbar spine (LS), total hip (TH), or femoral neck (FN). BMD was measured at baseline, M12 and M24. Here we evaluate subjects transitioning from DMAb to ALN at M12. A 3% BMD least significant change threshold identified subjects who lost, maintained, or gained BMD from M12 to M24. Characteristics were summarised using descriptive statistics.

Results: Of 126 subjects randomised to DMAb, 115 (91%) transitioned to ALN at M12. At baseline, the mean age was 65 years and mean BMD T-scores were −2.0, −1.6, and −2.0 at the LS, TH, and FN, respectively. BMD increased by 5.6%, 3.2%, and 3.1% with DMAb from M0 to M12 at the LS, TH, and FN, respectively, and changed by 0.6%, 0.4%, and −0.1% with ALN from M12 to M24. After transitioning to ALN, most subjects showed maintained or increased BMD (Table); 15.9%, 7.6%, and 21.7% lost BMD at the LS, TH, and FN, respectively, and only 1 subject (1.2%) lost BMD at all 3 sites. Baseline characteristics, M12 BMD, and adherence to ALN showed no trend with BMD change from M12 to M24. However, subjects who lost BMD from M12 to M24 on ALN showed greater BMD gains from M0 to M12 on DMAb, and few who lost BMD fell below their pre-study baseline BMD. No subject experienced clinical vertebral fracture.

Conclusion: These data highlight the need for oral BP therapy following DMAb cessation and BMD monitoring of patients transitioning from DMAb to ALN.

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Table: Selected baseline characteristics and percentage change in BMD for subjects who lost, maintained, or gained BMD after transitioning from denosumab to alendronate.

	Lumbar Spine (N = 82)			Total Hip (N = 92)			Femoral Neck (N = 92)		
Subjects stratified by BMD change category from M12 to M24	Lost (≤−3%)	Maintained (>−3% and <3%)	Gained (≥3%)	Lost (≤−3%)	Maintained (>−3% and <3%)	Gained (≥3%)	Lost (≤−3%)	Maintained (>−3% and <3%)	Gained (≥3%)
n (%)	13 (15.9)	52 (63.4)	17 (20.7)	7 (7.6)	75 (81.5)	10 (10.9)	20 (21.7)	56 (60.9)	16 (17.4)
Baseline characteristics	**n = 13**	**n = 52**	**n = 17**	**n = 7**	**n = 75**	**n = 10**	**n = 20**	**n = 56**	**n = 16**
Age (years), mean (SD)	63.8 (5.5)	65.5 (7.7)	64.3 (8.2)	66.4 (8.5)	64.5 (7.1)	68.5 (7.5)	67.1 (8.8)	64.2 (6.7)	65.8 (7.2)
BMD T-score, mean (SD)	−1.8 (2.0)	−2.1 (1.1)	−1.9 (0.8)[a]	−2.0 (0.5)	−1.4 (0.7)	−2.2 (0.7)	−1.9 (0.4)	−2.0 (0.5)	−2.2 (0.4)
History of fracture (yes), n (%)	7 (53.9)	23 (44.2)	10 (58.8)	4 (57.1)	37 (49.3)	3 (30.0)	7 (35.0)	26 (46.4)	11 (68.8)
M12 and M24 characteristics	**n = 12**	**n = 51**	**n = 14**	**n = 7**	**n = 71**	**n = 10**	**n = 19**	**n = 55**	**n = 14**
% change in BMD from M0 to M12, mean (SD)	7.1 (3.1)	5.9 (3.8)	3.1 (3.9)	6.2 (4.5)	3.0 (3.1)	2.8 (4.0)	7.0 (6.3)	2.7 (2.9)	0.6 (2.6)
	n = 12	**n = 51**	**n = 15**	**n = 7**	**n = 71**	**n = 10**	**n = 19**	**n = 55**	**n = 14**
BMD (g/cm^2) at M12, mean (SD)	1.0 (0.3)	0.9 (0.2)	0.9 (0.1)	0.8 (0.1)	0.8 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.6 (0.1)
	n = 12	**n = 51**	**n = 15**	**n = 7**	**n = 71**	**n = 10**	**n = 19**	**n = 55**	**n = 14**
BMD T-score at M12, mean (SD)	−1.2 (2.4)	−1.7 (1.2)	−1.7 (0.8)	−1.7 0.5)	−1.2 (0.7)	−2.1 (0.6)	−1.5 (0.6)	−1.9 (0.6)	−2.2 (0.5)
	n = 13	**n = 52**	**n = 17**	**n = 7**	**n = 75**	**n = 10**	**n = 20**	**n = 56**	**n = 16**
% change in BMD from M0 to M24, mean (SD)	2.1 (4.2)	6.7 (4.2)	7.7 (4.0)[a]	1.7 (4.1)	3.3 (3.2)	7.4 (3.8)	0.8 (5.4)	3.0 (2.8)	5.4 (2.3)
BMD at M24 below M0 value, n (%)	3 (23.1)	1 (1.9)	0 (0.0)[a]	2 (28.6)	5 (6.7)	0 (0.0)	10 (50.0)	8 (14.3)	0 (0.0)
ALN adherence at M24, n (%)	11 (84.6)	38 (73.1)	14 (82.4)	5 (71.4)	56 (74.7)	10 (100)	15 (75.0)	45 (80.4)	11 (68.8)

N = number of subjects with BMD values at M12 and M24; n = number of subjects in each BMD group with available data

BMD = bone mineral density; M = month; SD = standard deviation

[a] n = 16

6

An essential physiological role for MCT8 in bone in male mice

Victoria D Leitch[1, 2], Caterina Di Cosmo[3], Xiao-Hui Liao[3], Sam O'Boy[2], Thomas M Galliford[2], Holly Evans[4], Peter I Croucher[5], Alan Boyde[6], Alexandra Dumitrescu[3], Roy E Weiss[7], Samuel Refetoff[3], Graham R Williams[2], JH Duncan Bassett[2]

1. RMIT Centre for Additive Manufacturing, RMIT, Melbourne, VIC, Australia

2. Department of Medicine, Imperial College London, London, UK

3. The University of Chicago, Chicago, Illinois, USA

4. Sheffield Myeloma Research Team, University of Sheffield, Sheffield, UK

5. Bone Biology Laboratory, Garvan Institute of Medical Research, Sydney, NSW, Australia

6. Oral BioEngineering, Queen Mary University of London, London, UK

7. University of Miami, Miami, Florida, USA

T3 is an important regulator of skeletal development and adult bone maintenance. Thyroid hormone action requires efficient transport of T4 and T3 into target cells. We hypothesized that monocarboxylate transporter-8, encoded by *Mct8* on the X-chromosome, is an essential thyroid hormone transporter in bone. To test this hypothesis, we determined the juvenile and adult skeletal phenotypes of male *Mct8* knockout mice (*Mct8KO*) and *Mct8D1D2KO* compound mutants, which also lack the ability to convert the prohormone T4 to the active hormone T3. *Mct8KO* mice have mild central resistance to thyroid hormone with decreased T4 concentrations and slightly elevated T3 concentrations. By contrast, *Mct8D1D2KO* mice have severe central resistance to thyroid hormone with systemic hyperthyroidism. Intrauterine skeletal development was normal in both *Mct8KO* and *Mct8D1D2KO* mice, whereas postnatal endochondral ossification and linear growth were delayed in both *Mct8KO* and *Mct8D1D2KO* mice ($P<0.05$) and normalised by 12 weeks of age. This growth delay was accompanied by abnormal mineral content in *Mct8KO* and *Mct8D1D2KO* mice between 2 and 16 weeks of age ($P<0.001$). Adult *Mct8KO* and *Mct8D1D2KO* mice had decreased bone mass and mineralisation but only compound mutants had reduced bone strength with decreased yield and maximum loads ($P<0.05$). Bone resorption was increased in *Mct8D1D2KO* mice whereas bone formation parameters were not changed in either *Mct8KO* or *Mct8D1D2KO* mice. Delayed bone development and maturation in *Mct8KO* and *Mct8D1D2KO* mice is consistent with decreased thyroid hormone action in growth plate chondrocytes despite elevated serum T3 concentrations, whereas low bone mass and osteoporosis reflects increased thyroid hormone action in adult bone due to elevated systemic T3 levels. These studies demonstrate an essential role for the thyroid hormone transporter MCT8 in chondrocytes during skeletal development, and reveal the importance of other transporters in adult bone maintenance.

7

Multi-targeting DKK1 and LRP6 prevents myeloma-induced bone disease

Marija K Simic[1, 2], Sindhu T Mohanty[1], Ya Xiao[1], Tegan L Cheng[3], Feng Cong[4], Michael Daley[4], Peter I Croucher[1, 2], Michelle M McDonald[1, 2]

1. Healthy Ageing, Bone Biology Division, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

2. St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Kensington, NSW, Australia

3. Centre for Children's Bone and Musculoskeletal Health, The Children's Hospital at Westmead, Westmead, NSW, Australia

4. Novartis Institutes for Biomedical Research, Cambridge, MA, United States of America

An imbalance between bone resorption and bone formation underlies the devastating osteolytic lesions and subsequent fractures seen in more than 90% of multiple myeloma (MM) patients. Wnt-targeted therapeutic agents have the potential to address these skeletal complications, where they could rebuild lost bone and improve bone strength in affected individuals. We have demonstrated an anti-LRP6 agent, which potentiates Wnt signalling through binding the Wnt receptor LRP6, prevented the development of myeloma-induced bone loss primarily through preventing bone resorption. However, since MM patients present with both increased bone resorption and decreased bone formation, we hypothesised that combining anti-LRP6 with the bone anabolic anti-DKK1 (100mg/kg twice weekly i.v.) would lead to more robust improvements in bone structure than single treatment approaches. MicroCT demonstrated a 74% increase in femoral bone volume per tissue volume (BV/TV) in naïve mice given the combination treatment compared to control agents ($p<0.0001$). Mice injected with 5TGM1eGFP murine myeloma cells had a 34% reduction in femoral BV/TV compared to naïve controls ($p<0.0001$). Combination treatment drastically improved BV/TV in 5TGM1-bearing mice by 111% ($p<0.0001$), which was also superior to anti-LRP6 single treatment ($p<0.001$). Interestingly, these improvements in bone volume were primarily due to reduced bone resorption, with significant reductions in osteoclast numbers and osteoclast surface per bone surface demonstrated in 5TGM1eGFP-bearing mice treated with the combination strategy ($p<0.001$). Consequently, this combination significantly improved resistance to fracture in lumbar vertebrae in 5TGM1eGFP-bearing mice compared to their controls ($p<0.001$), and it provided greater protection against fracture compared to anti-LRP6 single agent treatment. Importantly, tumour activity was not altered with either single or combination strategies. This study defines a therapeutic strategy superior to current approaches, which will reduce fractures and improve quality of life in patients with MM.

8

Single-cell mapping of bone identifies key intercellular interactions within the endosteal bone microenvironment

James T Smith[1, 2], Ryan C Chai[1, 2], Alexander P Corr[1, 2], Matthew A Summers[1, 2], Weng Hua Khoo[1], John A Eisman[1, 2, 3], Peter I Croucher[1, 2], Paul A Baldock[1, 2, 3]

1. Healthy Aging Theme, Bone Biology Laboratory, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

2. Faculty of Medicine, St Vincent's Clinical School, UNSW Australia, Sydney, NSW, Australia

3. School of Medicine Sydney, University of Notre Dame Australia, Sydney, NSW, 2010

Bone remodelling is enacted by osteoclasts and osteoblasts, however, the endosteal surface, a major site for bone remodelling, is populated by a complex and incompletely defined cellular milieu. Consequently, our understanding of the cell types and signalling pathways that influence bone remodelling remains incomplete. We hypothesised that ligand-receptor mapping at the single-cell resolution can identify which cell types within the endosteal microenvironment may regulate bone remodelling.

Single-cell RNA sequencing was performed on 134,000 cells from the endosteal bone surface and bone marrow of 25x male C57BL/6 mice via the 10X Chromium platform to identify cell types present on the endosteal surface. Ligand-receptor screening was then used to identify putative intercellular interactions.

34 separate cell types were identified by their distinct expression patterns. Ligand-receptor screening determined that cells of the osteoblast lineage exhibit the greatest potential for intercellular interactions, displaying significant interactions with endothelial cells (73 ligand-receptor pairs), NK cells (29 pairs) and multiple populations of monocytes/macrophages (11 populations, 6-21 pairs).

Initial focus upon osteoblastic and monocyte/macrophage lineages identified 6 differentiation stages of osteoblasts and 14 monocyte/macrophage sub-clusters. Osteoblast/macrophage interactions were most enriched between Cxcl12+ mesenchymal stem cells (MSCs) and a population of Vcam1+ macrophages found only on the endosteal surface. All ligand-receptor pairs involved MSC ligands and macrophage receptors, with Gas6-Axl and Cxcl12-Sdc4 the top-ranking pairs. In contrast, interactions involving mature osteoblasts/macrophages were entirely dependent on ECM genes (e.g. Col1a1/2). No significant interactions were identified involving early-stage monocytes or their progenitors.

This combination of transcriptomic techniques and intercellular ligand-receptor screening has characterised key interaction partners of osteoblastic cells, implicating a subset of bone surface macrophages as potential key regulators of bone remodelling via their interactions with osteoblast precursors. This dataset could revolutionise our understanding of intercellular communications within the bone microenvironment, with numerous interactions still to be explored.

9

Osteal macrophage contributions to post-menopausal osteoporosis bone pathology

Lena Batoon[1], Susan Millard[1], Wenhao Sun[1], Andy Wu[2], Simranpreet Kaur[3], Cheyenne Sandrock[1], Martin Wullschleger[4, 5, 6], Liza Raggatt[1, 5], Nathan Pavlos[7], Allison Pettit[1, 5]

1. Mater Research Institute - The University of Queensland, Translational Research Institute, Woolloongabba, QUEENSLAND, Australia

2. Translational Research Institute, Woolloongabba, Queensland, Australia

3. The University of Queensland, Diamantina Institute, Translational Research Institute, Woolloongabba, Queensland, Australia

4. Gold Coast University Hospital, Southport, Queensland, Australia

5. The University of Queensland, Faculty of Medicine, Herston, Queensland, Australia

6. Griffith University, School of Medicine, Southport, Queensland, Australia

7. The University of Western Australia, Crawley, Western Australia, Australia

Osteal macrophages (osteomacs) support osteoblast function and promote bone anabolism, but their contributions to osteoporosis have not been explored. While mouse ovariectomy models have been repeatedly used, spontaneous osteoporosis in female C57Bl/6 mice limits utility of this strain and there are no comprehensive studies confirming ovariectomy recapitulated all pathological features of osteoporosis in other mouse strains. We characterised ovariectomy model of post-menopausal osteoporosis in C3H/HeJ mice. Ovariectomy caused reduced trabecular bone volume (-46%), thickness (-21%) and number (-23%) as well as reduced cortical thickness (-8%) with increased cortical porosity (17.5%) at 4-weeks post-surgery. High resolution micro-CT revealed enlargement of cortical vascular canals post-ovariectomy. Bone loss was associated with increased osteoclasts on trabecular ($p<0.0001$) and endocortical bone ($p<0.0016$), and decreased osteoblasts on trabecular bone ($p<0.0001$). While there was no impact on overall osteocyte number, the TRAP-expressing osteocyte frequency was increased in cortical bone ($p=0.0005$) which is suggestive of osteocytic osteolysis, especially when considered in the context of the increased cortical porosity. Unexpectedly, osteomac frequency was increased on both trabecular ($p<0.0001$) and endocortical bone ($p<0.0001$) post-ovariectomy. Dual F4/80 (pan-macrophage marker) and TRAP staining revealed osteomacs frequently located near TRAP+ osteoclasts and containing TRAP+ intracellular vesicles, suggesting osteomac-mediated phagocytosis of extracellular TRAP at resorption sites. Using an in vivo inducible macrophage depletion model (CD169-DTR mouse), that does not simultaneously deplete osteoclasts, we observed that osteomac loss was associated with elevated serum TRAP ($p=0.0017$). Using in vitro high-resolution confocal imaging of mixed osteoclast-macrophage cultures on bone substrate, we observed macrophages juxtaposed to osteoclast basolateral functional secretory domains scavenging degraded osteoclast by-products. These data demonstrate a role for osteomacs in supporting bone resorption through sequestering resorption byproducts. Overall, our data expose a novel role for osteomacs in supporting osteoclast function and provide the first evidence of their involvement in osteoporosis bone pathology.

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MCP-1 inhibition prevents breast cancer-induced bone loss

Bridie S Mulholland[1], Jelena Vider[1], Pierre Hofstee[1], Alan I Cassady[1], Ya Xiao[2], Michelle M McDonald[2], Nigel A Morrison[1], Mark R Forwood[1]

1. Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

2. Bone Biology Division, The Garvan Institute of Medical Research, Sydney, NSW, Australia

Despite great advances in the diagnosis, management and treatment of breast cancer (BC), its metastasis to distant organs still poses a significant clinical challenge. Secondary BC in the bone is a devastating progression of the original disease, remains incurable and has a poor prognostic outlook. In 2019, the 5-year survival rate of BC was 91% in Australia; for metastatic BC, the 5-year survival rate dropped to just 32%. Monocyte chemoattractant protein-1 (MCP-1) is a chemotactic protein that is implicated in bone resorption and BC progression. MCP-1 expression is increased in diseases of excess bone resorption, such as osteoporosis. Our aim was to identify a role of MCP-1 in BC bone metastasis, investigate the therapeutic effects of MCP-1 inhibition on metastatic burden within bone, and determine whether there is an increased risk of BC bone metastasis associated with pre-cancer osteoporosis. We hypothesised that increased MCP-1 expression is associated with BC bone metastatic disease.

Ovariectomised (OVX; n = 19) and sham-operated (SHAM; n = 18) female BALB/c mice were challenged with the 4T1.2 murine BC cell line. Mice were administered a plasmid DNA encoding 7ND, a mutant, dominant-negative form of MCP-1 that inhibits its action (OVX, n=9; SHAM, n = 9), or an empty vector as a control (OVX, n = 10; SHAM, n = 9). Mice were sacrificed 3-weeks post-4T1.2 challenge. Tibial microCT analysis showed significant increases in trabecular bone volume and trabecular number in 7ND-treated OVX mice ($p < 0.05$). Flow cytometry analysis of bone marrow showed changes in cell population abundance. Nanostring analysis showed significant downregulation of genes associated with cancer and osteoclastogenesis pathways in the 7ND-treated OVX mice ($p < 0.05$). Our findings suggest a critical role of MCP-1 in BC-related bone loss, presenting MCP-1 as a potential treatment target and introducing 7ND as a potential therapeutic.

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Hip structural analysis and incident fractures in a cohort of women

Kara B Anderson[1], Natalie K Hyde[1], Julie A Pasco[1, 2], Mark A Kotowicz[1, 3, 2], Kara L Holloway-Kew[1]

1. Deakin University, Geelong, VIC, Australia

2. Department of Medicine-Western Health, Melbourne Medical School, The University of Melbourne, Melbourne, VIC, Australia

3. Barwon Health, Geelong, Victoria, Australia

Publish consent withheld

The effect of denosumab on post-fracture mortality risk in the 45 and Up study

Dunia Alarkawi[1], Dana Bliuc[1], Thach Tran[1], Weiwen Chen[1, 2], Judy Simpson[3], Lyn March[4, 5, 6], Fiona M Blyth[7], Jackie Center[1, 2]

1. Bone Biology Division, Garvan Institute of Medical Research, University of New South Wales, Sydney, NSW, Australia

2. Clinical School, Faculty of Medicine, St Vincent's Hospital, University of New South Wales, Sydney, NSW, Australia

3. Sydney School of Public Health, University of Sydney, Sydney, NSW, Australia

4. Sydney Medical School, University of Sydney, Sydney, NSW, Australia

5. Institute of Bone and Joint Research, Kolling Institute, Sydney, NSW, Australia

6. Clinical School, Royal North Shore Hospital, St Leonards, NSW, Australia

7. Clinical School, Concord Repatriation General Hospital, Sydney, NSW, Australia

Bisphosphonates (BP), most commonly used antiresorptive agents, have been associated with mortality reduction possibly through reduction in bone loss and other extra-skeletal effects. However, the effect on mortality of Denosumab (DB), an increasingly used antiresorptive treatment has not been well explored.

This study determined the effect of DB, oral and intravenous BP (oBP and iBP) on post-fracture mortality. The 45 and Up Study is a prospective population-based cohort study with questionnaire data linked to i) mortality data, ii) hospital records by the Centre for Health Record Linkage (CHeReL) and, iii) the Pharmaceutical Benefits Scheme data provided by the Department of Human Services.

In this study 17,524 participants (15,604 not treated and 617 women and 154 men on DB, 615 women and 266 men on oBP and 176 women 90 men on iBP) aged 45+ years with an incident fracture were followed from 2006 to 2017. Propensity scores (PS) were calculated using pre-specified variables (age, comorbidities, socioeconomic status, lifestyle factors and comedications). DB, oBP and iBP users (treatment initiated after fracture) were matched 1:2 to non-users by PS, fracture type (hip and non-hip) and time to treatment initiation. Matching resulted in 90 -100% covariate balance except in iBP pairs in men (75%). Mortality risk was measured using pairwise multivariable Cox models, which accounted for any remaining imbalance post-matching.

DB in women but not in men was associated with 51% reduced mortality risk with the highest reduction observed in those who initiated treatment within a year post-fracture. oBP in women and men were also associated with 36-38% lower mortality risk while iBP's association with mortality was not significant (Table).

DB in women was associated with lower post-fracture mortality, possibly driven by a reduction in bone loss as shown for oBP. Further studies exploring the mechanisms of antiresorptive treatment and mortality is warranted.

Table: Hazard ratios of mortality for treated versus not treated matched 1:2

	Sample (n)	Deaths[a]		HR[b] (95% CI)	HR (Rx ≤1 year)[c]	HR (Rx >1 year)[b]
Women		**Treated**	**Not treated**			
Denosumab	1848	24 (4%)	163 (13%)	**0.49 (0.30-0.81)***	**0.45 (0.25-0.82)**	0.61 (0.24-1.57)
Oral Bisphosphonates	1844	90 (15%)	197 (16%)	**0.62 (0.46-0.84)**	**0.59 (0.42-0.82)**	0.78 (0.40-1.53)
i.v. Bisphosphonates	527	20 (11%)	43 (12%)	0.91 (0.46-1.78)	0.60 (0.23-1.56)	2.04 (0.46-8.98)
Men						
Denosumab	462	23 (15%)	88 (29%)	1.21 (0.70-2.08)	1.39 (0.63-3.04)	1.41 (0.63-3.17)
Oral Bisphosphonates	795	86 (32%)	177 (33%)	**0.64 (0.47-0.88)**	**0.65 (0.46-0.91)**	0.61 (0.28-1.34)
i.v. Bisphosphonates	270	23 (26%)	66 (37%)	0.82 (0.43-1.57)	0.66 (0.26-1.65)	1.80 (0.42-7.70)

*Bolded HRs are significant at p<0.05

[a] Number (%)

[b] Hazard ratios were adjusted for all variables which remained unbalanced after matching

[c] Rx - antiresorptive treatment, hazard ratios for those who initiated treatment within one year after fracture and after one-year post-fracture

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Heterogeneity in Microstructural Deterioration Following Spinal Cord Injury Reflects Site-Specificity of Mechano-transduction

Ali Ghasem-Zadeh[1], Mary Galea[2], Andrew Nunn[2], Maya Panisset[2], Xiao-Fang Wang[1], Sandra Iuliano[1], Steven Boyd[3], Mark Forwood[4], Ego Seeman[1]

1. Departments of Endocrinology and Medicine , Austin Health, the University of Melbourne, Heidelberg, VIC, Australia

2. Vicroian Spinal Cord Service, Austin Health, University of Melbourne, Melbourne, VIC, Australia

3. McCaig Institute for Bone and Joint Health, University of Calgary, Calgary, Calgary, AB, Canada

4. School of Medical Science and Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland, Australia

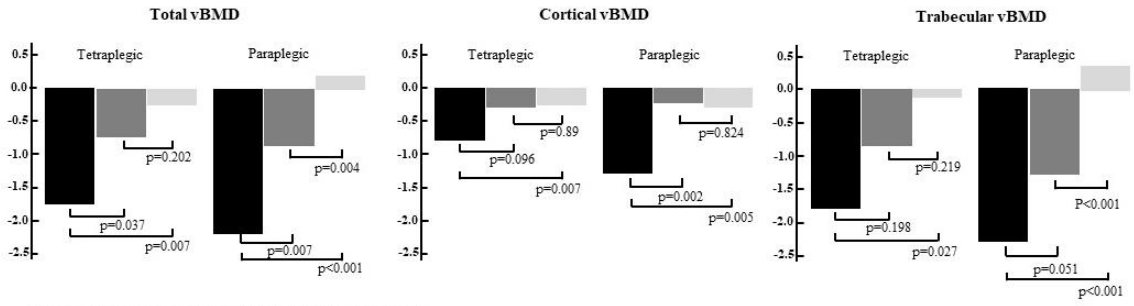
Background: Modeling and remodeling adapt bone morphology to accommodate strains encountered during usual loading. If strains exceed a threshold that increases the likelihood of fracture, bone formation increases bone volume reducing these strains. If unloading reduces strains below a threshold inhibiting resorption, bone resorption decreases bone volume restoring strains but perhaps at the price of compromised microstructure. We hypothesized that weight-bearing regions, usually adapted to greater strains, suffer more bone loss and structural deterioration following spinal cord injury than regions commonly adapted to low strains.

Methods: We quantified distal tibial, fibula and radius volumetric bone mineral density (vBMD) using high-resolution peripheral quantitative computed tomography in 32 men, mean age 43.5 years (range 23.5-75.0), 12 with tetraplegia and 20 with paraplegia of 0.5 to 18.6 years duration, and 102 healthy age-matched male controls. Differences in morphology relative to controls were expressed as standardized deviation (SD) scores (mean ± SD). Standardized mean differences in vBMD between the regions were expressed as SDs (95% confidence intervals, CI).

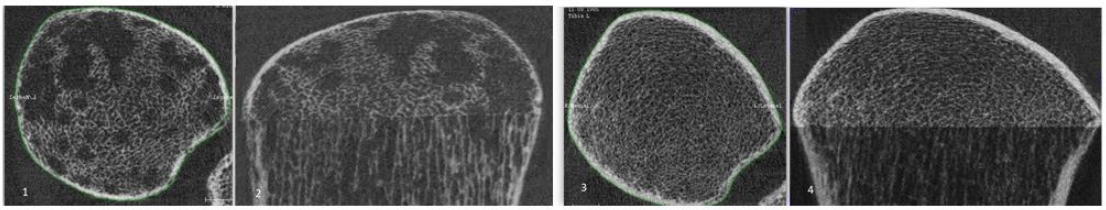
Results: Compared to controls, men with tetraplegia had deficits in total vBMD at the distal tibia (-1.72±1.38 SD) (p<0.001) and distal fibula (-0.68±0.69 SD) (p=0.041), not distal radius(-0.21±0.96 SD) (p=0.641), despite paralysis. Deficits in men with paraplegia were (-2.14±1.50 SD) (p<0.001) at the distal tibia and (-0.68±0.69 SD) (p=0.041) at the distal fibula while distal radial total vBMD was (0.23±1.02) (p=0.371), not increased, despite upper limb mobility. Comparing regions, in men with tetraplegia, distal tibial total vBMD was 1.04 SD (95%CI -2.01, -0.07) lower than the distal fibula (p=0.037) and 1.51 SD (95%CI -2.57, - 0.45) lower than the distal radius (p=0.007); the latter two sites did not differ from each other. Results were similar in men with paraplegia, but distal fibula total vBMD was 1.06 SD (-1.77, -0.35) lower than the distal radius (p=0.004).

Conclusion: Microarchitectural deterioration following spinal cord injury is heterogeneous, partly because the strain thresholds regulating the cellular activity of mechano-transduction are region specific.

Comparison of deficits total, cortical and trabecular volumetric bone mineral density (vBMD) at the distal tibia (black bar), distal fibula (deep grey bars) and distal radius (light grey bars) in men with tetraplegia and paraplegia. Deficits are expressed as standard deviations from the mean in age-matched controls.



Images of trabecular and cortical bone at the distal tibia



Axial and axial-coronal views of distal tibia of a 28 years male with SCI (left panels 1,2) and control (right panels 3, 4). Thinned trabeculae with loss of homogeneity in distribution and thin porous cortex.

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Fractures in T2DM independently predicted by insulin use and vascular complications (FIELD study)

Angela Sheu[1, 2, 3], Rachel O'Connell[4], Alicia Jenkins[4], Jackie Center[1, 2, 3], Christopher White[2, 3, 5], Tony Keech[4]

1. Endocrinology Department, St Vincent's Hospital, Sydney, NSW, Australia

2. Osteoporosis and Bone Biology Division, The Garvan Institute, Sydney, NSW, Australia

3. University of New South Wales Faculty of Medicine, University of New South Wales , Sydney

4. NHMRC Clinical Trials Centre, University of Sydney, Sydney

5. Department of Endocrinology and Metabolism, Prince of Wales Hospital, Sydney

Background: Type 2 diabetes mellitus (T2DM) is associated with increased risk of some fractures although the exact mechanisms are unclear. Bone fragility may be associated with T2DM severity (microvascular complications, longer duration, insulin use), although prospective studies evaluating their independent contributions are lacking.

Aims: To determine whether baseline micro- or macrovascular disease predict incident fractures in T2DM, and whether T2DM medications or clinical characteristics independently contribute to fracture risk.

Methods: The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial was a randomised controlled trial of fenofibrate therapy in T2DM patients (aged 50-75), with fractures collected as adverse events. In this post-hoc analysis, cox proportional hazards models were used to determine gender-specific predictors of fracture from baseline data of all enrolled patients.

Results: Over 49,470 person-years, 137/6138 men and 143/3657 women suffered a fracture. Men with fracture (vs without) were older (63.7±7.5 vs 62.4±6.8 years, p=0.03), more likely to have macrovascular disease (32.9% vs 23.4%, p=0.01), use insulin (21.9% vs 13.6%, p=0.005) and had longer T2DM duration (8.1±6.9 vs 6.9±6.2 years, p=0.03). Women with fractures had more neuropathy (9.8% vs 4.3%, p=0.002) and greater insulin use (22.4% vs 13.3%, p=0.002). Age was similar in women with and without fracture (61.8±6.8 vs 62.9±7.5 years, p=0.06). Overall, mean HbA1c was 7.1%.

In men, significant univariate predictors for fracture included age, macrovascular disease, T2DM duration, insulin use and serum triglycerides, but only insulin use (HR 1.69 (1.12-2.54), p=0.01) and macrovascular disease (HR 1.47 (1.02-2.12), p=0.04) were significant in multivariable modelling.

In women, significant univariate predictors included age, neuropathy, insulin use and serum creatinine, but only neuropathy (HR 2.16 (1.23-3.80), p=0.007) and insulin use (HR 1.65 (1.11-2.47, p=0.01) predicted fractures in multivariable modelling.

Conclusions: Insulin use and gender-specific vascular complications (macrovascular disease in men and neuropathy in women) predict fractures in T2DM. Studies evaluating insulin use and T2DM fracture risk are warranted.

Association between bone measures and use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers

Kara L Holloway-Kew[1], Amelia G Morse[1], Kara B Anderson[1], James Gaston[1], Wan-Hui Liao[2], Maciej Henneberg[4, 3], Julie A Pasco[1, 5, 6]

1. Deakin University, Geelong, VIC, Australia

2. Swiss Federal Institute of Technology in Zurich, Zurich, Switzerland

3. The University of Adelaide, Adelaide, Australia

4. Institute of Evolutionary Medicine, Zurich, Switzerland

5. The University of Melbourne, Melbourne, VIC, Australia

6. Barwon Health, Geelong, VIC, Australia

Publish consent withheld

Bone Compartment Characteristics in Patients with Atypical Femoral Fractures on 3D Dual X-Ray Absorptiometry (DXA)

Albert S Kim[2, 1], Jean Doyle[1], Lillias Nairn[1], Gareth Crouch[2], Maria-Liza Nery[1], Andrew Ellis[3], Roderick Clifton-Bligh[2, 1], Christian Girgis[4, 2, 1]

1. Department of Endocrinology, Diabetes and Metabolism, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Faculty of Medicine & Health, University of Sydney, NSW 2600, Australia

3. Department of Orthopedic and Trauma Surgery, Royal North Shore Hospital, St Leonards, NSW, Australia

4. Department of Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia

Introduction: Atypical femoral fractures(AFF) represent a rare but devastating complication related to long-term antiresorptive therapy for osteoporosis(1). While generalised increase in cortical thickness of the femoral diaphysis on X-ray has been associated with AFF(2), the underlying microarchitectural changes remain unclear. A three-dimensional(3D)-DXA software algorithm(3DSHAPER,GalgoMedical,Barcelona,Spain) was utilised to quantify cortical thickness and volumetric bone mineral density(BMD) of trabecular and cortical bone from DXA projections(3).

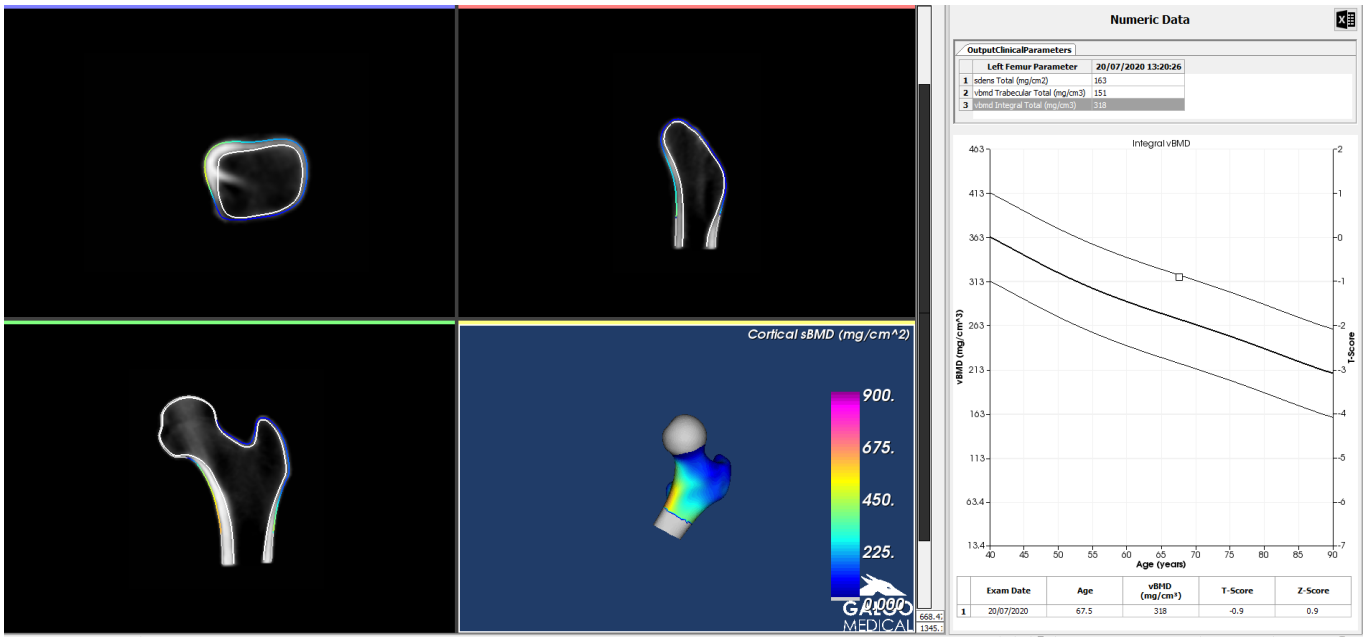
Methods: Retrospective review of areal BMD and 3Dassessment of trabecular and cortical parameters at the hip and femoral shaft in patients on long-term therapy(>5years) who sustained AFF and those who did not, in a case-control ratio of 1:2.

Results: 30 femoral models were obtained using the 3D-DXA algorithm from 10 patients with AFF and 20 patients without(example output in Figure 1). There were no significant differences in mean age (AFF 72.4±11.4 and Control 76.9±8.2 years,p=0.29) or duration of antiresorptive therapy (AFF 9.9±6.9and Control 11.4±4.6years,p=0.56). There were no significant differences in the areal BMD in the femoral neck, trochanter, shaft and total hip.

Femoral neck area was significantly smaller in patients with AFF compared to controls (4.64±0.25 and 5.05±0.26cm^2,p<0.001). Cortical bone mineral content was significantly lower in the AFF group at the neck (1.77±0.26 and 2.23±0.34g,p<0.001) and the shaft (6.77±1.80 and 8.57±1.56g,p=0.16). This difference was not seen in the trabecular compartment. There was no significant difference in mean cortical thickness (1.67±0.26 and 1.80±0.13g,p=0.16).

Cortical thickness was not correlated with age or duration of therapy. There was significant correlation between duration of treatment and total cortical volume(r=0.467,r^2=0.218,p=0.04) in the control group but this was not significant in the AFF group

Conclusion: Lower cortical bone mineral content but no significant cortical thickening was observed in AFF patients compared to those on a similar duration of antiresorptive agents. Reduced cortical bone mineralisation may increase the risk of developing AFF.



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DXA-derived Trabecular Bone Score and Hip Structural Analysis Parameters in Patients with Type 1 Diabetes Mellitus undergoing Simultaneous Pancreas Kidney Transplantation

Jasna Aleksova[1, 2, 3], Krishnapavan Echampati[4], Peter R Ebeling[5, 2], Frances Milat[5, 2, 3], Grahame J Elder[4, 6]

1. Endocrinology, Monash Health, Calyton, Vic, Australia

2. Department of Medicine, Monash University, Melbourne

3. Hudson Institute for Medical Research, Melbourne

4. Renal Medicine, Westmead Hospital, Sydney

5. Endocrinology, Monash Health, Clayton, Vic, Australia

6. Garvan Institute of medical Research, Sydney

Patients with chronic kidney disease (CKD) stage 5D (receiving dialysis) have heightened fracture risk and post-fracture mortality, and those with type 1 diabetes mellitus (T1DM) are at even greater risk. Bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) shows inferior fracture prediction in CKD-5D than in the general population. Recently we showed DXA-derived trabecular bone score (TBS) and hip structural analysis (HSA) parameters: femoral neck cortical thickness (CT) and the buckling ratio (BR; an index of femoral neck instability) to be markedly abnormal and associated with prevalent fracture in CKD-5D.

This study assessed TBS and HSA parameters in patients with T1DM and CKD-5D at the time of simultaneous pancreas kidney (SPK) transplantation compared with kidney only transplantation without T1DM (KTx). Of 226 patients, 64.8% were male and 58 (26%) were SPK recipients. SPK recipients were younger (42.3±7.6 vs. 50.8±13.8 years), had shorter dialysis duration (23±20 vs. 42±39 months), higher HbA1c (8±1.5% vs. 5.5±1.0%) and lower BMI (24.7±5.9 vs. 27.1± 4.8). SPK recipients had lower BMD Z-Scores at the spine, hip and ultradistal radius (all p≤0.001), but not at the 1/3 radius (p=0.298). SPK recipients had lower TBS (1.316±0.104 vs. 1.366±0.123; p=0.006), higher buckling ratios (10.5±7.5 vs. 7.7±4; p<0.001) and lower femoral neck, calcar and shaft CT (p=0.047, p=0.041 and p<0.001 respectively). In regression analyses adjusted for age, gender, dialysis vintage and weight, T1DM SPK transplant predicted a higher BR (β= -0.243= , p<0.001), lower femoral neck CT (β= 0.242, p<0.001) and lower femoral shaft CT (β=0.241, p <0.001), but not calcar CT.

Patients with T1DM undergoing SPK transplantation have reduced TBS, increased BR and reduced CT compared with KTx patients despite their younger age and shorter dialysis vintage. The utility of these DXA-derived parameters for fracture prediction should be assessed prospectively in patients with T1DM, CKD-5D and following transplantation.

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The Use of Denosumab in Central Giant Cell Granuloma: 5-year Institutional Experience

Yoon Ji Jina Rhou[1], Che-Jen (Jarren) Wang[2], Lydia Lim[3], Minh Nguyen[1], Hedley Coleman[4], Craig Munns[5], Jane Holmes-Walker[1], Christian Girgis[1]

1. Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

2. Oral and Maxillofacial Surgery, John Hunter Hospital, Newcastle, NSW, Australia

3. Oral and Maxillofacial Surgery, Westmead Hospital, Sydney, NSW, Australia

4. Anatomical Pathology, Douglass Hanly Moir Pathology, Sydney, NSW, Australia

5. Diabetes and Endocrinology, The Children's Hospital at Westmead, Sydney, NSW, Australia

Introduction: Central giant cell granuloma (CGCG) is a rare, benign tumour of the jaw typically occurring in children and young adults, characterised by osteoclast-like multinucleated giant cells and local RANKL overexpression by mononucleated stromal cells. Traditional management is surgery but with potential significant morbidity and recurrence.

Denosumab targets RANKL, inhibits osteoclastic bone resorption and when given 120mg monthly is effective in the management of a related condition, namely giant cell tumour of the bone. Experience in CGCG is limited to case reports and small case series. Optimal dosing and monitoring remain unclear.

Aims: To review treatment regimen, clinical, biochemical and imaging changes, and adverse effects during and post-denosumab for CGCG.

Methods: Patients with histologically confirmed CGCG managed by Westmead Hospital Oral-Maxillofacial Surgery and referred to Endocrinology for denosumab 2015-2020 were identified. Baseline data was obtained from medical records and external providers including demographics, comorbidities, symptoms, investigations (imaging, biochemistry, parathyroid hormone, 25-hydroxyvitamin D, bone turnover markers). Clinical features, investigations and adverse effects during and post-cessation of denosumab were obtained.

Results: We identified seven cases of CGCG treated with denosumab, the largest adult series in literature to our knowledge. Four (57%) were male. Three were mandibular, three were maxillary, one involved both. Three had multiple lesions. Mean 25-hydroxyvitamin D pretreatment was 69±11.8 nmol/L. Mean age at denosumab commencement was 24±9.0 years. Three were treatment-naïve, three had intralesional steroids, two had previous surgery. Denosumab was effective in reducing symptoms and size after 5±3.4 doses. Five ceased after 15±6.8 doses but three (60%) experienced recurrence 13±4.2 months post-cessation. One had rebound hypercalcaemia post-cessation. There were no cases of osteonecrosis of the jaw.

Conclusion: Denosumab has demonstrated efficacy for CGCG and is promising neoadjuvant therapy for improving ability to provide surgical intervention. Long-term safety of high dose denosumab in younger subjects needs evaluation.

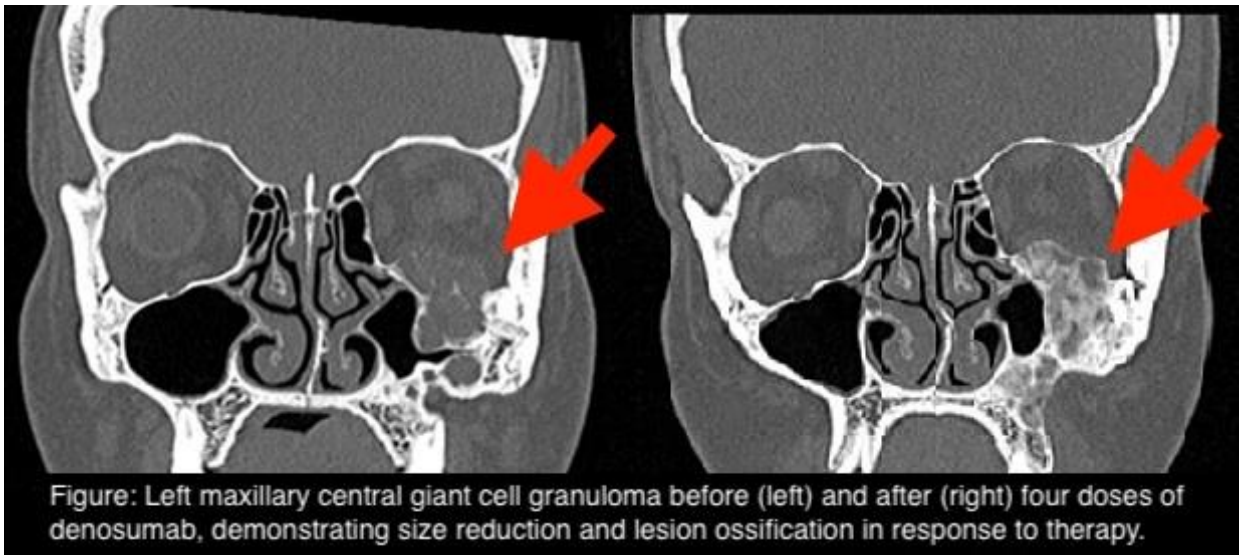

Figure: Left maxillary central giant cell granuloma before (left) and after (right) four doses of denosumab, demonstrating size reduction and lesion ossification in response to therapy.

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A global natural history study of fibrodysplasia ossificans progressiva (FOP): 12-month outcomes

Mona Al Mukaddam[1], Robert J Pignolo[2], Geneviève Baujat[3], Matthew A. Brown[4], Carmen De Cunto[5], Maja Di Rocco[6], Edward C. Hsiao[7], Richard Keen[8], Kim-Hanh Le Quan Sang[3], Andrew Strahs[9], Rose Marino[9], Frederick S. Kaplan[1], Shane Patella[10]

1. Departments of Orthopaedic Surgery & Medicine, The Center for Research in FOP and Related Disorders, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

2. Department of Medicine, Mayo Clinic, Rochester, MN, USA

3. Département de Génétique, Institut IMAGINE and Hôpital Necker-Enfants Malades, Paris, France

4. Guy's & St. Thomas' NHS Foundation Trust and King's College London , NIHR Biomedical Research Centre, London, UK

5. Pediatric Rheumatology Section, Department of Pediatrics, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

6. Unit of Rare Diseases, Department of Pediatrics, Giannina Gaslini Institute, Genoa, Italy

7. Division of Endocrinology and Metabolism, UCSF Metabolic Bone Clinic, Institute of Human Genetics, and UCSF Program in Craniofacial Biology, Department of Medicine, University of California-San Francisco, San Francisco, CA, USA

8. Centre for Metabolic Bone Disease, Royal National Orthopaedic Hospital, Stanmore, UK

9. Ipsen, Newton, MA, USA

10. Ipsen, Glen Waverley, VIC, Australia

Background: FOP is an ultra-rare genetic disorder characterized by cumulative heterotopic ossification ([HO]; often preceded by episodic flare-ups), leading to physical disability and shortened life expectancy. FOP is diagnosed and managed by multiple healthcare professionals.

Objective: A prospective, 36-month, global natural history study (NCT02322255) was designed to investigate FOP progression, HO, and impact on physical function. Here, we report 12-month outcomes.

Methods: Individuals with FOP aged ≤65years with documented *ACVR1*^{R206H} mutation were eligible. HO volume was assessed by low-dose whole-body computed tomography (WBCT). Physical function was evaluated using Cumulative Analogue Joint Involvement Scale (CAJIS) and FOP Physical Function Questionnaire (FOP-PFQ). Changes from Baseline (CfB) in HO volume, CAJIS and FOP-PFQ at Month 12 were evaluated.

Results: Of 114 participants with Baseline data, 99 (aged 4–56years, mean 17years; 56% male) had a Month 12 assessment; 93 had evaluable Baseline and Month 12 data. Over 12-months, 40% developed new HO; 48% reported ≥1 flare-up. Of participants with new HO, 65% reported ≥1 flare-up (mean 2.3/year); 35% reported no flare-up. Of participants without new HO, 43% reported ≥1 flare-up (mean 1.8/year). Across participants, mean (SD) new HO volume in those reporting flare-ups was 39,718 (91,969) mm^3 (n=48) vs 5,081 (14,582) mm^3 (n=45) in those who did not. Mean CfB in CAJIS and FOP-PFQ were minimal and similar across participants with/without new HO.

Conclusions: Among participants, HO volume increased over 12-months. In those with new HO, this was not preceded by flare-ups in over one third of cases. Across all participants, mean new HO volume in those reporting flare-ups was ~8 times higher than in those who did not. CAJIS and FOP-PFQ were not sufficiently sensitive to assess disease progression over 12-months. New HO volume can be used to measure FOP disease progression over the course of a clinical trial.

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The first 4 years of an osteoporosis re-fracture prevention (ORP) service at Royal North Shore Hospital (RNSH): Treatment and re-fracture rates among ORP attendees and non-attendees:

Lillias Nairn[1], Angela Bowman[4], Adrian Piotrowski[1], Sue Davies[4], Ewan Driver[2], Christian Girgis[3], Roderick Clifton-Bligh[3], Lyn March[4]

[1]Physiotherapy, [2]Neurosurgery, [3]Endocrinology, [4]Rheumatology Departments Royal North Shore Hospital (RNSH), St Leonards, NSW 2065

AIM

To review osteoporosis treatment and re-fracture rates among people identified by the RNSH Leading Better Value Care (LBVC) ORP service over the first 4 years between July 2016 and June 2020.

METHOD

An Automated Electronic Screening (AES) Tool was used to identify patients >=50 years with fracture attending RNSH. Eligible patients were also referred directly to ORP by GPs. Triage was performed by the Fracture Liaison Coordinator (FLC) (LN) and patients meeting criteria were referred for medical review (CG, RCB, LM). All data was collected in a purpose-built e-form in eMR with Klik sense data output and review of eMR to validate; re-fracture rate was determined by re-identification in the AES.

RESULTS

From 11,991 encounters 10, 058 patients were identified over the 4 years. 1326 represented due to a new fracture with crude re-fracture frequency of 13.2%. A total of 2483 patients were invited to the ORP service. Antiresorptive medication was recommended for 74% of those who attended. Among 1048 patients did not attend the ORP Service 10.7% re-fractured during the 4 years compared with 6.6% among 1435 patients who did attend the ORP Service ($p < 0.001$), representing an unadjusted 38% relative reduction and 4.1% absolute reduction.

CONCLUSION

Re-fracture frequency was significantly lower among those who attended ORP compared to those who did not. The high volume of patients with fractures identified by the AES Tool could not be managed by the single FLC and the existing medical FTEs. At RNSH where ~2,500 aged >= 50 years present with fractures each year a fully staffed ORP should prevent 100 re-fractures over 4 years.

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Can circulating Wnt antagonist DKK1 alter bone mass by endocrine signalling?

Ya Xiao[1], Sindhu T Mohanty[1], Michelle M McDonald[1, 2], Aaron Schindeler[3, 4], David G Little[3, 4], Peter I Croucher[1, 2], Paul A Baldock[1, 2, 5]

1. Healthy Ageing,Bone Biology Division, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

2. St Vincent's Clinical School, Faculty of Medicine, University of New South Wales, Kensington, NSW, Australia

3. Kids Research Institute, The children's Hospital Westmead, Westmead, NSW, Australia

4. University of Sydney, Camperdown, NSW, Australia

5. School of Medicine Sydney, University of Notre Dame Australia, Darlinghurst, NSW, Australia

Osteoblasts and bone marrow stromal cells modulate bone mass through secretion of the Wnt inhibitor DKK1, which negatively regulates osteoblast differentiation. Increased levels of circulating Dkk1 have been correlated with post-menopausal bone loss. The extent to which serum Dkk1 levels represent their functional regulation of bone anabolism or provide a biomarker of local bone changes remains to be determined. We aimed to examine the effect of altered local Dkk1 production on systemic bone accrual.

Limb-specific DKK1 KO mice were generated using Prxx1 Cre mice; where Dkk1 was deleted in the appendicular skeleton, but retained in the axial skeleton and circulation. Prx1/Dkk1 KO and control WT/Dkk1 floxed mice were culled at 10 and 16 weeks of age and lumbar vertebrae and femurs assessed by DXA and microCT.

DXA in Prx1/Dkk1 KO revealed greater limb bone mineral density (BMD) in male (17.4%)and female (11%) mice at 10 weeks of age and male (13.6%) and female (5.3%) at 16 week of age compared to controls ($p<0.001$). In contrast, vertebral BMD was not different to control for both sexes and ages.

MicroCT confirmed these findings, with greater distal femoral metaphyseal trabecular bone volume (BV/TV) in male (27.9%) and female (81.2%) mice at 10 weeks, and male (23%) and female (67.1%) at 16 weeks compared to controls ($p<0.001$). These changes were a result of increased trabecular number with no changes in trabecular or femoral shaft cortical thickness. Again, vertebral trabecular BV/TV was not different to control for both sexes and ages.

We have provided evidence that loss of Dkk1 in the appendicular skeleton increased bone mass, whereas the axial skeleton was not impacted. In conclusion our results indicate that circulating levels of Dkk1 do not reflect the local production and therefore activity of this potent Wnt antagonist.

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Molecular imaging at the multi-scale discloses novel regulators of matrix organisation during bone pathology

Aikta Sharma[1], Alice Goring[1], Peter B Johnson[2], Roger JH Emery[3], Eric Hesse[4], Alan Boyde[5], Bjorn R Olsen[6], Andrew A Pitsillides[7], Richard OC Oreffo[8], Sumeet Mahajan[2], Claire E Clarkin[1]

1. School of Biological Sciences, University of Southampton, Southampton, Hampshire, United Kingdom

2. School of Chemistry, University of Southampton, Southampton, Hampshire, United Kingdom

3. Faculty of Medicine, Imperial College London, London, United Kingdom

4. Institute of Molecular Musculoskeletal Research, LMU Munich, Hamburg, Germany

5. Dental Physical Sciences, Queen Mary University of London, London, United Kingdom

6. Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts, United States of America

7. Department of Comparative Biomedical Sciences, Royal Veterinary College, London, United Kingdom

8. Institute of Developmental Sciences, University of Southampton, Southampton, Hampshire, United Kingdom

Objectives: Deletion of vascular endothelial growth factor (VEGF) in osteoblasts (OBs) induces pathological and sex-specific alterations to the conformation and mineralisation of the bone matrix in addition to vascular organisation in males. Whether the sexual dimorphism of the bone vasculature is due to the divergent control of matrix architecture is unknown, and the focus of this study.

Methods: Raman spectroscopy (RS) and polarised-second harmonic generation (p-SHG) microscopy were performed to compare the composition and organisation of the bone matrix in male and female osteocalcin-specific *Vegf* knockout (OcnVEGFKO) long bones. *Vegf* expression was deleted *in vitro* in OBs from male and female $Vegf^{fl/fl}$ mice using a Cre-recombinase adenovirus prior to screening of mRNA transcripts of extracellular matrix (ECM) components.

Results: p-SHG revealed a sex-specific macro-level disorganisation of bone matrix in male OcnVEGFKO versus females, attributed by; regionalised alterations to collagen fibril number (-1.16-fold) extensive osteoid and enhanced cortical porosity at the tibiofibular junction. Polarisation anisotropy revealed reductions in fibrillar anisotropy around the endosteal regions in female OcnVEGFKOs (-4.19-fold) and periosteal and perivascular regions (-1.97-fold and -2.64-fold, respectively) in male OcnVEGFKO. RS detected nano-molecular sexual dimorphism in the levels of carbonate following OcnVEGFKO, with reductions detected in females (-2.79-fold) and elevations in males (1.21-fold) versus WTs. Collagen intra-strand stability was exclusively reduced in OcnVEGFKO males (-3.06-fold) versus WT controls. OcnVEGFKO also induced reductions in hydroxyapatite mineralisation in both males and females versus WTs (-1.07-fold and -1.26-fold). Further divergence in genes encoding ECM proteins and pro-angiogenic factors in male and female *Vegf* deficient cells were detected *in vitro* (*Spp1, Mmp13 and Thbs1*).

Conclusions: We demonstrate the utility of label-free and non-destructive approaches for the detection of multi-scale sex-specific variations in the bone matrix following VEGF deletion. Identification of sex-specific genetic regulators of the ECM could be targeted in bone disorders that present nano and macro-level matrix disorganisation.

Treatment with a chimeric long-acting CSF1 molecule enhances fracture healing of healthy and osteoporotic bones

Lena Batoon[1], Susan Millard[1], Kyle Williams[1], Wenhao Sun[1], Cheyenne Sandrock[1], Andy Wu[2], Katharine Irvine[1, 3], Vaida Glatt[4], Liza Raggatt[1, 3], Martin Wullschleger[6, 3, 5], David Hume[1, 3], Allison Pettit[1, 3]

1. Mater Research Institute - The University of Queensland, Translational Research Institute, Woolloongabba, QLD, Australia

2. Translational Research Institute, Woolloongabba, Queensland, Australia

3. The University of Queensland, Faculty of Medicine, Herston, Queensland, Australia

4. University of Texas Health Science Center, San Antonio, Texas, United States of America

5. Griffith University, School of Medicine, Southport, Queensland, Australia

6. Gold Coast University Hospital, Southport, Queensland, Australia

Macrophage colony-stimulating factor 1 (CSF1) controls osteoclast and macrophage proliferation, differentiation and function. Pharmacokinetic limitations of recombinant CSF1 that constrained clinical application and pre-clinical experimentation have been overcome by engineering a potent, long acting chimeric CSF1-Fc. Exogenous CSF1 has been shown to have anabolic effects on multiple tissues, including bone, and we have previously reported that it can enhance fracture callus formation. However, bone catabolic actions of CSF1, particularly in osteoporotic bone is a potential contraindication of CSF1-Fc use for promoting fracture repair. We tested weekly and biweekly systemic CSF1-Fc treatment regimens over a 4-week period in adult 12-16-week-old mice. Histomorphometric analysis showed F4/80+ osteal macrophage number was unchanged after either regimen, while TRAP+ osteoclasts were significantly increased only after biweekly CSF1-Fc treatment ($p=0.0071$), irrespective of gender. Hence, weekly CSF1-Fc treatment had minimal impact on bone-related myeloid cell populations under homeostatic conditions. To assess whether this non-myeloproliferative CSF1-Fc regimen primed regenerative mechanisms, we investigated the fracture therapeutic potential using the MouseFix internally plated femoral fracture model. Torsional strength testing of the fractured femora in healthy adult mice revealed CSF1-Fc treatment significantly increased fracture maximum torque (+44%). To determine whether a similar treatment strategy can improve osteoporotic fracture healing, ovariectomised (OVX) C3H/HeJ mice that had confirmed trabecular ($p<0.0001$) and cortical bone loss ($p<0.01$) were fractured using the MouseScrew intramedullary screw fixation system. Micro-CT assessment of fracture sites showed a 50% reduction in cortical bridging at 5 weeks post-fracture in OVX mice compared to sham controls, indicative of delayed bone repair associated with OVX. Importantly, weekly CSF1-Fc treatment of OVX mice post-fracture corrected this delayed healing phenotype. Collectively, our results demonstrate that a low dose CSF1-Fc treatment regimen is a promising fracture therapeutic to promote regeneration in healthy and osteoporotic bones.

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Neutrophils are dispensable for neurogenic heterotopic ossification development following spinal cord injury

Hsu-Wen HWT Tseng[1], Irina IK Kulina[1], Marjorie MS Salga[1, 2], Whitney WF Fleming[1], Cedryck CV Vaquette[3, 4], François FG Genêt[5, 2], Jean-Pierre JPL Levesque[1], Kylie KA Alexander[1]

1. Mater Research Institute-The University of Queensland, Brisbane, Queensland, Australia

2. Department of Physical Medicine and Rehabilitation, , Raymond Poincaré Hospital, APHP, CIC 1429, Garches, France

3. Regenerative Medicine, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia

4. The University of Queensland, School of Dentistry, Herston, Brisbane, Queensland, Australia

5. END:ICAP U1179 INSERM, UFR des Sciences de la Santé-Simone Veil, Université Versailles Saint Quentin en Yvelines, Montigny le Bretonneux, France

Neurogenic heterotopic ossifications (NHO) are a frequent complications after spinal cord injuries (SCI), which manifest as abnormal ossification in soft tissues. NHO leads to pain, joint deformation, ankylosis and vascular and nerve compression that largely compromises life quality of patients. NHO pathogenesis is poorly understood and the only treatment remains surgical resection. Our team developed the first mouse model of NHO following SCI, which mimics most clinical features of NHO to investigate the pathogenesis. Using this model, we have demonstrated that *1)* monocytes/macrophages are necessary for NHO development, *2)* SCI exacerbates macrophage infiltration into injured muscles and *3)* that oncostatin M (OSM), a pro-inflammatory cytokine produced by macrophages, is an important driver of NHO formation in both mouse model and patients. Indeed, inhibition of OSM downstream signalling with the JAK1/2 inhibitor ruxolitinib significantly attenuated NHO development in mice. We have now investigated the potential role of neutrophils, another major source of OSM in injured muscles. We find that granulocyte stimulating factor (G-CSF) is significantly upregulated by either SCI or muscle injury. However, mice defective for the G-CSF receptor gene *Csf3r*, which are neutropenic, have unaltered NHO development following SCI. As the administration of recombinant human G-CSF (rhG-CSF) has been trialed after SCI to increase neuroprotection and neuronal regeneration, we then investigated the impact of rhG-CSF treatment after SCI and muscle injury, on NHO development. Treatment with rhG-CSF significantly increased neutrophils in the blood, bone marrow and injured muscles however micro-computed tomography confirmed no change in NHO bone volumes compared to saline treated controls after 7 days of treatment post-surgery. Overall, our results establish that unlike macrophages, neutrophils are dispensable for NHO development and rhG-CSF treatment post-injuries does not impact NHO development. Therefore, G-CSF treatment to promote neuro-regeneration is unlikely to adversely promote NHO development in SCI patients.

Chondrocyte glucocorticoid receptor deletion attenuates cartilage damage in murine osteoarthritis

Eugenie Macfarlane[1], Jan Tuckermann[2], Markus J Seibel[1], Hong Zhou[1]

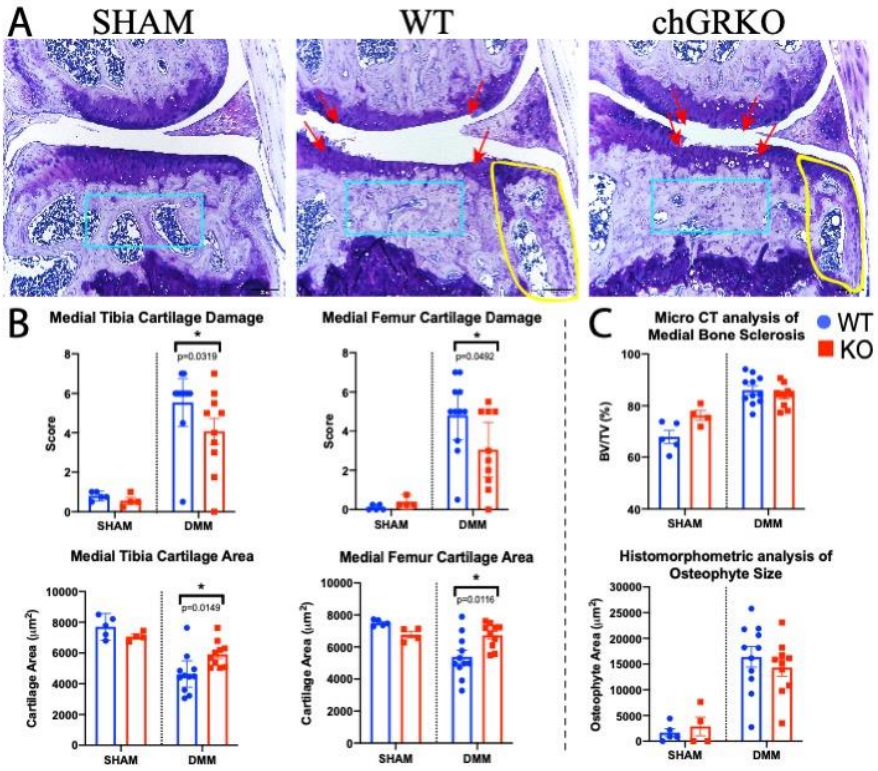
1. ANZAC Research Institute, University of Sydney, Concord, NSW, Australia

2. Institute of Comparative Molecular Endocrinology, University of Ulm, Ulm, Germany

Although glucocorticoids (GCs) are often used in the treatment of osteoarthritis (OA) for pain relief, the role of endogenous GCs in the pathogenesis of OA remains unknown. We have previously shown that disruption of endogenous GC signaling in osteoblasts and osteocytes mitigates OA in mice by reducing the severity of cartilage damage, subchondral bone sclerosis and osteophyte formation. This finding led us to investigate whether disruption of endogenous GC signaling specifically in chondrocytes also affects the development of OA.

Osteoarthritis of the knee joint was induced by surgical destabilization of the medial meniscus (DMM) in tamoxifen-inducible glucocorticoid receptor-knockout (chGRKO) mice and their wild-type (WT) littermates at 22-weeks of age (n=10-11 per group). Sham surgery was used as a control (n=4-5 per group). Both WT-DMM and chGRKO-DMM mice developed apparent OA 16-weeks after surgery, characterized by cartilage degradation, subchondral bone sclerosis and osteophyte formation. No obvious signs of inflammation were observed histologically at this time point. Histological semiquantitative scoring revealed that compared to WT-DMM mice, cartilage damage was significantly less pronounced in chGRKO-DMM mice, particularly at the medial tibial plateau and femur condyle (Fig. 1A, B). Medial tibial cartilage area assessed by histomorphometry was 5912µm2 and 4632µm2 in chGRKO-DMM and WT-DMM mice respectively (p=0.0149; Fig. 1B). Similarly, intact medial femoral cartilage area was 6728µm2 and 5384µm2 in chGRKO-DMM and WT-DMM mice respectively (p=0.0116; Fig. 1B). Deletion of the glucocorticoid receptor in chondrocytes appeared to have no impact on subchondral bone sclerosis, osteophyte formation or synovial inflammation as no differences were observed between chGRKO-DMM and WT-DMM mice by either micro-CT or histomorphometry analyses (Fig. 1C).

We conclude that endogenous GC signaling in chondrocytes promotes cartilage degradation but not abnormal bone formation (subchondral bone sclerosis and osteophytes) in a mouse model of surgically induced OA.



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Inhibition of Sclerostin by Sclerostin Antibody Does Not Affect Morphological or Transcriptional Endpoints Related to Atheroprogression, Plaque Calcification, or Inflammation in 2 Murine Models of Atherosclerosis

Rogely Boyce[1], Aimee Deaton[2], Jun Yin[3], Marina Stolina[1], Melanie Felx[4], Gabrielle Boyd[4], Jean-Guy Bienvenu[4], Aurore Varela[4], Martin Guillot[4], Gill Holdsworth[5], Alison Wolfreys[5], Denise Dwyer[1], Sheetal Kumar[2], Emily de Koning[2], Kathrin Locher[1], Luke Ward[1], Charles Glaus[1], Yudong He[3], James Turk[1]

1. Amgen Inc, Thousand Oaks, CA, USA

2. Amgen Inc, Cambridge, MA, USA

3. Amgen Inc, San Francsico, CA, USA

4. Charles River Laboratories Montreal ULC, Senneville, QC, Canada

5. UCB Pharma, Slough, UK

Sclerostin has been proposed to inhibit vascular calcification, atheroprogression, and inflammation. To investigate the effects of sclerostin inhibition by Scl-Ab (humanised sclerostin antibody) on vasculature in the presence of vascular disease, studies were conducted in ovariectomised ApoE knockout (ApoE KO-OVX) mice fed a high-fat diet—model of severe atherosclerosis and the bone-vascular axis of postmenopausal women, and angiotensin-infused male ApoE knockout (AngII ApoE KO) mice—model of atherosclerosis and aortic aneurysm.

First study: Scl-Ab (Scl-Ab VI; 10 mg/kg/wk) or vehicle was administered for 3, 8, or 16 wk to ApoE KO- and wild-type (WT)-OVX mice. Alendronate (0.02mg/kg/twice wk) or saline was administered for 16 wk to ApoE KO-OVX mice (comparator). Evaluated endpoints: aortic total and mineralised plaque volume (by µCT); plaque histopathology; serum biomarkers of inflammation, bone formation and endothelial/platelet activation; and aortic transcriptional changes (by RNA Seq). Second study: Male ApoE KO mice were infused with AngII at approximately 1 µg/kg/min for 4 wk, and administered Scl-Ab (Scl-Ab VI; 10 mg/kg/wk) or vehicle. WT mice were infused with and administered vehicle. At 4 wk, aortic total and mineralised plaque volume, serum inflammation biomarkers, bone mass, aortic transcriptional changes, and aortic aneurysm incidence were evaluated.

Collective data showed robust expected effects on cardiovascular endpoints in vehicle-treated ApoE KO-OVX and AngII ApoE KO vs WT mice. Scl-Ab promoted bone formation but did not affect total/mineralised plaque volume, plaque histopathology, serum biomarkers of inflammation and endothelial/platelet activation, atheroprogression-related signalling pathways, or aortic aneurysm incidence compared with respective vehicle-treated mice. Alendronate had no meaningful effects on atheroprogression-related endpoints.

Sclerostin inhibition by Scl-Ab at systemic exposures predicted to be 4-fold greater than romosozumab clinical exposure at 210 mg QM has no effect on atheroprogression-related endpoints, plaque calcification, or inflammation in 2 murine models of atherosclerosis.

Study was sponsored by Amgen Inc. and UCB Pharma

Myth-busting the NHMRC Investigator Grant scheme

David Scott[1, 2]

1. NHMRC Emerging Leadership Fellow, NHMRC

2. Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University

Associate Professor David Scott is an exercise scientist and musculoskeletal researcher who has held a National Health and Medical Research Council (NHMRC) Career Development Fellowship since 2017 and was awarded a NHMRC Investigator Grant (Emerging Leadership Level 2) in the inaugural round of the scheme in 2019. David has also served on grant review panels for Emerging Leadership Levels 1 and 2, as well as for NHMRC Postgraduate Scholarships.

In this presentation, David will discuss his experiences both as an applicant and a reviewer for Investigator Grants, and attempt to shed light on some of the mysteries of the scheme. Given the increased focus on research impact in Investigator Grant assessments, he will also highlight effective strategies for demonstrating impact across different types of research. While the content of the presentation will be targeted primarily at intending Investigator Grants applicants (whether applying for the first time or planning a resubmission), it will also provide useful tips for applicants to other fellowship and scholarship schemes.

Prescribing exercise for osteoporosis

Belinda Beck[1]

1. Griffith University, QLD, Australia

As therapy for osteoporosis, exercise has come of age. Previously considered to evoke only modest benefit for BMD, recent examination of higher intensity loading has produced more positive results. In fact, from an ancillary therapy playing a distant second fiddle to medications, there is now strong evidence that exercise should be first line therapy for osteoporosis for all who are physically capable. Not only can exercise improve bone density but bone morphology in strategic locations likely to improve the resistance of a bone to fracture. Furthermore, the vital role of exercise to promote neuromuscular adaptations in the lower extremity that reduce the propensity to fall cannot be overstated. Several clinical cases will be presented demonstrating the effects of high intensity exercise under a variety of conditions of low bone mass, including a 'standard' postmenopausal response, pre-menopause, breast cancer recovery, and cessation of bone medications. The principles behind effective exercise loading for bone will be discussed as the rationale for high intensity exercise prescription. A successful model of translation of high intensity exercise into clinical practice for osteoporosis will be described. Recognised challenges to high intensity exercise arising from prevalent comorbidities in the osteoporotic demographic will be addressed and practical solutions presented.

Bone health in obesity and weight loss

Jackie Center[1]

1. Garvan Institute of Medical Research and St Vincent's Hospital, Darlinghurst, NSW, Australia

The close positive relationship between bone and weight has been known for many years. However, it has recently become clear that obesity doesn't necessarily protect against fracture. Furthermore, with the exponential increase in obesity in today's society, there has been a rapid increase in bariatric surgery resulting in large weight losses. Weight loss is known to result in bone loss but the clinical iimplications of this in the morbidly obese have only been studied more recently.

Following a case presentation, this talk will focus on some of the evidence for fracture risk in the obese, on what happens to bone following various types of bariatric surgery, the data for fracture risk following bariatric surgery and what measures may be useful for optimal bone health post bariatric surgery. It will also touch briefly on some of the potential mechanisms for bone loss.

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Determining the role of maternal epigenetic inheritance in bone development and disease

Ruby Oberin[1], Heidi Bildsoe[1], Tesha Tsai[1], Natalie A Sims[2, 3], Patrick Western[1]

1. Hudson Institute of Medical Research, Clayton, VICTORIA, Australia

2. St. Vincent's Institute of Medical Research, Fitzroy , Vic, Australia

3. Department of Medicine at St. Vincent's Hospital, The University of Melbourne, Fitzroy , Vic, Australia

Exposure of the developing germline to environmental factors, such as diet and drugs, is thought to alter epigenetic programming in gametes and modify development, phenotype, and disease heritability in offspring. Embryonic Ectoderm Development (EED) is an essential subunit of the epigenetic modifier Polycomb Repressive Complex 2, which regulates genes controlling cell differentiation in bone, brain and haemopoiesis. In humans, germline mutations in *EED* result in Cohen-Gibson syndrome, characterized by fetal overgrowth, accelerated bone aging and skeletal defects. Whilst the role of EED in stem cell differentiation and development is well understood, its role in epigenetic programming of the oocyte and its consequent influence on offspring development is poorly understood.

To determine the role of EED in oocyte programming and epigenetic inheritance, we developed a mouse model in which *Eed* is specifically deleted in growing oocytes. This model facilitates the production of genetically identical heterozygous offspring from oocytes with differences in their epigenetic heritage. We predicted that offspring from oocytes lacking EED would have impaired bone development, as well as compromised bone maintenance and repair.

Heterozygous offspring produced from the oocytes lacking EED-dependent epigenetic programming exhibited increased bone length and greater bone mineral density compared to genetically identical heterozygous controls that retained programming. MicroCT analyses identified an 10% increase in mineralised bone length and 15% increase in mineralised bone width. Histological analyses revealed this was associated with lengthening of the growth plate hypertrophic zone, indicating fetal overgrowth due to accelerated bone development. This indicates that altered EED-dependent epigenetic programming in the oocyte has consequences for offspring bone development and phenotype, and provides a model of the skeletal defect associated with Cohen-Gibson syndrome. This model will be used to identify how inherited epigenetic information controls early life and long-term skeletal development, which is crucial for understanding the developmental origins of disease.

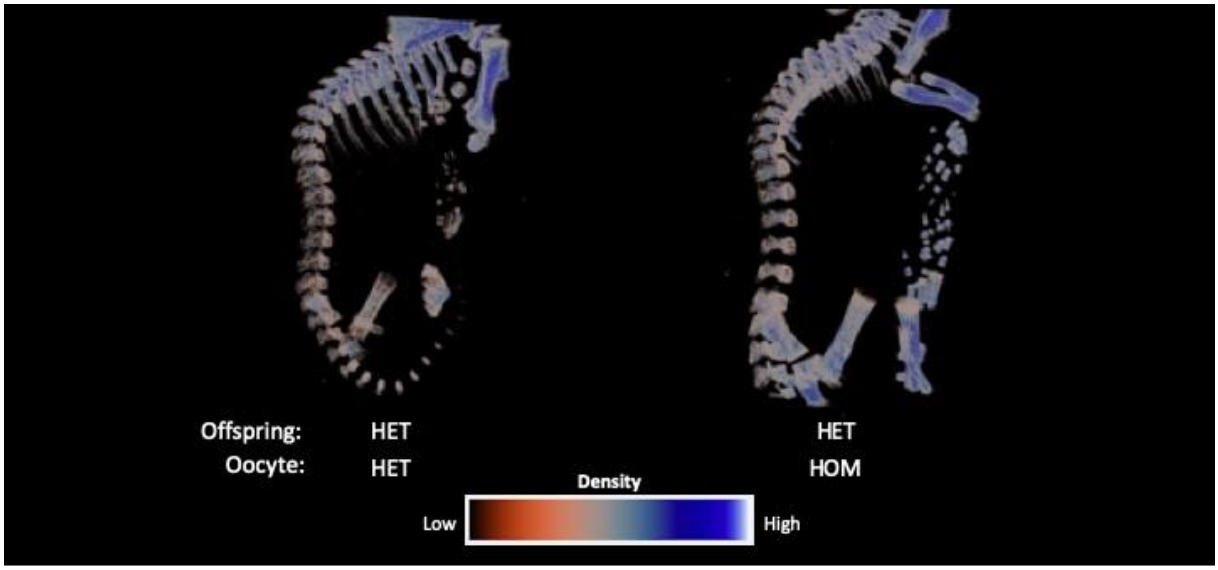

Figure 1: Density map images of genetically identical, yet epigenetically different, postnatal day 3 offspring.
Heterozygous offspring produced from oocytes lacking EED-dependent epigenetic programming (HOM oocyte) exhibit greater bone mineral density, increased femoral mineralised bone length and mineralised bone width compared to genetically identical heterozygous controls that retained EED-dependent epigenetic programming (HET oocyte).

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Extra-nuclear PTHrP induces HIF and drives tumor cell exit from dormancy in bone

Courtney M Edwards[1, 2], Jasmine A Johnson[1], Lawrence A Vecchi III[1], Rachelle W Johnson[1]

1. Clinical Pharmacology , Vanderbilt University Medical Center , Nashville, Tennessee , USA

2. Vanderbilt University Medical Scientist Training Program , Nashville , Tennessee, USA

Breast cancer cells that disseminate to the bone can either lie dormant or induce osteolytic bone destruction mediated, in part, through tumor cell expression of parathyroid hormone-related protein (PTHrP). Our lab found that PTHrP also promotes tumor cell exit from dormancy, since PTHrP overexpression downregulates known pro-dormancy genes (e.g. *LIFR*) and switches dormant MCF7 breast cancer cells to an osteolytic phenotype *in vivo*. PTHrP has biological domains that include a nuclear localization sequence (NLS) and C-terminal region with cytoplasmic activity, but the role of these domains in breast cancer dormancy and bone colonization is unknown. Since nuclear PTHrP stimulates cell cycle progression in muscle cells, we hypothesized that PTHrP NLS deletion would prevent tumor cell exit from dormancy *in vivo*. Surprisingly, we found that deletion of the PTHrP NLS (termed DNLS) or NLS + C-terminal region (DNLS+CTERM) in MCF7 cells dramatically increased radiographic bone destruction *in vivo* (up to 5.3-fold, $p<0.05$), even greater than overexpression of full-length secreted (FLSEC) PTHrP. RNAseq and Gene Set Enrichment Analysis revealed that hypoxia signaling was significantly enriched in DNLS (NES = 1.60, FDR = 0.017) and DNLS+CTERM (NES = 1.41, FDR = 0.058) cells, consistent with significant enrichment of additional hypoxia-inducible pathways (e.g. EMT). HIF1α mRNA was significantly elevated in the FLSEC cells only (1.7-fold, $p<0.01$), suggesting PTHrP may directly promote HIF1α transcription, since PTHrP is excluded from the nucleus in DNLS and DNLS+CTERM cells. Interestingly, HIF1α protein levels were significantly elevated in DNLS cells only (1.9-fold, $p<0.05$), suggesting that PTHrP in the cytoplasm stabilizes HIF1α protein. While PTHrP may drive HIF1α transcription, it is likely rapidly degraded, since protein levels were not elevated in FLSEC cells. These data reveal important insights into PTHrP regulation of HIF and a novel mechanism by which breast cancer cells exit dormancy and colonize the bone.

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Targeting Notch2 signalling to overcome methotrexate chemotherapy-induced bone loss and bone marrow adiposity in rats

Yaser Peymanfar[1], Yu-Wen Su [1], Cory Xian[1]

1. University of South Australia, UniSA Clinical and Health Sciences, Adelaide, SA, Australia

Publish consent withheld

Characterisation of osteoprogenitors within the periosteum

Ye Cao[1], Dorit Naot[2], Jillian Cornish[2], Brya G Matthews[1]

1. Molecular Medicine and Pathology, University of Auckland, Auckland

2. Medicine, University of Auckland, Auckland

Tissue-resident stem and progenitor cells support skeletal healing and regeneration throughout life. The periosteum is a major source of cells involved in fracture healing. We hypothesize that the periosteum is enriched for osteoprogenitor cells compared to other tissue compartments, and therefore periosteal cells more efficiently contribute to bone healing.

Bone marrow, endosteum and periosteum were isolated from mouse hindlimbs using enzymatic digestion. Most stem and progenitor markers examined were enriched in the periosteum, including Sca1 (7.0% ± 1.4% in the periosteum, <1% in the endosteum and bone marrow), CD51, CD90, PDGFRa, and CD200. After sorting of freshly isolated periosteal cells, Sca1+/CD51+ cells showed efficient CFU-F formation (9-fold increase compared with total non-haematopoietic cells) as did Sca1-/CD51+ cells (5-fold increase). Sca1+/CD51+ colonies could undergo both osteogenic and adipogenic differentiation in vitro, while Sca1-/CD51+ cells were osteogenic only.

We also investigated the response of periosteum to a local scratch injury, which causes formation of a callus. Sca1+/CD51+ and Sca1-/CD51+ were significantly expanded 7 days after injury. In addition, CD90+ cells were more abundant by day 3 (34.8% ± 4.4%) and day 7 (42.9% ± 4.2%) after injury compared with uninjured (22.4% ± 2.8%).

We also examined marker expression in human skeletal tissue: bone marrow from the femoral head; endosteum (or bone-associated cells) digested from cleaned trabecular bone; and periosteum dissected from the femoral neck, followed by collagenase digestion. Most markers were enriched in the periosteum, including CD51 (32.3% ± 17.2% in the periosteum and <1% in the endosteum and bone marrow), CD90 (32.8% ± 15.4%, <1% in the endosteum and bone marrow), and CD73 (52.5% ± 19.3%, <0.5% in the endosteum and bone marrow).

Using cell surface markers we identified populations of progenitors with dual lineage potential and osteoprogenitors. Our findings suggest that the periosteum is highly enriched for skeletal stem and progenitor cells, and some of these populations expand in response to injury.

Horizontal fissuring at the osteochondral interface: a novel and unique pathological feature in patients with obesity-related osteoarthritis

Lianzhi Chen[1], Felix Yao[1], Tao Wang[1], Guangyi Li[2], Peilin Chen[1], Max Bulsara[3], Jessica Jun Yi Zheng[4], Euphemie Landao-Bassonga[1], Marty Firth[5], Praveen Vasantharao[6], Yi-Gang Huang[2], Michelle Lorimer[7], Stephen Graves[8], Junjie Gao[2, 1, 9], Richard Carey-Smith[4], John Papadimitriou[1, 10], Changqing Zhang[2], David Wood[1], Christopher Jones[11, 12], Ming-Hao Zheng[1, 9]

1. Centre for Orthopaedic Research, University of Western Australia, Perth, Western Australia, Australia

2. Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

3. Institute for Health Research, University of Notre Dame, Fremantle, Western Australia, Australia

4. Department of Orthopaedic Surgery, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

5. Centre for Applied Statistics, University of Western Australia, Perth, Western Australia, Australia

6. Department of Orthopaedic Surgery, Royal Perth Hospital, Perth, Western Australia, Australia

7. South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

8. Australian Orthopaedic Association National Joint Replacement Registry , Adelaide, South Australia, Australia

9. Perron Institute for Neurological and Translational Science, Perth, Western Australia, Australia

10. Pathwest Laboratories, Perth, Western Australia, Australia

11. Medical School, Curtin University, Perth, Western Australia, Australia

12. Department of Orthopaedic Surgery, Fiona Stanley Hospital Group, Perth, Western Australia, Australia

Obesity is a well-recognized risk factor for osteoarthritis (OA). We aim to investigate if obesity is the causal antecedent of early joint replacement in patients with OA and characterize the body mass index (BMI)-associated pathological changes in the osteochondral unit. We analyzed the impact of BMI on the age at which patients underwent total knee replacement (TKR). A total of 41,023 cases of TKR from the Australian Orthopaedic Association National Joint Replacement Registry (AOANJRR) were assessed. It revealed that approximately 57% of TKR applied for patients with primary OA were obese. There was a significant progressive reduction in the age at which patients with increased BMI. The mean age of TKR in obese class III patients was 8 years younger than normal-weight patients. We then investigated the effect of BMI on pathological changes of knee joints in a representative cohort undergoing TKR. Histopathological examination revealed, for the first time, that horizontal fissuring at the osteochondral interface (Figure 1) was the major pathological feature of obesity-related OA. The frequency of horizontal fissure was strongly associated with increased BMI in the predominant compartment. An increase in one unit of BMI (1 kg/m^2) increased the odds of horizontal fissures by 14.7%. Over 80% of the horizontal fissures were attributable to obesity. Reduced cartilage degradation and alteration of subchondral bone microstructure were also associated with increased BMI. In conclusion, obesity is strongly associated with a younger age of TKR. Due to differences in the mechanical properties of cartilage, calcified cartilage and subchondral bone, secondary shear stress generated by high body weight in obesity-related OA induces horizontal fissures at the osteochondral interface. This key pathological feature may be the cause of earlier TKR in patients with obesity. This finding will draw a considerable attention to the osteochondral interface as a therapeutic target for obesity-related OA.

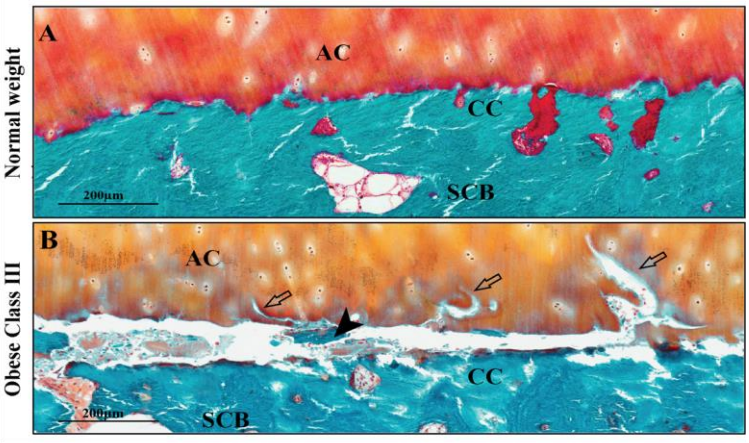

Figure 1. Representative images of horizontal fissures in the osteochondral units from OA patients with normal weight and morbid obesity. In patients with normal weight (A), cartilage is firmly attached on calcified cartilage (CC). In patients with obese class III (B), horizontal fissuring is observed at the osteochondral interface between the articular cartilage (AC) and subchondral bone (SCB). Free bone debris (black arrowhead) and cartilage erosion (empty arrow) are presented within the fissure.

Feasibility, safety and effectiveness of a pilot 16-week home-based, high-impact exercise intervention in post-menopausal women with low bone mineral density

Carrie-Anne Ng[1], Lachlan B McMillan[1], Ludovic Humbert[2], Peter R Ebeling[1], David Scott[1, 3, 4]

1. Department of Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia

2. Musculoskeletal Unit, Galgo Medical, Barcelona, Spain

3. Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia

4. Department of Medicine at Western Health, The University of Melbourne, Sunshine, Victoria, Australia

OBJECTIVE: High-impact exercise is recommended to improve bone health, but the feasibility and efficacy of home-based exercise in postmenopausal women with low bone mineral density (BMD) is unclear. We aimed to determine feasibility, safety and changes in BMD, bone microarchitecture and physical function following a pilot 16-week home-based high-impact exercise intervention in postmenopausal women with osteopenia or osteoporosis.

METHODS: 50 community-dwelling postmenopausal women with BMD T-scores <-1.0 participated in 16 weeks of home-based exercise progressively increasing to 50 multi-directional unilateral hops on each leg daily. Bone density and structure were assessed by lumbar spine and total hip dual-energy X-ray absorptiometry (DXA), 3D modelling algorithms (3D-SHAPER) of hip DXA scans, and distal tibial high-resolution peripheral quantitative computed tomography scans. Physical performance was assessed by repeated chair stand time and stair climb time.

RESULTS: 44 (88%) women (mean±SD age 64.5±7.5 years) completed the intervention, with adherence to exercise sessions of 84.7±18.0%. Six (12%) women withdrew from the study due to related soreness (n=2), unrelated injury (n=1) and loss of interest (n=3). Femoral neck areal BMD significantly increased by 1.13±3.76% (p=0.048). Trabecular volumetric BMD of the total hip and femoral neck estimated by 3D-SHAPER significantly increased by 2.27±7.03% (p=0.038) and 3.20±5.39% (p<0.001), respectively. Additionally, femoral neck integral (trabecular plus cortical) volumetric BMD increased by 1.81±4.33% (p=0.010). At the distal tibia, total volumetric BMD significantly increased by 0.32±0.88% (p=0.032) and cortical cross-sectional area significantly increased by 0.55±1.54% (p=0.034). Chair stand and stair climb time significantly improved by 2.3±1.88s (p<0.001) and 0.27±0.49s (p<0.001), respectively.

CONCLUSION: A home-based 16-week high-impact exercise intervention was feasible and effective in improving femoral neck areal BMD, total hip and distal tibial volumetric BMD, and physical function in postmenopausal women. Home-based high-impact exercise interventions may reduce risk factors for fracture in older populations with limited access to clinic- or gym-based programs.

Multidisciplinary Care Pathways Associated with Recovery of Quality of Life in Individual Fracture Sites: Analyses of the International Costs and Utilities Related to Osteoporotic Fractures Study (ICUROS)

Jason Talevski[2, 1], Kerrie Sanders[3, 2, 1], Lucy Busija[4], Alison Beauchamp[5, 2, 1], Gustavo Duque[2, 1], Fredrik Borgström[6], Axel Svedbom[7], John Kanis[8, 9], Amanda Stuart[10], Sharon Brennan-Olsen[3, 2, 1]

1. Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne and Western Health, Melbourne, VIC, Australia

2. Department of Medicine–Western Health, The University of Melbourne, Melbourne, VIC, Australia

3. School of Health and Social Development, Deakin University, Melbourne, VIC, Australia

4. School of Public Health and Preventive Medicine, Monash University, Melbourne, VIC, Australia

5. School of Rural Health, Monash University, Melbourne, VIC, Australia

6. Quantify Research, Stockholm, Sweden

7. ICON Plc, Stockholm, Sweden

8. Mary Mackillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, Australia

9. University of Sheffield, Sheffield, United Kingdom

10. The Institute for Mental and Physical Health and Clinical Translation (IMPACT), Deakin University, Melbourne, VIC, Australia

Aim: To identify combinations of health service use associated with recovery of health-related quality of life (HRQoL) 12-months post-major osteoporotic fracture (MOF) – specific to each MOF site (hip, distal forearm, vertebrae, humerus).

Methods: The analyses included 4126 adults aged ≥50 years with a MOF (1657 hip, 1354 distal forearm, 681 vertebral, 434 humeral) from the International Costs and Utilities Related to Osteoporotic fractures Study (ICUROS) conducted across Australia, Austria, Estonia, France, Italy, Lithuania, Mexico, Russia, Spain and the UK. HRQoL at pre-fracture and 12-months post-fracture was measured using the EuroQoL questionnaire (EQ-5D-3L). Health service use data were collected via participant interviews and medical record reviews and included: in-hospital care; outpatient care; community health services, medication use and imaging. The data analysis involved two stages: 1) latent class analyses to identify common combinations of health service use ("classes"); and 2) logistic regression to assess associations between classes and HRQoL recovery. Analyses were undertaken separately for each MOF site.

Results: The proportion of participants who recovered to their pre-fracture HRQoL at 12-month follow-up varied across sites: hip (37.3%), distal forearm (65.8%), vertebrae (48.9%) and humerus (49.5%). The latent class analyses determined eight, five, four and three distinct classes for hip, distal forearm, vertebral and humeral participants, respectively. We identified at least one class in each site associated with increased likelihood of HRQoL recovery and one class associated with decreased likelihood (Figure 1). Generally, the combination of hospital presentations without admission; primary care center visits; osteoporosis-related medication use; vitamin D/calcium supplementation and non-opioid analgesic use was associated with higher likelihood of HRQoL recovery.

Conclusion: We identified several, fracture site-specific health service use pathways associated with recovery of HRQoL. The widespread introduction of evidence-based, site-specific care pathways could potentially improve the management and health outcomes of patients treated for a MOF worldwide.


Hip fracture
Class 1
Hospital Admission
Residential Aged Care
Imaging (e.g. X-ray)
Class 2
Hospital Admission
Outpatient Department
Allied Health
HP / GP Home Visit
Phone Counselling
Informal Home Help
Home Modifications
Non-opioid Analgesics
Imaging (e.g. X-ray)
Class 3
ED Presentation
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 4
Hospital Admission
Outpatient Department
Home Modifications
Non-opioid Analgesics
Imaging (e.g. X-ray)
Class 5
Hospital Admission
Outpatient Department
PCC / GP Visit
Allied Health
Home Modifications
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 6
Hospital Admission
Outpatient Department
PCC / GP Visit
Allied Health
Formal Home Help
Informal Home Help
Home Modifications
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
DXA Scan
Imaging (e.g. X-ray)
HRQoL
Class 7
Hospital Admission
Outpatient Department
PCC / GP Visit
Formal Home Help
Informal Home Help
Home Modifications
Vitamin D & Calcium
Imaging (e.g. X-ray)
Class 8
Hospital Admission
Informal Home Help
Home Modifications
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Distal forearm fracture
Class 1
ED Presentation
Outpatient Department
PCC / GP Visit
Allied Health
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
Class 2
Hospital Admission
Operations
Outpatient Department
PCC / GP Visit
Allied Health
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 3
Hospital Admission
Operations
Outpatient Department
PCC / GP Visit
Allied Health
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 4
ED Presentation
Outpatient Department
PCC / GP Visit
Allied Health
Imaging (e.g. X-ray)
HRQoL
Class 5
ED Presentation
PCC / GP Visit
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Vertebral fracture
Class 1
Hospital Admission
Outpatient Department
PCC / GP Visit
Informal Home Help
Home Modifications
Imaging (e.g. X-ray)
HRQoL
Class 2
ED Presentation
Outpatient Department
PCC / GP Visit
Home Modifications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
Class 3
Hospital Admission
Outpatient Department
PCC / GP Visit
Informal Home Help
Home Modifications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
Class 4
ED Presentation
PCC / GP Visit
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
DXA Scan
Imaging (e.g. X-ray)
HRQoL
Humerus fracture
Class 1
Hospital Admission
Outpatient Department
PCC / GP Visit
Allied Health
Informal Home Help
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 2
ED Presentation
PCC / GP Visit
Osteoporosis Medications
Vitamin D & Calcium
Non-opioid Analgesics
Imaging (e.g. X-ray)
HRQoL
Class 3
ED Presentation
Outpatient Department
PCC / GP Visit
Allied Health
Non-opioid Analgesics
Imaging (e.g. X-ray)

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Antidepressant use, depressive symptoms and incident fracture: data from the PROFRAC study

Jasmine R Cleminson[1], Lana J Williams[1, 2], Julie A Pasco[1, 3, 2], Sharon L Brennan-Olsen[4, 5, 6], Michael Berk[1, 7, 8, 9]

1. School of Medicine, Deakin University, Geelong, Victoria, Australia

2. Barwon Health, Geelong, Victoria, Australia

3. Melbourne Medical School-Western Precinct, The University of Melbourne, St Albans, Victoria, Australia

4. School of Health and Social Development, Deakin University, Waterfront Geelong Campus, Victoria, Australia

5. Institute for Health Transformation, Deakin University, Waterfront Geelong Campus, Victoria, Australia

6. Department of Medicine, Western Health, St Albans, Melbourne, Victoria, Australia

7. The University of Melbourne, Parkville, Victoria, Australia

8. Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia

9. Orygen, The National Centre of Excellence in Youth Mental Health, Centre for Youth Mental Health, Parkville, Victoria, Australia

Background:

Bone deficiencies have been identified in individuals with depression, and antidepressants use. We aimed to investigate the association between antidepressant use and incident fracture utilizing data from the Predictors and Outcomes of incident FRACtures (PROFRAC) study and Geelong Osteoporosis Study.

Method:

Men and women who had sustained an incident fracture (cases, n=1458, 48.22% men) were recruited from the Barwon Statistical Division and participants of the Geelong Osteoporosis Study, with no history of adult fracture, selected as controls (n=1795, 53.54% men). Information on medication use, depressive and anxiety symptoms (HADS), falls history, previous fracture and lifestyle variables were obtained via questionnaire. Binary logistic regression models were used to test associations, after adjusting for covariates.

Results:

Women: Compared to controls, fracture cases were older, had lower BMI, were less active, more likely to have depressive symptoms and take antidepressant medication, otherwise the groups were similar. Antidepressant use was associated with an increased risk of fracture compared to non-users (OR 1.41, 95% CI 1.08-1.83). Associations persisted after further adjustment for physical activity, falls, previous fracture, anxiety symptoms, and medications known to affect bone (OR 1.52, 95% CI 1.10-2.10).

Men: Fracture cases were younger, had lower BMI, more likely to smoke, be active and take antidepressant medication compared to controls, otherwise, the groups were similar. Following age-adjustment, antidepressant use was associated with an increased risk of fracture compared to non-users (OR 2.25, 95% CI 1.56-3.26). These associations persisted after further adjustment for BMI, depressive and anxiety symptoms and medications known to affect bone (OR 1.90, 95% CI 1.24-2.90).

Conclusion:

These data suggest antidepressant use is associated with an increased likelihood of fracture, indicating the need for careful evaluation of risks and benefits when prescribing antidepressants. Further research to understand the underpinning mechanism is warranted.

Engineering the vasculature for bone engineering

Khoon S Lim[1]

1. Department of Othopaedic Surgery & Musculoskeletal Medicine, University of Otago Christchurch, New Zealand.

Bone grafting remains as one of the most common surgical procedure to treat fracture bones and bone injury, with over two million grafting procedures performed annually worldwide. To date, synthetic bone grafts are commonly used clinically to treat these bone injuries. Although these bone grafts have been previously validated and proven to support bone growth, the maintenance of their long-term survival remains a challenge. This phenomenon might be due to the lack of vascularization, where the slow and limited angiogenic ingrowth from the host tissue is insufficient to provide nutrients and oxygen to the newly formed bone. This might also subsequently affect the degree of osseointegration with the surrounding native bone. Therefore, there is a need to develop novel strategies to improve the vasculogenic capacity of these synthetic bone grafts. In this study, we evaluated the vasculogenesis and biofabrication potential of a photo-polymerisable thiol-ene gelatin based hydrogel. Gelatin (10wt%) was reacted with carbic anhydride (20wt%), at 50˚C for 24h with pH kept in the range of 7.5 – 8 to produce gelatin-norbornene (GelNor). 5wt% GelNor hydrogels were photo-polymerised (400-450nm, 30mW/cm2, 3min, Ruthenium (Ru)/Sodium Persulphate (SPS) as photoinitiators) with thiolated molecules as crosslinking agents. The physico-mechanical properties were characterised with varying crosslinking parameters (Nor/thiol ratio, Ru/SPS concentration and crosslinking agent). Human umbilical vein endothelial cells (HUVEC) were co-encapsulated within GelNor hydrogels with human mesenchymal stromal cells (MSCs). Co-cultures in casted hydrogel disks (Ø5x1mm) were maintained in endothelial growth media for 7 and 14 days, following fixation and immunohistochemical evaluation (CD31/F-actin). Scaffolds with interconnected channels were fabricated by casting GelNOR macromer over 3D plotted Pluronic127© which served as a sacrificial template. GelNOR was successfully synthesised with a 45% degree of modification. Varying Nor:SH (DTT) ratios, photoinitiator concentrations and crosslinking agents resulted in tailorable sol fractions, mass swelling ratios and compression modulus. HUVECs were co-encapsulated with MSCs in gelNOR hydrogels, showing high viability (>90%) and a retained HUVEC phenotype over the cell-culture period. These conditions were able to facilitate the formation and stabilisation of interconnected vessel-like structures. Interconnected channels were successfully generated. In conclusion, we have shown that GelNOR hydrogels, with tailorable physico-chemical properties, can be used to promote in vitro vasculogenesis for large biofabricated bone grafts.

Duration of OPG:Fc treatment determines the extent of rebound bone loss following its cessation – insight into rebound bone loss and fracture following Denosumab cessation

Jad Zamerli[1], Ya Xiao[1], Shelley Ma[1, 2], Albert Kim[3], Sindhu Mohanty[1], Peter Croucher[1], Jackie Center[4, 1], Christian Girgis[3, 5], Michelle M McDonald[4, 1]

1. Bone, Garvan Institute of Medical Research, Sydney, NSW, Australia

2. University of Technology, Sydney, NSW

3. Endocrinology and Metabolism, Royal North Shore Hospital, Sydney, NSW, Australia

4. University of New South Wales, Sydney, NSW, Australia

5. Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

Treatment cessation of the anti-resorptive denosumab (Dmab) can result in rebound loss in bone mineral density (BMD) and increased risk of pathological fracture. Bone turnover analysis reveals this results from increased osteoclast formation and activity, however predicting high-risk patients who will require preventative intervention is unclear. We hypothesized that a proportionality exists between treatment duration and magnitude of rebound. We aimed to compare Dmab-withdrawal rebound bone-loss following short-term and long-term therapy. Growing mice were treated thrice-weekly with osteoprotegerin (OPG:Fc) for 2 weeks (short-OPG) or 8-weeks (long-OPG) to mimic Dmab and bone mass changes assessed to 15 weeks post cessation.

Lower-limb BMD peaked at 6-weeks (short-OPG) and 8-weeks (long-OPG) post OPG cessation at 31% and 54% above vehicles, respectively ($p<0.0001$). 4-weeks later, BMD had normalized to vehicle levels for both groups. However, whilst short-OPG plateaued at control levels to 15 weeks, BMD continued to decline in the long-OPG group, reaching 13.5% below controls ($p<0.01$).

MicroCT at 15 weeks post treatment cessation confirmed normalization of femoral trabecular bone volume fraction (tbBV/TV) and cortical bone thickness (CBth) in the short-OPG group. However, despite BMD falling below vehicle levels at 15-weeks post-cessation, tbBV/TV remained elevated by 300% ($p<0.0005$) in long-OPG treated mice compared to vehicle ($p<0.01$). Conversely, CBth was reduced by 18% ($p<0.0001$) and CB-volume 15% in long-OPG mice compared to vehicles ($p<0.01$).

Our data indicates that longer OPG treatment led to greater BMD gains, however normalization time remained similar to short-OPG, indicating higher bone resorption rates. Longer treatment also led to rebound BMD below control levels, with cortical bone the primary contributor. Future analysis of bone turnover will confirm cellular mechanisms and mechanical testing will determine fracture resistance. Taken together, this data indicates treatment duration may be a factor in determining the magnitude of rebound bone loss and fracture-risk in patients ceasing Dmab.

Romosozumab (Romo) Improves Lumbar Spine Bone Mineral Density (BMD) and Bone Strength Greater Than Alendronate (ALN) as Assessed by Quantitative Computed Tomography (QCT) and Finite Element Analysis (FEA) in the ARCH Trial

Arkadi Chines[1], Jacques P Brown[2], Roland Chapurlat[3], Joseph Foldes[4], Xavier Nogues[5], Roberto Civitelli[6], Tobias De Villiers[7], Fabio Massari[8], Cristiano Zerbini[9], Wenjing Yang[1], Chris Recknor[10], Cesar Libanati[11]

1. Amgen Inc, Thousand Oaks, CA, USA

2. Laval University, Quebec City, QC, Canada

3. INSERM UMR 1033, University of Lyon, Lyon, France

4. Hadassah Hebrew University Medical Center, Jerusalem, Israel

5. IMIM Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain

6. Washington University School of Medicine, St. Louis, MO, USA

7. Stellenbosch University, Stellenbosch, South Africa

8. Instituto de Investigaciones Metabólicas, Buenos Aires, Argentina

9. Centro Paulista de Investigação Clinica, São Paulo, Brazil

10. United Osteoporosis Centers, Gainesville, GA, USA

11. UCB Pharma, Brussels, Belgium

Purpose: Recent evidence suggests treatment-achieved BMD is a reliable surrogate for fracture risk reduction. In ARCH (NCT01631214), Romo (bone-forming agent) followed by ALN achieved greater BMD gains and fracture risk reduction vs ALN alone. Here, we assess lumbar spine (LS) BMD and bone strength improvements by QCT and FEA.

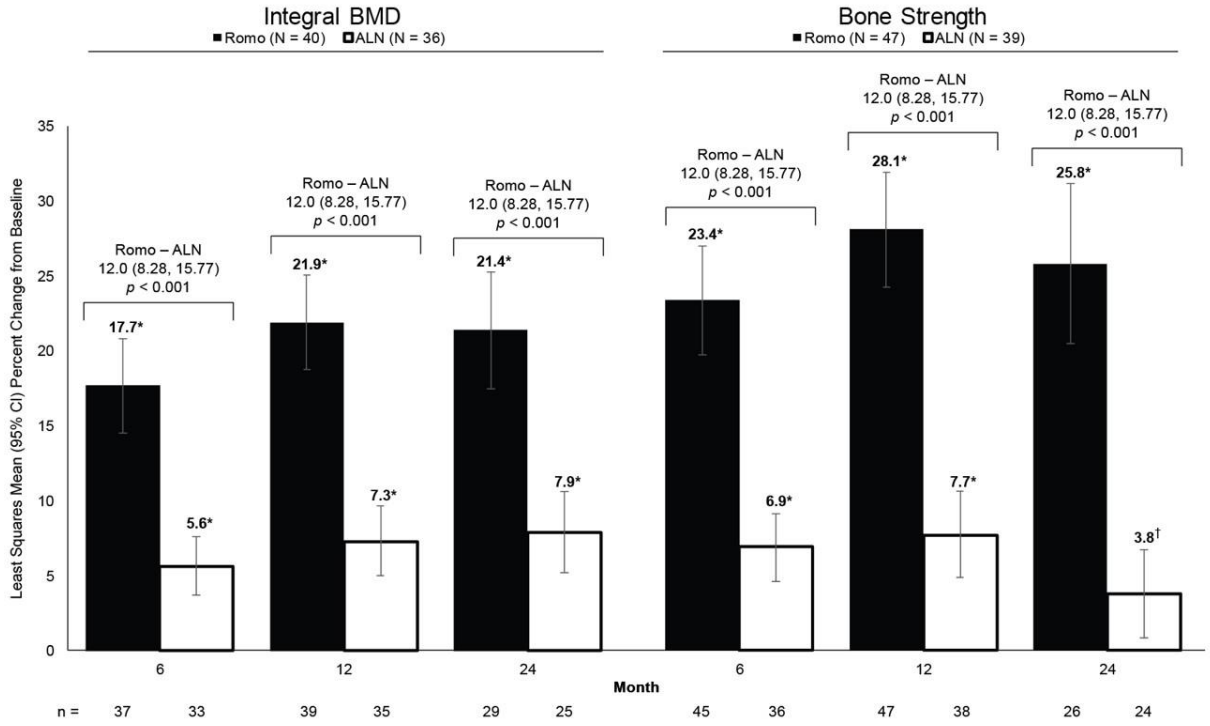
Methods: Postmenopausal women with osteoporosis and prior vertebral/hip fracture received Romo 210mg SC monthly or ALN 70mg PO weekly for 12 months (1:1), followed by open-label ALN 70mg PO weekly. In an imaging substudy, LS BMD was assessed by QCT and vertebral-estimated bone strength by FEA using QCT images obtained at baseline (BL) and months 6, 12, and 24. Correlation analyses evaluated the relationship between changes in FEA, QCT, and DXA.

Results: Post-hoc analysis included 90 subjects (49 Romo;41 ALN) with BL and ≥1 post-BL QCT/FEA assessment. At BL, mean (SD) age was 73 (7) years; LS, total hip, femoral neck DXA T-scores were 3.08 (1.09), –2.70 (0.68), –2.84 (0.45); and 97% had prior vertebral fracture, similar to core study. Both groups experienced significant integral and trabecular BMD gains from BL at all time points (except ALN in trabecular BMD; Fig and data not shown). Romo and ALN differences were significant at months 6, 12, and 24. QCT BMD increases were accompanied by significant LS bone strength increases in both groups at all time points; Romo increases significantly greater. Correlation between post-BL FEA change and QCT BMD (integral and trabecular) and DXA was similar (combined treatment arms; r=0.69–0.87, all $p<0.001$).

Conclusions: Romo significantly improved LS BMD (QCT) and bone strength (FEA) vs ALN. Effects were rapid (month 6), sustained (over 12 months), preserved upon transition (through 24 months), and consistent with greater fracture risk reduction observed in this trial with Romo-ALN vs ALN.

Funding: Amgen Inc., Astellas, and UCB Pharm

Figure: Percentage change from baseline and difference between romosozumab and alendronate treatment in integral BMD and lumbar spine bone strength through the primary analysis in ARCH



Months 6 and 12 measurements were during the double-blind period where subjects received Romo 210 mg SC QM or ALN 70mg PO QW for 12 months; the month-24 measurement were during the open-label period when subjects received open-label ALN 70mg PO QW for 12 months
N = number of subjects with values at baseline and at least one post-baseline visit at or before the primary analysis; n = number of subjects with values at that time point
Data based on ANCOVA model adjusting for treatment, presence of severe vertebral fracture at baseline, and baseline value
*$p < 0.001$, †$p = 0.013$ vs baseline
Missing values are imputed by carrying forward the last non-missing post-baseline value prior to the missing value and within the treatment period
ALN = alendronate; BMD = bone mineral density; CI = confidence interval; FEA = fine element analysis; QCT = quantitative computed tomography; Romo = romosozumab

Tissue Biobanking in Research

Jennifer Byrne[2, 1], A Rush[3, 4, 5], DR Catchpoole[3, 4], R Ling[6], A Searles[6], PH Watson[7, 8, 9]

1. School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW

2. NSW Health Pathology, Camperdown, NSW, Australia

3. Children's Cancer Research Unit, Kids Research Institute, The Children's Hospital at Westmead, Westmead, NSW, Australia

4. Children's Hospital at Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW

5. NSW Health Statewide Biobank, NSW Health Pathology, Camperdown , NSW

6. Health Research Economics, Hunter Medical Research Institute , New Lambton Heights, NSW

7. Tumor Tissue Repository, British Columbia Cancer Agency, Victoria, Canada

8. Office of Biobank Education and Research, Department of Pathology and Laboratory Medicine, University of British Columbia, Victoria, Canada

9. Canadian Tissue Repository Network, British Columbia Cancer Agency, Victoria, Canada

Human preclinical and translational research frequently relies upon the availability of quality human biospecimens and associated data. These materials are usually obtained from human tissue banks or biobanks, which are research support facilities located within hospitals, medical research institutes and universities. Despite the importance of human biospecimens in biomedical research, human biobanks frequently report financial sustainability challenges. We have proposed that these sustainability challenges may partly arise from a lack of information about biobank value (1). For a cohort of n=12 cancer biobanks in New South Wales, we performed an in-depth analysis of both annual monetary and in-kind costs and the publications that were supported by each biobank. Biobanks were compared according to their stated biospecimen access policies (n=6 open-access biobanks, n=6 restricted-access biobanks) (2) and their total annual costs (n=6 high-cost biobanks, n=6 low-cost biobanks). Median total costs, staffing and in-kind costs, as well as median numbers and predicted quality metrics of supported publications were similar for open-access and restricted-access cancer biobanks. A significantly higher proportion of the publications supported by open-access biobanks acknowledged the biobank's contribution. Similar numbers of publications were also supported by high-cost and low-cost biobanks, although high-cost biobanks supported publications with a significantly higher median Journal Impact Factor and Altmetrics score. A significantly higher proportion of the publications supported by high-cost biobanks included biobank staff as co-authors. In summary, our analysis of a small cohort of cancer biobanks identified few differences between the costs and publications supported by open-access versus restricted-access biobanks, whereas greater investments in biobanking were associated with supported publications in more prestigious journals that gained more on-line attention. Our results suggest that the availability of biobank cost and output data will allow more evidence-based approaches to biobank management, improving the capacity of human biobanks to support biomedical research.

(1) Rush A, Catchpoole DR, Ling R, Searles A, Watson P, Byrne JA (2020). More comprehensive measures of biobank value: A step towards improved biobank sustainability? Value in Health, epub ahead of print.

(2) Rush A, Christiansen JH, Farrell JP, Goode SM, Scott RJ, Spring KS, Byrne JA (2015). Biobank classification in an Australian setting. Biopreserv Biobank. 13: 212-8.

The pathogenesis and morphological basis of bone fragility

Ego Seeman[1]

1. Austin Health, University of Melbourne, Melbourne, VIC, Australia

Modelling-based bone formation upon bone's periosteal (outer), and the endocortical and trabecular components of the endosteal (inner) surfaces, slows after completion of longitudinal growth. Concurrently, balanced remodeling upon all three (intracortical, endocortical, trabecular) components of the endosteal surface renews matrix volume, morphology and composition. At some time during early adulthood, the volumes of bone resorbed and deposited by each bone multicellular unit (BMU) both decrease but less bone is deposited than was resorbed by each BMU. The resulting negative remodeling imbalance is the necessary and sufficient morphological basis of bone loss and structural deterioration. However, before menopause, any reduction in total bone volume, microarchitectural deterioration, or loss of bone strength is minimal because the birth rate of BMUs is slow, modest modeling-based bone formation upon the periosteal and perhaps endosteal surfaces offsets bone loss, and periosteal bone formation shifts cortical bone volume radially maintaining resistance to bending.

Around mid-life, oestrogen deficiency of menopause worsens remodeling imbalance and increases the birth rate of BMUs. Remodeling now becomes widespread and produces two types of deficit in mineralized matrix volume. The reversible (transient) deficit is due to the normal delay in onset and slowness of the matrix synthesis, deposition and mineralization. This deficit is *focally transient* because refilling of the excavated cavity eventually occurs, but it is *globally ever-present* because the refilling of cavities excavated weeks to months early is accompanied by concurrent excavation of new cavities at other locations. The magnitude of this 'remodeling space' deficit (excavated cavities, cavities with osteoid, cavities with incompletely mineralized bone) depends on the birth rate of BMUs, and so it is responsive to remodeling suppressants. The incomplete refilling of cavities caused by remodeling imbalance produces the second type of deficit – the irreversible (permanent) deficit in mineralized matrix volume which compromises total bone volume and its microarchitecture; cortices thin and become porous, trabeculae are completely resorbed, perforated and disconnected. Trabecular surface area available for remodeling decreases while intracortical and endocortical surface areas increase facilitating more remodeling of an ever-diminishing bone volume; 80% of bone loss is cortical. Remodeling suppression cannot reverse the reduced total cortical and trabecular bone volume and microstructural destruction; anabolic therapy is needed. Unbalanced remodeling upon the endocortical and trabecular surfaces may also remove modest concurrent modeling-based bone formation upon these two surfaces.

This cellular activity upon the periosteal and endosteal surfaces produce the macro-, micro-, nano- and pico-level structural changes causing bone fragility. Reduced periosteal apposition slows the radial drift of the cortex. Resistance to bending decreases to the 7^{th} power of cortical porosity and the 3^{rd} power of trabecular density so fragility increases disproportionate to the bone loss causing it. This partly explains why ~70% of fractures arise among women seemingly at low risk with 'osteopenia'. Changes at higher resolution also contribute to fragility. For example, incompletely refilled cavities, osteocyte death and lacunar mineralization produce stress concentrators predisposing to microcrack generation, increased inter-osteonal (interstitial) matrix mineral density, accumulating advanced glycation end products (AGEs) facilitate microcrack propagation while replacement of mineralized bone with young under-mineralized bone reduces osteonal matrix mineral density limiting crack propagation. AGEs reduce uncoiling of non-collagenous proteins which dissipate stress. Strains damage mineral platelets causing intra-osteonal diffuse damage. The heterogeneous cellular and morphological origins of bone fragility are rational targets for single, combined and sequential antiresorptive and anabolic therapy.

Advances in combination therapy for postmenopausal osteoporosis

Sabashini Ramchand[1]

1. Massachusetts General Hospital, Harvard University, Boston, United States

The prevalence of osteoporotic fractures continues to increase globally as the world population of older adults, in particular those aged 85 years and over, increases. Osteoporotic fractures are associated with significant morbidity, mortality, and health care expenditure. Although the number of osteoporosis therapies have expanded over the last few decades, the majority of available antiresorptive and anabolic drugs only modestly reduce non-vertebral fracture rates, which account for approximately 80% of all fragility fractures. Hence, there is an ongoing need for more effective drugs or therapeutic regimens with improved fracture risk reduction. In this presentation, I will describe advances in combination antiresorptive/anabolic treatment approaches, with a focus on combined denosumab and teriparatide regimens, which appears to be the most promising combination approach for postmenopausal osteoporosis, evaluated to date.

Predicting Mortality and Re-fracture Following an Initial Fracture

Thao P. Ho-Le[1], Thach S. Tran[1], Hanh M. Pham[2], Steven A. Frost[1], Jackie R. Center[1], John A. Eisman[1], Tuan V. Nguyen[1]

1. Garvan Institute of Medical Research, Darlinghust, NSW, Australia

2. Fertility Department, Andrology and Fertility Hospital of Hanoi, Hanoi, Vietnam

Background and Objective

A fragility fracture can lead to reduced life expectancy. There is, however, a large variation between individuals in terms of adverse health states following a fracture. This study sought to define the pattern of, and determinants for, the transition between fracture, re-fracture, and mortality.

Methods

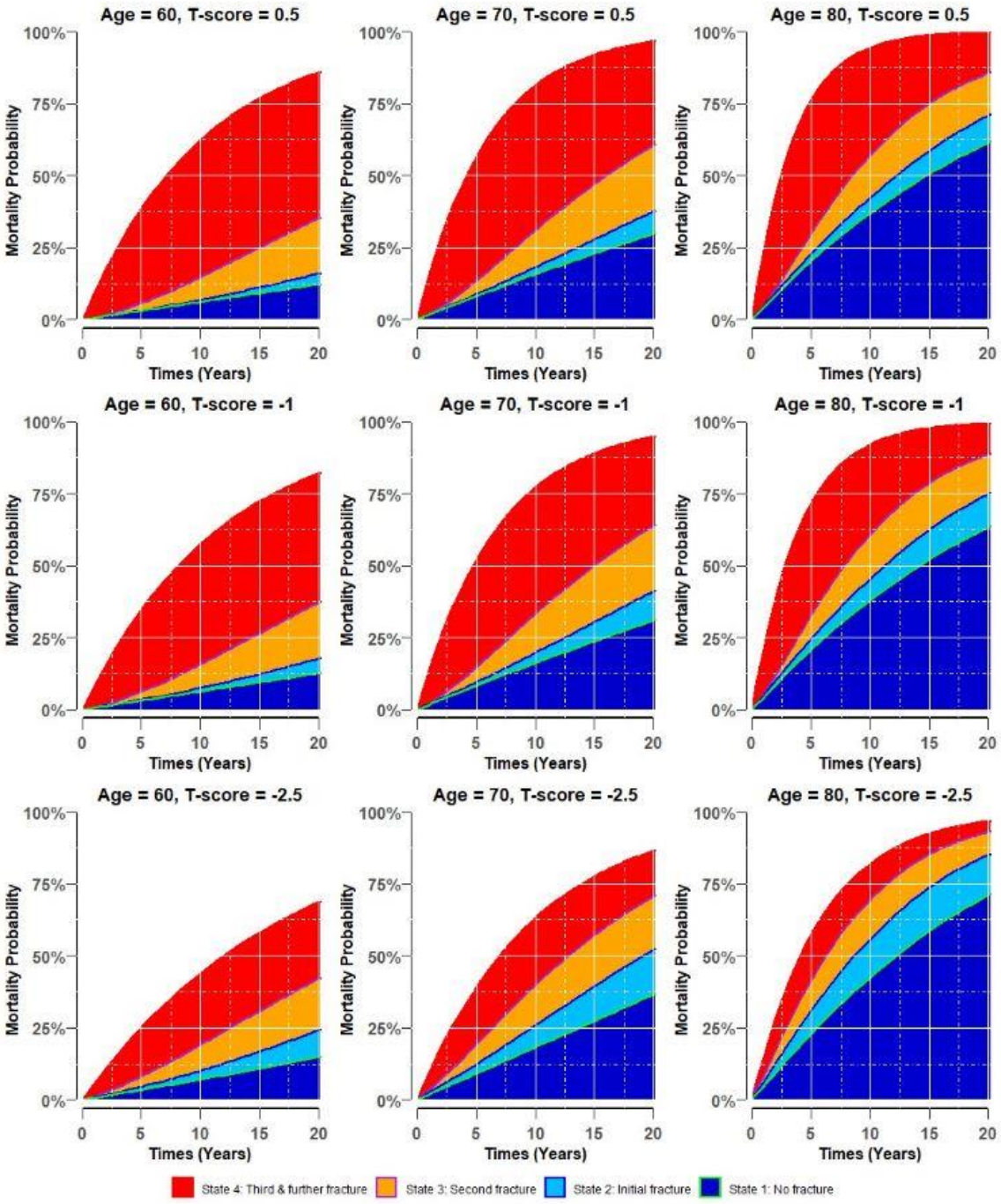
This cohort study involved 2046 women and 1205 men aged 60+, whose fracture status and health outcomes had been continuously monitored for up to 20 years. Fragility fractures were ascertained using X-ray report. The incidence of mortality was ascertained from the state birth, death and marriage registry. Femoral neck bone mineral density (FNBMD, GE-Lunar Prodigy) was measured at baseline. The transition probabilities and time between no fracture, initial fracture, second fracture, third fracture, and mortality were estimated using multi-state model.

Results

The average age at baseline of 2046 women and 1205 men was 70 years. During the [average] 10 years of follow-up, 31% (n=632) women and 15% (n=184) men had sustained a first fracture. Among those with the first fracture, the risk of sustaining a second fracture was 36% in women and 22% in men, and the risk of mortality was 25% in women and 41% in men. Key predictors of subsequent fracture risk included advancing age (HR 1.17; 95%CI, 1.08 to 1.26) and low BMD (1.41; 1.23 to 1.61). Predictors of fracture-associated mortality were male gender (HR 2.4; 95%CI, 1.79 to 3.21), age (1.67; 1.53 to 1.83), and femoral neck BMD (1.16; 1.01 to 1.33).

Conclusion

Individuals with an initial fracture have an increased risk of subsequent fractures and mortality, and the risk of fracture-associated mortality in men is greater than in women. The risks could be predicted by age and bone density. These results can aid patients and doctors make evidence-based treatment decisions.


Age = 60, T-score = 0.5
Age = 70, T-score = 0.5
Age = 80, T-score = 0.5
Age = 60, T-score = -1
Age = 70, T-score = -1
Age = 80, T-score = -1
Age = 60, T-score = -2.5
Age = 70, T-score = -2.5
Age = 80, T-score = -2.5
Mortality Probability
100%
75%
50%
25%
0%
0
5
10
15
20
Times (Years)
State 4: Third & further fracture
State 3: Second fracture
State 2: Initial fracture
State 1: No fracture

Prevalence of vitamin D deficiency in patients with hip fracture over 20 years: time trends and impact on in-hospital mortality

Shyan Goh[1], Wichat Srikusalanukul[1], Alex A Fisher[1, 2]

1. Departments of Geriatric Medicine and Orthopaedic Surgery, The Canberra Hospital, Canberra, ACT, Australia

2. Australian National University Medical School, Canberra, ACT, Australia

Introduction: Role of vitamin D status in osteoporotic fractures remains controversial; its effect on outcomes poorly defined.

Objectives: To assess in older hip fracture (HF) patients temporal trends in vitamin D status at admission and in-hospital mortality over a 20-year period (1999-2019).

Methods: Data on sociodemographic, clinical and laboratory parameters, including admitting 25-hydroxyvitamin D [25(OH)D] levels, and outcomes were collected prospectively from 3719 consecutive HF patients (mean age 82.8±8.1[SD] years; 76.4% females; 53.4% with cervical HF). Patients were stratified according to 25(OH)D levels: vitamin D deficiency (<25 nmol/L), moderate (25-50 nmol/L) and mild insufficiency (50-75 nmol/L). Trends were assessed in five 4-year periods using Poisson regression; models were adjusted for age, gender and various pre-fracture chronic conditions.

Results: Over 20 years, mean 25(OH)D levels increased by 68.4% (from 37.0 nmol/L in 1999-2002 to 62.3 nmol/L in 2015-2018), while post-operative in-hospital mortality rates decreased by 23.9% (from 6.7% to 5.1%, respectively). Every 4-year the prevalence of vitamin D deficiency decreased (age and gender adjusted) on average by 40% (incidence rate ratio [IRR] 0.60, 95% confidence interval [CI] 0.55-0.64, p<0.001), prevalence of moderate vitamin D insufficiency decreased by 27% (IRR 0.73, 95%CI, 0.69-0.76, p<0.001) and the mortality rate declined by 14% (IRR 0.86, 95%CI, p=0.031). Regression analysis demonstrated significant inverse relationship between low admission 25(OH)D levels and fatal outcome. Adjustment for pre-existing conditions including dementia, cardiovascular, lung, Parkinson's disease and diabetes (prevalence of each increased or unchanged), chronic kidney disease (decreased) and HF type did not appreciably explain declining mortality rates. Only vitamin D deficiency, elevated PTH levels (>6.8 pmol/L) along with advanced age and male gender were significantly associated with in-hospital death in multivariate regression analysis.

Conclusions: Over the last decades, in HF patients the prevalence of altered vitamin status and mortality rates are decreasing. Vitamin D deficiency is a significant independent determinant of fatal outcome. Further research is suggested to evaluate the effect of vitamin D supplementing on outcomes.

Higher dietary nitrate intake is associated with better muscle function in men and women independent of physical activity levels

Marc Sim[1, 2], Lauren C Blekkenhorst[1, 2], Nicola P Bondonno[2, 3], Simone Radavelli-Bagatini[2], Peter Peeling[5, 4], Catherine P Bondonno[1, 2], Dianna J Magliano[6, 7], Jonathan Shaw[7], Richard Woodman[8], Kevin Murray[9], Joshua R Lewis[1, 2, 10], Robin M Daly[11], Jonathan M Hodgson[1, 2]

1. Medical School, The University Western Australia, Perth, Western Australia, Australia

2. School of Medical & Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

3. School of Biomedical Science, The University Western Australia, Nedlands, Western Australia, Australia

4. Western Australian Institute of Sport, Perth, Western Australia, Australia

5. School of Human Sciences (Exercise and Sports Science), The University Western Australia, Perth, Western Australia, Australia

6. School of Public Health and Preventive Medicine Melbourne, Monash University, Melbourne, Victoria, Australia

7. Baker Heart and Diabetes Institute (HDI), Melbournce, Victoria, Australia

8. Flinders Centre for Epidemiology and Biostatistics, Flinders University, Adelaide, South Australia, Australia

9. School of Population and Global Health, The University Western Australia, Perth, WA, Australia

10. Centre for Kidney Research, Children's Hospital at Westmead, The University of Sydney, Sydney, New South Wales, Australia

11. Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC, Australia

Introduction: Short-term, high-dosage nitrate supplements can improve vascular and muscle function, but whether higher habitual dietary nitrate is associated with better muscle function remains underexplored. We examined if habitual dietary nitrate intake is associated with better muscle function in a large cohort of men and women across the adult lifespan, and also whether the association was dependent on levels of physical activity.

Methods: The sample (n=3759) was drawn from the national population-based, Australian Diabetes, Obesity and Lifestyle (AusDiab) Study (56% female; mean±SD age 48.6 ± 11.1 y, range 25-85 y). Habitual dietary intake was assessed over 12 years by obtaining an average (at time-points 2000/01, 2004/05 and/or 2011/12) from a food frequency questionnaire. Nitrate intake was calculated from a validated nitrate database. Muscle function was quantified in 2011/12 by knee extension strength (KES) and the 8ft-timed-up-and-go (8ft-TUG) test. Physical activity was assessed by questionnaire (sedentary, <150 min/week, ≥150 min/week).

Results: Median (IQR) total nitrate intake was 65 (52-83) mg/day, with ~81% derived from vegetables. There were non-linear multivariable-adjusted dose-response relationships between total nitrate intake and both KES and 8ft-TUG (**Figure 1**). Individuals in the highest tertile of nitrate intake (median intake 91 mg/d) had 2.6 kg stronger KES (11%) and 0.24 sec faster 8ft-TUG (4%) compared to individuals in the lowest tertile of nitrate intake (median intake 47 mg/d; both p<0.05). Physical activity did not influence the relationship between nitrate intake and KES (p for interaction=0.864) or 8ft-TUG (p for interaction=0.997).

Conclusion: Higher habitual dietary nitrate intake, predominantly from vegetables, was associated with better long-term lower-limb muscle strength and physical function in men and women across the adult lifespan, independent of physical activity levels. Higher nitrate intake from vegetables may be an effective way to limit age-related declines in muscle function; a major contributor towards fall and fracture risk.

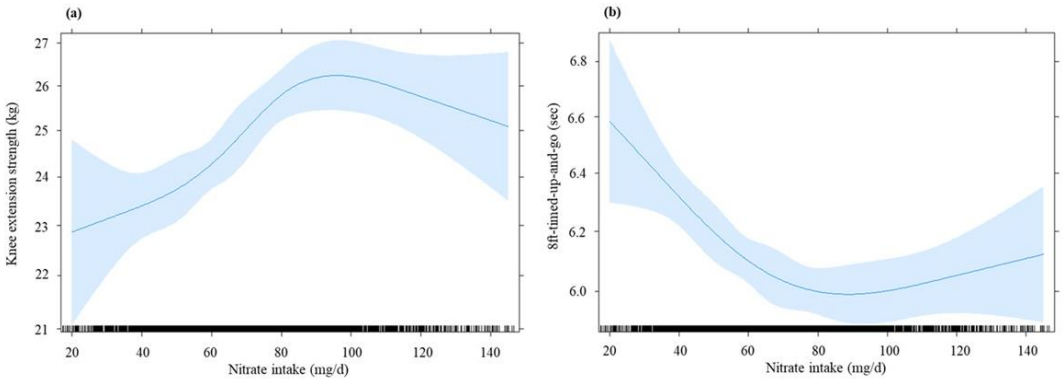


Figure 1. Multivariable-adjusted dose-response relationship between total nitrate intake and (a) knee extension strength, and (b) 8ft-timed-up-and-go obtained by generalized regression models with the exposure included as a restricted cubic spline. Blue shading represents 95% confidence intervals. The rug plot along the bottom of each graph depicts each observation.

Heel bone structure is associated with all cause and cardiovascular disease mortality independent of hip bone mineral density

Abadi Kahsu Gebre[1, 2], Richard L Prince [3, 4], John T Schousboe[5, 6], Douglas P Kiel[7], Peter L Thompson[3, 8], Kun Zhu[3, 4], Wai H Lim[3, 9], Marc Sim[1, 3], Joshua R Lewis [1, 3, 10]

1. School of Medical and Health Sciences , Edith Cowan University, Joondalup, WESTERN AUSTRALIA, Australia

2. School of Pharmacy , Mekelle University, Mekelle , Tigray , Ethiopia

3. Medical School , The University of Western Australia, Perth, Western Australia, Australia

4. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

5. Park Nicollet Osteoporosis Centre and HealthPartners Institute, HealthPartners, Minneapolis, Minnesota, USA

6. Division of Health Policy and Management, University of Minnesota, Minneapolis, Minnesota, USA

7. Marcus Institute for Aging Research, Hebrew SeniorLife, Department of Medicine, Beth Israel Deaconess Medical Centre, Boston, Commonwealth of Massachusetts, USA

8. Department of Cardiology, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

9. Renal department, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

10. Centre for Kidney Research, Children's Hospital at Westmead School of Public Health, Sydney Medical School, The University of Sydney, Sydney, New South Wales, Australia

Osteoporosis is a prominent cause of disability and poor quality of life which has been linked to increased risk of cardiovascular disease (CVD) and mortality. We investigated the association between calcaneal quantitative ultrasound (QUS) measurements and 15-year mortality in 1,404 older women [mean age 75.2±2.7 years]. Calcaneal QUS bone ultrasound attenuation (BUA) and speed of sound (SOS) were obtained using a Lunar Achilles Ultrasound machine at baseline, while hip BMD was obtained by dual-energy X-ray absorptiometry (Hologic Acclaim 4500A) at either baseline or year 1. Mortality records including cause of death were obtained from linked health records. Cox proportional hazard models were used to investigate the relationship between QUS measures, all cause, CVD, cancer and 'other' causes of mortality, with no violations of the proportional hazards assumptions being detected. Over the 15-years of follow-up (17,955 person years), 584 (42%) died from any cause. This included 223 (16%), 158 (12%) and 203 (14%) deaths from CVD, cancer and 'other' causes, respectively. Consistent and robust associations were observed for BUA with all-cause and CVD mortality but not SOS (**Table 1**). For every standard deviation (SD) reduction in BUA in minimally and multivariable-adjusted models, there was a 15% and 20% increase in the relative hazards of all-cause mortality and CVD mortality, respectively. These relationships persisted in multivariable adjustment models after the inclusion of hip BMD (**Table 1**). No consistent associations were seen for SOS or BUA measures with cancer or "other" mortality. In conclusion, the association between calcaneal BUA, a measure of osteoporosis, with all-cause and CVD mortality in older women suggests a link between bone disease and other disorders, particularly CVD. However, due to the observational nature of this work, further intervention studies are needed to demonstrate causality.

Table 1. Hazard ratio per standard deviation (SD) reduction in calcaneal quantitative ultrasound measures.

		All-cause mortality (n = 584)		CVD mortality (n = 223)	
	SD	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Model 1					
BUA, db/Mhz	8.0	**1.15 (1.05-1.25)**	**0.002**	**1.21 (1.05-1.38)**	**0.008**
SOS, m/s	25.6	**1.14 (1.05-1.24)**	**0.002**	1.12 (0.98-1.28)	0.090
Model 2					
BUA, db/Mhz	8.0	**1.15 (1.06-1.26)**	**0.001**	**1.20 (1.04-1.38)**	**0.010**
SOS, m/s	25.6	**1.13 (1.04-1.23)**	**0.004**	1.11 (0.97-1.26)	0.146
Model 3#					
BUA, db/Mhz	8.0	**1.20 (1.07-1.32)**	**0.001**	**1.29 (1.07-1.55)**	**0.006**
SOS, m/s	25.6	1.09 (0.98-1.22)	0.108	1.08 (0.90-1.29)	0.434

Abbreviations: CVD, Cardiovascular disease; BUA, broadband ultrasound attenuation; SD, standard deviation; SOS, speed of sound. Model 1: age, BMI and treatment (calcium/placebo); Model 2: Model 1 plus smoking history, diabetes, prevalent CVD, prevalent cancer. #Total hip BMD at 1998/1999 assessed in 1,130 participants. P value represents from Cox proportional hazards regression with values in bold, $p<0.05$. Primary cause of death data was used for; CVD death (codes included: ICD-9-CM codes 390-459 and ICD-10-AM codes I00-I99); cancer death (codes included ICD-9-CM code 140–239 excluding 210–229 and ICD-10-AM code C00-D48 excluding D10-D36) and other deaths (all other codes).

Is high-intensity exercise associated with vertebral fracture in middle-aged and older men with osteopenia and osteoporosis? A secondary analysis of the *LIFTMOR-M* trial

Amy T Harding[1, 2], Benjamin K Weeks[1, 2], Conor Lambert[1, 2], Steven L Watson[1, 2], Belinda R Beck[1, 2, 3]

1. Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD, Australia

2. School of Allied Health Sciences, Griffith University, Gold Coast, QLD, Australia

3. The Bone Clinic, Brisbane, QLD, Australia

Purpose

Although several trials have examined exercise effects on vertebral morphology in postmenopausal women, none have done so in men. The purpose of the current analysis was to examine the prevalence and incidence of vertebral fracture (VF) following eight months of either high-intensity progressive resistance and impact training (HiRIT) or machine-based isometric axial compression exercise (IAC).

Methods

Men (≥ 45yrs) with low aBMD were randomised to either eight months of supervised, twice-weekly HiRIT or IAC in the *Lifting Intervention For Training Muscle and Osteoporosis Rehabilitation for Men* (LIFTMOR-M) trial. VF assessment using the Genant semi-quantitative method was determined from DXA-derived lateral thoracolumbar spine morphology (Medix DR, Medilink, France). Vertebral deformities were classified by type and grade. Prevalent VFs were those identified at baseline, and incident VFs were new fractures detected at follow-up. Worsening VFs were those that showed reduced height at follow-up at the site of a prevalent fracture.

Results

Forty participants (HiRIT n=20, IAC n=20; 66.1±7.8yrs; lumbar spine T-score -0.1±0.8) underwent lateral thoracolumbar spine DXA and VF assessment at baseline and following completion of the intervention. Four HiRIT participants had five prevalent VFs and six IAC participants had nine prevalent VFs. Over the eight months, no incident VFs nor progression of prevalent VFs occurred for HiRIT. Five incident thoracic wedge VFs and progression of one wedge VF from grade one to grade two occurred for IAC participants.

Conclusions

HiRIT did not cause incident VFs or progression of prevalent VFs, but there was evidence of progression of VF severity and incident VFs for some IAC participants. Clearly, larger trials are required to confirm the observations of this exploratory analysis, however, supervised HiRIT appeared to be safe, whereas IAC may need to be applied with caution in middle-aged and older men with low bone mass if VFs are to be avoided.

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The functional role of Scleraxis in enthesis formation

Yuki Yoshimoto[1]

1. Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Enthesis between tendon or ligament and bone consists of four layers, tendon/ligament, fibrocartilage, calcified fibrocartilage, and bone. This graded structure gives enthesis strength and protects from rupture. During embryonic development, tendon/ligament, skeletal muscle and cartilage primordia appear independently. Through the subsequent interaction between these primordia, tendon and ligament joints skeletal muscle and cartilage are established as one functional unit. We identified progenitor cells expressing both Scleraxis (Scx) and SRY-box 9 (Sox9), which contributes to the formation of the junctional region between tendon/ligament and cartilage. Scx is a basic helix-loop-helix transcription factor predominantly expressed in tendons and ligaments and is required for maturation of these tissues. *Scx* deficient mice show hypoplastic formation of tendons and ligaments. Sox9 is an essential transcription factor for chondrogenesis, and mutations in the *SOX9* gene causes skeletal dysplasia in human. $Scx^+/Sox9^+$ progenitors give rise to chondrocytes and tenocytes or ligamentocytes in and around the attachment sites constructing the prospective enthesis in the axial and the appendicular skeleton. In *Scx* deficient mice, at embryonic day 13.5, cartilage primordia appeared to be normally formed, but the number of $Sox9^+$ cells decreased in the attachment region of tendons and ligaments, such as patella and deltoid tuberosity of humerus. In the same region, phosphorylation of Smad 1/5 and 3 was also reduced. In addition to defects in tendons and ligaments, we found hypoplastic formation or loss of cartilaginous attachment sites in neonatal *Scx* deficient mice. At 4 weeks of age, the hierarchal structure observed in control mice was lost at the patella tendon enthesis of *Scx* deficient mice. Our findings indicate that *Scx* expression in the $Scx^+/Sox9^+$ progenitors is required for establishment of the prospective entheseal region. Taken together with recent reports, I would like to introduce the functional role of Scx in enthesis formation.

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Loss of Tsc2 in Cathepsin k expressing cells results in a high bone mass phenotype: not the usual suspects

Renee T Ormsby[1], Nicola Alesi[2], Molly Zgoda[1], Elizabeth Henske[2], Julia F Charles[1]

1. Orthopedic Research, Brigham and Womens Hospital, Boston, MA, United States

2. Department of Medicine, Brigham and Womens Hospital, Boston, MA, United States

Tuberous sclerosis is a congenital disorder that occurs due to loss of function of the tuberous sclerosis complex, most commonly due to mutation of *TSC2*. Patients with this disorder develop tumours and various pathologies including sclerotic bone lesions. Loss of *Tsc2* in mice similarly results in sclerotic bone lesions. In order to test whether loss of *Tsc2* in osteoclasts was sufficient to cause sclerotic bone lesions, we crossed *Tsc2*$^{fl/fl}$ mice with cathepsin K Cre promoter (CtskCre) mice to specifically target osteoclasts. Micro-CT analysis of the femurs of both male and female *Tsc2*$^{fl/fl}$*;CtskCre* mice showed increased cortical BV/TV, increased periosteal diameter and increased trabecular bone volume 1mm below the growth plate. Unexpectedly, histological analysis using TRAP staining showed no decrease in osteoclast number. In contrast, serum analysis showed increased expression of P1NP, a marker of bone formation, and dynamic histomorphometry revealed increased periosteal and trabecular bone formation rates. It has been shown that osteoblast lineage cells can express cathepsin K under some conditions. Cathepsin K is expressed by osteocytes during lactation and periprosthetic osteolysis, and by a population of periosteal stem cells during development, suggesting that the *Tsc2*$^{fl/fl}$*;CtskCre* bone phenotype could reflect the function of TSC2 in one of these cell types. To determine the cellular location of Cre-mediated deletion, *Tsc2*$^{fl/fl}$*;CtskCre* was crossed to the ROSA26^{mTmG} reporter line. Immunofluorescence using GFP showed green fluorescence which indicated Tsc2 deletion in both osteocytes and periosteal lining cells. Furthermore, bone marrow chimera experiments demonstrated that the cortical phenotype is independent of *Tsc2* genotype in hematopoietic stem cells. The *Tsc2*$^{fl/fl}$*;CtskCre* bone phenotype depends on intact mTORC activity as it is abrogated by deletion of *Raptor*. Taken together, our data suggests a crucial role for TSC2 in regulating the periosteal bone formation function in osteoblast lineage cells via regulation of mTORC, linking energy sensing to bone growth.

Utilization of a microtissue-engineered 3D model of osteoblastic metastases to unravel the effects of anti-androgen therapies in the bone microenvironment of metastatic prostate cancer

Nathalie Bock[1], Thomas Kryza[2], Ali Shokoohmand[1], Joan Rohl[1], Akhilandeshwari Ravichandran[1], Marie-Luise Wille[1], Colleen Nelson[1], Dietmar W Hutmacher[1], Judith A Clements[1]

1. School of Biomedical Sciences, Queensland University of Technology, Woolloongabba, QLD, Australia

2. Cancer Biology, Mater Research Institute - University of Queensland, Brisbane, Qld, Australia

Osteoblastic metastatic lesions are found in over 90% of patients with metastatic castrate resistant prostate cancer (mCRPC). While current drugs in that stage present with poor survival advantages (< 5 months), androgen receptor (AR)-targeted therapies (ATT) continue to be the gold standard for recurrent advanced disease, even when the cancer has established in the bone. Yet, ATT ultimately involve both cancer cell and stroma adaptation through a combination of AR reactivation and paracrine signalling, with most processes currently unknown in the bone microenvironment. To address this issue, adequately modelling the pathology of the disease in the laboratory becomes paramount. In our previous work, using additive biomanufacturing, we have developed a reproducible microtissue-engineered human 3D model of osteoblastic metastases, that comprises primary osteoblasts, osteocytes and cancer cell lines, able to display some functional and molecular features, as observed in clinical androgen-deprived cancer. In the current study, we used the bone metastasis model to determine the quantitative effects of a first-generation (bicalutamide) and a second-generation (enzalutamide) ATT, combining 4D live microscopy, cell morphometry, gene and protein analysis (Figure 1). We showed how anti-androgen treatments increased cancer cell volume and reduced sphericity, correlating with a more adaptive phenotype, and how reduced mineralization increased cancer cell migration. Anti-androgen treatments also affected the proliferation and migration of AR-dependent-, but not AR-independent- cell lines, at both 48h and after 3 weeks of co-culture. Dysregulation of markers under these treatments was significant with AR-dependent LNCaP cell-line, including included RUNX2, OPN and BSP upregulation, correlated with increased osteoblastic activity, while also increasing epithelial-to-mesenchymal (SLUG) and neuroendocrine markers (DDC), linked to a more adaptive phenotype. Ultimately the model and innovative quantitative methodologies unravelled the detrimental effects anti-androgens therapies may have on AR-dependent bone metastases, presenting a powerful platform to study cancer cell behaviour in any therapeutic context.

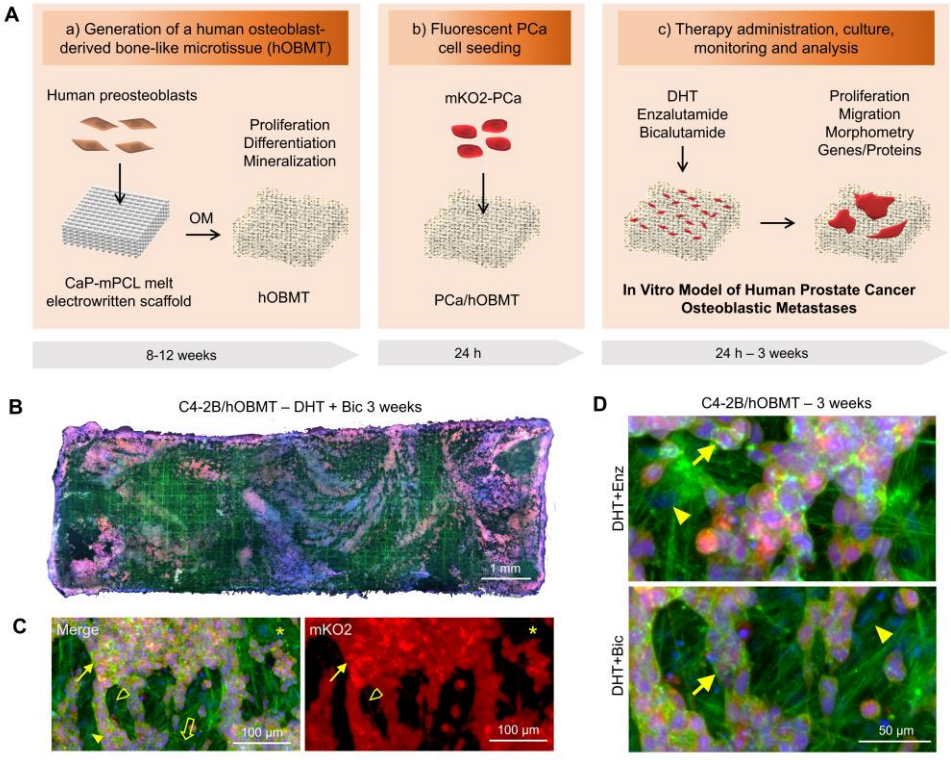

Figure 1. Development of an *in vitro* microtissue-engineered model of human prostate cancer osteoblastic metastases. (**A**) Schematic overview of manufacturing involving; (a) A calcium phosphate coated melt electrowritten polycaprolactone (CaP-mPCL) scaffold seeded with primary human preosteoblasts, followed by 8-12 weeks culture in osteogenic media (OM), leading to a human osteoblast-derived bone-like mineralized microtissue (hOBMT); (b) Co-culture with prostate cancer (PCa) cell lines for 24h, and (c) Therapy administration, culture for 24h to 3 weeks, monitoring and analysis. (**B-D**) Confocal microscopy images of the 3D metastatic microtissues after 3 weeks hOBMT co-culture with C4-2B cells under dihydrotestosterone (DHT, 10 nM) and bicalutamide (Bic, 10 µM) or enzalutamide (Enz, 10 µM) showing cancer cell coverage of hOBMT and formation of micrometastases (maximum projections shown, 50 µm *z*-stacks). Split channels show C4-2B cells (mKO2 in red), cell nuclei (DAPI in blue) and actin filaments (Phalloidin in green). Asterisks show hOBMT, full arrows show cancer cells, open arrows show scaffold fibers, full arrow heads show osteoblasts, open arrow heads show 70 µm-long cancer cell filopodia.

Age-related mesenchymal stromal cell senescence is associated with progression from MGUS to multiple myeloma

Natalya Plakhova[1, 2], Krzysztof Mrozik[1, 2], Vasilios Panagopoulos[1, 2], Stan Gronthos[2, 3], Kate Vandyke[1, 2], Andrew CW Zannettino[1, 2]

1. Myeloma Research Laboratory, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia, Australia

2. Precision Medicine Theme, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, South Australia, Australia

3. Mesenchymal Stem Cell Laboratory, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia, Australia

Senescent mesenchymal stromal cells (sMSCs) accumulate in the bone marrow (BM) with age. sMSCs display an altered secretory phenotype, which includes the key multiple myeloma (MM) growth factor interleukin-6 (IL-6). Notably, the risk of progression of monoclonal gammopathy of undetermined significance (MGUS) to MM increases with advancing age and may be driven by extrinsic microenvironmental changes within the BM microenvironment. We hypothesise that age-related increases in BM-MSC senescence lead to increased proliferation of plasma cells (PC) in MGUS patients, resulting in progression to MM.

We assessed BM-MSCs isolated from BM trephine biopsies from MGUS (n=20) and MM (n=8) patients and healthy controls (n=10). We show that BM-MSCs from MM (age: 66.5 [52-81]), and MGUS (age: 63.4 [37-84]) patients exhibit a senescent phenotype characterised by increased β-galactosidase activity, flattened cell morphology, decreased proliferation, increased gene expression of IL-6 and senescence markers such as *CDKN2A* when compared with healthy BM-MSCs (age: 24.6 [19-32]). The percentage of sMSCs significantly correlates with donor age in MGUS and MM patients. Notably, the risk of progression to MM is significantly elevated in MGUS patients with increased BM-MSC senescence (p=0.0294,HR; 0.18 (95% CI 0.04-0.84) and high IL-6 gene expression. Moreover, co-culture with MM and MGUS (age≥65) BM-MSCs significantly increases the proliferation of KMM1 MM cell line compared with co-culture with BM-MSCs from healthy donors or younger MGUS patients (age<65). Induction of senescence in healthy BM-MSCs via irradiation or replicative exhaustion also increased expression of IL-6 and the proliferation of murine 5TGM1 and human KMM1 MM cell lines in co-culture assays.

Collectively, we showed for the first time that the accumulation of senescent BM-MSCs precedes progression from MGUS to MM and that BM-MSC senescence promotes MM proliferation. Moreover, elevated BM-MSC senescence at MGUS may be associated with more rapid progression to MM, which may be mediated by IL-6.

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Skeletal abnormalities in *Csf1r* knockout rats are rescued by transplant of total bone marrow

Lena Batoon[1], Melanie Caruso[1], Sahar Keshvari[1], Omkar Patkar[1], Katharine Irvine[1, 2], Allison Pettit[1, 2], David Hume[1, 2]

1. Mater Research Institute - The University of Queensland, Translational Research Institute, Woolloongabba, QUEENSLAND, Australia

2. The University of Queensland, Faculty of Medicine, Herston, Queensland, Australia

Macrophage and osteoclast proliferation, differentiation and survival depend on colony-stimulating factor-1 receptor (CSF1R) signaling. Human homozygous *CSF1R* mutations are associated with flattened and diffusely dense vertebral bodies, metaphyseal and epiphyseal osteosclerosis and thin cortices. Studies of the phenotypic consequences of *Csf1r* knockout *(Csf1rko)* in mice were limited by early postnatal lethality. We recently generated *Csf1rko* rats which are viable as adults on an outbred background. Here, we explore *Csf1rko* impacts on postnatal bone development. *Csf1rko* rats are indistinguishable from littermates at birth but exhibit postnatal growth retardation. However, Alcian blue-Alizarin red staining of newborn skeletons already showed delayed mineralisation of small paw bones and digits. At 1 week, osteopetrosis was evident. The muscle fibers formed around bones were reduced in diameter. By 3 weeks, mutant rats had intriguing site-specific skeletal defects: profound delay in ribcage ventral segment mineralisation, vertebral body development, secondary ossification center formation in long bones and digits, and subarticular ossification of small paw bones and carpal-tarsal bones. At 7 weeks, the cranial case of mutants lacked calcification and suture closure was impaired. All these skeletal features were shared with human *CSF1R* mutation. We examined macrophage and osteoclast distribution by immunohistochemical localisation of IBA1 and TRAP, respectively. Abundant macrophages in the paw, vertebral body and hind limbs were detected in 1- and 3-week-old wild-type rats and were almost absent in *Csf1rko*. Osteoclasts were also undetectable. Macrophages were partly restored in 7-9-week-old *Csf1rko* rats, but osteoclasts remained absent. Wild-type bone marrow cell transplantation into unconditioned 3-week-old *Csf1rko* rats reconstituted macrophages and osteoclasts, reversed skeletal abnormalities as early as 7 weeks post-transplant, promoted skeletal and somatic growth, and long-term survival. Our findings have important implications for understanding the consequences of CSF1R mutation for the human skeleton and reveal the therapeutic potential of targeting the CSF1-CSF1R axis in related bone diseases.

Role of Eph and ephrin interactions in mediating cross-communication in the neuro-osteogenic network during development

Nethmi Abeynayake[1, 2], Agnieszka Arthur[1, 2], Stan Gronthos[1, 2]

1. Mesenchymal Stem Cell Laboratory, Adelaide Medical School , The University of Adelaide, Adelaide, South Australia, Australia

2. Precision Medicine , SAHMRI, Adelaide, South Australia, Australia

Background and Aims: While there is evidence of cross-communication between the skeletal and neural systems, knowledge of the underlying molecular mechanisms is limited. The Eph receptors, EphA and EphB, are the largest family of receptor tyrosine kinases that interact with their corresponding ephrinA and ephrinB ligands. Eph/ephrin signalling mediates bone homeostasis and neuronal patterning. We hypothesise Eph/ephrin interactions mediate communication within the neuro-osteogenic network. We assessed the Eph /ephrin expression profile of neural cells and investigated the effect of manipulating these Eph/ephrin interactions on neural differentiation, adhesion and migration.

Methodology: A human neuroblastoma line was cultured under basal and neural-inductive conditions, to determine changes in Eph and ephrin gene expression levels during neural differentiation by qPCR. Cells adhesion and neurite extension was assessed in the presence of bound Eph-Fc fusion proteins over time, following staining with DAPI and Phalloidin. Chick embryo dorsal root ganglia (DRG) were cultured with bound Eph-Fc then stained with anti-Neurofilament Medium Chain (NF-M) to determine total neurite growth and percentage of invading sensory neurites.

Results: Gene expression levels of ephrinA2, ephrinA5, ephrinB1 and ephrinB2 were significantly upregulated in neural differentiated cells, which bind to EphA3, EphA4, EphB2 and EphB4 expressed by bone marrow stromal cells, respectively. During neural differentiation, *NESTIN* and *PERIPHERIN* expression were decreased in response to EphA4-Fc and EphB2-Fc, whereas *NESTIN* and *NF-M* expression were upregulated in response to EphB4-Fc. In addition, *β-III TUBULIN* expression was reduced in response to EphB2-Fc. In the neural differentiated cells, neurite extension was enhanced in response to EphA4-Fc, while EphB4-Fc inhibited neural adhesion. There was a trend of EphA3-Fc inhibiting DRG neurite extension that did not reach statistical significance.

Conclusion: Human neuroblastoma expressed ephrinA2, ephrinA5, ephrinB1 and ephrinB2 with increasing neural differentiation. EphA4-Fc, EphB2-Fc and EphB4-Fc fusion proteins differentially affected neural differentiation, while EphB4-Fc inhibited neural adhesion suggesting Eph/ephrin interactions mediate crosstalk between neural and stromal populations.

Lipid Signaling Mediators Regulate Bone-Muscle Crosstalk During Ageing

Ahmed Al Saedi[1, 2], Zhiying Wang[3], Marco Brotto[3], Gustavo Duque[1, 2]

1. Department of Medicine – Western Health, The University of Melbourne, St Albans, VIC, Australia

2. Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne and Western Health, St Albans, VIC, Australia

3. Bone-Muscle Research Center, College of Nursing and Health Innovation, The University of Texas-Arlington, Arlington, TX 76019, USA

Due to its association with adverse outcomes, the simultaneous concurrence of sarcopenia and osteoporosis, a condition termed osteosarcopenia, has raised attention and concern. Fat infiltration contributes to age-related bone and muscle decline. This effect could be explained by fat-secreted factors, which could be locally secreted in the muscle and bone milieu thus affecting cell-cell interactions, and cell function and survival. However, the specific fat-secreted factors that simultaneously affect those tissues remain unknown. Using new targeted-lipidomic approach via liquid chromatography with tandem mass spectrometry, we comprehensively quantified fat composition (lipid metabolites [LMs]) in gastrocnemius, serum, and bone marrow flushes from tibia and femur obtained from young (6W) and old (24W) C57BL6 mice. Compared to young mice, all tissues in older mice showed significantly higher levels of arachidonic acid (AA) (p=0.042) and AA-derived eicosanoids, PGs2α (p=0.036), PGsα(p=0.021), which are known to affect muscle and bone function. Moreover, Lipoxin B4, another AA product and an enhancer of bone turnover and negative regulator for muscle, showed significantly lower values in older mice compared to young mice in both genders (p= 0.0092). Furthermore, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) autoxidation products (20-HDoHE, 11-HDoHE, 7-HDoHE and 4-HDoHE), an omega-3 fatty acids that negatively regulate bone and muscle health were significantly higher in older mice (p=0.003, p=0.020, p=0.025, p=0.045 respectively). Additionally, γ-aminobutyric acid (GABA) and β-aminoisobutyric acid (BAIBA), which they are muscle-derived osteocyte survival factors, were significantly lower with older mice and higher in females than males (p<0.05). In conclusion, elucidation of those LMs that are present in both ageing muscle and bone could provide valuable evidence on the role of fat infiltration in osteosarcopenia. These results suggest that LMs could play a role in modulating musculoskeletal function during aging, which might relate to sarcopenia and osteoporosis, and could become therapeutic targets in the future.

The loss of ephrinB1 in osteoprogenitors delays endochondral ossification during the fracture repair process

Agnieszka Arthur[1, 2], Sharon Paton[1, 2], Andrew Zannettino[2, 3], Stan Gronthos[1, 2]

1. Mesenchymal Stem Cell Laboratory, Adelaide Medical School, Faculty of Health Science, University of Adelaide, Adelaide, SA, Australia

2. Precision Medicine Theme, South Australian Health and Medical Research Institute, Adelaide, SA, Australia

3. Myeloma Research Laboratory, Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, SA, Australia

Background: The process of bone healing comprises mechanical, cellular, and molecular cues. The contact-dependent, membrane-bound Eph receptors and ephrin ligands are implicated in numerous processes required during skeletal development, homeostasis, and bone healing. However, the function of ephrinB1 in fracture repair is unknown.

Aim: To identify the cellular and molecular involvement of ephrinB1 during fracture repair.

Methods: An internal fixation femoral fracture model in conditional male mice lacking ephrinB1 (*EfnB1)* in the osteo/chondrogenic lineage ($EfnB1_{OB}^{fl/O}$), driven by the *Osterix* (*Osx*) promoter and their *Osx:Cre* controls was utilised. Micro computed tomography (mCT) and histomorphometric analyses were used to analyse bone parameters, while osteogenic cells were isolated from femora 2 weeks post-fracture (PF) to determine mineral forming potential *in vitro*. To evaluate ephrinB1 downstream signalling, human bone marrow stem/ stromal cells (hBMSC) were cultured with soluble EphB2-Fc or control IgG-Fc. The phosphorylation status of downstream signalling target TAZ was determined by immunoprecipitation and Western Blot.

Results: In C57Bl/6 wild-type mice *ephrinB1* gene expression was elevated 1 week (haematoma) and 2-weeks (soft callus formation/ remodelling) PF. A significant decrease in bone volume/ tissue volume within the fracture callus 2 weeks PF was observed in $EfnB1_{OB}^{fl/O}$ mice. Histomorphometric analysis confirmed a significant increase in chondrogenic tissue and decrease in calcein labelled bone in $EfnB1_{OB}^{fl/O}$ compared to *Osx:Cre* mice. Mineral synthesis and osteogenic transcription factor *Osterix* gene expression were significantly reduced in $EfnB1_{OB}^{fl/O}$ cultured BMSC compared to *Osx:Cre* controls. This study also confirmed a molecular mechanism of TAZ de-phosphorylation in response to ephrinB1 activation in hBMSC, which was consistent with ephrinB1 signalling in mouse stromal cells.

Conclusion: The present study identified that ephrinB1 expressed by the chondrogenic and osteogenic lineages is important for correct soft callus formation and remodelling during the fracture repair process.

Antipsychotic medications use and bone mineral density in women from the Geelong osteoporosis study

Behnaz Azimi Manavi[1], Amanda Stuart[1], Julie Pasco[1, 2, 3], Jason Hodge[1, 4], Lana Williams[1, 2]

1. School of Medicine, Deakin University, Geelong, VIC, Australia

2. Barwon Health, Geelong, VIC, Australia

3. Department of Medicine-Western Health, The University of Melbourne, St Albans, VIC, Australia

4. Geelong Centre for Emerging Infectious Diseases, Geelong, VIC, Australia

Background: Previous research has shown patients with schizophrenia to have reduced bone mineral density (BMD) and increased fracture risk. However, less is known regarding the effects of antipsychotic treatment independent of disease state. Thus, we aimed to investigate the association between antipsychotic use and bone mineral density in a population-based sample of women.

Methods: Current antipsychotic users (n=39) and non-users (n=750) aged from 23 to 95 years were drawn from the Geelong Osteoporosis Study (GOS). BMD (g/cm^2) was measured at the spine, hip and total body using dual-energy absorptiometry (DXA). Anthropometry and socio-economic status (SES) were determined, and information on medication use, and lifestyle was obtained via questionnaire. Regression analysis was used to test associations after adjusting for potential covariates.

Results: Antipsychotic users were less active and more likely to use antidepressants and hormone therapy and drink less alcohol; otherwise the groups were similar in regard to age, height, weight, SES, smoking status and use of thyroid and bone active medications. Age was an effect modifier in the relationship between antipsychotic use and BMD. Among women aged <50 years (n=319), age?-adjusted mean BMD for antipsychotic users was 6.0% lower at the spine [1.305 (1.173-1.437) vs. 1.388 (1.283-1.494) g/cm^2, p=0.047] and 6.1% lower at the femoral neck [0.937 (0.876-0.998) vs. 0.998 (0.980- 1.016) g/cm^2, p=0.058] compared to non-users. Associations persisted following further adjustment for alcohol consumption, smoking, SES and medications known to affect bone. There was no relationship detected at the total body and for ages ≥50 years.

Conclusion: These population-based data suggest antipsychotic use is associated with lower BMD in younger but not older women. Further research is required to investigate the underlying mechanisms involved.

Male preptin knockout mice have increased trabecular bone volume with advancing age

Emma J Buckels[1], Maureen Watson[2], Huai L Hsu[1], Dorit Naot[2], Jillian Cornish[2], Christina M Buchanan[1], Brya G Matthews[1], Kate L Lee[1]

1. Department of Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand

2. Department of Medicine, The University of Auckland, Auckland, New Zealand

Preptin is a 34-amino acid peptide derived from the E-peptide of pro-IGF-II. Preptin is co-secreted with insulin from β-cells, can increase glucose-stimulated insulin secretion, and promotes proliferation and differentiation of osteoblasts. Understanding the function and mechanism of action of preptin *in vivo* may enable development of novel osteoporosis therapeutics. We tested the hypothesis that preptin deficiency alters bone metabolism by evaluating a preptin knockout (KO) mouse.

Experimental KO and wild type (WT) mice were generated by heterozygous breeders. RT-qPCR on adult livers (n=4-9) confirmed similar *Igf2* expression between genotypes, whereas KO mice had undetectable preptin expression.

Metabolic phenotypes were evaluated by weekly fasting blood glucose measurements, intraperitoneal insulin tolerance tests (ITT) at 9, 29, and 44-weeks of age, and an oral glucose tolerance test (GTT) at 45-weeks of age (n=12-14/sex/genotype). Bone phenotypes were evaluated by femoral microCT at 6-weeks (n=8-12/sex/genotype), 14-weeks (n=10-12/sex/genotype), and 1-year of age (n=7, males only).

Bodyweights were similar between genotypes at all ages. Blood glucose concentrations returned to baseline quicker following ITT in female KO compared to WT mice at 9-weeks of age only. Female KO had increased blood glucose concentrations 15- and 30-minutes post-glucose during GTT compared to WT mice. There were no metabolic differences in males.

There were no differences between genotypes in trabecular or cortical bone parameters at 6-weeks of age. By 14-weeks of age, trabecular bone had a 21% increase in bone volume fraction (BV/TV), a 17% increase in trabecular number, and an 8% increase in cortical bone area in male KO compared to WT mice. These effects were absent in females. At 1-year of age, BV/TV was increased 47%, and trabecular number was increased 45% in male KO mice.

Male preptin KO mice demonstrated increased bone volume in the absence of overt metabolic dysfunction. Mechanistic evaluation of this phenotype is ongoing.

Intramuscular injection of Botox induces tendon atrophy and senescence of tendon-derived stem cells

Peilin Chen[1], Ming-Hao Zheng[1], Tao Wang[1], Christopher Mitchell[1], Junjie Gao[1], Lianzhi Chen[1], Ziming Chen[1], Jun Yuan[1], Delin Liu[1], Euphemie Landao[1]

1. University of Western Australia, Nedlands, WA, Australia

Background:

Botulinum toxin (Botox) injection is in widespread clinical use for the treatment of muscle spasms and tendinopathy but the mechanism of action is poorly understood.

Hypothesis:

We hypothesised that the reduction of patellar-tendon mechanical-loading following intra-muscular injection of Botox results in tendon atrophy that is at least in part mediated by the induction of senescence of tendon-derived stem cells (TDSC).

Study Design:

Controlled laboratory study

Methods:

A total of 36 mice were randomly divided in 2 groups (18 Botox-injected and 18 vehicle-only control). Mice were injected into to right *vastus lateralis* of quadriceps muscles with Botox to either induce mechanical stress deprivation of the patellar tendon or with normal saline (controls). At 2 weeks post-injection, animals were euthanized prior to harvesting of tissues for either evaluation of tendon morphology or *in vitro* studies. Tendon-derived stem cells were isolated by cell-sorting prior determination of viability, differentiation capacity and markers of senescence, as well as assessing their response to mechanical loading in a bioreactor. Finally, to examine the mechanism of tendon atrophy *in vitro*, key proteins in the PTEN/AKT pathway were evaluated in TDSCs derived from either Botox-injected or vehicle-only control mice.

Results:

A single injection of Botox into the right vastus lateralis muscle of the quadriceps in mice caused loss of muscle contractility and reduction in loading on the patellar tendon. Two weeks after Botox injection, patellar tendons display atrophic features; including reductions in tissue volume and extracellular matrix and collagen fibre crimping and misalignment. The atrophic tendon tissues showed increased degradation of collagen fibres. The colony formation assay revealed that the numbers of TDSCs colony forming units in the Botox injected group were significantly reduced in comparison to vehicle-only controls. Multipotent differentiation capacity of patellar tendon TDSCs has also diminished after Botox injection. To examine if population of TDSC that has suffered from mechanical deprivation is capable of forming tendon tissue, we used an isolated bioreactor system to culture 3D TDSCs constructs. The result showed that TDSCs from the Botox-treated group fail to restore tenogenic differentiation after appropriate mechanical loading. Examination of PTEN/AKT signalling pathway revealed that injection of Botox into quadriceps muscle causes PTEN/AKT mediated cell senescence of TDSCs.

Conclusion:

Intramuscular injection of Botox interferes with tendon homeostasis by inducing tendon atrophy and senescence of TDSCs. Botox injection may have long-term adverse consequences for the treatment of tendinopathy.

Clinical relevance:

Intramuscular Botox injection for tendinopathy and tendon injury could cause adverse effects in human tendons and re-evaluation of its long-term efficacy is warranted.

Preclinical Bacterial Bone Infection Models for Orthopaedic Research and Antimicrobial Efficacy Testing

Aiken Dao[2, 1], Justin Bobyn[2, 1], Emily Vasiljevski[2, 1], David Little[2, 1], Aaron Schindeler[2, 1]

1. Orthopaedic Research & Biotechnology Unit, The Children's Hospital at Westmead, Sydney, NSW, Australia

2. The Discipline of Paediatrics and Child Health, Faculty of Medicine, The University of Sydney, Sydney, NSW, Australia

Introduction: Osteomyelitis remains a major clinical challenge and high burden disease in orthopaedic and trauma patients, particularly when the infection is associated with antimicrobial resistance and biofilm contamination. Multiple strategies are being explored to improve infection prophylaxis and treatment, including novel antibiotics, antimicrobial implant coatings, and bacteriophage therapy. Due to the costs and risks associated with clinical trials, effective preclinical screening is essential.

Aim: To test the efficacy of these new therapies, we aim to develop a reliable and economical preclinical model of bone infection in mice. A relevant model would feature a local long bone defect seeded with a pathogenic microorganism associated with orthopaedic infection.

Methods: Tibial drilled-hole surgeries were conducted in the tibiae of C57BL/6 mice (N=100) with the introduction of *Staphylococcus aureus* (ATCC-12600). Variables included drilled-hole position, route of inoculation, and presence of a metal pin to act as a biofilm surface. In a variant of the surgery, a needle was used to create the initial hole. Animals were monitored for physiological or radiographic evidence of infection without prophylactic antibiotics. At the endpoint, blood, bone swabs, soft-tissue biopsies and pins were taken for bacterial culture. X-ray and micro-CT scans were performed along with histology analysis.

Results: The presence of an implant was found to be essential for reliable infection (80-100% infection rate), suggesting that formation of a local biofilm is critical for infection at low doses of *S. aureus* inoculation. Midshaft drilled-holes led to the frequent fracture of the tibiae, making this an unfavourable model in terms of biomechanical strength. Endpoint analyses showed a lack of sepsis despite the detection of bacteria within the local site. Micro-CT and X-ray imaging were found to be advantageous when used in concert to visualise osteolysis at the infected defect.

Conclusions: These studies indicate the nuance and complexity associated with model development but confirm the mouse can be a useful model organism for orthopaedic infection. Future work will employ bioluminescent bacterial strains that enable detection of infection progress in real-time.

Dietary Behaviors That Place Young Adults at Risk for Future Osteoporosis

Alyse Davies[1], Anna Rangan[1], Margaret Allman-Farinelli[1]

1. Sydney University, University Of Sydney, NSW, Australia

Dietary behaviors during adolescence and emerging adulthood have important consequences for peak bone mass (PBM) attainment. This study aimed to examine dietary factors that are either beneficial or detrimental to bone health and determine the major sources of calcium in the diets of a sample of young adults. A cross-sectional survey was conducted among 189 Australians aged 18–30 years. Three-day dietary intakes were collected using consecutive 24 h recall interviews. Daily totals for energy and nutrients and serves for food groups were computed. The proportion contribution of calcium (mg) from different food groups as well as calcium (mg) per portion and per 100 g were calculated. Females and males failed to meet the recommendations for dairy (91%, 82%), fruit (89%, 94%) and vegetables (74%, 86%). Eighty percent were above the recommended daily intake range for sodium. For calcium, 53% of females and 48% of males had intakes below the estimated average requirement (EAR). Milk products and dishes made the highest mean calcium contribution per portion (mg) mean standard deviation (SD), 204 mg (212) and accounted for 30% of calcium intake in females and 35% in males. As young adulthood is the final chance for dietary manipulation before PBM is achieved, these dietary risk factors should be addressed.

Endoplasmic reticulum stress induced inhibition of mitochondrial transfer and cathepsin K secretion in osteocyte network - a novel mechanism of glucocorticoid induced bone loss

Junjie Gao[1, 2], Jun Yuan[3], Delin Liu[3], Changqing Zhang[2], Ming-Hao Zheng[3]

1. Perron Institute for Neurological and Translational Science , Nedlands, WA, Australia

2. Department of Orthopaedics, Shanghai Sixth People's Hospital, Shanghai Jiaotong University, Shanghai, China

3. The University of Western Australia, Crawley, WA, Australia

Glucocorticoid (GC) associated osteoporosis is common but GC-triggered bone loss remains largely unclear. Accounting for 90% of cell population, osteocytes and their network may play critical role in response to GC administration. Previous we showed that endoplasmic reticulum (ER) regulated activity and transfer of mitochondria within osteocyte dendritic processes is critical for bone hemostasis. Here we showed for the first time that GC inhibits osteocyte hemostasis by suppressing mitochondrial transfer between osteocytes and induced secretion of cathepsin K through the induction of mitophagy of osteocytes. We first showed that dexamethasone (Dex) modified the configuration of ER in osteocytes from a tubular-like form to a stressed condensed sheet-like pattern. The change of configuration lead to the arrest of MitoTracker® Green labelled mitochondrial movement along the dendritic network and compromise mitochondrial transfer within the osteocyte dendritic network. Degradation of Mfn2 by GC induced PINK1 activation is responsible for the inhibition of mitochondrial transfer evidenced by siRNA study in MLO-Y4 cells. We further showed that PINK1-mediated mitophagy has resulted in the induction of cathepsin K in mouse calvaria culture. Knocking down PINK1 gene abolished the GC-triggered cathepsin K production, and this GC activation effects towards cathepsin K was not impeded when inhibiting the canonical autophagy via Atg5 suppression, suggesting that a non-canonical autophagy pathway promotes the production of cathepsin K in osteocytes following GC treatment. In summary, our results indicate the novel finding that GC-induced PINK1 modulates both of the mitochondrial transfer, and the production of cathepsin K in osteocytes, thereby contributing to the GC-induced bone loss.

Comparison between adjusting DXA-derived lean mass for body mass index (BMI) or height^2 Associations with physical performance and clinical outcomes

Ebrahim Bani Hassan[1, 2], Fernan Munandar Putra[1, 2], Sara Vogrin[1, 2], Mahdi Imani[1, 2], Julie A Pasco[2, 4, 3], Mark A Kotowicz[2, 4, 3], Gustavo Duque[1, 2]

1. Australian Institute for Musculoskeletal Science (AIMSS),), The University of Melbourne and Western Health, St. Albans, Victoria , Australia

2. Department of Medicine-Western Health, The University of Melbourne, St. Albans, Victoria , Australia

3. University Hospital Geelong, Barwon Health, Geelong, Victoria , Australia

4. School of Medicine, Deakin University, Geelong , Victoria , Australia

Background: In previous cross-sectional (n=70) and retrospective (n=260) studies of older people (>65 yr) it was shown that correcting dual x-ray absorptiometry (DXA)-derived appendicular lean mass and mid-thigh lean mass for Body Mass Index (BMI) better associates with outcomes such as grip strength (GSt), gait speed (GSp) and timed-up-and-go (TUG) tests. In this study, we investigated whether the same is observed in a larger population of community-dwelling men and women of a wider age range.

Methods: Using whole-body DXA scans at 15 yr. follow up of the Geelong Osteoporosis Study, a longitudinal study of men and women (n=1322), lean mass in five regions of interest (ROIs) was calculated: 2.6 cm mid-thigh, 13 cm mid-thigh, whole thigh, whole calf and forearm. The lean masses in these ROIs as well as the appendicular lean mass (ALM) were corrected for BMI, (as recommended by the American consensus [FNIH]) and height squared (h^2, recommended by the European consensus [EWGSOP2]). The correlation among these variables was evaluated using Pearson's correlation coefficient and their association with available clinical outcomes (TUG, 1-year retrospective falls history and 5-year retrospective fracture history) was compared using linear and logistic regression.

Results: After adjusting for age and sex, muscle indices of all ROIs adjusted for BMI showed significant associations with TUG (p<0.01) and falls (p<0.027; except whole-calf and whole-forearm muscles [p=0.067 and 0.112]). None of the indices corrected for height squared was associated with TUG or falls. Fractures were only associated with ALM/BMI (p=0.016).

Conclusions: Adjusting DXA-derived lean mass for BMI better associates with physical performance and clinical outcomes than adjustment for h^2. Considering the adoption of the European definition of sarcopenia by Australia, future Australian consensuses should consider the above findings. This may improve sarcopenia as well as osteosarcopenia diagnostic algorithms.

Histopathology/immunopathology in high-prevalence bisphosphonate-related osteonecrosis of the jaw around implants in rat maxillae

Maaya Inoue[1], Chihaya Matsumoto[2], Kazunori Nakajima[1], Hiroki Hayano[1], Ryohei Kozutsumi[1], Shinichiro Kuroshima[1], Takashi Sawase[1]

1. Department of Applied Prosthodontics, Graduate School of Biomedical Sciences, Nagasaki University

2. Department of Prosthodontics, School of Dentistry, Ohu University

Objective: Bisphosphonate-related osteonecrosis of the jaw (BRONJ) around dental implants, which is rare but severe adverse effect of bisphosphonates, has been increased in clinical situations. However, histopathology and immunopathology of BRONJ around implants are unclear due to the lack of animal models of BRONJ around implants. The aim of this study was to create the animal model of BRONJ-like lesions around implants (Implant-BRONJ) and to investigate histopathology and immunopathology of Implant-BRONJ.

Materials and Methods: Eight-week-old female, wistar rats were used. Grade IV titanium implants with 2.0 mm in diameter and 3.5 mm in length were used. Tooth extraction of right maxillary first molars were performed. Drug administration was carried out at 4 weeks after tooth extraction. Alendronate (ALN) monotherapy, dexamethasone (DEX) monotherapy, ALN/DEX combination therapy, and saline injection (VC) were performed. Implants were placed in the healed extraction sites at 16 weeks post-extraction. All rats were euthanized at 2 weeks after implant placement. Gross healing, structural analysis, histopathological and immunopathological analyses of soft and hard tissue around implants were quantitatively evaluated.

Results: Impaired wounds with exposed bone around implants were observed in all ALN/DEX-treated rats. ALN/DEX significantly increased necrotic bone and empty lacunae, and decreased living bone and osteocyte density when compared with VC. Moreover, ALN/DEX significantly decreased collagen production with the infiltration of polymorphonuclear cells when compared with VC. Thus, compromised soft and osseous healing around implants were defined as Implant-BRONJ-like lesions. ALN/DEX significantly increased macrophages in the connective tissue around implants with suppressed angiogenesis when compared with VC. Interestingly, the ratio of M1/M2 ratio in ALN/DEX was significantly larger than that in VC.

Conclusions: An available animal model of implant-BRONJ-like lesions were created in this study. Suppressed angiogenesis and accumulation of M1 macrophages in the connective tissue around implants may contribute to development of Implant-BRONJ.

Surface modification of dental implants improves bone quantity and quality in rabbit tibiae

Riho Kanai[1], Shinichiro Kuroshima[1], Yusuke Uto[1], Yusuke Uchida[1], Masayoshi Suzue[1], Takashi Sawase[1]

1. Department of Applied Prosthodontics, Graduate School of Biomedical Sciences, Nagasaki University, Japan

Objective: Morphology of implant surface plays important roles in osseointegration of dental implants. Recent studies have reported that hierarchical structured surface showed favorable effects on bone quantity around implant. However, the effects on bone quality remains unclear. The aim of this study was to investigate the effects of implant surface modification with oxalic acid on bone quantity and quality around dental implants in rabbit tibiae.

Materials and Methods: Grit-blasted CP grade Ⅳ titanium implants treated with HCl/H_2SO_4 and oxalic acid were used as test implants. The implants without oxalic acid treatment was used as a control. Two sets of each implant were placed in the tibial proximal metaphysis of 14 female-Japanese-white rabbits. Euthanasia was performed at 4 and 8 weeks after implant placement (n = 14/group). Villanueva Goldner staining and picrosirius red staining were carried out for histomorphometric analyses to evaluate bone quality and quantity around implant.

Results: No infection was noted in all placement sites. Bone-to-implant contact increased in test group at 4 and 8 weeks post-implantation, whereas bone area fraction and total collagen production were not changed between groups. Bone maturity around test implants was significantly larger than that around control at 8-weeks after implant placement. Increased type I collagen and decreased type III collagen production was significantly observed 8-weeks post-implantation. Moreover, positive effects of morphological changes with oxalic acid treatment on bone area fraction, mature bone, total collagen and type I collagen, and negative effects of it on immature bone and type III collagen was significantly noted in the later 4 weeks post-implantation.

Conclusions: Our findings reveal that the hierarchical structure on implant surface treated with oxalic acid increases bone formation from the early stages and improves bone quality from the late stages, which may contribute to the long-term success of dental implant treatment in clinical situations.

Gene expression profiling and histopathology/immunopathology in high-prevalence bisphosphonate-related osteonecrosis of the jaw in mice

Shinichiro Kuroshima[1], Muneteru Sasaki[1], Kazunori Nakajima[1], Hiroki Hayano[1], Maaya Inoue[1], Rhohei Kozutsumi[1], Takashi Sawase[1]

1. Nagasaki University, Nagasaki, NAGASAKI, Japan

Publish consent withheld

Spatial mapping of *N*-glycans on human osteoarthritis cartilage-bone tissue using mass spectrometry imaging

Yea Rin Olivia Lee[1, 2, 3], Matthew Briggs[2], Julia Kuliwaba[3], Peter Hoffmann[2], Dzenita Muratovic[3], Paul Anderson[1]

1. Clinical and Health Sciences, Health and Biomedical Innovation, University of South Australia, Adelaide, South Australia, Australia

2. Future Industries Institute, University of South Australia, Mawson Lakes, South Australia, Australia

3. Discipline of Orthopaedics and Trauma, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia, Australia

Objective:

The alterations of *N*-glycans on proteins contribute to the pathophysiology and progression of various diseases. However, the biomolecular distribution of *N*-glycans on osteoarthritis cartilage-bone tissue is poorly understood. Thus, the aim of this study was to spatially compare *N*-glycans from formalin-fixed paraffin-embedded (FFPE) cartilage-bone tissue of knee osteoarthritis (KOA) patients and cadaveric controls (CTL).

Methods:

Human FFPE cartilage-bone tissue from end-stage KOA patients (2-Female; aged 58 and 79 years) and CTL individuals (2-Female; 44 and 54 years) was analysed by matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI). Based on the theoretical masses, *N*-glycan peaks were manually selected, and ion intensity maps were generated using FlexImaging and SCiLS Lab software. Putative *N*-glycan structures were annotated using the following tools: GlycoMod, which calculates the theoretical monosaccharide composition, and Glycoworkbench to create individual *N*-glycan structures.

Results:

MALDI-MSI revealed differential *N*-glycan profiles between KOA patients and CTL individuals within the cartilage region only. Overall, 13 *N*-glycans were identified in KOA cartilage compared to 9 *N*-glycans in CTL cartilage, with approximately a 3-fold increase in the signal intensity (Figure 1-A). Interestingly, ion intensity maps of KOA cartilage-specific hybrid/complex-type *N*-glycans, *m/z* 1501.7 ±0.5 Da, *m/z* 1647.2 ±0.5 Da, and *m/z* 1663.4 ±0.5 Da, showed higher intensity localisation to the superficial fibrillated area of degraded cartilage (OARSI grade 2-2.5) with underlying bone sclerosis, compared to the adjacent region with less damaged cartilage tissue (OARSI grade 0-1), associated with non-sclerotic bone (Figure 1-B).

Conclusion:

Our preliminary results demonstrate the novel application of MALDI-MSI to identify and localise KOA cartilage-specific *N*-glycans. The alterations of these hybrid/complex-type *N*-glycans could evolve into a potential cartilage degradation marker and may play an important role in the development of underlying bone sclerosis. Future work will identify which specific proteins the *N*-glycans are attached to using liquid chromatography/tandem mass spectrometry (LC-MS/MS).

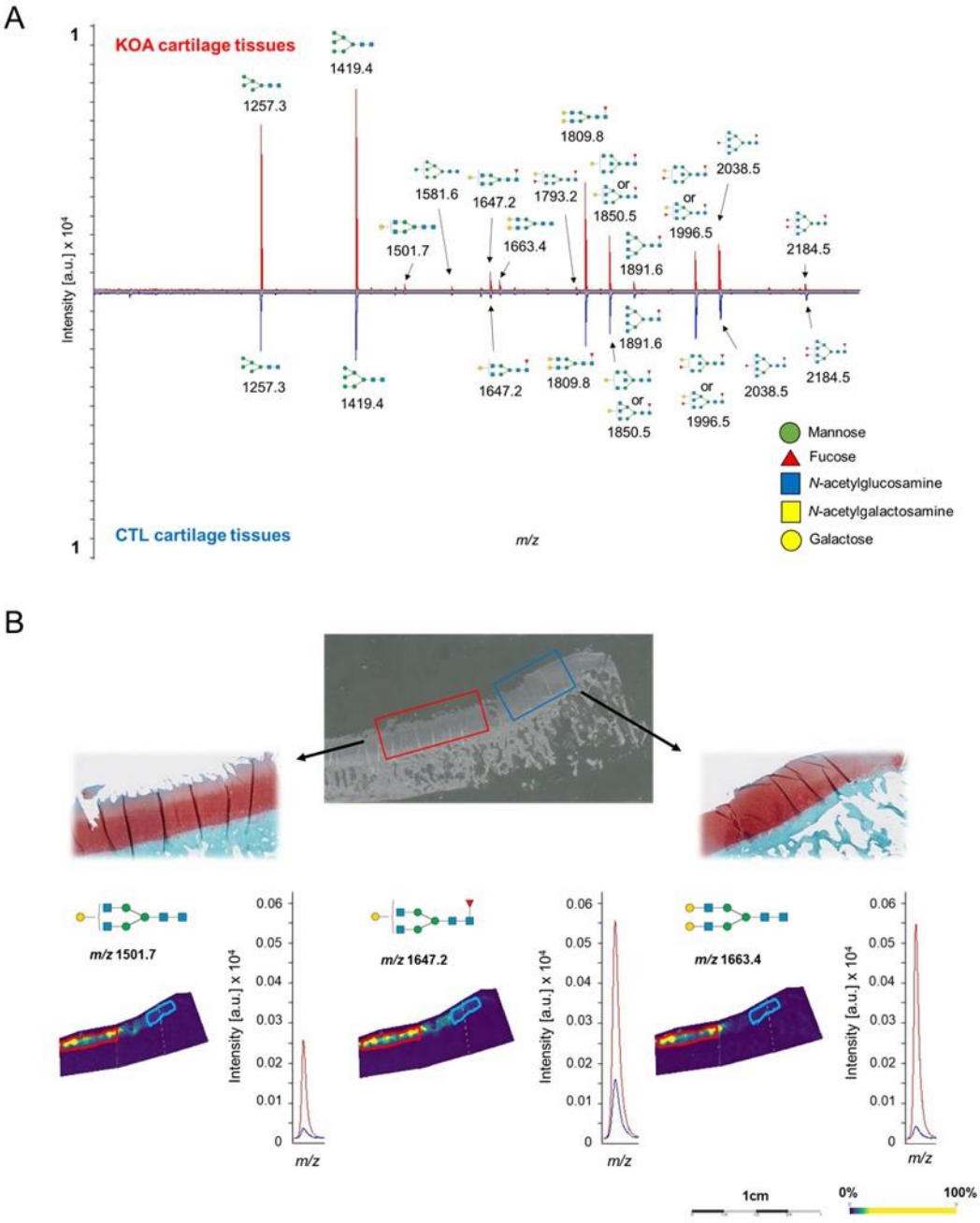

Figure 1: (A) Overall comparison of *N*-glycans identified on FFPE cartilage-bone tissue sections between KOA patients (top) and CTL individuals (bottom). Raw data were normalised to total ion count (TIC) using SCiLS Lab software and the region of interest was manually selected only in the cartilage for analysis. (B) Higher ion intensity maps of KOA cartilage-specific hybrid/complex-type *N*-glycans; *m/z* 1501.7 ±0.5 Da, *m/z* 1647.2 ±0.5 Da, and *m/z* 1663.4 ±0.5 Da were localised in the superficial fibrillated area of degraded cartilage tissue region (red), compared to the adjacent region with less damaged cartilage tissue (blue).

Influence of stem cells in an *in vitro* model of an osteoarthritic joint

Jiao Jiao Li[1, 2], Christopher Little[2]

1. University of Technology Sydney, Ultimo, NSW, Australia

2. Kolling Institute, St Leonards, NSW, Australia

Background: Osteoarthritis is a leading cause of chronic pain and disability, for which there is no cure. Mesenchymal stem cells (MSCs) have recently brought new hope for treating osteoarthritis due to their ability to send anti-inflammatory and trophic signals to surrounding tissues. Interestingly, the few available clinical trials utilising MSCs to treat knee osteoarthritis have not demonstrated consistent benefits.

Aim: This study aims to unravel the mechanisms behind the variable efficacy of stem cell injections for osteoarthritis using an *in vitro* model of a human osteoarthritic joint.

Methods: MSCs were co-cultured with human synovial fibroblasts (HSFs) isolated from osteoarthritic joint tissues, for up to 21 days in growth, osteogenic and chondrogenic media (simulating the relevant conditions in a joint environment). Cell interactions were assessed using RT-PCR (n=4) and histology (n=2).

Results: MSCs co-cultured with osteoarthritic HSFs showed increased inflammation (MMP2, ADAMTS5 upregulation) and impaired ability to form new bone (reduced BSP, SPP1 expression and calcium deposition) and cartilage (reduced COL2A1, ACAN expression and proteoglycan deposition) at 21 days, suggesting that the osteoarthritic joint is a highly inhibitory environment that negatively influences MSCs and reduces their therapeutic effects. Furthermore, short-term (3 days) exposure of the osteoarthritic HSFs to MSCs ('pre-conditioning') was insufficient for sustained modifications to their diseased phenotype. The osteoarthritic HSFs, whether previously exposed to MSCs or not, had similar expression profiles of inflammatory markers, and also had similar negative effects on MSCs, including inflammatory marker upregulation e.g. IL-8, ADAMTS4 and impaired chondrogenesis.

Conclusion: Diseased cells in an osteoarthritic joint create an inflammatory environment that impairs healing. Although MSCs have anti-inflammatory and trophic functions, they may develop a diseased phenotype similar to osteoarthritic cells following injection and show reduced therapeutic effects. Future regenerative therapies for osteoarthritis may have greater success by focusing on the biological derivatives of stem cells.

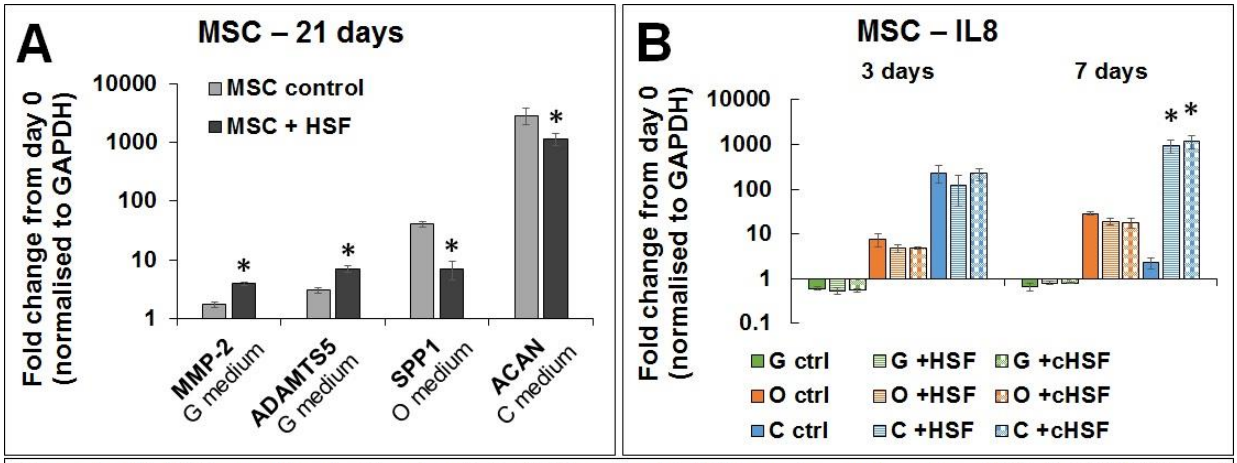

Figure 1: (A) MSCs co-cultured with osteoarthritic HSFs upregulated inflammatory markers (e.g. MMP-2, ADAMTS5) and downregulated osteogenic (e.g. SPP1) and chondrogenic (e.g. ACAN) markers (*P < 0.01). (B) HSFs, pre-conditioned or not, had similar effects on MSCs (e.g. IL-8; *P < 0.001). G=growth medium; O=osteogenic medium; C=chondrogenic medium.

Undercarboxylated osteocalcin and ibandronate improve hindlimb immobilisation-induced atrophy and muscle insulin resistance in a muscle-specific manner in mice

Xuzhu Lin[1], Cassandra Smith[1], Navabeh Zare-Kookandeh[1], Alan Hayes[1], Tara Brennan-Speranza[2], Itamar Levinger[1]

1. Victoria University, Footscray

2. The University of Sydney, Camperdown

Background: Muscle disuse leads to muscle atrophy and insulin resistance. Recent reports suggest that a bone-produced hormone undercarboxylated osteocalcin (ucOC), and bisphosphonates - a commonly used drug for osteoporosis, may improve muscle mass and insulin sensitivity. We tested whether ucOC and/or bisphosphonate (ibandronate) treatments prevents muscle wasting and insulin resistance during disuse conditions in mice.

Methods: Male C57BL/6 mice were subjected to single-leg immobilisation, or provided with free movement, for 14 days. During the immobilisation, mice were injected with placebo, ucOC, ibandronate, or ucOC combined with ibandronate. Insulin tolerance tests and oral glucose tolerance tests were performed on Day 10 and 12, respectively. After euthanasia, extensor digitorum longus (EDL) and soleus muscles were isolated and weighed, then immediately used in glucose uptake analysis. Tibialis anterior (TA), gastrocnemius, and quadriceps muscles were also isolated and weighed, then snap frozen for further analyses including Western Blot.

Results: ucOC exerted an overall anti-atrophic effect only on immobilised quadriceps muscle (ANOVA $p < 0.001$). Ibandronate had overall anti-wasting effects specifically on soleus muscle (ANOVA $p = 0.0062$) and TA muscle (ANOVA $p = 0.049$). The administration of ucOC combined with ibandronate exhibited better therapeutic effect than single administrations, improving soleus and quadriceps muscle mass by 31.7% and 19.9%. Furthermore, only the combination administration enhanced Akt phosphorylation in quadriceps muscle.

Hindlimb immobilisation did not affect whole-body glucose tolerance or insulin sensitivity, albeit reducing insulin response in soleus muscle. UcOC, but not ibandronate, had overall improving effects on glucose tolerance (ANOVA $p = 0.014$) and insulin sensitivity in immobilised soleus muscle (ANOVA $p = 0.020$).

Conclusion: ucOC and ibandronate reduce hindlimb immobilisation-induced muscle wasting and muscle insulin resistance in a muscle-specific manner, with combined administration demonstrating better therapeutic potential. Therefore, the effects of such administration on human muscle cells or tissue need to be further investigated.

Stress distribution in the third metacarpal condyle (MC3) of a racehorse with focal subchondral bone lysis

Fatemeh Malekipour[1], Chris R. Whitton[2], Peter VS Lee[1]

1. Department of Biomedical Engineering, The University of Melbourne, Parkville, VIC, Australia

2. U-Vet Equine Centre, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences,, The University of Melbourne, werribee, VIC, Australia

Microdamage accumulation and adaptation of subchondral bone (SB) subjected to intensive cyclic loading are associated with catastrophic bone failure in athletic horses. At the tissue-level, they lead to a spatial variation in bone mineral density (BMD), affecting the mechanical response of the bone. We used CT-based finite element (FE) modelling to investigate the effect of BMD variation at the joint-level. CT images were taken from the MC3 joint of a standing racehorse. Sesamoid bones were rotated in the sagittal plane to simulate the mid-stance position during galloping where the maximum compression occurs (Fig.A). A single slice through the joint was cropped to develop FE models. The metacarpal condyle, sesamoid bones and cartilage were segmented based on their grey scale. The area between sesamoids was segmented as the intersesamoidian cartilage. Mesh volume was exported to ABAQUS. A BMD-based elastic modulus (E) was assigned to bone to estimate a gradient of E in the proximal SB based on our previous studies on explants from distopalmar condyles (Fig.C). 15 MPa compression was applied to the intersesamoidian cartilage. Stresses were compared to those of the same model with a homogenous E. The homogenous model exhibited peak stresses and strains at the parasagittal grooves with the orientation mostly parallel to the joint surface (Fig.D). The heterogenous model also predicted peak stresses and strains at the joint surface (Fig.E). However, within focal lytic areas stress was lower, with high surrounding stresses where focal sclerosis was observed. High stresses correspond to areas of sclerosis consistent with modelling in response to the loading environment, however this was not the case for the strain measurements suggesting that the relationship between CT data and material properties need further investigation. Future FE models of horses with various CT-identified lesions and longitudinal scans would better explain the MC3 stress fractures.

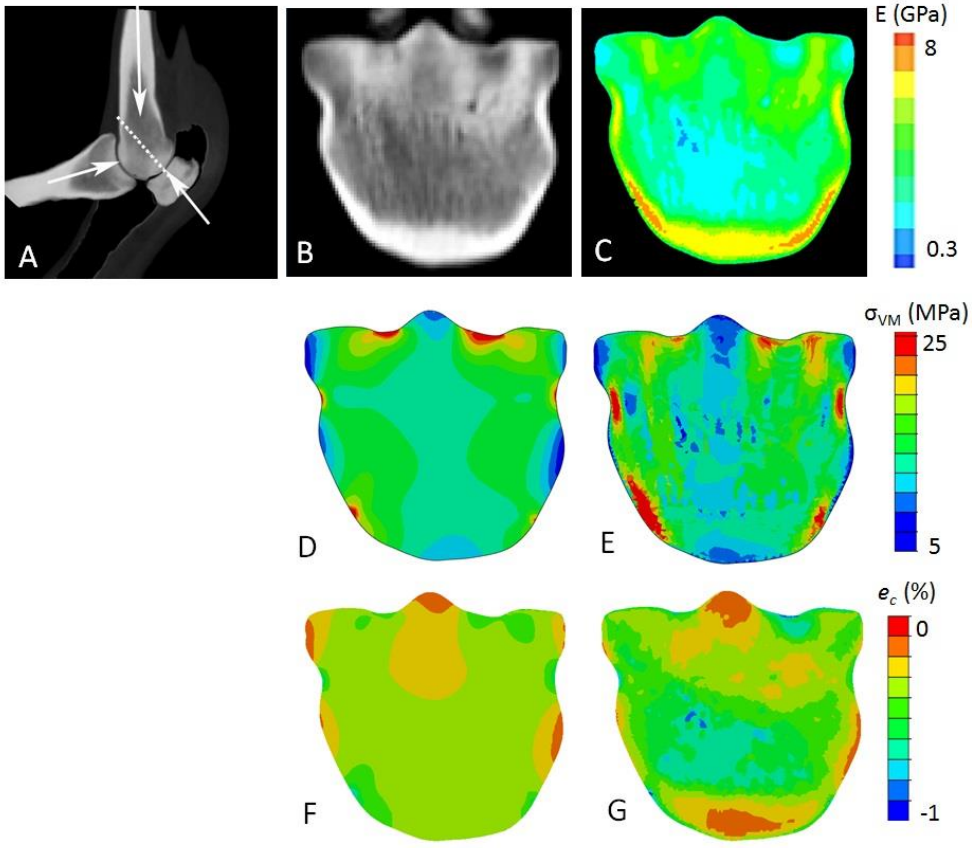

Fig.: (A) CT image of the fetlock joint at midstance, (B) the cross-section which was used for FE model development, (C) the density-based elastic modulus distribution, FE-predicted von Mises stress (σ_{VM}) and compressive strain (e_c) in homogenous (D,F) and heterogenous (E,G) models.

The (un)expected influence of pesticides exposure in bone cancer development and transgenerational epigenetic effects in bone metabolism

Sílvia Moreira[1], Sónia Oliveira[2, 3], Vicente Seco-Rovira[4], Pedro Fontes Oliveira[5], Maria de Lourdes Pereira[1, 2]

1. Department of Medical Sciences, University of Aveiro, Aveiro, Portugal

2. CICECO-Aveiro Institute of Materials, University of Aveiro, Aveiro, Portugal

3. HMRI, Hunter Medical Research Institute & HCRA, Hunter Cancer Research Association, New Lambton, New South Wales, Australia

4. Department of Cell Biology and Histology, Ageing Institute, IMIB-Arrixaca, University of Murcia, Murcia, Spain

5. QOPNA & LAQV, Department of Chemistry, University of Aveiro, Aveiro, Portugal

In the past few decades an increasing number of cases of diseases related to endocrine disrupting chemicals (EDCs) have been reported. More than 350 synthetic molecules are present in the environment, many of them found in pesticides formulations. Carcinogenesis is a slow process, which can start very early (even in uterus) under the action of initiating agents as a result of a mutation or epigenetic modification, expressing itself later in life as the effect of promoting agents, such as estrogens in the case of women and androgens in men.
Considering epidemiological and agricultural health studies associated with exposure to pesticides, different types of neoplasms have been reported, including bone, brain carcinomas, among others, as well as non-Hodgkin lymphomas. In the specific case of bone sarcomas, an interesting collaborative European study found that workers that use pesticides have a greatest risk of developing it. Also, there is evidence of increased risk of childhood osteosarcoma associated with the father working in farming, horticulture and animal husbandry. Moreover, exposure to tetradifon, an organochlorine pesticide, was found to impact bone metabolism. Nowadays, chronic exposure to low-doses of pesticides, through diet, is considered one of the main risk factors of suffering cancer. Here, we aim to investigate the harmful associations between EDCs and bone-related neoplasms. A review search on several databases was undertaken using keywords that we considered relevant for this theme. The literature was critically screened and several evidences and mechanisms underlying the impact of EDCs in bone metabolism and/or bone cancer were found. Thus, despite the fact bone sarcomas are rare, which difficult their study, and are mainly a childhood cancer, investigations point exposure to EDCs, namely pesticides, as the cause for its development. Parental or perinatal exposure to these chemicals can disturb the normal bone metabolism and, ultimately conduct to bone cancer.

Elevated Levels of active TGFβ1 in the Subchondral Bone Associate with the Severity of Human Osteoarthritis

Dzenita Muratovic[1], David M Findlay[1], Halimatul Hajimasri[1], Catherine J.M Stapledon[1], Yea-Rin Lee[1], Julia S Kuliwaba[1]

1. Centre for Orthopaedic & Trauma Research, The University of Adelaide, Adelaide, South Australia, Australia

Introduction

Over-activity of transforming growth factor beta (TGFβ1) in subchondral bone has a direct causal role in rodent knee osteoarthritis (OA) which can be blocked by TGFβ neutralisation. The aim of this study was to investigate whether the level of active TGFβ protein in the subchondral bone associates with the structural, cellular and molecular parameters that are characteristic of human knee OA.

Methods

Tibial plateaus were collected from 35 knee OA arthroplasty patients (15 men, aged 66±9 years; 20 women, aged 70±8 years). Subchondral trabecular bone was sampled from regions below macroscopically present cartilage (CA+) and regions with denuded cartilage (CA-). Bone samples were processed for ELISA measurement of active and total TGFβ1 protein concentration and gene-specific mRNA expression by RT-PCR, synchrotron micro-CT imaging to obtain bone quality parameters (Figure 1), and histology to determine OARSI grade, osteocyte density and TRAP+ cell density.

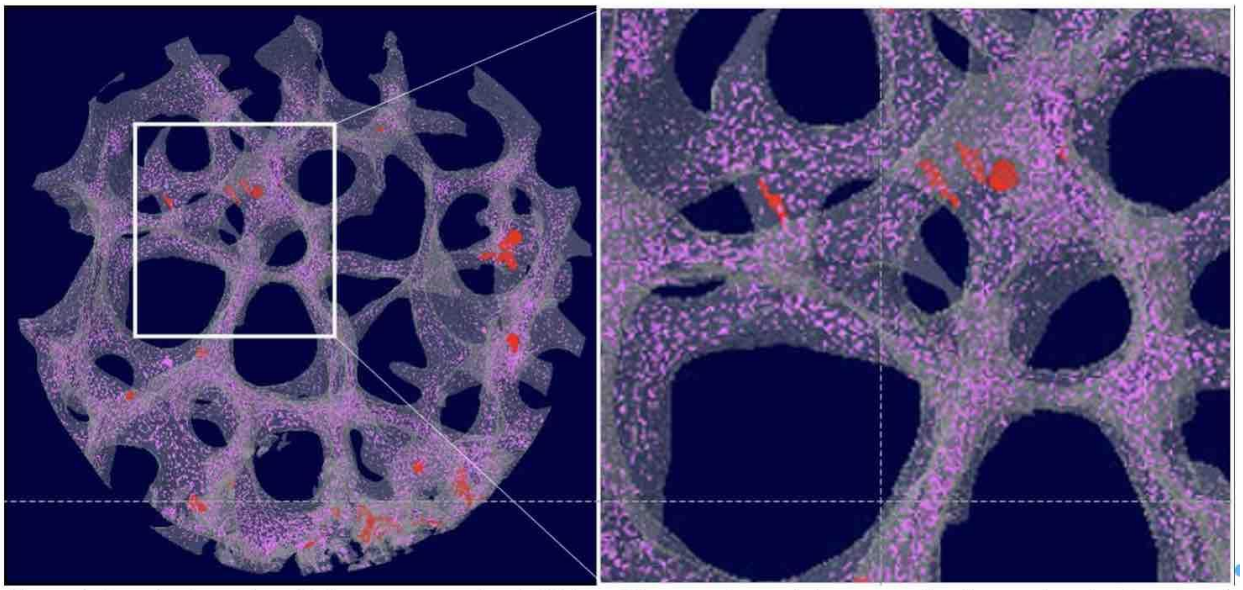

Figure 1. Synchrotron micro-CT images reconstructed into a 3D computer model, representing bone microstructure (grey), osteocyte lacunar density (pink) and vascular canal density (red) of trabecular bone.¶

Results

Bone samples collected below CA- regions (mean OARSI=6) had increased concentration of active TGFβ1 protein and RANKL/OPG mRNA ratio compared to bone samples from CA+ regions (mean OARSI=3.3). Subchondral bone from CA- regions was characterised by sclerotic microarchitecture, increased bone mineral density, increased osteocyte and TRAP+ cell density, larger and greater numbers of osteocyte lacunae that were less spherical shape, and increased bone marrow and bone matrix vascular density. Further, OARSI grade positively associated with active/total TGFβ1 ratio, total TRAP+ cell density, osteocyte density, lacunar volume and shape, and bone marrow and bone matrix vascular density.

Discussion

Together, these findings suggest regional cellular and molecular mechanisms involved in bone remodelling in human knee OA. Further, increased concentration of active TGFβ in the subchondral bone closely associates with impaired overall bone quality and the severity of human OA. Importantly these findings may open up new options for therapeutic approaches for human OA, through the inhibition of TGFβ1.

Generating a pipeline for screening genetic variants associated with bone fragility disorders

Alexandra O'Donohue[1, 2], Genevieve Dammery[1, 3], Craig F Munns[4, 2], Andrew Biggin[4, 2], Aaron Schindeler[1, 2]

1. Bioengineering & Molecular Medicine Laboratory, The Children's Hospital at Westmead, Sydney, New South Wales, Australia

2. Discipline of Child & Adolescent Health, The University of Sydney, Sydney, New South Wales, Australia

3. Applied Medical Science, The University of Sydney, Sydney, New South Wales, Australia

4. Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, Sydney, New South Wales, Australia

Background: The expansion of genetic screening is increasingly identifying new variants of unknown significance (VUSs), and there is currently a major gap in terms of functional genomics. Osteogenesis imperfecta (OI) classically features collagen mutations, however the list of causative genes has expanded over the past two decades. Our team is developing streamlined methods for CRISPR-based gene editing to reproduce OI patient VUSs in cell lines, and then perform functional assays to evaluate their pathogenicity.

Aims: Prior to testing patient VUSs, it is critical to evaluate the editing efficacy of our system and confirm the phenotypic changes in functional assays. Thus our aims are (1) To create a bank of human osteoblast lines with knockout, pathogenic and benign variants; (2) To functionally assess differentiated gene-edited cells.

Methods: Patient VUSs were screened using the PolyPhen2 database to identify likely pathogenic mutations and create a priority list. CRISPR guides were generated using crispr.mit.edu and ligated into Cas9 plasmids. Guides were screened using the T7E1 assay in HEK293 and ASC52telo cells to confirm editing efficiency. Subsequently, individual cells were picked for clonal expansion. Differentiation of ASC52telos was assessed after 14 days using alkaline phosphatase and alizarin red S staining.

Results: Assessment of VUSs from a cohort of 120 bone fragility disorder patients identified *COL1A1*, *LRP5, BMP1,* and *FKBP10* as high priority genes. CRISPR guides were screened in a 2-step process, initially in HEK293 and again in ASC52telos to confirm mutational efficiency. Following validation, clonal populations were generated and are being expanded. Initial assays have demonstrated that differentiated ASC52telo cells have a high level of alkaline phosphatase activity and matrix mineralisation, ideal for a functional pipeline.

Discussion: This approach is currently being expanded to trial additional functional assays, with the goal to streamline the process for use in a NATA-accredited hospital laboratory within 2 years.

LIF/OSM/CNTF Activate Distinct Pathways in Breast Cancer

Tolu Omokehinde[1, 2], Alec Jotte[2, 3], Rachelle W Johnson[2, 4]

1. Graduate Program in Cancer Biology, Vanderbilt University, Nashville, TN, United States

2. Vanderbilt Center for Bone Biology, Vanderbilt University Medical Center, Nashville, TN, United States

3. Department of Biochemistry, Vanderbilt University, Nashville, TN, United States

4. Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, TN, United States

Bone-disseminated breast cancer cells oftentimes home to the osteogenic niche, where they are exposed to glycoprotein130 (GP130) cytokines secreted by osteoblast-lineage cells. Leukemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotropic factor (CNTF) are GP130 cytokines that can all signal through LIF receptor (LIFR). Our lab previously showed that breast cancer cell expression of LIFR and JAK/STAT signaling promotes tumor dormancy in bone. We hypothesized that LIF, OSM, and CNTF induce pro-dormancy signaling pathways and promote tumor suppression. Treatment with recombinant LIF, OSM or CNTF (50ng/ml) robustly stimulated pSTAT3 (up to 34-fold, $p<0.01$-0.0001), LIF and OSM induced pERK (up to 4-fold, $p<0.01$-0.001), and only OSM induced pAKT (up to 9-fold, $p<0.0001$) in MCF7 breast cancer cells by Western blot. LIF and CNTF induced no signaling in MDA-MB-231 breast cancer cells, which have a non-functional LIFR, but OSM robustly stimulated pSTAT3 and pAKT (up to 26-fold, $p<0.05$-0.0001) in MDA-MB-231b cells, suggesting OSM may signal through OSM receptor (OSMR) on breast cancer cells. Reverse phase protein array (RPPA) analysis of MCF7 cells treated with recombinant LIF, OSM, and CNTF revealed several previously unknown signaling pathways and effectors to be activated, including ATM, CREB, HSP27, and N-RAS. Overexpression of OSM in MCF7 cells reduced the expression of 4/18 the pro-dormancy genes *BMP7*, *FOXA1*, *IGFBP5* and *TGFB2* (by 41-97.5% $p<0.05$-0.0001) *in vitro*, while treatment with recombinant cytokines or CNTF overexpression resulted in no significant changes. When orthotopically inoculated into mice (n=10/group), neither OSM nor CNTF overexpressing MCF7 cells altered the progression of tumor formation, tumor volume or weight *in vivo.* Together, these data suggest that the GP130 cytokines robustly stimulate multiple signaling pathways with tumor-suppressive and tumor-promoting activity, but further studies are needed to determine their effect on tumor progression and dissemination to bone.

Localised alterations in bone geometry and cortical porosity predispose fracture site in a mouse model of Paget's disease of bone

Alisha C Sharma[1], Jacob J Trend[1], Kaz Wanelik[2], Stephen Brown[3], Stuart H Ralston[4], Philipp Schneider[1], Bram G Sengers[1], Claire E Clarkin[1]

1. University of Southampton, Southampton, Hampshire, United Kingdom

2. Diamond Light Source, Didcot, United Kingdom

3. Institute of Genetic and molecular Medicine, Edinburgh, Scotland

4. University of Edinburgh, Edinburgh, United Kingdom

Femoral fractures are an impediment in Paget's Disease of Bone (PDB), with clinical studies demonstrating limitations of bone mineral density measurements for fracture prediction. Recent attention has focused on the impact of bone geometry and microstructure on bone strength. Our aim was to examine whether alterations in longitudinal cortical microstructure comprising of osteocyte lacunae (La) and vascular canals (Ca) underlie fracture risk in PDB. A murine model exhibiting a proline to leucine mutation at codon 394 of Sequestostome-1 (P394L), equivalent to the P392L mutation in humans was used. Synchrotron X-ray computed tomography (CT) focused on microstructural components of the femoral cortex in wildtype (WT) and homozygotes ($P394L^{+/+}$), at multiple sites (Fig. 1). Bone geometry measurements and four-point bending tests were also undertaken. Differences between WT and $P394L^{+/+}$ bone microstructure and shape were region-specific. At 30% femoral length $P394L^{+/+}$ exhibited a larger cortical area fraction (WT 0.776% ± 0.132 and $P394L^{+/+}$ 1.167% ± 0.047; $p<0.05$) and increased Lc volume density (WT 0.637% ± 0.100 and $P349^{+/+}$ 0.935% ± 0.036; $p<0.05$) versus WT. $P394L^{+/+}$ Lc had reduced circumference at 60-70% (WT 16.160µm ± 0.437 and $P394L^{+/+}$ 14.695µm ± 0.377 $p<0.05$) versus WT. At 80% there was increased maximum Ca. thickness (WT 20.359µm ± 0.479 and $P349L^{+/+}$ 25.145 µm ± 1.771; $p<0.05$) associated with increased cortical thickness (WT 0.4625mm + 0.06 and $P394L^{+/+}$ 0.3195mm + 0.4; $p<0.001$) and reduced polar moment of inertia (WT 1.725mm^4 + 0.485 and $P394L^{+/+}$ 0.396 mm^4+ 0.096 ;$p<0.05$) versus WT. Mechanical testing revealed a lesser force required to break $P394L^{+/+}$ femora (36.6N) versus WT (42.8N; $p<0.05$) with $P394L^{+/+}$ exhibiting fracture point range between 66-68% femoral length versus 48-53% in WT ($p<0.05$; Fig.1). Elucidation of skeletal microstructure could be used in combination with DXA scans to better predict fracture risk sites in clinical settings including PDB.

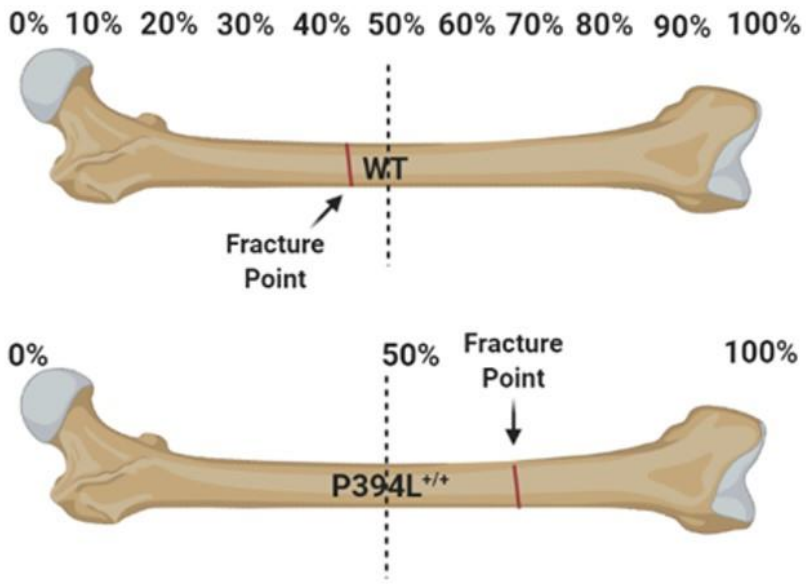

Figure 1. Longitudinal analyses of cortical microstructure and whole bone geometry in this study represented as % femoral length. Regional alterations in bone composition link to localised fracture points in WT and $P394L^{+/+}$ femora.

The influence of undercarboxylated osteocalcin on endothelial function in normal and high glucose conditions

Alexander Tacey[1, 2], Tawar Qaradakhi[1], Alan Hayes[1, 2], Anthony Zulli[1], Itamar Levinger[1, 2]

1. Institute for health and sport (IHES), Victoria University, Melbourne, Victoria, Australia

2. Australian Institute for Musculoskeletal Science (AIMSS), Western Health, Melbourne University, Melbourne, Victoria , Australia

Purpose: The bone derived protein undercarboxylated osteocalcin (ucOC) improves glucose regulation and is associated with a reduced risk of diabetes. In animals, *in vivo* treatment with ucOC improves vascular function, but this may be due to improved glucose control rather than a direct influence on blood vessels. Moreover, there are conflicting reports on the association of ucOC with cardiovascular outcomes in humans, including reports of adverse effects. The aim of this study was to examine whether ucOC directly influences (positively or negatively) endothelial function in rabbit arteries following incubation in different glucose concentrations.

Methods: Male New Zealand white rabbit arteries were fed a normal or atherogenic diet for 4-weeks. Arteries were incubated *ex vivo* in 11mM or 20mM glucose solutions for 2-hours and the vasoactivity of ucOC was assessed via several different techniques. Isometric tension analysis was use to assess the vasoactivity of abdominal aortic rings to ucOC pre-incubation (10ng/ml or 30ng/ml). To replicate physiological conditions, perfusion myography techniques were used to assess the vasoactivity of carotid artery segments to ucOC dose response curves (0.3 – 45ng/ml).

Results: In the abdominal aorta, both 10ng/ml and 30ng/ml doses of ucOC did not significantly alter endothelium-dependent (acetylcholine) or endothelium-independent (sodium nitroprusside) blood vessel relaxation in aortic rings following incubation in 11mM or 20mM glucose solutions after either the normal or atherogenic diet ($p > 0.05$). In the carotid artery, ucOC treatment did not cause any alteration in vasoactivity at any dose of ucOC, compared to a control treatment following either the normal or atherogenic diet ($p > 0.05$).

Conclusion: ucOC does not have a direct adverse effect on endothelial function in rabbit arteries. The improvement in endothelial function previously reported with ucOC may be a result of improved glucose regulation and not a direct regulatory effect on the vasculature.

Effects of weight loss on bone health in mice

Senthil Thillainadesan[1], Soren Madsen[1], Samantha Hocking[1], David James[1]

1. University of Sydney, Camperdown, NSW, Australia

Introduction:

Human and animal studies have demonstrated that some of the deleterious health consequences of obesity such as insulin resistance and hypertension can be reversed by weight loss. The effects of weight loss through dietary modification on bone health are unclear.

Aim:

Assess the effects of weight loss on bone health in a mouse model of diet induced obesity.

Method:

C57Bl/6J mice were divided into 3 groups: 1)Mice fed high fat diet for 16 weeks (HH); 2)Mice fed low fat diet for 16 weeks (LL); 3) Mice fed high fat diet for 8 weeks and then switched to low fat diet for 8 weeks (HL). Bone density was monitored with DXA. Tibia was collected for *ex vivo* microCT analysis of trabecular architecture

Results:

After switching from high fat to low fat diet at 8 weeks the body weight of the HL group returned to the weight of LL group within 2-3 weeks. DXA analysis at 8 weeks showed high-fat fed mice had reduced bone density (79.9±1.1 vs 75.3±1.1 g/cm^2, $p<0.01$). At 16 weeks the HL and LL groups had significantly higher bone density than the HH group (LL=81.9±1.2, HL=82.5±1.1, HH=72.8±1.3g/cm^2). The change in bone density over this 8 week period was significantly higher in the HL group compared to HH group($p<0.01$) and between the HL and LL groups($p=0.02$).

MicroCT analysis of tibia collected at 16 weeks showed that the HL and LL groups had significantly higher trabecular bone volume (BV/TV) compared to the HH group but there was no significant difference between HL and LL groups.

Conclusions:

These data suggest that deterioration in bone quality and density caused by high-fat feeding are reversible after returning to a healthier diet.

Loss of HIF signaling in breast tumors promotes outgrowth in the lung but not bone

Vera M Todd[1], Lawrence A Lawrence [2], Rachelle W Johnson[2]

1. Vanderbilt University, Nashville, TENNESSEE, United States

2. Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, United States

As tumors outgrow their blood supply, they frequently become hypoxic, activating hypoxia-inducible factor (HIF) signaling. Previous studies suggest that HIF signaling in breast cancer cells promotes lung dissemination in genetic models and bone colonization following intracardiac inoculation, but the impact of HIF1α in the primary tumor on spontaneous dissemination to bone has never been evaluated. Thus, we hypothesized that HIF1α deletion in the primary tumor would reduce spontaneous dissemination to bone. To test this, we generated MMTV-Cre.Hif1αf/f.PyMT mice, which spontaneously develop mammary carcinomas with conditional deletion of HIF1α. $Cre^{T/w}$/$Hif1\alpha^{f/f}$/$PyMT^{T/w}$ ($Hif1\alpha^{-/-}$) and $Cre^{w/w}$/$Hif1\alpha^{f/f}$/$PyMT^{T/w}$ ($Hif1\alpha^{f/f}$) littermate and cousin controls were used. Our data indicate that despite similar levels of intratumoral hypoxia (assessed by pimonidazole staining), $Hif1\alpha^{-/-}$ mice (n=21 mice) had an 85% reduction in *Hif1α* expression, but not *Hif2α*, in whole mammary tumors compared to $Hif1\alpha^{f/f}$ mice (n=19 mice) ($p<0.0001$). Surprisingly, histological inspection of lung sections revealed that $Hif1\alpha^{-/-}$ mice had *greater* incidence of macroscopic tumor nodules (n=15/21 $Hif1\alpha^{-/-}$, n=6/19 $Hif1\alpha^{f/f}$, $p<0.05$), and *greater* average lesion number (1.9-fold increase, $p<0.05$) and area (2.2-fold fold increase, $p<0.05$), although there was no difference in proliferative capacity of the $Hif1\alpha^{-/-}$ tumors (assessed by Ki-67 staining). In contrast, there was no significant difference in tumor dissemination to bone (tibia or spine), as determined by histological inspection, flow analysis for EpCAM+ tumor cells, and qPCR detection of tumor specific *Pymt* mRNA, nor were there differences in dissemination to liver or brain. These data suggest that blocking HIF1α signaling in primary breast tumors and metastatic tumor cells may promote tumor cell dissemination or outgrowth in the lung, where tumor cells only encounter hypoxia as they grow, but not in the physiologically hypoxic environment of the bone.

Automated regional segmentation of the murine bone cortex exposes microstructural diversity in porosity

Jacob Trend[1], Alisha Sharma[1], Katrin Deinhardt[1], Philipp Schneider[1], Claire Clarkin[1]

1. University of Southampton, Southampton, HAMPSHIRE, United Kingdom

Objective: Cortical bone is densely vascularised and exceptional to study as it provides physical compartmentalisation of mineral and vascular compartments allowing 3D reconstruction of vessel spatial heterogeneity. Previously, we provided the first evidence that age-related effects on murine bone porosity depend on the locality of the intracortical vasculature[(1).] Herein, our aim was to develop an automated means to segment cortical bone for regional assessment of microstructural heterogeneity.

Method: Synchrotron X-Ray computed tomography scans were undertaken at the tibiofibular junction of 13-month c57BL6 mice and reconstructed to form 8-bit greyscale image stacks. To standardise region separation across bone samples into anterior posterior, lateral, and medial regions, the Bone J moment of interia function[(2)] was used to align each bone to its principle axis (Fig.1). Porosity was extracted as described[(1)] and sorted into osteocyte lacunae (La.) and vascular canals (Ca.).

Results: Cortical porosity was identified as highest within the posterior (2.04%±0.008) and medial regions (1.65%±0.339) and lowest in the lateral (1.19%±0.192) and anterior (1.09%±0.1198) compartments. Intra-region variability was further exposed in Ca. circumference, with posterior Ca. larger than medial (65.39µm±3.03 vs 36.37µm± 6.22; p<0.01). Ca. circularity was less in the anterior versus all other regions, while Ca. volume density was greater in the posterior than the medial (0.51% ±0.099 vs 0.29%±0.056, p<0.05). La. circumference was greatest in the medial and lowest in the anterior region (23.27µm ±1.43 vs 18.3µm ± 1.17, p<0.05). La. volume density and La. number density were also highest in the medial and lowest in the anterior region (1.64±0.34 vs 2.47±0.11, p=0.019, and 0.66±0.13, 1.81±0.028, p=0.001, respectively).

Conclusion: Our study highlights an importance for regional assessment of cortical heterogeneity in the study of bone phenotypes, which could improve understanding into alterations in structure-function characteristics of cortical porosity during disease progression.

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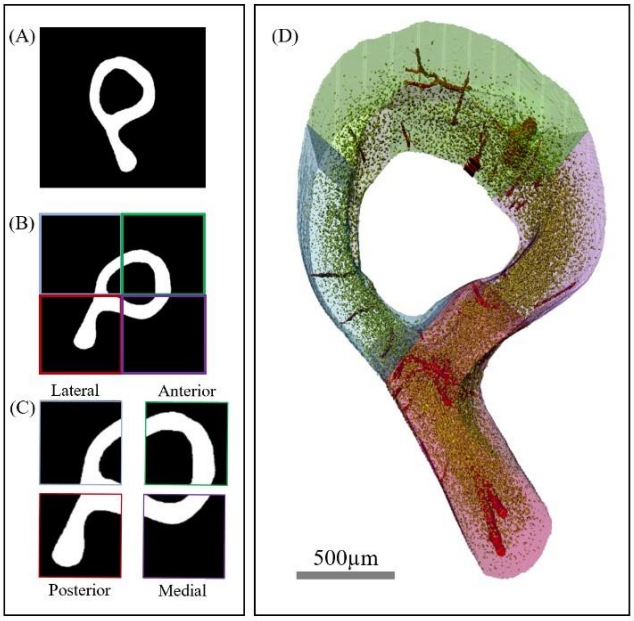


Figure 1) Regional analysis of the murine tibio-fibula junction. (A) BoneJ moment of inertia function used to align bones to their principle axis. (B, C) Separation of bone into quadrants; lateral, anterior, posterior and medial. (D) 3D reconstruction of regional porosity sorted into intracortical canals (red) and osteocyte lacunae (yellow).

Intermittent administration of parathyroid hormone has different effects on peri-implant bone in a site-dependent manner.

Yusuke Uchida[1], Shinichiro Kuroshima[1], Yusuke Uto[1], Riho Kanai[1], Masayoshi Suzue[1], Takashi Sawase[1]

1. Department of Applied Prosthodontics, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki-shi, Nagasaki-ken, Japan

Objective: Intermittent administration of parathyroid hormone (PTH) is used to treat osteoporosis by enhancing bone architecture. The purpose of this study was to investigate the detail effects of intermittent administration of PTH on bone quality and quantity around implants in rat tibia.

Methods: The titanium threaded implants were placed in 12-week-old-female wistar rat tibiae, and then, they were randomly divided into two groups at 3 weeks post-implantation; intermittent PTH administration group (80 μg/kg, every other day; PTH) and saline group as a control (vehicle control; VC) (n=7 per group). Rats were euthanized at five weeks after drug administration. Tibiae including placed implants were harvested. Three-dimensional structural, histomorphometric and immunohistochemical analyses were conducted inside and outside areas of implant threads.

Results: Micro-CT analysis showed that intermittent administration of PTH was effective on bone in this study. PTH significantly increased bone mass around implants inside areas of the threads, whereas PTH did not increase it outside area of the threads. PTH significantly increased osteocyte numbers inside area of all threads. PTH increased the number of osteoblasts and osteoclasts inside areas of almost threads, but not outside areas of the threads. Moreover, administration of PTH induced both increased and decreased total collagen amounts inside areas of some threads. On the other hand, it increased type III collagen amounts inside areas of some threads.

Conclusions: Our findings suggests that intermittent administration of PTH has different effects on bone quality in a site-dependent manner. Application of PTH administration may contribute to upregulating bone quality around dental implants in clinical situations.

Unexpected skeletal phenotype in a mesenchymal-targeted Calcitonin-like receptor (CLR) knockout model

Natalie K Wee[1, 2], Sanja Novak[2], Ivo Kalajzic[2]

1. Bone Cell Biology and Disease, St Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia

2. Reconstructive Sciences, University of Connecticut Health Center, Farmington, Connecticut, USA

The calcitonin peptide family exerts a range of effects on bone cells. Calcitonin gene-related peptide (CGRP), adrenomedullin and intermedin promote osteoblast proliferation and differentiation through actions mediated by Calcitonin receptor-like receptor (CLR; gene: *Calcrl*) and Receptor Activity-Modifying proteins (RAMPs; genes: *Ramp1,2,3*). CGRP is a signal in pain transmission and is released from nerves at sites of trauma. While CGRP stimulates osteoblast proliferation and differentiation *in vitro*, there is limited evidence that endogenous CGRP directly regulates the skeleton. The periosteum, outer surface of bone, is richly innervated with CGRP+ nerves, therefore CGRP may regulate periosteal bone apposition. Our aims were to: 1) evaluate how periosteal cells respond to CGRP and 2) deplete CGRP-CLR signalling in mesenchymal cells.

We identified CGRP receptor components, *Calcrl* and *Ramp1*, were expressed in periosteal cells, with *Ramp1* expression increased 7-fold during osteoblastic differentiation *in vitro*. CGRP treatment of periosteal cells led to cAMP activation, increased cell number by 20% and increased mineralisation by 40%. We also generated a mesenchymal-specific constitutive deletion model of CLR using Prxcre. Although CLR deletion was expected to reduce bone formation, we observed significantly 27% higher trabecular bone mass in 10-week-old male mice (Prxcre+,*Calcrl*$^{fl/fl}$) compared to Cre- littermates. Cortical thickness and maximum moment of inertia were significantly greater (10% and 16% respectively) in these mice compared to controls, suggesting greater bone formation on the periosteal surface.

These findings *in vivo* using Prxcre-induced CLR deletion differ from known CGRP effects on mesenchymal cells and appear more in line with observations from the calcitonin/CGRP double knockout and calcitonin receptor knockout models that each have increased bone formation. Targeting CLR deletion using a constitutive promoter (Prxcre) that is broadly expressed in multiple lineages within bone makes the model difficult to interpret. Further investigation of CLR cell specificity and signalling may uncover novel mechanisms that increase bone mass.

Corylin inhibits RANKL-induced osteoclastogenesis: signaling mediated by NF-κB pathways

Anna Xiao-Dan Yu[2, 1], Xiang-Peng Kong[2, 1], Shi-Zheng Zhao[2], Kenneth Kin-Leung Kwan [2, 1], Tina Tingxia Dong [2, 1], Karl Wahkeung Tsim[2, 1]

1. Shenzhen Key Laboratory of Edible and Medicinal Bioresources, HKUST Shenzhen Research Institute, Nanshan, Shenzhen, China

2. Division of Life Science and Center for Chinese Medicine, Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, Hong Kong, China

Publish consent withheld

MicroRNA control in the reduced osteogenesis but increased adipogenesis in the bone marrow following methotrexate treatment in rats

Yali Zhang[1], Yu-wen Su[1], Cory Xian[1]

1. Clinical and Health Sciences , University of South Australia, Adelaide , SA, Australia

Methotrexate (MTX) is commonly used in cancer chemotherapy to treat childhood leukaemia and osteosarcoma. Although the application of MTX chemotherapy improves the population of survivors, the prevalence of chronic bone-related complications has increased. Reduced bone formation (osteogenesis) and increased marrow fat formation (adipogenesis) have been observed through a "switch-like" change in commitment of bone marrow stromal cells (BMSCs) following MTX treatment. However, the underlying molecular mechanisms of this bone/fat switch are not fully elucidated. MicroRNAs participate in regulating BMSC differentiation by targeting the 3' untranslated region of osteogenic/adipogenic related genes. Here, in bone samples from MTX-treated rats, we found some differentially expressed microRNAs by microRNA array and RT-PCR. In addition, we found some microRNAs which have a negative correlation with expression of TGF-β signalling molecule Smad2 or Wnt antagonist sFRP-1. *In vitro* cell models were applied to validate the effects of microRNA agomir and antagonist delivery. Results suggest that overexpression of these microRNAs (microRNA-6315 & microRNA-542-3p) enhanced osteogenic differentiation, while their inhibition had the opposite effects. In addition, we found that the expression of these microRNAs was positively correlated with bone formation marker genes RUNX2, ALP, OCN and OSX. Furthermore, target prediction and dual-luciferase assays identified Smad2 and sFRP-1 as the direct targets for these microRNAs. Since Smad2 and sFRP-1 are the essential regulators involved in TGF-β and Wnt/β-catenin signalling pathways which are associated with osteogenesis and adipogenesis in BMSCs, the microRNAs we identified are likely to regulate osteogenesis and adipogenesis and the bone/fat switch after MTX treatment via the TGF-β and Wnt/β-catenin signalling pathways.

A vexing case of bone pain

Matthew Balcerek[1], Syndia Lazarus[1]

1. Department of Endocrinology and Diabetes, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

Case

A 68-year-old male presented in May 2020 with a 6-week history of severe, diffuse bone pain, which was refractory to analgesics. He appeared frail and mobilised, with difficulty, with a four-wheeled walker. This was a marked deterioration from his baseline function just three months prior (fully independent with mobility and activities of daily living).

His background was significant for high-risk acute myeloid leukaemia, diagnosed 9 months prior, with induction and consolidation chemotherapy being complicated by severe *Aspergillus fumigatus* pneumonia, despite posaconazole prophylaxis. Voriconazole 200mg BD was commenced in October 2019, and he underwent allogeneic stem cell transplant in January 2020.

Technetium-labelled bone scintigraphy showed diffuse periarticular radiotracer uptake (Fig.1), and initial biochemistry is shown in Table 1. Skeletal survey revealed areas of increased uptake on bone scintigraphy corresponding to sites of multifocal periosteal reaction and soft tissue calcification/ossification, affecting both the axial and appendicular skeleton (Fig. 2).

The differential diagnosis included chemoradiation versus voriconazole-induced periostitis. Notably, the voriconazole dose was increased to 400mg BD in February 2020 due to subtherapeutic voriconazole levels, with pain onset six weeks later. A serum fluoride level demonstrated a toxic result of 26 µmol/L (1-4 µmol/L). Voriconazole was ceased, and pain improved within two weeks, confirming a diagnosis of voriconazole-induced periostitis (skeletal fluorosis).

By eight weeks, his pain had resolved, and he was mobilising independently. Serum fluoride levels reduced to <10 µmol/L and bone turnover markers had decreased (Table 1). Repeat bone scintigraphy showed interval decrease in osteoblastic activity.

Discussion

Awareness of this debilitating condition, which manifests as severe bone pain, elevated serum alkaline phosphatase and periostitis on imaging, is of paramount importance in patients on long-term voriconazole. Serum fluoride levels are helpful in confirming the diagnosis. Pathophysiology and predisposing risk factors for voriconazole-induced skeletal fluorosis will be further discussed.

Figure 1. Technetium-labelled bone scintigraphy (performed two weeks prior to initial metabolic bone clinic review), showing intense uptake in the posterior 6th-8th ribs bilaterally, diffuse periarticular uptake in bilateral knees, elbows and wrist joints, and focal uptake in bilateral femoral diaphyses, proximal tibiae and bilateral proximal forearms.

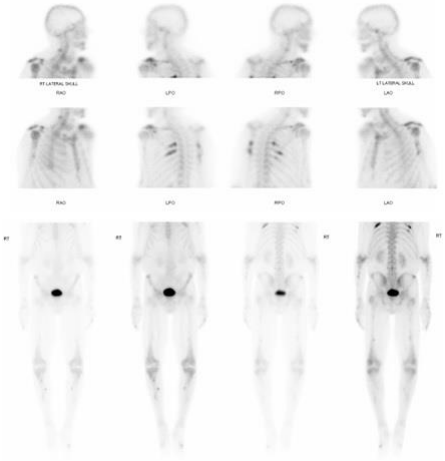
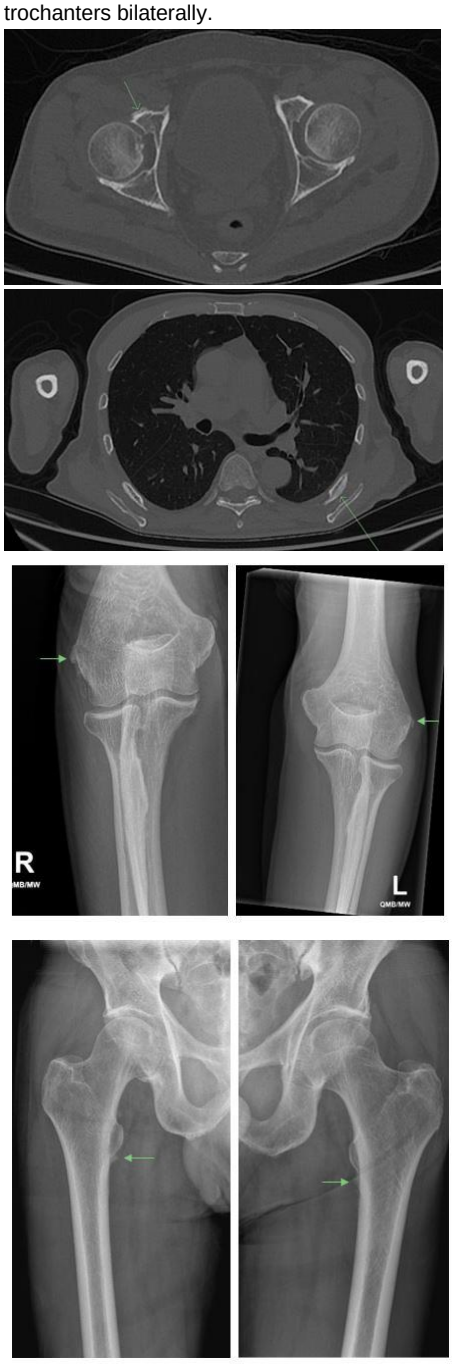


Table 1. Baseline biochemistry and post voriconazole cessation

	Baseline Value	Post voriconazole cessation	Reference Range
Alkaline phosphatase	217	99	30-110 U/L
eGFR	44	60	>90 mL/min/1.73m^2
Creatinine	139	108	64-108 µmol/L
Corrected calcium	2.30	2.31	2.10 – 2.60 mmol/L
Phosphate	1.50	1.13	0.75 – 1.50 mmol/L
25(OH)D	66	-	>50 nmol/L
Serum CTX	1480	900	100-600 ng/L
Serum total P1NP	132	48	15-80 µg/L

Figure 2. Skeletal survey (x-ray and computed tomography) highlighting multifocal solid periosteal reaction, as well as focal soft tissue calcification/ossification most prominent adjacent to the lateral epicondyles bilaterally (right > left), and lesser trochanters bilaterally.



Associations between household income and proinflammatory cytokines related to bone early in the life-course: A Systematic Review

Stefanie Bird[1, 2], Jason Talevski[1, 2], Jack Feehan[1, 3], Ghazala Naureen[1, 2], Joanne May Ling Tay[1, 2], Pryia Goyal[1, 2], Morgan Scott-Berman[1, 2], Gustavo Duque[1, 2], Rachel L Duckham[1, 4], Sharon L Brennan-Olsen[1, 4]

1. Australian Institute of Musculoskeletal Science, Melbourne, St Albans, Australia

2. University of Melbourne, Melbourne, Victoria, Australia

3. Victoria University, Melbourne, Victoria, Australia

4. Deakin University, Melbourne, Victoria, Australia

The life-course association between socioeconomic inequalities and bone health is becoming well-documented. Yet, little is known about the impact of socioeconomic factors on bone-related proinflammatory cytokines in the years before the achievement of peak bone mass. We aimed to systematically identify and synthesise existing data to investigate the relationship between household income, an important parameter of socioeconomic status (SES) known to influence child health, and bone-related pro-inflammatory cytokines during the years prior to peak bone mass attainment (ages 6 to 30 years).

We applied a defined e-search strategy to the PubMed, Ovid (Medline), EMBASE, PsycINFO and CINAHL databases, with no set limits of publication date. All full-text peer-reviewed articles written in English that encompassed household income data and levels of proinflammatory cytokines associated with bone accrual (C-reactive protein [CRP], interleukin-6 [IL-6], and tumour necrosis factor-alpha [TFN-a]) were screened for eligibility; epidemiological cross-sectional, case-control, and/or cohort studies in children and young adults aged 6-30 years, and baseline data from randomised control trials, where relevant, were included. The Lievense et al.[1] scoring system was used to determine the level of evidence.

In total, 14 studies met the inclusion criteria; 4 cohort studies (from USA and Australia) and 10 cross-sectional studies (from USA, Puerto Rico and Canada). These studies included a total of 52,586 participants (~54% female). We found limited evidence for an association between low household income and higher levels of IL-6. However, moderate-strong evidence was observed regarding a lack of association between TFN-a and CRP, respectively.

This review suggests that an association may exist between low household income with increased levels of IL-6, although based on a paucity of data. Future analyses should investigate associations between alternate parameters of SES and proinflammatory cytokines, given the importance of early life influences on later life bone health.

[1] Lievense A, Bierma-Zeinstra SMA, Vergahen AP, van Baar ME, Verhaar JAN, Koes BW (2002) Influence of obesity on the development of osteoarthritis of the hip: a systematic review. Rheumatol 41:1155–1162

A comparison of two electronic search tools to identify patients with osteoporotic fractures

Kenrick D Blaker[1], Ayanthi Wijewardene[2], Eleanor White[2], Gabrielle Stokes[3], Serena Chong[1], Lillias Nairn[2], Kirtan Ganda[1], Lloyd Ridley[4], Shane Brown[5], Christopher White[3], Roderick Clifton-Bligh[2], Markus J Seibel[1]

1. Department of Endocrinology, Concord Hospital, Concord, NSW, Australia

2. Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia

3. Department of Endocrinology, Prince of Wales Hospital, Sydney, NSW, Australia

4. Department of Radiology, Concord Hospital, Concord, NSW, Australia

5. Abbot Diagnostic Division, Abbot Diagnostics, Sydney, NSW, Australia

Introduction: Most patients who suffer a minimal trauma fracture (MTF) remain undiagnosed and untreated[1]. Osteoporosis refracture prevention (ORP) services are implementing different models of care for early identification of patients with MTFs. Case finding may be assisted by electronic search tools to automatically screen medical records for fracture. Our study assessed the efficacy of two new tools in identifying MTFs at two tertiary hospitals in Sydney.

Methods: 'XRAIT' uses natural language processing of imaging reports to detect fractures while the 'AES' tool identifies fractures through hierarchic disease code and text-based search of the electronic Medical Record (eMR) and radiology reports, respectively. Data were collected from 1/7/2018 to 31/12/2018. A sample of the extracted reports was then manually reviewed to determine the specificity and sensitivity of each search tool in detecting MTFs. The eMR was accessed to determine the fracture mechanism and treatment status.

Results: The true positive rate was similar for both tools (76.6-88.4%). However, in terms of detecting MTFs (rather than just fractures or a code-based diagnosis of osteoporosis) the tools performed differently at different sites, with 55.3-87.7% correct identification. Each tool identified separate subsets of patients. When all patients detected by both tools were combined, the AES tool identified 52.3-55.3% of patients with a MTF, while the XRAIT tool identified 87.7-93.2% of these patients (similar in both centres). Less than half (43-45.4%) of patients with a MTF were detected by both tools.

Conclusion: Both tools had a high true positive rate of identifying fractures, however one tool missed almost half of all MTFs. A hybrid tool that combines the methodology of both XRAIT and AES could improve patient identification. In the meantime, ORP service providers should be cognisant that subsets of patients continue to be missed with both tools.

Targeting the Fracture Prevention Message

Robert D Blank[1], Yoonah Choi[2], Joon Kiong Lee[3]

1. Garvan Institute, Darlinghurst, NSW, Australia

2. Evidencia Medical Communications, Sydney, NSW, Australia

3. Orthopedic Surgery, Beacon Hospital, Petaling Jaya, Selangor, Malaysia

Background

The impact of fracture on human suffering is underappreciated. Fractures increase mortality and the risk of recurrent fracture. Notwithstanding these facts, only a minority of patients suffering osteoporotic fractures receive fracture preventing therapy. Education about the burden of disease and the benefits of prompt treatment are important means to increase the treatment rate. We propose that the focus of the educational message should be varied for distinct audiences.

Method

We searched existing literature to determine perceptions about the importance of treating fractures and osteoporosis among the following groups: the general population, primary care physicians, specialist physicians, hospital administrators, and government health officials.

Result

Extensive data focusing on patients and primary care physicians exist about barriers to treatment initiation. In these groups, lack of perceived treatment benefit is a widespread, common finding. To address this gap, we have produced a primary care education toolkit and patient booklet as Asia Pacific Fragility Fracture Alliance (APFFA)-sponsored initiatives.

Data are more limited for specialist physicians, hospital administrators, and government officials. Health economic arguments have been found to be persuasive among policy makers.

Conclusion

Information about the importance of treating fractures and osteoporosis is far more abundant with reference to patients and primary care providers than to specialty physicians, hospital administrators, and government officials. Understanding the state of knowledge and belief is an essential first step in developing educational materials that address the concerns and misperceptions of each key constituency. Existing data suggest that patients will be more receptive to materials emphasising independence and quality of life, while policy makers will be more receptive to materials highlighting the burden of disease.

Mediterranean diet adherence and its associations with circulating cytokines, musculoskeletal health and incident falls in community-dwelling older men

Mavil May Cervo[1], David Scott[1], Markus Seibel[2], Robert Cumming[2], Vasi Naganathan[2], Fiona Blyth[2], David Le Couteur[2], David Handelsman[2], Louise Waite[2], Vasant Hirani[2]

1. Medicine, Monash University, Clayton, VICTORIA, Australia

2. University of Sydney, Sydney, New South Wales, Australia

Objective*:* This study aimed to determine associations of Mediterranean diet adherence with circulating cytokine levels, musculoskeletal health and incident falls in community-dwelling older men.

Methods*:* 794 community-dwelling men with mean age 81.1+4.5 years, who participated in the 5y follow-up of the Concord Health and Ageing in Men Project(CHAMP) were included in the cross-sectional analysis, and 616 attended follow-up 3y later. Mediterranean diet adherence was assessed using MEDI-LITE (literature-derived Mediterranean diet) score. 24 evaluable circulating cytokines were analysed using Bio-Plex Pro Human Cytokine 27-plex Assay kit. Appendicular lean mass (ALM) and bone mineral density (BMD) were measured using dual-energy x-ray absorptiometry (DXA). 3y changes in gait speed and hand grip strength were assessed by walking a 6-meter course and using a dynamometer respectively. Incident falls over 3y were determined through telephone interviews every 4 months.

Results*:* A higher MEDI-LITE score, indicating greater adherence to Mediterranean diet, was associated with higher appendicular lean mass adjusted for body mass index (ALM_{BMI}) (β: 0.004 kg/kg/m^2; 95% CI: 0.000, 0.008), lower interleukin-7 (IL-7) (β: -0.017 pg/mL; 95% CI: -0.031, -0.003), and incident falls rates (IRR: 0.94; 95% CI: 0.89, 0.99). Higher consumption of monounsaturated fatty acids (IRR: 0.76; 95% CI: 0.59, 0.98) and monounsaturated fatty acids to saturated fatty acids ratio (IRR: 0.72; 95% CI: 0.57, 0.90) were associated with 24%, and 28% lower falls risk in older men respectively. Association between MEDI-LITE scores and 3y incident falls was mediated by IL-7 by 10%. MEDI-LITE scores were not associated with BMD or physical function parameters.

Conclusion: Mediterranean diet adherence is associated with higher ALM_{BMI}, lower levels of IL-7, and fewer falls in older men. Monounsaturated and saturated fatty acids were the most important contributors to the association between Mediterranean diet and falls risk. Association between MEDI-LITE score and incident falls was partly mediated by IL-7.

Burden of end-stage osteoarthritis in Australia: A population-based study on the incidence of total knee replacement attributable to overweight/obesity

Lianzhi Chen[1], Monica Zheng[2], Ziming Chen[1], Michelle Lorimer[3], Christopher Jones[4, 5], Stephen Graves[6], Peilin Chen[1], Richard Carey-Smith[7], Toby Leys[7], John Papadimitriou[1, 8], Christopher Mitchell[1], Yi-Gang Huang[9], David Wood[1], Max Bulsara[2], Ming-Hao Zheng[1, 10]

1. Centre for Orthopaedic Research, University of Western Australia, Perth, Western Australia, Australia

2. Institute for Health Research, University of Notre Dame, Fremantle, Western Australia, Australia

3. South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

4. Medical School, Curtin University, Perth, Western Australia, Australia

5. Department of Orthopaedic Surgery, Fiona Stanley Hospital Group, Perth, Western Australia, Australia

6. Australian Orthopaedic Association National Joint Replacement Registry , Adelaide, South Australia, Australia

7. Department of Orthopaedic Surgery, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia

8. Pathwest Laboratories, Perth, Western Australia, Australia

9. Department of Orthopaedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

10. Perron Institute for Neurological and Translational Science, Perth, Western Australia, Australia

Increasing global obesity has been attributable to the increased prevalence of osteoarthritis (OA) but its risk on end-stage OA is still not clear. This population-based study aimed to define population attributable risk of total knee replacement (TKR) associated with obesity in Australia. A total of 191,723 TKRs for primary OA from 2015-2018 and estimated populations with BMI distribution were collected from the Australian Orthopaedic Association National Joint Registry and Australia Bureau of Statistics, respectively. Age- and gender-specific incidence rate (IR) and incidence rate ratio (IR) were calculated for each BMI category. We investigated the time-trend change in incidence of TKR in each BMI category and assessed the influence of obesity on the incidence of TKR in different age and gender groups. Population attributable fraction (PAF) was calculated to infer the effect of obesity on TKR incidence. In total, the number of TKR for primary OA increased 28.33% from 40954 cases in 2015 to 52555 cases in 2018. The greatest increase in number of TKRs performed occurred in individuals with BMI greater than 40.00. Obesity has resulted in the greatest risk of TKR at young population (aged 18-54). There are 3.722-, 13.704- and 18.463-times higher risk in patients with overweight, obesity class I & II and obesity class III than patients with normal weight, respectively. A greater risk of TKR was observed in the female population in obesity class III, at approximately 1.7-time higher than male population. The PAFs of TKR associated with obesity were 34.98% in 2015 and increased by 2% in 2018, with 12156 cases of TKR being attributable to obesity in 2018. In conclusion, obesity contributed to the largest proportion of TKR in young (less than 54) and female patients. Weight loss strategies can be implemented in these populations to reduce or delay the need for TKR.

"Age Before Frailty": Why Do Some People Fracture their Hips at a Younger Age?

Cellina Ching[1], Christian Girgis[1, 2], Cristina Ciobanu[3], Robert Pascoe[4]

1. Dept of Diabetes & Endocrinology, Westmead Hospital, Westmead, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

3. Department of Aged Care Medicine, Westmead Hospital, Westmead, NSW, Australia

4. Westmead Hospital, Westmead, NSW, Australia

Introduction

Osteoporotic hip fractures cause significant burden to the patient and healthcare system but are primarily considered an elderly issue. Data is lacking comparing demographics and risk factors of neck of femur (NOF) fractures in younger versus older populations. A clear understanding of these factors would facilitate an early and targeted approach in aggressive fracture prevention in high-risk individuals.

Method

Patients who had minimal impact NOF fractures at Westmead Hospital in the past year were included and groups divided at 70 years of age, the cut-off for geriatric care at Westmead Hospital. Data regarding their demographics, risk factors, bone mineral density (BMD) scans and outcomes were compared.

Results

Participants included 31 younger and 26 older patients. There was a greater proportion of males in the young NOF group (48% versus 42%). Smoking and alcohol abuse was more prevalent in young patients (38% versus 19%; 23% versus 12% respectively). Young NOF patients were more likely to have a history of previous fractures (45% versus 27%). There were similar proportions of vitamin D deficiency and other risk factors across both groups. The mean femur BMD was 0.71g/cm2 and T-score was -2.24 for young patients compared to 0.75g/cm2 and -2.47 for older patients (p=0.68 and p=0.80) with mean FRAX risk of osteoporotic fracture 4.8% and 17.1% respectively (p=0.37). All patients underwent surgery, but the average length of stay was 14.8 days for younger patients and 24 days for older patients with more older patients having reduced (68% versus 85%).

Conclusion

This retrospective review indicates that smoking and alcohol excess predispose to the development of NOFs in younger patients who likely have a critical degree of bone fragility as evidenced by previous fracture. Specific strategies to mitigate the risk of hip fracture in this group of patients is underway.

Risk factors for atypical femoral fracture: a retrospective radiographic and demographic study

Gareth Crouch[1], Nitesh D Dhanekula[2], Sue-Lynn Lau[1, 3], Edward Graham[2], Maria-Liza Nery[4], Jean Doyle[4], Lillias Nairn[5], Karen Byth[1, 6], Andrew Ellis[1, 7], Roderick Clifton-Bligh[1, 4], Christian Girgis[1, 3, 4]

1. Sydney Medical School, University of Sydney, Sydney, NSW, Australia

2. Department of Orthopaedic Surgery, Westmead Hospital, Westmead, NSW, Australia

3. Department of Endocrinology, Westmead Hospital, Westmead, NSW, Australia

4. Department of Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

5. Department of Physiotherapy, Royal North Shore Hospital, St Leonards, NSW, Australia

6. Research and Education Network, Western Sydney Local Health District, Westmead, NSW, Australia

7. Department of Orthopaedic Surgery, Royal North Shore Hospital, St Leonards, NSW, Australia

Background

Atypical femoral fractures (AFFs) are a serious complication of anti-resorptive therapies, the first line treatment for osteoporosis. It is important to elucidate predictive markers of AFF to clarify the risk:benefit ratio of osteoporosis therapy in patients requiring long-term treatment.

Aims

This study sought to identify demographic risk factors and femoral geometric features associated with AFF development. Additionally, predictors of a subtrochanteric or diaphyseal fracture location were examined.

Methodology

Radiographs of femoral shaft and subtrochanteric fractures treated from January 2008 - May 2017 were retrieved using electronic medical record coding. Subsequently, 413 anteroposterior pelvic radiographs with morphological characteristics of AFFs were reviewed by three expert adjudicators and classified as AFFs/impending AFFs or non-AFFs. Included fractures were further limited to those in females with a low-energy mechanism of injury and no osteo-metabolic or metastatic disease. Geometric, pharmaceutical and demographic data were extracted, and correlated with fracture location and AFF status.

Results

80 AFFs/impending AFFs in 65 individuals and 45 non-AFFs in 45 individuals were included. AFFs occurred in smaller, thinner femurs, with smaller femoral neck widths (35.26vs38.44mm,p<0.001), head diameters (51.45vs54.04mm,p=0.001) and medullary canal widths (16.15vs18.93mm,p<0.001). This may reflect greater bisphosphonate accumulation in smaller femurs and consequent increased microfracture accumulation. Geometries associated with force transfer also differed; Femoral neck shaft angle (133.3vs129.6degrees,p=0.033) and lateral cortical width at the lesser trochanter (5.19vs4.45mm,p=0.004) were larger and hip axis length (120.36vs125.81mm,p=0.037) smaller in patients with AFFs than controls. AFFs were associated with younger age (76.4vs81.9years,p<0.001), absence of dementia and greater number of medications. No variables studied were associated with fracture location.

Conclusions

This study represents a large Australian cohort of AFFs and suggests femoral geometry and comorbidities are associated with development of these fractures. Further characterisation of these risk factors may lead to the development of a predictive tool for AFF in susceptible patients.

Blood borne bone: Circulating Osteoprogenitors Associated with Bone and Muscle quality.

Jack Feehan[1, 2], Cassandra Smith[2, 3], Alexander Tacey[2, 3], Ahmed Al Saedi[1, 2], Gustavo Duque[1, 2], Itamar Levinger[2, 3]

1. Department of Medicine - Western Health, The University of Melbourne, Melbourne, Victoria, Australia

2. Australian Institute of Musculoskeletal Science, Melbourne, Victoria, Australia

3. Institute for Health and Sport, Victoria University, Melbourne, Victoria, Australia

Circulating osteoprogenitors (COP) cells, found within the peripheral blood, express characteristics of mesenchymal stem cells (MSCs) with the capacity to proliferate and differentiate into bone, muscle and fat. While some aspects of their *in vitro* and *in vivo* behaviour as mesenchymal progenitors has been established, much remains unknown about their role in age-related musculoskeletal diseases such as osteoporosis and sarcopenia. In this study, our aim was to identify if a relationship exists between COP cells and important muscle and bone parameters.

Thirty-eight healthy older people [mean age 72 (±6yrs), mean BMI 28.4] were recruited from the community. Exclusion criteria included recent history of fracture, or initiation of osteoporosis medication, type 2 diabetes mellitus, haematological, myelodysplastic or myeloproliferative disorders. Participants underwent a series of assessments including a blood sample, anthropometry and body composition analysis via densitometry. Peripheral blood samples were analysed for via flow cytometry, with COP cells defined as peripheral blood mononuclear cells (PBMCs) co-expressing the CD45 and osteocalcin markers. COP cell number is expressed as a percentage of the total PBMC population. Regression analysis was used to identify relationships between variables.

Increased COP cell number was associated with increased femoral (r=0.442, p=0.008,) and total BMD (r=0.376, p=0.025) and tended to correlate with ALM (r=0.314, p=0.06). These results remained consistent after adjustment for age and BMI.

COP cell number is linked to bone and muscle parameters *in vivo.* This raises the hypothesis that COP cells have a role in the maintenance and cross talk between these tissues. Future studies should evaluate whether COP cells can be used as a novel biomarker for diseases such as osteosarcopenia.

Vitamin D Dependent Rickets Type 1A: A Case Report

Aviva Frydman[1], Jeffrey Zajac[2, 3], Christopher Yates[1]

1. Department of Endocrinology, Western Health

2. Department of Endocrinology, Austin Health

3. Department of Medicine, University of Melbourne

We report the case of a 32-year-old female with vitamin D dependent rickets. She was diagnosed with congenital rickets at 18 months of age. Initial symptoms were inability to walk, bowed legs, failure to thrive and hypocalcaemic seizures. She was found to have low serum calcium, low phosphate, elevated parathyroid hormone (PTH), elevated alkaline phosphatase (ALP), normal 25 hydroxyvitamin D (25OHD), and very low 1,25 dihydroxyvitamin D ($1,25(OH)_2D$). X-rays revealed gross rachitic changes in the skeleton, hands and knees at 21 months. She was treated with oral calcitriol, but had recurrent hypocalcaemia requiring intravenous calcium. By age 12, X-rays revealed anterior bowing of bilateral mid to lower tibiae and lateral bowing of bilateral femora. At age 22 an iliac crest biopsy revealed marked reduction in mineralized bone consistent with severe osteomalacia/rickets with scattered healing microfractures. Throughout her 20s she remained intermittently adherent to oral calcitriol and calcium supplementation. Her rickets has been complicated by a right femoral diaphyseal fracture, bilateral femoral osteotomies for severe bowing and she requires a scooter for mobilization. She has had two successful pregnancies.

She is one of eight children of non-consanguiness parents. Two brothers were diagnosed with rickets 6-7 months of age (figure 1). Family genetic testing in 2016 revealed a CYP27B1 gene mutation in one allele. She may be a compound heterozygote for CYP27B1 mutations, however no mutations have been identified in the other allele to date.

There are 3 main forms of Vitamin D Dependent Rickets (VDDR) (1). VDDR type 1A is an autosomal recessive condition resulting from biallelic mutations in the CYP27B1 gene which codes for 1a hydroxylase, the enzyme responsible for conversion of 25OHD to $1,25(OH)_2D$. Impaired hydroxylation leads to normal or high levels of 25OHD but low levels of $1,25(OH)_2D$, consistent with our case. Treatment requires oral replacement of calcitriol.

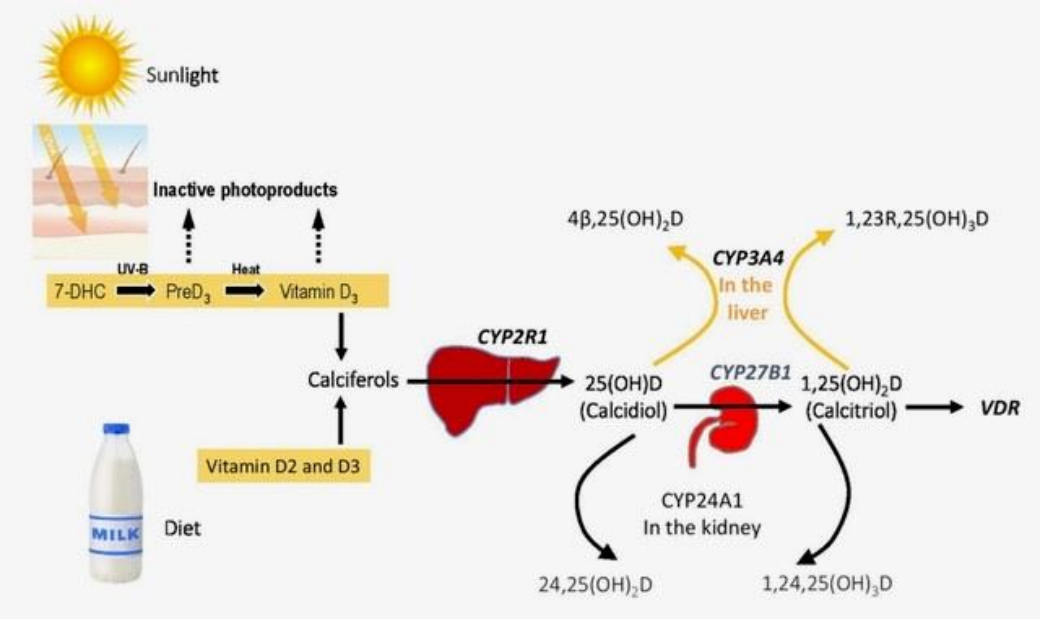


Figure 1: Vitamin D metabolism

Post-Fracture Care (PFC) Programs: A Literature Assessment of Where Secondary Fracture (Fx) Prevention is Today

Kirtan Ganda[1, 2], Kristina Åkesson[3], Cynthia Deignan[4], Amy Volpert[5], Keyla Brooks[6], David Lee[4], Andrea Singer[7]

1. Concord Repatriation General Hospital, Concord, NSW, Australia

2. Universsity of Sydney, Sydney, NSW, Australia

3. Lund University and Skåne University Hospital, Malmö, Sweden

4. Amgen Inc., Thousand Oaks, California, USA

5. BioScience Communications, New York, NY, USA

6. UCB Pharma, Brussels, Belgium

7. MedStar Georgetown University Hospital, Washington, D.C., USA

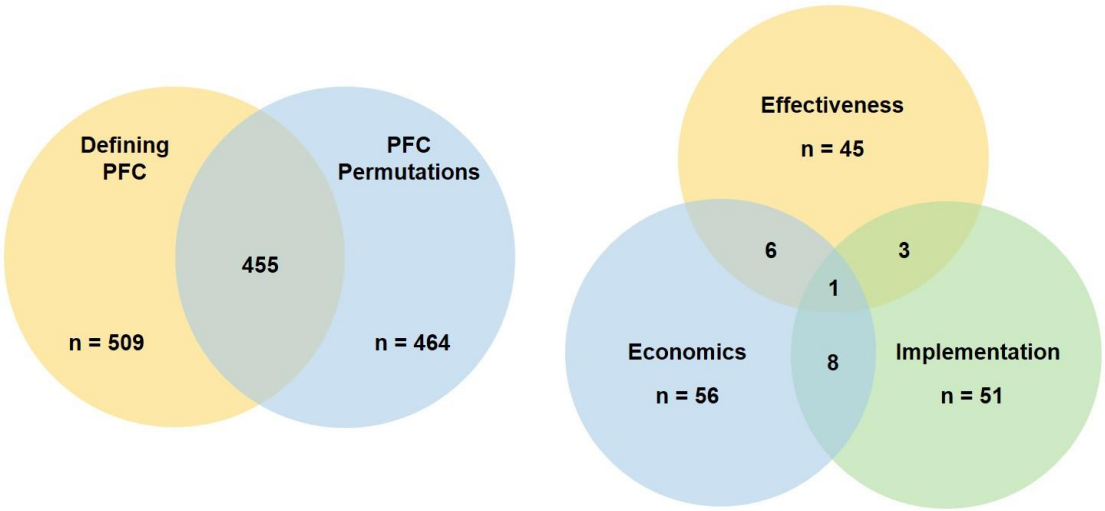
Purpose: PFC programs, notably Fx liaison services (FLSs), identify, investigate, and manage Pts for secondary Fx prevention. Despite an increase in PFC program literature in the last decade, limited information exists on their diversity and success. This literature review describes PFC program types, distribution and outcomes.

Methods: This analysis included peer-reviewed articles from 2003—2018; search terms related to adult, secondary Fx prevention programs. Articles belonged to 5, non-mutually exclusive areas: PFC definition i.e. intervention types and goals; PFC permutations in real-world practice; clinical effectiveness; economics; and implementation science. Primary objective was quantitative and qualitative assessment of PFC programs in these areas; geographic variations included.

Results: 647 of 729 articles were included (primary Fx prevention excluded; Figure); most published since 2013. Most programs fall into 2 categories—FLS out-Pt and orthogeriatric Fx, which differ in target populations, Fx types, and Pt outcomes. Most data on FLSs focussed on effectiveness and cost-benefit analyses rather than quality of life and social/disease burden. More intensive and coordinated FLS programs were associated with better outcomes (Ganda OI 2013). Some lower intensity interventions e.g. providing Pts with literature to discuss with primary care providers, also improved outcomes (Majumdar OI 2017). The number of articles per country did not correlate with population size, osteoporosis prevalence, or hip Fx incidence. Most articles originated from Europe and North America—several countries had few PFC programs but high hip Fx incidence.

Conclusions: PFC programs today comprise primarily of FLS and geriatric Fx programs. While primary goals and Pt populations differ, both demonstrated Pt benefit. Areas of improvement include expansion/adaptation of PFC for lower population density regions/limited resources and improvements in established programs. Data gaps remain around sustainability and standardisation of PFC program outcomes, including long-term Fx data. Future work may consider remote digital programs and trends among age subgroups.

Figure. Post-fracture Care Program Articles Published Between 2003 and 2018: Overlap Among Five Topic Areas



The "Defining PFC" and "PFC Permutations" categories explored the breadth of the field, including the program design and execution, and therefore, encompassed most of the articles from the search. By comparison, the "Economics", "Effectiveness" and "Implementation" categories were more narrowly focussed on PFC outcomes. 110 articles did not fall into any of these five categories and are not included in this diagram.

Falls, fractures and areal bone mineral density in older adults with sarcopenic obesity: A systematic review and meta-analysis

Anoohya Gandham[1], Jakub Mesinovic[1], Paul Jansons[2, 1], Ayse Zengin[1], Maxine P Bonham[3], Peter R Ebeling[1], David Scott[2, 1]

1. Department of Medicine, Monash University, Clayton, Victoria, Australia

2. Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences, Deakin University, Geelong , Victoria, Australia

3. Department of Nutrition, Monash University, Clayton, Victoria, Australia

Background: Sarcopenia and obesity are two common conditions in older adults that may have differing effects on falls and fracture risk. This systematic review and meta-analysis aimed to determine whether older adults with sarcopenic obesity (SO) have increased risk of falls and fractures, or lower areal bone mineral density (aBMD), compared with sarcopenic non-obese (SNO), non-sarcopenic obese (NSO) or non-sarcopenic non-obese counterparts (NSNO).

Methods: An electronic literature search of four databases (MEDLINE, Web of Science, EMBASE and Scopus) was performed using relevant search terms from inception to June 2020. Random-effects meta-analyses determined mean differences (95% confidence intervals) in aBMD, and differences in falls risk (risk ratios; RR) and fracture rates (incidence rate ratios; IRR), between SO, NSO, SNO and NSNO older adults.

Results: Twenty-five cross-sectional and cohort studies (n=36,941) were included in the systematic review and 15 (n=31,189) were included in the meta-analysis. SO older adults had lower femoral neck aBMD compared with NSO and NSNO counterparts, but had higher aBMD compared with SNO counterparts (all $P<0.05$). SO older adults also had higher aBMD at the total hip and lumbar spine (both $P<0.05$), but higher non-vertebral fracture rates (IRR: 1.88; CI:1.09, 3.23), compared with SNO individuals. SO older adults had a significantly higher falls risk compared with NSNO (RR: 1.33; CI: 1.08, 1.65) and NSO older adults (RR: 1.17; CI: 1.01, 1.36), but not compared with SNO (RR: 1.07; CI: 0.83, 1.38).

Conclusion: SO older adults have lower femoral neck aBMD and increased falls risk compared with NSO and NSNO counterparts but have increased fracture rates only when compared with SNO counterparts. These data support the need for further investigation of the underlying mechanisms of fracture risk in older adults with sarcopenic obesity, and for interventions to improve bone health and physical function in this population.

Primary care management of knee osteoarthritis prior referral to orthopaedic clinic and its concordance with published national guidelines and recommendations

Jack H Gilmore[1], Andrew Dorman[1], Shyan Goh[1]

1. Department of Orthopaedic Surgery, Redland Hospital, Brisbane, QLD, Australia

Introduction: Knee pain related to osteoarthritis (OA) is a common musculoskeletal condition managed in primary care. Despite widely available national, there is persistently significant variation in treatment attempted prior to referral to orthopaedic specialist clinic.

Objectives: To determine prevalence of knee OA in adults referred to orthopaedic clinic for knee pain, define previous treatment trialled prior referral and its concordance with national guidelines.

Methods: 99 consecutive adults (> 40 years) referred for knee pain in 2016-7 to orthopaedic outpatient clinic at Redland Hospital (250 beds, in South Brisbane) were reviewed over a 12-month period . Referral letters, clinical history, assessment (including imaging report) and management determined at outpatient clinic were recorded. For those with referral diagnosis of OA, pre-referral treatment was compared to then current NHMRC guideline (2009). We consider any management trialing at least 80% of therapies listed by guideline as guideline compliant.

Results: 72% of patients referred (51% female, mean 56.2 years) had OA diagnoses. Most common treatment recorded in referrals is oral analgesics (12%), 86% referral letters (RL) did not mention any treatment attempted prior referral. Previous treatment self reported by patients (SR) revealed oral analgesic use in 91%, 49% attempted weight loss, 36% tried physiotherapy. Corroboration of history and treatment of knee OA between RL vs SR shows only 24% concurrence. SR treatment is more likely to be compliant with NHMRC's recommendation as compared to those recorded by RL (39% vs 6%) but neither achieve better than 39% in adherence to recommendation, hence none of pre-referral management were guideline compliant.

Conclusions: Despite the availability of NHMRC's guideline for knee OA management for almost a decade, few patients have actually full experience of the recommended management before referral to specialist clinic. This revelation suggests potential success of conservative measures if instituted by both general practitioners and orthopaedic surgeons, if the majority of knee pain is correctly diagnosed as caused by OA.

Ongoing bone loss at the hip occurs in young adults with transfusion-dependent thalassemia major and is associated with increased urinary calcium and haemoglobin over time

Madhuni Herath[1, 2, 3], Pierre-Antoine Dugue[4], Zane Kaplan[5, 6], Jasna Aleksova[1, 2, 3], Nisal Punchihewa[2, 7], Frances Milat[1, 2, 3], Phillip Wong[1, 2, 3]

1. Department of Endocrinology, Monash Health, Melbourne, VIC

2. Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Melbourne

3. Department of Medicine, School of Clinical Sciences, Monash University, Melbourne, Australia

4. Precision Medicine, School of Clinical Sciences, Monash University, Melbourne, Australia

5. Department of Haematology, Monash Health, Melbourne, Australia

6. Inherited Blood Disorders Clinic, Monash Health, Clayton, VIC, Australia

7. Clinical School, Monash University, Melbourne, VIC, Australia

Background: Thalassaemia major (TM), an inherited disorder of ineffective erythropoiesis, requires lifelong transfusions and iron chelation therapy. Osteoporosis is a complication of thalassaemia; chelation therapy and hypercalciuria have been associated with reduced areal bone mineral density (aBMD) in cross-sectional studies.

Methods: An open-label, prospective study was conducted to investigate the effect of hydrochlorothiazide and iron chelation (deferasirox or deferoxamine) on aBMD in TM. Enrolled patients (n=111) were followed for eight years and aBMD was measured by dual energy x-ray absorptiometry (DXA) biennially. Time was assessed as a categorical variable. Patients with >1 missing DXA result were excluded; 92 patients were analysed. Mixed model and linear regression analyses were performed.

Results: There were 44 (48%) men: mean baseline age was 38±9 years, mean haemoglobin and ferritin levels were 103g/L (normal 115-165g/L) and 1248µg/L (normal 30-600µg/L) respectively. Seventy-two patients (78%) received deferasirox; 20 (22%) received deferoxamine. Twenty-five (27%) patients were treated with hydrochlorothiazide (12.5mg or 25mg). At the study completion eleven (12%) patients sustained fractures, 33 (36%) were hypogonadal and 15 (16%) had exposure to anti-resorptives.

Femoral neck (FN) aBMD declined (right: -0.0048g/cm^2/year, 95%CI [-0.0067, -0.0028], P=3x10^{-6}; left: -0.0038g/cm^2/year, 95%CI [-0.0056, -0.0020] P=4x10^{-5}) independent of chelation therapy. Urine calcium/creatinine ratio was associated with decline in FN aBMD (-0.011g/cm^2 [-0.022, -0.001] per unit change, P=0.04) but treatment with hydrochlorothiazide was not. An increase in haemoglobin over follow-up, but not ferritin, was associated with decline in aBMD at the LFN and lumbar spine (-0.0016g/cm^2 [-0.0029, -0.0004], P=0.01, and -0.0018g/cm^2 [-0.0034, -0.0004], P=0.02, respectively).

Conclusion: Increased urine calcium/creatinine was associated with bone loss at the FN but the study was underpowered to assess hydrochlorothiazide effect. The association of haemoglobin increment over time with bone loss may have implications for long term transfusion therapy targets in TM and deserves further study.

Mastocytosis, an unusual case of osteoporosis.

Ana Maria Delgado Lucio[1]

1. Hospital Universitario Burgos, Spain

Introduction:

Osteoporosis is defined as a systemic skeletal disorder characterized by low bone mass and damage to the bone tissue microarchitecture, with the consequent increase in bone fragility and increased susceptibility to fractures. It is usually classified as primary (postmenopausal/senile/idiopathic) and secondary. In this review we will treat, from a clinical case, systemic mastocytosis and its impact on bone mineral metabolism.

Clinical case:

A 53-year-old male was sent to medical specialist in 2016 due to multiple nontraumatic vertebral fractures during the last years (D1, D4, D9, D12 and L3). Neither pathological personal history of interest nor chronic treatment. His risk factors for osteoporosis include obesity grade 2 (BMI 36.2) and smoking habit (Pack Years Index 2,5). Patient provides bone densitometry with a spine T-score of -3.7 DS and -2.6 DS at the femoral neck. We completed the study of secondary endocrinological causes, discarding dysfunction in the adrenal, thyroid, parathyroid, pituitary, gonadal, diabetes mellitus, as well as malabsorptive origin. We start treatment with bisphosphonates associated with calcifediol as well as exercise recommendations.

During the ambulatory follow-up, he refers monthly evening episodes of erythema located in the forehead and ears. It accompanies with a heat sensation that lasts several hours, this nuisance released with Ibuprofen. Given the suspicion of a vasodilator substances mediated disorder, a study is conducted towards a possible neuroendocrine tumor which is negative. Suspecting a hematological cause, serum tryptase is requested, which is high, completing a study through bone marrow biopsy resulting in systemic mastocytosis. Nowadays, after treatment, the patient has a good densitometric and clinical evolution in the follow-up.

Conclusions:

1-Mastocytosis is an uncommon entity with abnormal cell proliferation from stem cells (CD34 +). 70% of cases cause bone damage due to the release of histamine, heparin and prostaglandins.

2-The low prevalence of this entity derives in the difficulty of its diagnostic. It's very important a detailed clinical history and examination for the screening of secondary causes of osteoporosis.

3-A right diagnostic allows us to start a targeted treatment on time, improve bone architecture and reduce its impact on quality of life.

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A survey on common side effects and incidence of bone pain and fractures among the patients taking long term anti-ulcerant therapies in Bangladesh

Md. Saddam Hussain[2, 1], Shuvo Mitra[1], Yu-Wen Su[2], Cory J. Xian[2]

1. Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Chittagong, Bangladesh

2. UniSA Clinical and Health Sciences, UniSA Cancer Research Institute, City West Campus, University of South Australia, Adelaide, South Australia, Australia

Proton pump inhibitors (PPIs), H_2 blockers, and antacids are commonly prescribed medications to treat ulcers in the stomach and the upper part of the small intestine and also prescribed for some other common GIT complications such as gastroesophageal reflux disease, esophagitis, irritable bowel syndrome, and dyspepsia. Previous studies claimed that, apart from other side effects, these anti-ulcerant therapies significantly altered bone mineral density (BMD) by interfering with intestinal reabsorption of calcium and phosphate, and the most widely prescribed PPIs were significantly associated with increased risks of hip and spine fractures. However, potential skeletal side effects of these antiulcerants are unknown in Bangladesh. To examine safety concerns of anti-ulcer therapies and their impact on bone health among patients in Bangladesh, the present work surveyed 200 patients in five different hospitals from December 2019 to February 2020.

Among the 200 patients, those taking PPIs alone or with other anti-ulcerants (>5 years; 95% respondents) claimed some unusual side effects, such as weakness, flank pain, spasm of hands and feet, muscle aches, numbness, and tremor. About 61% of patients taking PPIs experienced low back pain whereas 22% of respondents experienced dull pain and the respondents with the neck pain and knee joint pain were 10% and 7%, respectively. Although further studies are required to confirm the impact of these antiulcerants on the bone, these patient responses suggest that these musculoskeletal-related side effects might have some links with altered bone metabolism. It is possible that anti-ulcerant therapies may worsen the bone metabolism of patients suffering from osteoporosis or other bone disorders, and awareness and precautions should be raised among the patients and clinicians for the careful administration of PPIs to patients suffering from bone disorders.

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Age of introduction to solids is not associated with bone mineral density in childhood: Data from the Vitamin D in Pregnancy Study

Natalie K Hyde[1], Mia A Percival[2], Rachel L Duckham[3], John D Wark[4], Veronica Francis[1], Julie A Pasco[1]

1. School of Medicine, Deakin University, Geelong, Victoria, Australia

2. Pharmacy Department, Alfred Health, Melbourne, Victoria, Australia

3. Insititute of Physical Activity and Nutrition , Deakin University, Burwood, Victoria, Australia

4. Medicine, University of Melbourne (Royal Melbourne Hospital), Parkville, Victoria, Australia

Introduction: Early introduction of solids (4-6 months) may be associated with a reduction in allergic outcomes; however less is known about musculoskeletal outcomes, particularly in an Australian context. Thus, we aimed to determine if age of introduction to solids was associated with childhood bone outcomes in an Australian cohort.

Methods: Participants were recruited within the Vitamin D in Pregnancy study. At birth there were 402 mother child pairs, and 209 returned at the 11 year follow-up. At age one year, mothers self-reported the age of introduction to solids. At age 11 years, children underwent total body (TBLH) and lumbar spine DXA scans. Final linear regression models were adjusted for child height, lean mass, fat mass, pubertal stage and sex.

Results: There were 177 children who had complete information. Median age of introduction to solids was 5.0 (IQR: 4.0-6.0) months. There were 161 (91.0%) who were introduced to solids at or after four months and 62 (35.0%) who were introduced at or after six months. There was no association between beginning solids at or after four months and either TBLH or spine BMD (adjusted-β: -0.002 95% CI: -0.025,0.022 & adjusted-β:-0.014 95% CI: -0.060,0.031, respectively). Nor was there an association between beginning solids at or after six months and either TBLH or spine BMD (adjusted-β: -0.000 95% CI: -0.014,0.014 & adjusted-β:0.007 95% CI: -0.020,0.034, respectively). Similarly, holding 4-5 months referent, there was no difference in BMD to those introduced to solids at six months and above compared to those introduced at 4-5 months (adjusted-β: 0.003 95% CI: -0.013,0.019 & adjusted-β:0.013 95% CI: -0.018,0.034, respectively).

Conclusion: Within this cohort, there was no evidence of an association between the age of introduction of solids and childhood bone outcomes. This association will be re-examined in an upcoming young adulthood follow-up.

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Mid-thigh bone, lean and fat mass are reliable indices of tissue mass in predicting osteosarcopenia-associated outcomes – a validation study

Ebrahim Bani Hassan[1, 2], Fernan Munandar Putra[1, 2], Sara Vogrin[1, 2], Julie A Pasco[2, 4, 3], Mark A Kotowicz[2, 4, 3], Gustavo Duque[1, 2]

1. Australian Institute for Musculoskeletal Science (AIMSS),), The University of Melbourne and Western Health, St. Albans, Victoria , Australia

2. Department of Medicine-Western Health, The University of Melbourne, St. Albans, Victoria , Australia

3. University Hospital Geelong, Barwon Health, Geelong, Victoria , Australia

4. School of Medicine, Deakin University, Geelong , Victoria , Australia

Background: Mid-thigh has been recognized as a surrogate to assess bone, muscle and fat. Mid-thigh tissue measures associate well with both strength and adverse outcomes. In this study, we further investigated mid-thigh region of interest (ROI)'s ability to assess musculoskeletal health in a large cohort of community-dwelling participants.

Methods: Whole body DXA scans from 1322 participants of the Geelong Osteoporosis Study were analysed for bone, lean and fat mass in five ROIs: 2.6 cm mid-thigh, 13 cm mid-thigh, whole thigh, whole calf and forearm. Tissue masses in these ROIs were compared to conventional indices of musculoskeletal assessment (hip, spine and femoral neck BMD; appendicular lean mass adjusted for BMI or height^2; gynoid and android fat) using Pearson's correlation coefficient. Their associations with physical performance (timed-up-and-go [TUG] test), 1-year retrospective falls history and 5-year retrospective fractures was compared using regression.

Results: Mid-thigh tissue masses were moderately to highly correlated with their counterpart conventional indices: bone (r=0.4–0.49, p<0.001), muscle (corrected for BMI, r=0.86–0.94, p<0.001) and fat (r=0.57–0.94, p<0.001). For every 10% change in the tissue masses of mid-thigh, TUG changed between 0.5%–3.5% (p<0.01). Other ROIs were variable and mostly did not show significant associations with TUG (0.06–1.75, p=0.01 to 0.767). Mid-thigh BMD had comparable association with fractures vs neck of femur, hip and spine BMD (0.84% vs 0.80-0.86% difference in fracture rate per 10% difference in BMD, p<0.034). Whole-thigh and mid-thigh lean mass were also comparable to ALM/BMI (0.87–0.91 vs 0.90% difference in falls rate per 10% change in lean mass, p<0.027).

Conclusions: Data suggest that the mid-thigh is the best potential ROI out of selected regions to study muscle while whole thigh is better for bone. Both ROIs are equivalent or better than conventional indices when assessing concurrent physical performance (including falls) and fractures.

Dual energy x-ray absorptiometry reporting displays lack of adherence to international guidelines

Alicia R Jones[1, 2], **Maylyn Goh**[2], **Fran Milat**[1, 3], **Peter R Ebeling**[1, 4], **Amanda J Vincent**[1, 2]

1. Department of Endocrinology and Diabetes, Monash Health, Clayton, VIC, Australia

2. Monash Centre for Health Research and Implementation (MCHRI), Monash University, Clayton, Victoria, Australia

3. Hudson Institute of Medical Research, Melbourne, Vic, Australia

4. Department of Medicine, Monash University, Clayton, Victoria, Australia

Introduction: Bone mineral density (BMD), measured by dual X-ray absorptiometry (DXA), is the gold standard for diagnosis of osteoporosis, however the utility of DXA relies on adequate reports. The International Society for Clinical Densitometry (ISCD) has published minimum reporting guidelines.[1] This study assessed whether DXA reports for patients attending an academic teaching hospital adhere to ISCD reporting standards and to identify any differences related to patient factors or imaging service.

Methods: Patients aged ≥18 years, attending outpatient clinics between 01/01/2018 and 31/12/2019, with a DXA report, were included. DXA reports were reviewed for adherence to ISCD guidelines, with each criteria scored as one point, and the score converted to a percentage. Statistical analysis included frequencies and Poisson regression analysis to compare DXA report performance between imaging services.

Results: Of 459 DXA scans included, 214 were performed internally at our institution and 245 performed externally at 23 imaging services. Mean (SD) patient age was 60 (16.3) years; 75.8% were female. The overall median (IQR) DXA report score was 57.1% (39.5). ISCD criteria with the lowest scores were 'recommendation and timing of future DXA scans' (included in 1.1% of reports) and 'investigation for secondary causes of osteoporosis' (included in 1.2% of reports). Reports performed internally had significantly higher scores than those performed externally, after adjusting for age, sex, indication and type of scan (IRR 1.83, 95% CI 1.77, 1.89). Baseline DXA reports had higher scores than repeat DXA scans (IRR 1.05, 95% CI 1.02, 1.09, p<0.001), and, among external imaging services, rural services had higher scores than metropolitan services (IRR 1.16, 95% CI 1.06, 1.28, p=0.001).

Conclusion: The largest comprehensive evaluation of DXA reports, this study highlights significant deficiencies and variation in report standards between imaging services. This has potential implications for osteoporosis diagnosis and management.

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The effect of exercise intensity on postmenopausal BMD: a meta-analysis

Melanie Kistler-Fischbacher[1, 2], Benjamin K Weeks[1, 2], Belinda R Beck[1, 2, 3]

1. Menzies Health Institute Queensland , Griffith University, Gold Coast, Queensland, Australia

2. School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia

3. The Bone Clinic, Brisbane, Queensland, Australia

Background: Exercise trials often report unremarkable effects on bone. While a strong positive relationship exists between load magnitude and bone response in animal research, human trials rarely test high intensity exercise. Meta-analysing according to exercise intensity was required to fully elucidate exercise effects.

Objectives: To determine the effects of intensity on exercise effect on aBMD in postmenopausal women.

Methods: Electronic databases and reference lists were searched for RCTs reporting the effect of exercise on DXA-derived lumbar spine, femoral neck or total hip aBMD in healthy postmenopausal women. Interventions were classified and pooled as low, moderate or high intensity. Mean differences (MD) were calculated using random effects models and risk of bias analyses were undertaken.

Results*:* Fifty-three trials, testing 63 interventions (19 low, 40 moderate, 4 high intensity) were included. At the lumbar spine, high intensity exercise yielded greater BMD effects (MD = 0.031 g/cm^2 95% CI [0.012, 0.049], p=0.002) than moderate (MD = 0.012 g/cm^2 95% CI [0.008, 0.017], p<0.001) and low intensity (MD = 0.010 g/cm^2 95% CI [0.005, 0.015], p<0.001). Low and moderate intensity exercise was equally effective at the femoral neck (low: 0.011 g/cm^2 95% CI [0.006, 0.016], p<0.001, moderate: 0.011 g/cm^2 95% CI [0.007, 0.015], p<0.001), but no effect of high intensity exercise was observed. Moderate intensity exercise increased total hip aBMD (0.008 g/cm^2 95% CI [0.004, 0.012], p<0.001), but low intensity did not. There were insufficient data to meta-analyse high intensity exercise effects at the total hip.

Conclusion: High intensity exercise is a more effective stimulus for lumbar spine aBMD than low or moderate intensity. While data from high intensity interventions are limited, this meta-analysis demonstrates the same positive relationship between load magnitude and bone response in humans as observed from animal research. Findings have implications for optimal exercise prescription for osteoporosis in postmenopausal women.

Forgotten fractures - the extremes of Paget's disease

Sneha Krishna[1], Ann Robinson[1], Aakansha Zala[1], Katherine Griffin[1]

1. Gold Coast University Hospital, Southport, QLD, Australia

Introduction

Paget's disease of bone (PDB) is a disorder of accelerated bone turnover, leading to focal disorganised skeletal structure. The incidence of PDB has declined significantly in the past 30 years, leading to lower clinical familiarity with the condition. A short course of bisphosphonate therapy is first-line and has long term efficacy in many cases. We examine two cases of Paget's disease, one under-treated and one over-treated with bisphosphonates.

Cases

We present the cases of two elderly men with Paget's Disease, each presenting to a tertiary hospital with a femoral fracture. The first patient was under-treated as evidenced by multiple osteolytic lesions and prolonged elevation of alkaline phosphatase. The second patient was treated with daily oral bisphosphonate therapy for over 20 years, and presented with atypical femoral fracture.

Discussion

PDB is a disorder of skeletal remodelling that can result in localised bone pain, deformity, and fractures. Treatment with bisphosphonate therapy is indicated for a brief period in symptomatic disease and particular asymptomatic cases. These two cases highlight the risks of under and over treatment, and the importance of long term follow up.

Skeletal health in patients with traumatic spinal cord injury: a retrospective cross-sectional study from an Australian tertiary centre

Joshua L Lee[1, 2], Jean Doyle[3], Maria-Liza Nery[3], Roderick Clifton-Bligh[3, 4], Lianne Nier[1, 4], Christian Girgis[3, 5, 4]

1. Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney, NSW, Australia

3. Department of Endocrinology and Diabetes, Royal North Shore Hospital, St Leonards, NSW, Australia

4. Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

5. Department of Endocrinology and Diabetes, Westmead Hospital, Westmead, NSW, Australia

Objectives:
Traumatic spinal cord injury (tSCI) is associated with reduced bone mineral density from denervation and reduced load-bearing. We aimed to evaluate bone mineral density (BMD) in patients with tSCI, effects of bisphosphonates, and fracture rates by body region.

Methods:
Retrospective cross-sectional study including consecutive patients with BMD imaging at a tertiary hospital in Sydney, Australia from October 2004 to June 2018 was undertaken. Explanatory variable data extracted from electronic medical records; bisphosphonate usage confirmed using clinic data. BMD modelled using multivariate linear regression adjusting for baseline risk factors.

The outcome variable was femoral neck BMD measured in g/cm^2. Potential tSCI risk factors adjusted for BMD included bisphosphonate usage, ASIA score, Australian National Sub-acute and Non-acute Patient (AN-SNAP) coding, time since tSCI. Other adjusted explanatory variables included age, sex, smoking, alcohol, fractures by location, renal/bladder calculi, osteoarthritis.

Multivariable analysis performed using backwards elimination method and χ^2 likelihood ratio test. Absolute reduction in BMD with 95% confidence intervals reported using alpha of 0.05.

Results:
60 participants with tSCI were included. After multivariable analysis, BMI, bisphosphonate use, alcohol, ASIA, AN-SNAP, previous fracture location, were significantly associated with BMD. Previous bisphosphonate use compared to no previous antiresorptive treatment was associated with 0.21g/cm^2 lower BMD (p=0.0004). ASIA score A had 0.03g/cm^2 lower BMD than D (p=0.02). Worse AN-SNAP impairment was associated with lower BMD (C1-C4 0.19g/cm^2 lower than incomplete paraplegia, p=0.01), lower BMI (0.02g/cm^2 lower per unit, p<0.0001), and alcohol use (0.40g/cm^2 lower if ≥2 standard drinks daily, p<0.0001). Fractures were associated with lower BMD – particularly axial/upper limb and knee fractures (0.27 and 0.29g/cm^2 lower, p=0.0003).

Conclusions:
Bisphosphonates were used in tSCI patients with statistically lower femoral neck BMD. Independent risk factors for lower BMD in tSCI were ASIA, AN-SNAP coding, lower BMI, alcohol use. BMD was associated with fractures in tSCI patients, despite adjustment for bisphosphonate treatment.

Table 3. Multivariate analysis – adjusted multiple regression for factors predicting femoral neck bone mineral density in patients with traumatic spinal cord injury (n = 60)

Factor		BMD (g/cm^2)	95% CI	P-value
Increased BMI (kg/m^2)		0.02	0.01 – 0.03	<0.0001
Bisphosphonate use	No previous bisphosphonate use	Referent		0.0004
	Use of bisphosphonates previously	-0.21	-0.32 – -0.09	0.001
	Use of denosumab previously	-0.37	-0.58 – -0.16	0.0007
Alcohol use	Less than 2 standard drinks per day	Referent		
	2 or more standard drinks per day	-0.40	-0.56 – -0.23	<0.0001
ASIA score	A	Referent		0.02
	B	0.08	-0.07 – 0.24	0.29
	C	-0.14	-0.28 – -0.004	0.04
	D	0.03	-0.14 – 0.19	0.75
AN-SNAP coding	C1 – C4	Referent		0.01
	C5 – C8	0.17	0.06 – 0.29	0.004
	Complete paraplegia	0.13	0.006 – 0.26	0.04
	Incomplete paraplegia	0.19	0.04 – 0.34	0.01
Location of previous fracture	Nil previous fracture	Referent		0.0003
	Axial / upper limb fracture	-0.27	-0.47 – -0.07	0.0083
	Hip (pelvic / proximal femoral) fracture	-0.05	-0.19 – 0.08	0.42
	Knee (distal femoral / proximal tibial) fracture	-0.29	-0.44 – -0.14	0.0002
	Other lower limb fracture	0.41	0.11 – 0.72	0.009

$F = 26.6$, $p < 0.0001$, $R^2_{adj} = 0.83$

Does exercise attenuate bone mineral density losses during diet-induced weight loss in overweight and obese adults? A systematic review and meta-analysis of randomised control trials

Jakub Mesinovic[1], Paul Jansons[1, 2], Ayse Zengin[1], Barbora De Courten[1], Alexander J Rodriguez[1], Robin M Daly[2], Peter R Ebeling[1, 3], David Scott[1, 2, 3]

1. Department of Medicine, Monash University, Melbourne, Victoria, Australia

2. Institute for Physical Activity and Nutrition (IPAN), Deakin University, Melbourne, Victoria, Australia

3. Department of Medicine and Australian Institute of Musculoskeletal Science, University of Melbourne, Melbourne, Victoria, Australia

Objective: Weight loss is effective for improving cardiometabolic health in overweight and obese individuals, but can also result in areal bone mineral density (aBMD) losses and increased fractures. aBMD losses during weight loss may be attenuated by exercise. Our objective was to compare aBMD changes in overweight and obese adults undertaking weight loss alone, and in combination with exercise.

Methods: The Cochrane Central Register of Controlled Trials, PubMed, Web of Science, EMBASE and Scopus databases were searched for eligible articles until June 2020. Random effects meta-analyses determined mean difference (95% confidence intervals) in percentage aBMD change between groups.

Results: Nine randomised controlled trials (n=821) were included in the systematic review and six were included the meta-analysis (n=359). There were no significant differences between the weight loss alone and weight loss plus exercise groups for percentage changes in aBMD at the femoral neck (net difference: -0.72% [95%CI: -1.71, 0.26], P=0.15), lumbar spine (0.37% [95%CI: -0.32, 1.05], P=0.29) and whole-body (-0.27% [95%CI: -0.65, 0.12], P=0.17). Subgroup analyses revealed no differences in aBMD changes at any skeletal site in studies that used resistance exercise, aerobic exercise, or combined aerobic and resistance exercise during weight loss (all P>0.05). There were also no differences in aBMD changes in studies that were <6 versus >6 months in duration, or in studies that included individuals aged <60 versus >60 years.

Conclusion: This meta-analysis suggests that exercise does not attenuate aBMD losses during weight loss. However, very few included studies prescribed well-designed exercise interventions performed at sufficient intensities and for sufficient durations to maintain or improve aBMD. Additional long-term randomised controlled trials utilising targeted, osteogenic exercise interventions during weight loss, particularly in overweight and obese populations at risk for falls and fracture, are warranted.

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Risk factors for incident falls and fractures in older men with and without type 2 diabetes mellitus: the concord health and ageing in men project

Jakub Mesinovic[1], David Scott[1, 2, 3], Markus J Seibel[4], Robert G Cumming[5, 6, 7], Vasi Naganathan[6], Fiona M Blyth[6], David G Le Couteur[6, 8], Louise M Waite[6], David J Handelsman[9], Vasant Hirani[6, 10]

1. Department of Medicine, Monash University, Melbourne, Victoria, Australia

2. Institute for Physical Activity and Nutrition (IPAN), Deakin University, Melbourne, Victoria, Australia

3. Department of Medicine and Australian Institute of Musculoskeletal Science, University of Melbourne, Melbourne, Victoria, Australia

4. Endocrinology and Metabolism, University of Sydney, Sydney, New South Wales, Australia

5. School of Public Health, University of Sydney, Sydney, New South Wales, Australia

6. Centre for Education and Research on Ageing and Alzheimer's Institute, University of Sydney, Sydney, New South Wales, Australia

7. The Australian Research Council Centre of Excellence in Population Ageing Research, University of Sydney, Sydney, New South Wales, Australia

8. ANZAC Research Institute and Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

9. Department of Andrology, University of Sydney, Sydney, New South Wales, Australia

10. School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia

Background: Type 2 diabetes mellitus (T2DM) increases falls and fracture risk. Our objective was to compare falls and fracture incidence in community-dwelling older men with and without T2DM, and to determine whether known risk factors affect falls and fractures differently in these individuals.

Methods: A total of 1,705 men (471 with T2DM; 1234 without T2DM) aged >70 years from the Concord Health and Ageing in Men Project (CHAMP) were assessed at baseline (2005–2007) and 5 years later (2010–2013). At both time-points, measurements included fat mass, appendicular lean mass (ALM), handgrip strength, upper-limb muscle quality and gait speed. Men were contacted every 4 months for 6.0 + 2.2 years to ascertain incident falls and fractures. Hip fractures were ascertained via data linkage (follow up: 8.8 + 3.6 years). Risk factors for falls and fractures included physical activity and function, body composition, medications and vision.

Results: Men with T2DM had lower handgrip strength, upper-limb muscle quality and gait speed than men without T2DM ($P<0.05$). Over five years, men with T2DM lost more ALM (-0.34kg [95%CI: -0.533, -0.152]), but had similar fat mass losses (-0.40kg [95%CI: -0.938, 0.144]) compared to men without T2DM. Men with T2DM had similar falls (IRR: 0.90 [95%CI: 0.69, 1.17]) and fracture (HR: 0.86 [95%CI: 0.56, 1.32]) rates compared to men without T2DM after adjustment for significant risk factors. Interaction terms demonstrated that increases in ALM over 2 years was independently associated with lower 2-year falls rates (IRR: 0.66 [95%CI:0.52, 0.83]), and better contrast sensitivity was independently associated with lower fracture rates (HR: 0.14 [95%CI:0.02, 0.85]) in men with T2DM compared to men without T2DM.

Conclusion: Older men with T2DM lose more lean mass than men without T2DM. Maintaining muscle mass and improving vision may reduce fracture risk in older men with T2DM.

Estimating the osteogenic potential of physical activity and its associations with five-year bone mineral density changes, incident falls and fractures in older men: the Concord health and ageing in men project

Carrie-Anne Ng[1], David Scott[1, 2, 3], Markus J Seibel[4], Robert G Cumming[5, 6, 7], Vasi Naganathan[6], Fiona M Blyth[6], David G Le Couteur[6, 8], Louise M Waite[6], David J Handelsman[9], Vasant Hirani[5, 6, 10]

1. Department of Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia

2. Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria, Australia

3. Department of Medicine at Western Health, The University of Melbourne, Sunshine, Victoria, Australia

4. Bone Research Program, ANZAC Research Institute, The University of Sydney, Concord Campus, Sydney, New South Wales, Australia

5. School of Public Health, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia

6. Centre for Education and Research on Ageing, Concord Hospital, University of Sydney, Sydney, New South Wales, Australia

7. The ARC Centre of Excellence in Population Ageing Research, University of Sydney, Sydney, New South Wales, Australia

8. ANZAC Research Institute & Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

9. Department of Andrology, Concord Hospital & ANZAC Research Institute, University of Sydney, Sydney, New South Wales, Australia

10. School of Life and Environmental Sciences, Charles Perkins Centre, University of Sydney, Sydney, New South Wales, Australia

OBJECTIVE: Physical activity which generates high bone strain may benefit bone health and reduce fracture risk. This study aimed to investigate the relationships between force intensities and application rate estimated from self-reported physical activity with (1) changes in bone mineral density (BMD) over five years, (2) incident falls over two years and (3) long-term incident fractures in older men.

METHODS: 1613 men (mean age 76.8±5.4) from the Concord Health and Ageing in Men Project (CHAMP) were assessed at baseline, 2-year follow-up and 5-year follow-up. At each time point, hip and lumbar spine BMD were estimated by dual-energy x-ray absorptiometry, and physical activity was self-reported using the Physical Activity Scale for the Elderly (PASE) questionnaire. Sum effective load ratings (ELRs) and peak force were computed from the PASE questionnaire reflecting the total and highest intensity and frequency of ground reaction forces for physical activity modalities respectively. Participants were contacted over two years to self-report incident falls, and over 6.0±2.2 years for fractures.

RESULTS: Compared to sum ELR and PASE scores, peak force demonstrated the greatest standardised effect size of BMD increases at the spine (β=9.77mg/cm^2) and total hip (β=14.14mg/cm^2) after adjustment for covariates including PASE components (all $p<0.01$). Only PASE scores were significantly associated with reduced falls risk (incident rate ratio=0.89 per standard deviation, p=0.03). In adjusted categorical analyses, only high peak force was significantly associated with decreased risk of any self-reported fractures compared to low peak force (hazard ratio=0.60, 95% confidence interval=0.35, 0.99).

CONCLUSION: Older men who engage in physical activity of sufficiently high and rapid impact maintain higher BMD and have reduced fracture risk, while physical activity of high metabolic expenditure reduced falls risk. Coupling traditional physical activity questionnaire outcomes with bone-loading estimates may improve understanding of relationships between physical activity and bone health in older adults.

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Sleep disturbance and risk of falls and fractures: a 10.7-year cohort study

Feng Pan[1], Jing Tian[1], Flavia Cicuttini[2], Graeme Jones[1]

1. Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

2. Department of Epidemiology and Preventive Medicine, Monash University Medical School, Melbourne , Victoria, Australia

Objective: Sleep problems are common in general population and have substantial adverse health outcomes including type 2 diabetes and depression which may increase risk of falls and fractures. However, no study has investigated the associations of sleep problems with risk of falls and fractures in general population. This study sought to examine the associations between sleep disturbance and risk of falls and fractures.

Methods: Data from a population-based prospective cohort study with 1,099 participants (aged 50–80 years) enrolled at baseline were analysed. 875, 768 and 563 participants attended subsequent three follow-ups at 2.6, 5.1 and 10.7 years, respectively. Self-reported sleep disturbance was recorded at each visit. The Short-Form Physiological Profile Assessment was used to measure falls risk expressed as Z-score. Fractures were self-reported at each visit. Mixed-effects linear and logistic regression were used for the analyses.

Results: There was a dose-response relationship between the extent of sleep disturbance and falls risk score (β=0.05, 95%CI 0.02-0.09) and reported-fractures at any site (odds ratio [OR]=1.11, 95%CI 1.01-1.22). After adjusting for covariates including age, sex, body mass index, physical activity, smoking history, and comorbidities, participants reporting more severe sleep disturbance had greater falls risk score (β ranging from 0.11 to 0.15, all $p<0.05$) compared to those without sleep disturbance. In multivariable analyses with adjustment for covariates, bone mineral density and falls risk, participants reporting being awake most of the night had highest odds of fractures at any site (OR=1.47, 95%CI 1.04-2.08). In addition, more severe sleep disturbance was associated with fractures at vertebral (OR=1.43, 95%CI 1.04-1.97), but not non-vertebral site.

Conclusion: Sleep disturbance was independently associated with risk of falls and fractures, highlighting that sleep intervention has potential to reduce falls risk and fractures. Inconsistent associations of sleep disturbance with fractures at vertebral and non-vertebral may reflect a difference in underlying mechanisms.

Operational definitions of sarcopenia that involve tests of muscle performance in women should consider depressive symptoms

Julie A Pasco[1, 2], Sophia X Sui[1], Emma C West[1], Kara L Holloway-Kew[1], Natalie K Hyde[1], Amanda L Stuart[1], Lana J Williams[1]

1. Deakin University, Geelong, VICTORIA, Australia

2. Department of Medicine-Western Health, University of Melbourne, St Albans, VICTORIA, Australia

Background*:* Originally, sarcopenia meant 'poverty of flesh', but recent operational definitions have brought measures of poor muscle performance to the fore. None has considered psychological wellbeing. We aimed to compare the muscle performance components of EWGSOP2, FNIH and SDOC algorithms for women with and without depressive and anxiety symptoms.

Methods*:* This cross-sectional study involved 348 women (ages 60-94 years) from the Geelong Osteoporosis Study. Hospital Anxiety and Depression Scale scores for depression (HADS-D) and anxiety (HADS-A) >=8 indicated depressive and anxiety symptoms, respectively. Handgrip strength (HGS) was measured by dynamometry and physical performance by timed up-&-go (TUG; n=320). Measures of gait speed were not available. According to EWGSOP2, low-HGS <16kg; for FNIH, low-HGS <16kg and low-HGS/BMI <0.56; for SDOC low-HGS <20kg. Slow-TUG (3m) was >20s (EWGSOP2). Chi-squared test (applying Fisher's exact test for cell counts<5) identified differences in proportions and logistic regression models identified poor muscle performance in association with depressive symptoms.

Results*:* Twenty-nine (8.3%) women had depressive and 83 (23.9%) had anxiety symptoms. Proportions with low-HGS were greater for those with depressive symptoms according to different criteria [EWGSOP2 11/29(37.9%) vs 34/319(10.7%), p<0.001], [FNIH 11/29(37.9%) vs 34/319(10.7%), p<0.001] and [SDOC 15/29(51.7%) vs 85/319(26.7%), p=0.006]; and low-HGS/BMI [FNIH 13/29(44.8%) vs 50/319(15.7%), p<0.001]. Slow-TUG [EWGSOP2 3/24(12.5%) vs 4/296(1.4%), p=0.011]. No differences were detected for those with and without anxiety symptoms.

In multivariable models adjusted for age, women with depressive symptoms were 2-5 fold more likely to have low-HGS [EWGSOP OR 4.77 (95%CI 1.83-12.45) p=0.001] and [FNIH OR 4.77 (95%CI 1.83-12.45) p=0.001] and [SDOC OR 2.59 (95%CI 1.10-6.07) p=0.029], and low-HGS/BMI [FNIH OR 3.92 (95%CI 1.69-9.07) p=0.001]; and 11-fold more likely to have a slow-TUG [EWGSOP OR 10.99 (95%CI 2.03-59.7) p=0.005]. No interactions were detected.

Conclusion*:* Operational definitions should consider depressive symptoms at the time of evaluation when assessing muscle performance in women.

Measuring outcomes in ultra-rare diseases: methodology of the palovarotene fibrodysplasia ossificans progressiva (FOP) clinical development program

Robert J. Pignolo[1], Geneviève Baujat[2], Matthew A. Brown[3], Carmen De Cunto[4], Maja Di Rocco[5], Edward C Hsiao[6], Richard Keen[7], Mona Al Mukaddam[8], Andrew Strahs[9], Donna R. Grogan[9], Rose Marino[9], Frederick S. Kaplan[8], Shane Patella[10]

1. Department of Medicine, Mayo Clinic, Rochester, MN, USA

2. Département de Génétique, Institut IMAGINE and Hôpital Necker-Enfants Malades, Paris, France

3. Guy's & St. Thomas' NHS Foundation Trust and King's College London , NIHR Biomedical Research Centre, London, UK

4. Pediatric Rheumatology Section, Department of Pediatrics, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

5. Unit of Rare Diseases, Department of Pediatrics, Giannina Gaslini Institute, Genoa, Italy

6. Division of Endocrinology and Metabolism, UCSF Metabolic Bone Clinic, Institute of Human Genetics, and UCSF Program in Craniofacial Biology, Department of Medicine, University of California-San Francisco, San Francisco, CA, USA

7. Centre for Metabolic Bone Disease, Royal National Orthopaedic Hospital, Stanmore, UK

8. Departments of Orthopaedic Surgery & Medicine, The Center for Research in FOP and Related Disorders, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

9. Ipsen, Newton, MA, USA

10. Ipsen, Glen Waverley, VIC, Australia

Background: FOP is an ultra-rare genetic disorder characterized by heterotopic ossification (HO) of soft and connective tissues (often preceded by flare-ups), cumulative disability, and shortened life expectancy. Palovarotene is a highly selective retinoic acid receptor-gamma agonist under investigation for treatment of FOP. Conducting FOP trials have multiple challenges, including low number of confirmed cases worldwide, limited understanding of disease progression, the need to identify disease-specific biomarkers and optimize assessment of HO progression.

Objective: To describe the challenges encountered when conducting clinical trials in FOP.

Methods: Here, we describe the methodology of: a non-interventional, prospective, protocol-specified, longitudinal natural history study (NHS; NCT02322255); a multicenter, randomized, double-blind, placebo-controlled phase II trial (NCT02190747); an ongoing open-label extension (OLE) to the phase II trial (NCT02279095) **(Table)**. Studies were designed adaptively. Palovarotene doses were administered episodically (high dose for 2 or 4-weeks, followed by low dose for 4 or ≥8-weeks, from flare-up onset) or daily. In the double-blind period of the phase II trial, participants were randomized in two cohorts: 0:3:1 (aged ≥15years) or 3:3:2 (≥6years) to palovarotene 5/2.5mg, palovarotene 10/5mg or placebo, episodically for 2-weeks then 4-weeks. HO incidence and volume are assessed annually by low dose whole-body computer tomography and/or at 12-weeks during the course of flare-ups (defined as ≥2 [≥1 in OLE Part C] or pain, swelling, stiffness, decreased range of motion, redness, or warmth). Other clinical, functional and patient-reported outcomes are assessed using FOP-specific measures, including the Cumulative Analogue Joint Involvement Scale (CAJIS) and FOP Physical Function Questionnaire (FOP-PFQ). Studies were approved by independent ethics committees. 151 unique participants were enrolled.

Conclusions: Novel methodological approaches are needed to develop disease-modifying treatments for serious, ultra-rare diseases. HO is the main cause of disability in patients with FOP. This program may inform development of new treatments in rare diseases.

Table: Palovarotene dosing and trial participants

	NHS N=114	Phase II trial N=40	Phase II OLE			
			Part A N=40 [a]	Part B N=54 [b]		Part C N=48 [c]
Patient age/ skeletal maturity	0–65 years	≥6 years	≥6 years	≥6 years	≥6 years	≥6 years
Palovarotene dosing regimen	N/A	Episodic (2/4 weeks)	Episodic (2/4 weeks)	Episodic (≥4/8 weeks) [d]	Chronic (daily) + Episodic (≥4/8 weeks) [e]	Chronic (daily) + Episodic (≥4/8 weeks)
Palovarotene dose (mg)	None	5/2.5; 10/5; PBO	10/5	20/10	Chronic 5 + Episodic 20/10	Chronic 5 + Episodic 20/10
Randomization	N/A	Age ≥15 years: 0:3:1 Age ≥6 years: 3:3:2	N/A	N/A		N/A

Palovarotene doses were weight-adjusted in skeletally immature children; [a] Including all 40 pts from the phase II trial; [b] Including 36 pts from OLE Part A and 18 new pts; 52 pts received palovarotene [c] 48 pts from OLE Part B; [d] <90% skeletal maturity on hand/wrist radiography; [e] ≥90% skeletal maturity on hand/wrist radiography. FOP: fibrodysplasia ossificans progressiva; NHS: natural history study; OLE: open-label extension; PBO: placebo; pts: participants.

Management of hypercalcaemia due to hypervitaminosis D

Chamath Premawardena[1], Shanal Kumar[1, 2], Chin Yao Tan[1, 3]

1. Department of Medicine, Peninsula Health, Frankston, VIC, Australia

2. Monash Centre for Health Research and Implementation, Monash Health, Clayton, VIC, Australia

3. Diabetes and Vascular Medicine Unit, Monash Health, Clayton, VIC, Australia

Hypervitaminosis D is an uncommon cause of hypercalcaemia and is usually due to excessive oral supplementation. We report a case of an elderly woman who presented with symptomatic severe hypercalcaemia secondary to hypervitaminosis D that did not respond well to conventional therapy. Administration of single dose denosumab resulted in rapid improvement of hypercalcaemia which remained stable at outpatient follow-up with a decline in vitamin D level as well. There is little evidence for use of denosumab in hypercalcaemia not due to malignancy, so this case highlights its safe and effective use in this setting, which may be useful when other treatment is contraindicated.

Case Series: Denosumab and Atypical Femoral Fractures

Chamath Premawardena[1], Shanal Kumar[1, 2], Chin Yao Tan[1, 3]

1. Department of Medicine, Peninsula Health, Frankston, VIC, Australia

2. Monash Centre for Health Research and Implementation, Monash Health, Clayton, VIC, Australia

3. Diabetes and Vascular Medicine Unit, Monash Health, Clayton, VIC, Australia

Atypical femoral fracture (AFF) is a well-recognised adverse effect of long-term bisphosphonate use necessitating the incorporation of drug holidays during treatment of osteoporosis. Less commonly reported is AFF secondary to denosumab. Use of denosumab for the treatment of osteoporosis and prevention of skeletal related events in the primary care and oncological settings respectively is increasing. We present a series of three cases where AFF occurred in individuals treated with denosumab. This is followed by a discussion of potential risk factors and complexities surrounding the management of AFF, particularly with regards to cessation of denosumab. Our case series contributes to increasing clinician and consumer awareness of AFF secondary to denosumab use.

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Clots and bleeds in patients with hyperparathyroidism; What is the evidence?

Kavindra N Ratnaweera[1], Alexander J Rodriguez[2, 3]

1. School of Medicine, Griffith University, Southport, QLD, Australia

2. Bone and Muscle Health Research Group, School of Clinical Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia

3. Disorders of Mineralisation Research Group, School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia, Australia

Calcium is critical in coagulation. Parathyroid hormone (PTH) maintains calcium homeostasis. Elevated PTH increases cardiovascular risk, but it is unclear if these patients are predisposed to thrombosis possibly related to PTH action on calcium. We aimed to determine if PTH was associated with increased clots or markers of coagulation in a systematic review. We searched MEDLINE and EMBASE (29 March, 2020) for studies that were non-randomized; directly measured PTH levels or specifically enrolled patients with hyper/hypo-parathyroidism; measured any clotting factor; or reported thromboembolic or haemorrhagic events. We excluded interventional studies, case reports, studies of surgical correction of hyperparathyroidism or therapies that interfere with mineral metabolism or haemostasis. Primary outcome was the association between PTH and clotting factors and the association of PTH with the incidence of thromboembolic or haemorrhagic events. Continuous data were meta-analysed if reported in at least 100 patients in more than one study. Random-effects models were fitted and reported as standardized mean difference (SMD) with 95% confidence intervals (95%CI). Heterogeneity was determined by the I^2 statistic. All data were computed using R (4.0.0). 2404 records were screened. Eight were eligible for inclusion. Seven studies were cross-sectional analyses of patients with primary (PHPT) or secondary (SHPT) hyperparathyroidism compared to controls. Study quality was poor. In pooled analyses comparing PHPT to controls, there was no statistical difference in fibrinogen [SMD=0.01 (-0.92–0.94); k=3 trials; n=133 patients; I^2=86%]; D-dimer [0.46 (0.03–0.97); 3; 133; 52]; PAI [0.01 (-0.54–0.57); 3; 181; 69]. Other outcomes were reported in less than 100 patients as were outcomes in studies involving SHPT patients. There was little evidence to support an association between PTH and increased coagulation. Prospective data are needed to understand what role if any, PTH plays in coagulation and if patients with elevated or reduced PTH are predisposed to clots or bleeds.

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The effect of pathogen associated molecular patterns in skeletal muscle osteogenic cultures

Selwin G Samuel[1]

1. Mater Research Institute - UQ, Woolloongabba, Brisbane, QLD, Australia

Neurogenic Heterotopic Ossification (NHO) is a very incapacitating condition where ectopic bones form in skeletal muscles and joints of individuals who suffer spinal cord injury (SCI) and traumatic brain injury (TBI). The pathophysiology isn't still completely understood. As a result, clinicians are left with limited therapies and no prophylactic management. Due to this unmet clinical need, we aim to understand the mechanisms involved and identify the causative and exacerbating factors of NHO.

To elucidate NHO pathogenesis, we developed the first murine model of SCI-NHO, in which NHO is induced via a combination of cardiotoxin induced muscle injury and a SCI. Using this model, we recently established that fibroadipoprogenitors (FAP) are the locally derived cells-of-origin in muscles developing NHO.

Recent retrospective studies in SCI and TBI patients show that the presence of local or systemic infections is a significant risk factor of developing NHO. Pathogen Associated Molecular Patterns (PAMPs) are molecular structures found in microbes, which are recognised by our innate immune system via Pattern Recognition Receptors (PRRs). To investigate whether PAMPs exacerbate NHO directly via FAPs, we established *in vitro* osteogenic cultures of FAPs supplemented with purified synthetic and natural PAMPs from bacteria, fungi and virus.

After two weeks of osteogenic culture, we observed that PAMPs such as Pam2CSK4 which activates TLR2/TLR6 dimers, Pam3CSK4 which activates TLR1/TLR2 dimers and Zymosan which activates Dectin 1, Dectin 2 and TLR2/TLR6 dimers induced a dose-dependent increase in mineralisation. These studies suggest that PAMPs have the potential to exacerbate FAP mineralisation in vitro but further studies are needed to confirm a similar response *in vivo*.

New vertebral deformities lead to clinically important impairments in function and worsening quality of life in older women but not men over 10.7 years

Anuj Shah[1], Feitong Wu[1], Li Shean Toh[2], Flavia Cicuttini[3], Graeme Jones[1], Laura Laslett[1]

1. Menzies Institute for Medical Research, Univeristy of Tasmania, Hobart, TAS, Australia

2. School of Pharmacy, University of Nottingham, Nottingham, UK

3. Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

Purpose To examine changes in health-related quality of life (HRQoL) and functional ability associated with incident vertebral deformities (VD) in community dwelling older adults over 10.7 years.

Methods Participants (n=780) underwent whole-body dual-energy X-ray absorptiometry (DXA) scans at baseline, 2.5, 5.1 and 10.7 years later. VD was defined as ≥20% reduction in anterior height relative to posterior height of vertebrae from T4-L4. An incident VD was defined as new VD at any follow-up visit. Assessment of Quality of Life (AQoL-4D) questionnaire and Health Assessment Questionnaire Disability Index (HAQ-DI) were used to assess HRQoL and functional impairment. Change in AQoL and HAQ-DI scores associated with incident VD was analysed using multilevel mixed-effects linear regression and log binomial regression. Log binomial regression was also used to examine effects of severity and number of VD.

Results Incidence of VD was 53% over 10.7 years. Incident VD were associated with annual reduction in AQoL utility score (β=-0.003,95%CI -0.01 - -0.001) in women, but not men. Incident VD increased the risk of clinically significant reduction in HAQ-DI among women (RR=1.50,95%CI 1.02-2.20), not men (RR=1.24,95%CI 0.74-2.09). Women showed increased risk of functional impairment with mild VD (RR=1.61,95%CI 1.05-2.46), but not moderate or severe (RR=1.36,95%CI 0.83-2.21). There was a dose-response relationship with increasing number of incident VD and risk of functional impairment and among women (RR=1.19 (95%CI 0.77-1.85) for one, RR=2.08 (95%CI 1.25-3.46) for two and RR=2.30 (95%CI 1.03-5.11) for ≥3VD), but not men for one (RR=1.10(95%CI 0.58-2.09) for one, RR=1.70 (95%CI 0.89-3.25) for two and RR=1.10 (95%CI 0.41-2.99) for ≥3VD).

Conclusions Incident VD are associated with clinically significant functional impairment and worsening HRQoL in older women, but not men. Increasing number of incident VDs were associated with increased risk of functional impairment in women, but not men.

How well do low population-specific values for poor muscle strength and lean mass associate with indices of poor health? Cross-sectional data from the Geelong Osteoporosis Study

Sophia X. Sui[1], Kara L. Holloway-Kew[1], Natalie K. Hyde[1], Lana J. Williams[1], Monica C. Tembo[1], Sarah Leach[2], Julie A. Pasco[1, 3, 4, 5]

1. Deakin University, Geelong, VIC, Australia

2. GMHBA, 2Geelong Medical and Hospital Benefits Association (GMHBA), Geelong, Vic, Australia

3. St Albans, The university of Melbourne, Melbourne, Vic, Australia

4. Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, VIC, Australia

5. University Hospital Geelong, Barwon Health, Geelong, VIC, Australia

Background: We aimed to examine associations between measures of skeletal muscle deficits and indices of poor health, using cut-points that identify low values in the distribution of the same population in comparison with cut-points from EWGSOP2 and FNIH.

Methods: Participants (n=665; 323 women) aged 60-96yr were from the Geelong Osteoporosis Study. Handgrip strength (HGS) was measured by dynamometers and appendicular lean mass (ALM) by DXA. Cut-points equivalent to 2SDs below the mean young reference range from the same population were (for men and women): low ALM/height^2 <5.30kg/m^2, <6.94kg/m^2; low ALM/BMI <0.512m^2, <0.827m^2; low HGS <16kg, <31kg. Cut-points for EWGSOP2 were ALM/height^2 <5.50kg, <7.0kg/m^2; HGS <16kg, <27kg. For FNIH, ALM/BMI <0.512m^2, <0.789m^2; HGS <16kg, <26kg. Indices of poor health included poor physical performance, fractures, falls and hospitalisations. Poor physical performance was identified as timed-up-and-go (TUG, 3m) >10s. Falls (at least one) in the past 12 months, fractures (at least one since age≥20yr, all sites and causes) and hospitalisations in the past month were self-reported. Logistic regression models (age and sex-adjusted) were used to identify to examine associations.

Results: There were 177 (25.3%) participants with falls, 189 (28.1%) with fractures including 20 rib 17 humerus, 15 tibia, 28 forearm/wrist, 14 ankle, 14 toe, and 34 others, 48 (6.9%) with hospitalisations and 233 (34.9%) with slow TUG. For all cut-points, low HGS was consistently associated with falls and slow TUG (Figure). Low ALM/BMI, using both population-specific and FNIH criteria, was associated with slow TUG. There was little evidence to support an association between ALM/height^2, using any cut-point, and indices of poor health.

Conclusions: Overall, muscle strength performed better than lean mass measures for indicating poor indices of health. These findings add to the growing evidence base to inform decisions regarding the selection of skeletal muscle parameters and their optimal cut-points for identifying sarcopenia.

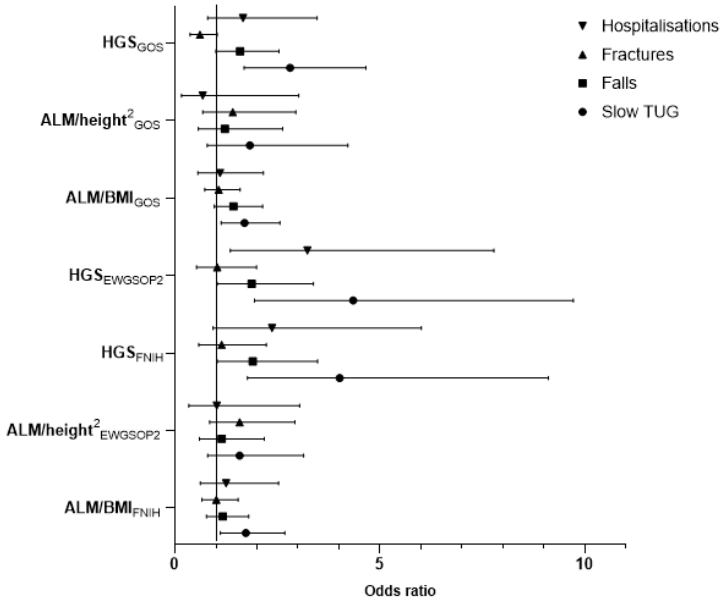

Figure Odds ratios (OR 95% confidence interval) of binary logistic age and sex-adjusted models for the associations between low muscle parameters and indices of poor health.
GOS: Geelong Osteoporosis Study

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Yoga- A Complementary Medicine for Juvenile Idiopathic Arthritis (JIA)?

Sukesh Sukumaran[1], Vini Vijayan[1], Aiswarya Premkumar[2], Niveditha Sukesh[3], Devi Asokan[4]

1. Valley Children's Health Care, Madera, CALIFORNIA, United States

2. Medical College Paripally, Medical Student , Kollam, Kerala , India

3. Student , Buchanan High School, Clovis, California, USA

4. Pulmonology, Lisie Hospital, Ernakulam, Kerala, India

Background: Yoga is practiced in ancient India for more than 5,000 years. The description of yoga is seen in Hindu textbooks as old as Vedas and Upanishads. Yoga is defined as the union of one with many. It is also described as the union of athma, the consciousness in one, with paramathma, the super consciousness or super soul. Yoga is becoming increasingly popular in the western world.

Objectives: We present a case study on a 15 year old Hispanic female with RF positive, CCP positive Poly articular Juvenile Idiopathic Arthritis. We also performed a PubMed search for the past 20 years. The purpose of this review is to identify the heterogeneity of its practice.

Methods: We evaluated the impact of Ashtanga Yoga with pranayama intervention over 6 month in this patient. We investigated the usefulness of Yoga in stiffness and pain, independence, health related quality of life and disease activity. Patient participated in weekly Yoga session and daily practice for 15- 20 minutes. She completed questionnaires assessing psychosocial functioning and disease activity, daily pain scale and pre- and post-intervention at six month follow-up.

Results: The outcomes were evaluated using quasi-experimental single-case design structure. Her case showed yoga reduced pain and stiffness intensity and duration of morning stiffness. Disease activity and psychosocial outcome measures also suggested improvements. This anecdotal report indicated acceptability of Yoga and improvements in pain and stiffness due to yoga intervention.

Conclusion: Yoga can be used as a complementary practice along with conventional treatment for JIA. The use of yoga is increasing in western world compared to the early 1990s. Yoga is used mainly in the chronic pain conditions and also in stress related conditions associated with JIA. However, there was significant heterogeneity in the study designs, interventions and type of the yoga practice and research methods.

Prevalence of frailty in older men and women: cross-sectional data from the Geelong Osteoporosis Study

Monica C. Tembo[1], Kara L. Holloway-Kew[1], Sophia X. Sui[1], Trisha Dunning[1], Adrian C.H. Low[2], Shi-Jynn Yong[2], Boon L. Ng[2], Sharon L. Brennan-Olsen[1, 3, 4], Lana J. Williams[1], Mark A. Kotowicz[1, 2], Julie A. Pasco[1, 2]

1. Deakin University, Geelong, VICTORIA, Australia

2. Barwon Health, Geelong, Victoria, Australia

3. Australian Institute for Musculoskeletal Science (AIMSS), The University of Melbourne, St Albans, Melbourne, Victoria, Australia

4. Melbourne Medical School- Western Campus, The University of Melbourne,St Albans, Melbourne, Victoria, Australia

Objectives

We aimed to determine the prevalence of frailty in a population-based sample of older adults (ages ≥60yr) and examine the relationship between frailty and comorbid conditions.

Methods

Men (n=347) and women (n=360) were from the Geelong Osteoporosis Study (GOS). A modified Fried frailty phenotype identified frailty, including unintentional weight loss, weakness, low physical activity, exhaustion and slowness; frail ≥3 items and pre-frail 1-2 items. Prevalence estimates were standardised to the 2011 Australian population. Kruskal-Wallis test and chi-square test identified intergroup differences. Binary logistic regression models, using the robust group as referent, adjusted for age and body mass index (BMI) were constructed to investigate associations between frailty groups and comorbidities.

Results

For women, mean standardised prevalence estimates were 18.3% (14.1-22.5) for frail, 54.1% (47.3-60.8) pre-frail and 22.9% (18.9-26.8) robust; for men estimates were 13.1% (9.8-16.3), 47.8% (42.0-53.6) and 27.3% (22.7-31.8) respectively. Women who were frail were older, shorter, tended to have a higher BMI and used more medications than other groups. Compared to robust women, those who were frail were more likely to have cardio-metabolic (OR 3.5 (95%CI 0.7-20.0)), pulmonary (3.5 (1.5-8.4)) and musculoskeletal (10.1 (2.1-48.0)) conditions. Frail men were older, had a higher BMI and tended to be from a lower SES. Frail men were more likely to have musculoskeletal conditions (5.8 (2.8-12.3)) compared to robust men. No other associations were observed.

Conclusion

Approximately half of adults aged ≥60yr were pre-frail, and 14.1-22.5% of women and 9.8-16.3% of men were frail. Frailty was associated with musculoskeletal conditions for both sexes; however, associations with cardio-metabolic and pulmonary comorbidities were evident in women only. These observations have important implications as they highlight areas of possible interventions for decreasing frailty progression, given the association between frailty and comorbid conditions in the context of an ageing population.

Population vitamin D stores are increasing in Tasmania and this is associated with less BMD loss over 10 years

Michael Thompson[1], Dawn Aitken[1], Saliu Balogun[1], Flavia Cicuttini[2], Graeme Jones[1]

1. Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

2. Musculoskeletal Unit, Monash University, Melbourne, Victoria, Australia

Introduction

Vitamin D deficiency is a prevalent, modifiable determinant of poor musculoskeletal health. Public health interventions have aimed to improve population vitamin D status. However, there is minimal longitudinal data assessing whether these programs have improved vitamin D sufficiency or evaluating the skeletal health outcomes of maintaining or achieving vitamin D sufficiency.

Methods

Community-dwelling adults aged 50-80 years had 25-hydroxyvitamin D (25(OH)D) assessed by radioimmunoassay at baseline (*n*=1096), 2.5 (*n*=870) and 10 (*n*=565) years in a Tasmanian population-based prospective cohort study. Sun exposure was quantified by questionnaire, supplement use at clinic review and BMD by DXA. 25(OH)D <50nmol/L was considered deficient.

Results

Over 10 years mean 25(OH)D increased (52.4±18.7 *to* 62.5±23.5 nmol/L, $p<0.001$) and the percentage of vitamin D deficient participants decreased (48.5% *to* 29.8%, $p<0.001$). Participants with baseline deficiency had larger 25(OH)D increases than baseline sufficient participants (19.2±25.3 *vs* 1.6±23.3 nmol/L, $p<0.001$). The proportion of participants taking vitamin D supplements increased (2.3% *to* 37.1%, $p<0.001$). Longitudinal change in 25(OH)D was associated with baseline summer ($\beta=1.46$, $p<0.001$) and winter ($\beta=1.29$, $p=0.003$) sun exposure, change in summer ($\beta=1.27$, $p=0.002$) and winter ($\beta=1.47$, $p<0.001$) sun exposure and vitamin D supplement use ($\beta=24.9\text{-}34.8$, $p<0.001$). Participants who were always vitamin D sufficient retained significantly more BMD at all sites ($\beta=0.019 – 0.030$, $p=0.006 – 0.047$ *vs* always deficient group). Participants who achieved vitamin D sufficiency had greater BMD gains at the lumbar spine ($\beta=0.019$, $p=0.02$) and similar BMD outcomes elsewhere compared to those who were always sufficient.

Conclusions

Cohort 25(OH)D concentration increased and vitamin D sufficiency was associated with less BMD loss over 10 years. This suggests that (1) intervention can improve population vitamin D status, (2) vitamin D sufficiency may ameliorate BMD loss in older adults and (3) vitamin D repletion of deficient individuals can achieve similar longitudinal BMD outcomes as persistent sufficiency.

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Deletion of the non-coding exon 1 in *AMER1* causes osteopathia striata with cranial sclerosis

Jingyi Mi[1], Padmini Parthasarathy[1], Benjamin J Halliday[1], Tim Morgan[1], John Dean[2], Malgorzata JM Nowaczyk[3], David Markie[1], Stephen P Robertson[1], Emma M Wade[1]

1. University of Otago, Dunedin, OTAGO, New Zealand

2. North of Scotland Regional Genetics Service, NHS Grampian, Aberdeen, Scotland

3. Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Osteopathia striata with cranial sclerosis (OSCS) is an X-linked dominant condition characterised by metaphyseal striations, macrocephaly, cleft palate and developmental delay in affected females. Males have a more severe phenotype with multi-organ malformations, and rarely survive. To date, only frameshift and nonsense variants in the single coding exon of *AMER1*, or whole gene deletions have been reported to cause OSCS. In this study, we describe two families with phenotypic features typical of OSCS. Exome sequencing and multiplex ligation-dependent probe amplification (MLPA) did not identify pathogenic variants in *AMER1*. Therefore genome sequencing was employed which identified two deletions containing the non-coding exon 1 of *AMER1* in the families. These families highlight the importance of considering variants or deletions of upstream non-coding exons in conditions such as OSCS, noting that often such exons are not captured on probe or enrichment based platforms because of their high G/C content.

Adult-onset hypophosphatasia diagnosed following bilateral atypical femoral fractures after denosumab

Annabelle M Warren[1], Shoshana Sztal-Mazer[1]

1. The Alfred Hospital, Melbourne, VIC, Australia

Hypophosphatasia (HPP) is a rare genetic disorder affecting mineralisation of bone due to a defect in the ALPL gene leading to deficiency of alkaline phosphatase (ALP). There is a spectrum of severity from severe childhood-onset form occurring in 1/100,000, to more common and less severe forms detected in adulthood in 1/2500. Hypophosphatasia is increasingly recognised in adults misdiagnosed with severe osteoporosis. These patients are known to have a significantly increased risk of atypical femoral fracture (AFF) after bisphosphonate therapy, but AFF after denosumab in hypophosphatasia is a less established risk.

A 40-year-old woman was referred to our osteoporosis clinic after sustaining bilateral AFFs after 4 years of denosumab therapy after minimal trauma. A symptomatic complete right midshaft femoral fracture was surgically fixed in December 2018, and an asymptomatic left contralateral partial AFF was conservatively managed. Denosumab was discontinued. Past medical history included metatarsal fracture age 25, complicated by delayed healing, and seizure at age 28 manged with valproate.

DXA in February 2019 showed lumbar spine T-score of 0.8 and a left total hip T-score of -1.7. Bloods October 2019 showed normal serum calcium, parathyroid hormone and renal function, and replete vitamin D. Serum ALP was low at 11 U/L (30 – 110). Vitamin B6 was elevated >2000 nmol/L (35 – 110) which, together with the low ALP, supports a diagnosis of hypophosphatasia. Confirmatory ALPL gene testing revealed two ALPL variants consistent with recessive hypophosphatasia. The patient commenced teriparatide treatment with plans to apply for special access to asfotase alpha enzyme therapy in the future.

Hypophosphatasia is a rare but under-diagnosed genetic cause of osteomalacia which can masquerade as osteoporosis. Low serum ALP is suggestive of this diagnosis. Clinicians should be aware that hypophosphatasia can be misdiagnosed as antiresorptive therapy related AFF and that both bisphosphanates and denosumab are contraindicated in this condition.

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Prolactinoma causing osteoporosis in a young male

Chamica W Gunawardena[1]

1. Endocrinology, Gold Coast University Hospital, Southport, QLD, Australia

A 58 year man presented with multiple rib and vertebral fractures secondary to minor trauma. His Bone Mineral density (BMD) in August 2018 showed severe osteoporosis with a femoral neck T score of -4.22 and lumbar spine T score of -2.04.

His secondary screening showed borderline low testosterone at 9.0nmol/L (9-35nmol/L) and low normal FSH of 4.6U/L and LH 3.5U/L. His prolactin was elevated at 6420mU/L (56-278mU/L) which was more than 10 times the upper limit of normal. He subsequently underwent an MRI of his pituitary which showed a 15mm macroprolactinoma

He was commenced on cabergoline 0.25mg twice weekly and Alendronate weekly. His prolactin levels normalized to 17mU/L and his testosterone improved to 13nmol/L.

Hyperprolactinaemia is an unusual cause of secondary osteoporosis in men. The pathogenesis is uncertain. It is unclear whether prolactin directly enhances bone loss or it is mediated by hypogonadism. In females the incidence of secondary osteoporosis correlates with amenorrhoea. However one small study that included male patients with prolactinomas showed no association between the incidence of osteoporotic fractures and serum testosterone levels, suggesting that prolactin has direct effect on bone loss.

Management includes normalization of prolactin levels. However normalization of prolactin is inadequate to correct the osteoporosis. One study showed that despite correction of hyperprolactinaemia and normalization of gonadal function bone density improvement was minimum. Therefore treatment in these patients should not only include dopamine agonists but also antiresorptive agents to improve fracture risk.

Bipolar disorder and bone quality

Lana J Williams[1], Amanda L Stuart[1], Michael Berk[1], Sharon L Brennan-Olsen [1], Jason M Hodge[2], Stephanie P Cowdery [1], Veena Chandrasekaran [1], Jasmine R Cleminson[1], Behnaz A Azimi Manavi[1], Mark A Kotowicz [2], Julie A Pasco[1]

1. Deakin University, Geelong, VIC, Australia

2. Barwon Health, Geelong, VICTORIA, Australia

Aim: Bipolar disorder is associated with significant psychological and physical comorbidity. Yet little is known about the bone health of adults with bipolar disorder. Thus, we aimed to investigate the association between bipolar disorder and bone quality in women.

Method: Women with a history of bipolar disorder (n=117) were recruited from the Barwon Statistical Division. Controls, without bipolar disorder, were drawn from the Geelong Osteoporosis Study (n=909). Bipolar disorder was identified using a semi-structured clinical interview (SCID-I/NP). Bone quality was determined by Quantitative heel ultrasound (Achilles Express, GE Medical Systems) and included the following parameters: Speed of Sound (SOS), Broadband Ultrasound Attenuation (BUA) and Stiffness Index (SI). Weight and height were measured and information on medication use and lifestyle variables were obtained via questionnaire. Linear regression models were used to test associations, after adjusting for age and weight.

Results: Those with bipolar disorder were heavier, less active, more likely to smoke, take psychotropic medication and have lower SOS and SI compared to controls (all p<0.003); otherwise the groups were similar in age, height and BUA. After adjustments, bipolar disorder was associated with lower adjusted mean SOS [1559.1 (95%CI 1552.4-1565.8) vs 1576.2 (95%CI 1573.8-1578.6) m/sec, p=<0.001], BUA [109.2 (95%CI 106.5-111.8) vs 112.8 (95%CI 111.9-113.7) dB/MHz, p=0.01] and SI [88.7 (95%CI 85.4-92.1) vs 96.6 (95%CI 95.4-97.7) %, p=<0.001] compared to controls. These associations persisted after further adjustment for smoking, physical activity and psychotropic medications.

Conclusion: These population-based data suggest bipolar disorder is associated with poor bone quality, as measured by QUS, in women. Given the dearth of literature, replication and research into underlying mechanisms are warranted.

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Atypical femur fracture with short term bisphosphonate and 36 months of denosumab therapy

Yu-Fang Wu[1], Christian Girgis[2], Veronica Preda[1]

1. Department of Endocrinology, Macquarie University Hospital, Sydney, NSW, Australia

2. Department of Endocrinology, Westmead Hospital, Sydney, NSW, Australia

Atypical femur fractures (AFFs) are rare adverse events linked to chronic bisphosphonate therapy for osteoporosis (median treatment seven years)(1). AFFs have also been reported in patients on denosumab, a monoclonal antibody that reduces osteoclastic activity. Patients typically present with prodromal symptoms of thigh or groin pain and diagnosis is made on plain x-ray, bone scan, CT or MRI. Immediate management of AFFs includes cessation of antiresorptive therapy, calcium and vitamin D supplementation, modified physical activity, analgesia and surgical intervention as required. Ongoing management of bone health post AFF is challenging, particularly in patients treated with denosumab where discontinuation results in acute bone loss (2). Options for further osteoporosis therapy include raloxifene and calcitriol, both have been shown to improve bone mineral density (BMD) and not associated with AFF. Parathyroid hormone has been used to advance healing (3) and drug holiday may be appropriate. Regardless, the benefit of antiresorptive therapy for prevention of osteoporotic fractures outweighs the risk of AFFs (4). We present a case of a 59 year old menopausal woman of Sri Lankan heritage with an atypical left subtrochanteric fracture in the context of a single zoledronic acid infusion followed by 36 months of denosumab therapy for osteoporosis. A cortical lesion in the right medial femur was found incidentally albeit an unusual location for an AFF. Acutely she was managed with prophylactic left intramedullary nailing. A therapeutic dilemma ensued from the cessation of denosumab for her AFF and risk of contralateral femoral fracture, and mitigating the risk of rebound bone loss and osteoporotic fracture. She was commenced on raloxifene, calcitriol and calcium supplementation. Interval dual-energy x-ray absorptiometry (DEXA) at 12 months showed stable BMD. Regular follow up on metabolic bone biochemistry and BMD along with close monitoring of at risk changes on the contralateral side are crucial.

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Severe urticarial rash secondary to internet-bought strontium citrate for osteoporosis management

Hannah Youn[1], Rinky Giri[1], Frances Milat[2, 3, 1]

1. Department of Endocrinology, Monash Health, Clayton, Victoria, Australia

2. Hudson Institute of Medical Research, Clayton, Victoria, Australia

3. Department of Medicine, School of Clinical Sciences, Clayton, Victoria, Australia

Strontium ranelate (SR) rose to popularity in the early 2000s with its promising benefits in osteoporosis management and fracture reduction. However, subsequent studies demonstrating increased risk of thrombo-embolism, cardiovascular disease and stroke have led to discontinuation of SR worldwide.(1-3) Cases of drug rash with eosinophilia and systemic symptoms (DRESS) were additionally reported with SR use. Patients with DRESS present with worsening rash, facial oedema, lymphadenopathy, elevated eosinophils and inflammatory markers which commence three to four weeks after SR initiation and require several weeks of tapering high-dose steroids to resolve.(4,5)

An alternative formulation of strontium, strontium citrate (Algaecal Strontium Boost), is currently sold over the internet as a supplement for managing low bone mineral density. Few studies have examined the safety of strontium citrate (SC); a 7-year longitudinal study funded by Algaecal reported nil adverse effects.(6) We present a case of a 30-year-old woman who obtained Algaecal Strontium Boost online for treatment of her osteoporosis, on a background of cerebral palsy, epilepsy, chronic kidney disease (secondary to reflux nephropathy) and subclinical hypothyroidism. She presented to the emergency department with worsening urticarial, maculopapular rash and mild lip swelling after two weeks of daily intake. She was afebrile, had normal inflammatory markers and normal eosinophil count. A skin biopsy demonstrated changes consistent with a drug reaction. She was commenced on oral prednisolone 100mg daily which was weaned and discontinued over one to two weeks. SC was also discontinued and the patient was prescribed topical Diprosone and Hydrocortisone therapy. Outpatient review one month afterwards reassuringly found almost complete resolution of her symptoms. Patients should be aware of the potential risk of skin eruptions with strontium citrate and should discontinue and urgently seek medical advice if it occurs. Patients with osteoporosis are encouraged to discuss management options with their treating clinician.

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In Pediatric X-linked Hypophosphatemia (XLH), Burosumab Improved Clinical Outcomes Versus Higher and Lower Doses of Oral Phosphate and/or Active Vitamin D

Erik A Imel[1], Francis H Glorieux[2], Michael P Whyte[3], Anthony A Portale[4], Craig F Munns[5], Ola Nilsson[6], Jill H Simmons[7], Raja Padidela[8], Noriyuki Namba[9, 10], Hae Il Cheong[11], Pisit Pitukcheewanont[12], Etienne Sochett[13], Wolfgang Högler[14], Koji Muroya[15], Hiroyuki Tanaka[16], Gary S Gottesman[3], Andrew Biggin[5], Farzana Perwad[4], Angel Chen[17], Mary Scott Roberts[17], Leanne Ward[18]

1. Indiana University School of Medicine, Indianapolis, IN, United States

2. Shriners Hospitals for Children, Montreal, Canada

3. Shriners Hospitals for Children St Louis, St Louis, MO, United States

4. Department of Pediatrics, University of California, San Francisco, CA, USA

5. The Children's Hospital at Westmead, Westmead, NSW, Australia

6. Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

7. Department of Pediatrics, Division of Endocrinology and Diabetes, Vanderbilt University School of Medicine, Vanderbilt University, Nashville, TN, United States

8. Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, United Kingdom

9. Osaka Hospital, Japan Community Healthcare Organization and Osaka University Graduate School of Medicine, Osaka, Japan

10. Tottori University Faculty of Medicine, Yonago, Japan

11. Seoul National University Children's Hospital, Seoul, Korea

12. Center of Endocrinology, Diabetes and Metabolism, Children's Hospital of Los Angeles, Los Angeles, CA, United States

13. Department of Pediatrics, Hospital for Sick Children, Toronto, ON, Canada

14. Department of Paediatrics and Adolescent Medicine, Johannes Kepler University Linz, Linz, Austria

15. Kanagawa Children's Medical Center, Yokohama, Japan

16. Okayama Saiseikai General Hospital Outpatient Center, Okayama , Japan

17. Ultragenyx Pharmaceutical Inc., Novato, CA, USA

18. University of Ottawa, Ottawa, Ontario, Canada

In a phase 3 trial in children with XLH (CL301; NCT02915705), burosumab resulted in greater improvements in hypophosphatemia and related morbidities than continuation with oral phosphate and active vitamin D (Pi/D). Here, we present a post-hoc analysis assessing the response to burosumab vs higher or lower average daily doses of Pi/D over 64 weeks.

Children (1-12 years-old) with XLH receiving Pi/D with Rickets Severity Score ≥2.0 were randomized 1:1 to 64 weeks of burosumab starting at 0.8 mg/kg subcutaneously Q2W or to resume Pi/D. Pi/D dose categories were assessed as: phosphate >40 mg/kg (HPi), phosphate ≤40 mg/kg (LPi), alfacalcidol >60 ng/kg or calcitriol >30 ng/kg (HD), and alfacalcidol ≤60 ng/kg or calcitriol ≤30 ng/kg (LD). We used the Radiographic Global Impression of Change (RGI-C) to assess rickets healing at 64 weeks based on Pi/D dose categories before (pre-baseline) and during study.

The distribution of children in the Pi/D dose categories pre-baseline were: 8 HPi, 21 LPi, 7 HD, and 22 LD in the burosumab group (n=29), and 3 HPi, 29 LPi, 7 HD, and 25 LD in the Pi/D group (n=32). On-study, the distribution was 12 HPi, 20 LPi, 14 HD, and 18 LD in the Pi/D group.

Regardless of Pi/D doses administered pre-baseline, children receiving burosumab had an approximately two-fold greater improvement in least square (LS) mean RGI-C score for rickets healing vs those continuing Pi/D (Table). Further, at Week 64, the LS mean RGI-C score with burosumab (2.1 ±0.1) was greater vs on-study treatment with HPi (1.0 ±0.2), LPi (1.0 ±0.2), HD (1.5 ±0.2), or LD (0.7 ±0.2). The LS mean improvement in lower limb deformity RGI-C with burosumab (+1.3 ±0.2) was greater vs HPi (+0.3 ±0.2), LPi (+0.3 ±0.1), HD (+0.4 ±0.2), or LD (+0.2 ±0.1). The LS mean alkaline phosphatase (U/L) decrease from baseline with burosumab was -174 ±14 vs HPi (-43 ±29), LPi (-19 ±28), HD (-59 ±30), or LD (-4 ±24). The mean parathyroid hormone (PTH) percent change from baseline with burosumab was +10.5 ±13.3 vs HPi (+45.2 ±41.0), LPi (-9.5 ±13.1), HD (-29.3 ±15.5), or LD (+42.5 ±27.2). Adverse events on burosumab included mild to moderate hypersensitivity and injection site reactions. No discontinuations occurred.

In children with XLH, improvements in rickets, lower limb deformity, and decreases in ALP were greater during burosumab than with either higher or lower on-study doses of phosphate or active vitamin D. The increase in PTH was greatest in the HPi and LD groups.

Table: Radiographic Global Impression of Change (RGI-C) for rickets healing by treatment categories

	Burosumab (N=29)		Pi/D (N=32)	
Pre-baseline Dose Categories				
	HPi (n=8)	**LPi (n=21)**	**HPi (n=3)**	**LPi (n=29)**
Week 64 RGI-C, LS mean ±SE	1.8 ±0.2	2.1 ±0.1	0.7 ±0.2	1.1 ±0.1
	HD (n=7)	**LD (n=22)**	**HD (n=7)**	**LD (n=25)**
Week 64 RGI-C, LS mean ±SE	1.9 ±0.2	2.1 ±0.1	1.1 ±0.2	1.0 ±0.2
On-study Dose Categories				
	Burosumab (n=29)		**HPi (n=12)**	**LPi (n=20)**
Week 64 RGI-C, LS mean ±SE	2.1 ±0.1		1.0 ±0.2	1.0 ±0.2
			HD (n=14)	**LD (n=18)**
Week 64 RGI-C, LS mean ±SE			1.5 ±0.2	0.7 ±0.2

HPi: phosphate >40 mg/kg, LPi: phosphate ≤40 mg/kg, HD: alfacalcidol >60 ng/kg or calcitriol >30 ng/kg, LD: alfacalcidol ≤60 ng/kg or calcitriol ≤30 ng/kg; LS: least square.

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- Andrew Biggin has no conflicts of interest to declare.
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Non-invasive imaging in fracture risk assessment

Mary L Bouxsein[1]

1. Professor, Department of Orthopedic Surgery, Harvard Medical School

Bone mineral density measurements by DXA are a key component in managing osteoporosis patients.[1] For decades we have known that low bone mineral density values predict increased fracture risk.[2,3] Yet, more than 50% of the fractures occur in women who do not have "osteoporosis" by BMD testing.[4] As bone strength is determined not only by bone mass, but also by geometry, microstructure and the intrinsic properties of the bone matrix, this observation suggests that in addition to low bone mass, deficits in these other traits may contribute to skeletal fragility.[5] Recent technologies allow non-invasive assessment of bone microstructure and bone strength, via finite element analysis.[6] Indeed, recent studies indicate that deficits in bone microstructure contribute to skeletal fragility independently of DXA-BMD.[7] We have used machine-learning approaches to demonstrate that individuals exhibit different "fragility phenotypes" — some with preserved cortical bone, but trabecular deficits and vice-versa. Recent studies have also demonstrated that bone strength measures from computed tomography (CT) predict fracture as well as, or better than BMD.[8,9] Accordingly, opportunistic CT — namely the use of CT scans acquired for other medical reasons — may be an efficient means to identify individuals at high risk for fracture.[10] Finally, this presentation will review recent work by the Foundation for the NIH SABRE (Study to Advance BMD as a Regulatory Endpoint) project, which aims to validate total hip BMD as a surrogate endpoint for fracture in order to facilitate future clinical trials in osteoporosis.

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