PAR2 response in fluid flow-stimulated chondrocytes

Huesa, Carmen; Williamson, E; Kreuser, U; Litherland, Gary; Lockhart, John; Ferrell, William R.

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September 1-3, 2015
Edinburgh, UK

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The Bone Research Society and British Society for Matrix Biology are extremely grateful to the following organisations for their support. The Scientific Programme has been developed independently by the Organisers under no influence from any supporting organisation or their employees. Any funding offered has been used for the sole purpose of supporting the educational aspects of the meeting.

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www.boneresearchsociety.org

The Society (formerly known as the Bone and Tooth Society) is the oldest and largest national scientific society in Europe dedicated to further research into clinical and basic science problems related to mineralised tissues. The BRS Annual Meeting attracts a wide audience from throughout the UK and also from continental Europe and further afield. The presentations are traditionally a balance between clinical and laboratory-based studies. The participation of young scientists and clinicians is actively encouraged. For membership enquiries email info@brsoc.org.uk

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BRS 2016
Liverpool, 29 June – 1 July 2016

For further details on all BRS activities and events please see www.boneresearchsociety.org

British Society for Matrix Biology

www.bsmb.ac.uk

Membership
For membership enquiries please see website.

Future meetings
Advances in Tendon Research: From Bench to Bedside
London, 7-8 September 2015

BSMB Spring Meeting
Chester 4-5 April 2016

For further details on all BSMB activities, awards and events please see www.bsmb.ac.uk

2015 Meeting

Local Organising Committee
Tom Van Agtmael (Co-chair)
Vicky MacRae (Co-chair)
Stuart Ralston (Co-chair)
Faisal Ahmed
David Hamilton
Hamish Simpson
Katherine Staines
Award winners

Best Oral Communication

OC22 – Simon Roberts (Edinburgh) - TRIM32 knockout mice develop accelerated osteoarthritis of the knee joint after destabilisation of the medial meniscus (DMM) surgery and upon ageing

OC24 - Aaron Murphy (Bristol) - High bone mass is associated with bone-forming features of osteoarthritis in non-weight bearing joints and independent of body mass index

OC28 - John Logan (London) - The origins of bone and cartilage disease: high throughput bone phenotype screen to identify new genes that determine bone structure and strength

Best Oral Poster

OP3 - Kim Askew (Lexington) - ENPP1 enzyme replacement therapy for generalized arterial calcification of infancy

Best Poster

LB2 - Mark Ditzel (Edinburgh) - Regulation of articular cartilage homeostasis by the N-end rule ubiquitin-protein ligase UBR5

LB6 - Owen Davies (Loughborough) - Modulation of ectopic ossification in tissue engineered skeletal muscle by an inflammatory environment

Rare Bone Diseases Workshop – Best Presentation

P53 - Leah Taylor (Liverpool) - Progression of osteoarthropathy in alkaptonuria patients monitored by 18F–NaF PET

Muscle and Bone Workshop – Best Presentation

P45 - Niina Hopper (Cambridge) - The role of osteocytes in targeted remodelling of third metacarpal bone in the TB racehorse

New Investigator Awards:
(awarded prior to the meeting, based on scores achieved during the blind review process)

OC6 - Siobhan Webb (Oxford) - The anti-diabetic drug metformin reduces tumour burden and osteolytic bone disease in multiple myeloma in vivo

OC11 - Neil Thomas (Liverpool) - Identification of high density mineralised protrusions (HDMPs) in ex-vivo human knee joints

OC14 - Pradeep Sacitharan (Oxford) - Loss of SirT1 dysregulates chondrocytes and leads to an arthritic phenotype in vivo, through decreased control of autophagy

OC17 - Mark Edwards (Southampton) - Relationships between DNA methylation and bone mineral content from an epigenome wide association study in the Hertfordshire Cohort Study

OC35 - Karla Oldknow (Edinburgh) - Endocrine role of bone: PHOSPHO1 a novel regulator of energy metabolism

ASBMR Travel Grants:
(awarded prior to the meeting to New Investigator ASBMR members residing outside of the UK to attend the Edinburgh meeting. Awards based on scores achieved during the blind review process)

OC10 - Megan Weivoda (Rochester, USA) - Reduced osteoclast TGFβ signaling with age impairs the coupling of bone resorption to bone formation

OC23 - Jawed Siddiqui (New York, USA) - Regulation of PTH-induced bone loss: a role for monocyte chemoattractant protein-1
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>12:00-13:35</td>
<td><strong>New Investigator Session</strong></td>
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<tr>
<td></td>
<td><em>Pentland</em></td>
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<tr>
<td>12:00</td>
<td>How to make the internet work for you in research</td>
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<tr>
<td></td>
<td><strong>Brooke Simmons (Oxford, UK)</strong></td>
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<tr>
<td>12:55</td>
<td>CV clinic</td>
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<td></td>
<td><strong>Short presentations on good and bad practice for CVs and an opportunity for New Investigators to have their CVs appraised</strong></td>
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<tr>
<td></td>
<td><strong>Mark Edwards (Southampton, UK)/Adam Taylor (Lancaster, UK)</strong></td>
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<tr>
<td>13:15-13:50</td>
<td>Registration and coffee</td>
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<tr>
<td>13:50-14:00</td>
<td>Welcome and opening remarks</td>
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<td></td>
<td><em>Pentland</em></td>
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<td></td>
<td><strong>Vicky MacRae (Edinburgh, UK)/Tom Van Agtmael (Glasgow, UK)</strong></td>
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<tr>
<td>14:00-15:30</td>
<td><strong>Symposium 1 (plenary)</strong></td>
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<td><em>Pentland</em></td>
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<td></td>
<td><strong>Genetics of musculoskeletal disease</strong></td>
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<tr>
<td>14:00</td>
<td>IS1</td>
</tr>
<tr>
<td></td>
<td>Sclerosing bone dysplasias: low prevalence but high relevance</td>
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<td></td>
<td><strong>Wim Van Hul (Antwerp, Belgium)</strong></td>
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<tr>
<td>14:30</td>
<td>IS2</td>
</tr>
<tr>
<td></td>
<td>Genetics of osteoarthritis</td>
</tr>
<tr>
<td></td>
<td><strong>Joyce van Meurs (Rotterdam, Netherlands)</strong></td>
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<tr>
<td>15:00</td>
<td><strong>Oral communications</strong></td>
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<tr>
<td>15:00</td>
<td>OC1</td>
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<tr>
<td></td>
<td>Targeted sequencing of the Paget’s disease associated 14q32 locus identifies several missense coding variants in RIN3 that predispose to Paget’s disease of bone</td>
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<tr>
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<td><strong>OME Albagha (Edinburgh, UK)</strong></td>
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<tr>
<td>15:15</td>
<td>OC2</td>
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<tr>
<td></td>
<td>Molecular mechanisms provide new insight on genotype to phenotype correlations in type II collagenopathies</td>
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<tr>
<td></td>
<td><strong>RM Jackson (Newcastle upon Tyne, UK)</strong></td>
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<tr>
<td>15:30-16.00</td>
<td>Coffee</td>
</tr>
<tr>
<td>16:00-16:45</td>
<td><strong>BRS Dent Lecture</strong></td>
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<td><em>Pentland</em></td>
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<td></td>
<td><strong>Chairs: Miep Helfrich (Aberdeen, UK)/Eugene McCloskey (Sheffield, UK)</strong></td>
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<tr>
<td></td>
<td>Advances in imaging and their application to musculoskeletal disease</td>
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<tr>
<td></td>
<td><strong>Judith Adams (Manchester, UK)/Ignac Fogelman (London, UK)</strong></td>
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<td>Time</td>
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<tr>
<td>17:00-18:30</td>
<td><strong>Reception and posters</strong></td>
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<tr>
<td>18:30</td>
<td>Buffet served <em>(for those registered for Rare Bone Diseases and Muscle and Bone Workshops)</em></td>
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<td><em>Pentland</em></td>
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<table>
<thead>
<tr>
<th>Time</th>
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</table>
| 19:00-22:30/21:50 | **BRS Rare Bone Diseases Workshop**  
|               | *Pentland East*                                                       |
|              | Supported by Alexion Pharmaceuticals                                     |
| 19:00        | Chairmen’s introductions                                               |
|              | Jim Gallagher *(Liverpool, UK)*/Kas Javaid *(Oxford, UK)*              |
| 19:10        | Rare bone disease research in the past, present and future             |
|              | *Wim Van Hul (Antwerp, Belgium)*                                       |
| 19:30        | Severe hypophosphatasia: from the cradle                                |
|              | *Raja Padidela (Manchester, UK)*                                       |
| 19:50        | Diagnosis of hypophosphatasia in adults: needle in a haystack          |
|              | *Richard Eastell (Sheffield, UK)*                                      |
| 20:00        | Oral Communications                                                    |
| 20:10 P53    | Progression of osteoarthropathy in alkaptonuric patients monitored by  |
|              | ¹⁸F–NaF PET                                                            |
|              | *LF Taylor (Liverpool, UK)*                                            |
| 20:16 P38    | Establishing reference intervals for pyridoxal 5'-phosphate: the National Health and Nutrition Examination Survey 2007-2008 data |
|              | *P Nicklin (Sheffield, UK)*                                            |
| 20:22 P47    | An anatomical investigation of a suspected case of Ollier’s disease    |
|              | *AP Bond (Liverpool, UK)*                                              |

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<tr>
<th>Time</th>
<th>Event</th>
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</table>
| 19:00-22:30/21:50 | **BRS Muscle and Bone Workshop**  
<p>|               | <em>Pentland West</em>                                                       |
| 19:00        | Chairmen’s introductions                                               |
|              | Kate Ward <em>(Cambridge, UK)</em>/Alex Ireland <em>(Manchester, UK)</em>            |
| 19:10        | The importance of muscle contraction for prenatal skeletal development |
|              | <em>Niamh Nowlan (London, UK)</em>                                            |
| 19:40 P13    | Is the human fibula responsive to disuse?                              |
|              | <em>A Ireland (Manchester, UK)</em>                                            |
| 19:50 P45    | The role of osteocytes in targeted remodelling of third metacarpal     |
|              | bone in the TB racehorse                                               |
|              | <em>N Hopper (Cambridge, UK)</em>                                              |
| 20:00        | Break                                                                  |
| 20:20 P2     | Dietary protein intake is associated with better physical function and |
|              | muscle strength among elderly women                                    |
|              | <em>M Isanejad (Kuopio, Finland)</em>                                         |
| 20:30 P20    | The influence of the Female Athlete Triad on bone quality in elite    |
|              | endurance runners                                                      |
|              | <em>J Coulson (Manchester, UK)</em>                                            |
| 20:40 P10    | High-intensity interval training: a potential novel method for         |
|              | improving bone mass                                                    |
|              | <em>FM Guppy (Brighton, UK)</em>                                              |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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</table>
| 19:00-22:30  | 20:28 P56 Ultrastructure of bone revealed by serial block face imaging scanning electron microscopy
MH Helfrich (*Aberdeen, UK*) |
|              | 20:34 P55 Osteopetrosis-distinct morphological changes in a rare skeletal disease
J Zusin (*Stanmore, UK*) |
|              | 20:40 Break                                                             |
|              | 21:00 Bisphosphonates in rare diseases: friend or foe?
Stuart Ralston (*Edinburgh, UK*) |
|              | 21:20 Lenz Majewski syndrome
Philip Stanier (*London, UK*) |
|              | 21:40 Genomics and phenotyping research: the UK framework
Kas Javaid (*Oxford, UK*) |
|              | 22:00 Panel discussion and questions                                    |
|              | 22:30 Close                                                             |
| 08:30-22:30  | Registration opens                                                       |
| 09:15-10:45  | Symposium 2
Pentland East
Stem cells and regenerative medicine
Chairs: Fraser Coxon (*Aberdeen, UK*)/Colin Farquharson (*Edinburgh, UK*)
09:15 IS3 Gene-cell therapy approaches in the treatment of osteogenesis imperfecta
Antonella Forlino (*Pavia, Italy*)
09:45 IS4
Matthew Dalby (*Glasgow, UK*)

Symposium 3
Pentland West
Muscle and bone
Chairs: Nick Harvey (*Southampton, UK*)/Kate Ward (*Cambridge, UK*)
09:15 IS5 Assessment of sarcopenia
Cyrus Cooper (*Southampton/Oxford, UK*)
09:45 IS6 Restoring muscle health in muscle wasting diseases
Estelle Trifilieff (*Basle, Switzerland*)
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:15</td>
<td>IS7</td>
<td>Neuroendocrine basis of sarcopenia</td>
<td>Marco Narici (Nottingham, UK)</td>
</tr>
<tr>
<td>10:15</td>
<td>OC3</td>
<td>Temporal and spatial drug targeting of fractures using nanoparticles for bone repair and regeneration</td>
<td>ND Evans (Southampton, UK)</td>
</tr>
<tr>
<td>10:30</td>
<td>OC4</td>
<td>The role of Wt1 in bone and bone marrow, and its response to hypoxia</td>
<td>SL McHaffie (Edinburgh, UK)</td>
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<tr>
<td>10:45-11:15</td>
<td>Coffee</td>
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<tr>
<td>11:15-12:45</td>
<td>Symposium 4</td>
<td>Intracellular pathways for matrix diseases</td>
<td>Isabel Orriss (London, UK)/ Donald Salter (Edinburgh, UK)</td>
</tr>
<tr>
<td>11:15</td>
<td>IS8</td>
<td>New insight into the structural and functional properties of matrilin-3; implications for disease mechanisms</td>
<td>Mike Briggs (Newcastle upon Tyne, UK)</td>
</tr>
<tr>
<td>11:45</td>
<td>IS9</td>
<td>SLRP signalling in the kidney</td>
<td>Liliana Schaefer (Frankfurt, Germany)</td>
</tr>
<tr>
<td>12:15</td>
<td>OC5</td>
<td>Coupling of angiogenesis and osteogenesis in bone</td>
<td>A Kusumbe (Muenster, Germany)</td>
</tr>
<tr>
<td>12:30</td>
<td>OC6</td>
<td>The anti-diabetic drug metformin reduces tumour burden and osteolytic bone disease in multiple myeloma in vivo</td>
<td>SL Webb (Oxford, UK)</td>
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<tr>
<td>12:45-14:00</td>
<td>Lunch</td>
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<td>Posters</td>
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<tr>
<td>12:45</td>
<td>CC1</td>
<td>Clinical Case: Clinical and genetic analysis in a unique systemic skeletal disorder characterised by high bone turnover and bone expansion</td>
<td>CL Gregson (Bristol, UK)</td>
</tr>
<tr>
<td>12:30</td>
<td>CC2</td>
<td>Successful use of denosumab to treat osteoporosis in a patient with severe anorexia nervosa</td>
<td>A Jamieson (Livingston and Glasgow, UK)</td>
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<td>12:45-13:15</td>
<td><strong>BRS AGM (Pentland East)</strong></td>
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<td>14:00-15:30</td>
<td><strong>Symposium 6</strong> Pentland East Scaffolds</td>
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<td><strong>Chairs:</strong> Catherine Shanahan <em>(London, UK)</em> / Gudrun Stenbeck <em>(Uxbridge, UK)</em></td>
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<td>14:00 IS12 New materials-based approaches to engineer and study mineralised tissue</td>
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<td><strong>Molly Stevens</strong> <em>(London, UK)</em></td>
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<td>14:30 IS13 Engineered extracellular matrices for regenerative medicine</td>
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<td><strong>Manuel Salmeron-Sanchez</strong> <em>(Glasgow, UK)</em></td>
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<td></td>
<td><strong>Oral communications</strong></td>
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<td>15:00 OC7 E11 stabilisation controls osteocytogenesis and is disrupted in osteoarthritic subchondral bone thickening</td>
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<td><strong>KA Staines</strong> <em>(Edinburgh, UK)</em></td>
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<td>15:15 OC8 The Collagen Toolkits: applications in tissue engineering</td>
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<td><strong>RW Farndale</strong> <em>(Cambridge, UK)</em></td>
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<tr>
<td>15:30-16:30</td>
<td><strong>Posters (odd numbers presented)</strong></td>
<td>Supported by Internis</td>
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<tr>
<td>16:30-17:45</td>
<td><strong>Plenary Lecture and Oral Posters</strong> Pentland</td>
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<td></td>
<td><strong>Chairs:</strong> Ray Boot-Handford <em>(Manchester, UK)</em> / Allie Gartland <em>(Sheffield, UK)</em></td>
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<tr>
<td>16:30</td>
<td>IS17 ACP5, autoimmunity and the skeleton</td>
<td><strong>Tracy Briggs</strong> <em>(Manchester, UK)</em></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td>OP1 The role of poly(ADP ribose) in bone mineralization</td>
<td><strong>R Rajan</strong> <em>(Cambridge, UK)</em></td>
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<tr>
<td>17:07</td>
<td>OP2 The role of CRELD2 in skeletal development and homeostasis.</td>
<td><strong>EP Dennis</strong> <em>(Newcastle-Upon-Tyne, UK)</em></td>
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<tr>
<td>Time</td>
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| 17:14 | OP3          | ENPP1 enzyme replacement therapy for generalized arterial calcification of infancy  
**KL Askew (Lexington, USA)** |
| 17:21 | OP4          | Peripheral blood derived mononuclear cells increase chondrocyte migration  
**N Hopper (Cambridge, UK)** |
| 17:28 | OP5          | Type 2 Diabetes is not associated with increased bone sclerostin expression, despite enhanced serum expression levels at onset of diabetes  
**M Pereira (London, UK)** |
| 17:35 | OP6          | Characterising the spotty osteomalacia in Phospho1 knockout mice  
**A Boyde (London, UK)** |
| 17:45-18:30 | BSMB John Scott Lecture  
**Pentland**  
**Chair: John Couchman (Copenhagen, Denmark)**  
**Vivien Coulson-Thomas (Cambridge, UK)**  
Winner of the BSMB Young Investigator Award 2015 |
| 19:30 | Dinner and Ceilidh  
**South Hall, Pollock Halls Campus**  
With the Canongate Cadjers Ceilidh Band |

### Thursday 3 September

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Details</th>
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<tbody>
<tr>
<td>08:30</td>
<td>Registration opens</td>
</tr>
</tbody>
</table>
| 09:00-10:30 | **BRS Oral Communications**  
**Pentland East**  
**Chairs: Stuart Ralston (Edinburgh, UK)/Wim Van Hul (Antwerp, Belgium)**  
09:00 OC9  
No association between RANK signalling pathway variants and bone mineral density in the Aberdeen Prospective Osteoporosis Screening Study  
**LJ Hocking (Aberdeen, UK)**  
09:10 OC10  
Reduced osteoclast TGFβ signaling with age impairs the coupling of bone resorption to bone formation  
**MM Weivoda (Rochester, USA)** |
| 09:00-10:30 | **BSMB open session + Symposium 8**  
**Pentland West**  
**Chairs: Peter Bell (Newcastle upon Tyne, UK)/Simon Tew (Liverpool, UK)**  
09:00 IS18  
Collagen VI is a critical regulator of nerve structure and function  
**Paolo Bonaldo (Padova, Italy)**  
09:30 OC18  
Hypertrophic differentiation of cartilage is dependent on low level fluid flow  
**A Crawford (Sheffield, UK)** |
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
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<tbody>
<tr>
<td>09:20</td>
<td>OC11</td>
<td>Identification of high density mineralised protrusions (HDMPs) in ex-vivo human knee joints</td>
<td>NP Thomas</td>
<td>Liverpool, UK</td>
</tr>
<tr>
<td>09:30</td>
<td>OC12</td>
<td>Ablation of the androgen receptor in vascular smooth muscle cells demonstrates a role for testosterone in vascular calcification</td>
<td>D Zhu</td>
<td>Edinburgh, UK</td>
</tr>
<tr>
<td>09:40</td>
<td>OC13</td>
<td>Sexual dimorphism in bone shape, rather than mass, is associated with Osteoarthritis in Str/ort mice</td>
<td>B Javaheri</td>
<td>London, UK</td>
</tr>
<tr>
<td>09:50</td>
<td>OC14</td>
<td>Loss of SirT1 dysregulates chondrocytes and leads to an arthritic phenotype in vivo, through decreased control of autophagy</td>
<td>PK Sacitharan</td>
<td>Oxford, UK</td>
</tr>
<tr>
<td>10:00</td>
<td>OC15</td>
<td>Identification of miRNAs involved in osteoblastic differentiation and regulation of sclerostin expression in osteosarcoma cell lines</td>
<td>OM Azuraidi</td>
<td>Liverpool, UK and Serdang, Malaysia</td>
</tr>
<tr>
<td>10:10</td>
<td>OC16</td>
<td>Ethnic differences in the relationship between muscle strength and tibial bone mineral density in ageing UK men</td>
<td>A Zengin</td>
<td>Cambridge, UK</td>
</tr>
<tr>
<td>10:20</td>
<td>OC17</td>
<td>Relationships between DNA methylation and bone mineral content from an epigenome wide association study in the Hertfordshire Cohort Study</td>
<td>MH Edwards</td>
<td>Southampton, UK</td>
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10:30-11:00 Coffee
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<tr>
<th>Time</th>
<th>Event Description</th>
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</table>
| 11:00-12:30 | **BRS Oral Communications**  
Chairs: Bente Langdahl (Aarhus, Denmark)/Katherine Staines (Edinburgh, UK)  
11:00 OC22  
TRIM32 knockout mice develop accelerated osteoarthritis of the knee joint after destabilisation of the medial meniscus (DMM) surgery and upon ageing  
**SB Roberts** (Edinburgh, UK)  
11:10 OC23  
Regulation of PTH-induced bone loss: a role for monocyte chemotactant protein-1  
**JA Siddiqui** (New York, USA)  
11:20 OC24  
High bone mass is associated with bone-forming features of osteoarthritis in non-weight bearing joints and independent of body mass index  
**A Murphy** (Bristol, UK)  
11:30 OC25  
Increased dentine tubule areal density in a mouse model of osteogenesis imperfecta, oim  
**M Doube** (London, UK)  
11:40 OC26  
Osteoporosis and atherosclerosis: higher bone density is associated with greater carotid intima-media thickness in middle-aged women  
**M Frysz** (Bristol, UK)  
11:50 OC27  
Early life motor ability is positively associated with adolescent bone strength  
**A Ireland** (Manchester, UK) | **BSMB open session**  
Chairs: Blandine Poulet (Liverpool, UK)/Jo Adams (Bristol, UK)  
11:00 OC31  
Nuclear magnetic resonance spectroscopy fingerprinting of tissues allows detailed analysis of glycation reactions  
**MJ Duer** (Cambridge, UK)  
11:15 OC32  
Importance of glycosylation state and aggregation in ER-associated degradation of mutant matrilin proteins and induction of UPR  
**PA Bell** (Newcastle upon Tyne, UK)  
11:30 OC33  
Embryo movement drives cellular progression through the developing growth plate  
**AS Pollard** (London, UK)  
11:45 OC34  
A role for MRTF-A in mechanical stress-associated monocyte/macrophage activation  
**A Tam** (London, UK)  
12:00 OC37  
Ectosteric protease inhibitors as substrate selective drugs: their mechanism and efficacy in matrix diseases  
**Dieter Bromme** (Vancouver, Canada)  
12:15 OC38  
Latent transforming growth factor-β binding protein 1 forms independent oligomeric structures  
**Helen Troilo** (Manchester, UK) |
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<td>12:30-13:00</td>
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<td>14:00</td>
<td>IS19 The initiation and regulation of skeletal mineralisation</td>
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<td>IS20 Mechanisms of vascular calcification</td>
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<td>OC35 Endocrine role of bone: PHOSPHO1 a novel regulator of energy metabolism</td>
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<td>OC36 Extracellular nucleotides inhibit vascular calcification in vitro: evidence for dual inhibitory mechanisms involving both the P2Y2 receptor and pyrophosphate.</td>
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**Session Details**

**OC28**

The origins of bone and cartilage disease: high throughput bone phenotype screen to identify new genes that determine bone structure and strength

**JG Logan (London, UK)**

**OC29**

The role of LC3 and autophagy in bone resorption by osteoclasts

**FP Coxon (Aberdeen, UK)**

**OC30**

PAR2 response in fluid flow-stimulated chondrocytes

**C Huesa (Paisley, UK)**

**Posters (even numbers presented)**

Supported by Internis

**Symposium 9 (Pentland) Mineralisation**

Supported by Synageva BioPharma

**Chairs:** Jim Gallagher (Liverpool, UK)/Andy Pitsillides (London, UK)

**IS19**

The initiation and regulation of skeletal mineralisation

**Colin Farquharson (Edinburgh, UK)**

**IS20**

Mechanisms of vascular calcification

**Catherine Shanahan (London, UK)**

**OC35**

Endocrine role of bone: PHOSPHO1 a novel regulator of energy metabolism

**KJ Oldknow (Edinburgh, UK)**

**OC36**

Extracellular nucleotides inhibit vascular calcification in vitro: evidence for dual inhibitory mechanisms involving both the P2Y2 receptor and pyrophosphate.

**IR Orriss (London, UK)**
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<tr>
<td>15:30</td>
<td>LB1</td>
<td>Late breaking abstract</td>
<td>Genome-wide analysis identifies significant predictors of therapeutic response to teriparatide in severe osteoporosis</td>
<td>N Alonso (Edinburgh, UK)</td>
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<td>15:40-16:00</td>
<td>Awards and close of meeting</td>
<td>Pentland</td>
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<td>John Couchman (Copenhagen, Denmark)/Eugene McCloskey (Sheffield, UK)</td>
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Targeted sequencing of the Paget’s disease associated 14Q32 locus identifies several missense coding variants in RIN3 that predispose to Paget’s disease of bone

M. Vallet¹, D. C. Soares², S. Wani¹, A. Sophocleous¹, J. Warner³, D. M. Salter¹, S. H. Ralston¹ and O. M. E. Albagha¹*

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2. Centre for Molecular Medicine, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK
3. South East Scotland Clinical Genetic Service, NHS, Western General Hospital, Edinburgh, UK

Objectives: Paget’s disease of Bone (PDB) is a common skeletal disorder with a strong genetic component. We identified a susceptibility locus for PDB on chromosome 14q32 by GWAS in 2011 (1), tagged by rs1049863 within the RIN3 gene. Here we investigate the candidacy of RIN3 as a predisposing gene for PDB.

Methods: We conducted re-sequencing of the 14q32 locus in PDB cases and controls and studied the expression of RIN3 in mouse tissues and bone cells by quantitative PCR, Western Blotting and immunohistochemistry.

Results: We detected 16 missense variants in RIN3, including 12 rare potentially damaging variants, 7 of which were detected only in PDB. A common coding variant (p.R279C) in LD with the GWAS hit (r² = 0.96) was strongly associated with PDB (OR = 0.64, P = 1.4 × 10⁻⁹). The rare variants were also strongly associated when combined (OR = 3.72; P = 8.9 × 10⁻¹⁰). mRNA for RIN3 was strongly expressed in bone, lung, and kidney and there was an increased expression of RIN3 mRNA during the osteoclast differentiation from cultured bone marrow (P = 0.02). Expression of RIN3 also increased at the protein level during osteoclast differentiation in vitro.

Conclusion: These findings indicate that RIN3 is the causal gene for PDB on 14q32 and suggest that its susceptibility is mediated by a combination of common and rare coding variants. While the function of RIN3 in bone biology is incompletely understood the data are consistent with a model whereby RIN3 acts to inhibit osteoclast formation and that the predisposing variants damage its ability to do so, resulting in the osteoclast activation characteristic of PDB.

Grants: European Research Council (311723-GENEPAD); Arthritis Research UK (19799&19520); Arthritis Research UK (18304&18163); Medical Research Council (09-800-05).

Reference:
Temporal and spatial drug targeting of fractures using nanoparticles for bone repair and regeneration

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Objectives: Bone fractures are a significant economic and societal burden that continues to grow as the average age of our populations increases. Current therapies, particularly for fractures that fail to heal naturally such as non-unions, are suboptimal and are largely based on surgical interventions and implanted prostheses or biomaterials. We are investigating the alternative notion that fracture sites might be targeted systemically with drugs, including those that target the Wnt signalling pathway, by using nanotechnology. We hypothesise that particular periods in fracture healing may be open to nanoparticle targeting due to vascular changes at the injury site.

Methods: Bone marrow mononuclear cells were isolated by Lymphoprep from patients undergoing hip arthroplasty at Southampton General Hospital. Isolates were incubated in serum-containing medium overnight in rotation suspension culture at 37°C and 5% CO2, in the presence of 0.1 ug/mL Wnt3A or vehicle control. Cell suspensions were then assayed for colony formation by CFU-F and CFU-O assay or osteogenic differentiation was assayed in stromal cells arising from these colonies. Polymersomes (PEG-PCL) or liposomes (dimyristolphosphatidylcholine) were fabricated by self-assembly or extrusion, respectively, followed by protein or Wnt agonist loading, and activity was tested on a Wnt-responsive cell line (Leading LightTM). Cortical defects were created in the femurs of adult MF1 mice, and fluorophore-labelled nanoparticles were injected post-surgery. Fluorophore accumulation was measured using an IVIS imager.

Results: To demonstrate the feasibility of this approach, we show that transient Wnt stimulation of human bone marrow mononuclear cells promotes an expansion in the frequency of osteoprogenitors (60% increase, p < 0.01, 7 patients), and a subsequent increase in osteogenesis. Both Wnt proteins (Wnt3A) and GSK3 inhibitors (6-bromoindirubin-3’ oxime) stably associated with liposomal and polymersomal nanoparticles of diameter 98.6 ± 21.7 nm and 83.46 ± 36.46 nm, respectively and remain active in serum-containing medium, as shown by activity assays using a Wnt-responsive reporter cell line. Finally, whole animal IVIS imaging showed that DiR-labeled nanoparticles accumulate specifically at the fracture site, but also in the spleen and liver, following administration after experimental bone fracture in cortical or segmental bone injury defects in mice.

Conclusion: These results demonstrate that drug-associated nanoparticles may have promise as a novel method of stimulating fracture healing following injury.
The anti-diabetic drug metformin reduces tumour burden and osteolytic bone disease in multiple myeloma in vivo

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2. Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

Multiple myeloma (MM) is a fatal haematologic malignancy characterised by accumulation of malignant plasma cells in bone marrow (BM), and severe lytic bone disease. Metformin is widely prescribed in diabetes, and recently associated with improved outcomes in diabetic patients with MM, suggesting a potential anti-myeloma effect. Our aim was to investigate the effect of metformin within the myeloma-bone microenvironment. C57Bl/KaLwRij mice were inoculated with 5TGM1MM cells and treated with metformin from time of tumour inoculation (met-cont) or from time of established tumour (met-delay). MM-bearing mice treated with metformin exhibited a decrease in myeloma-specific serum paraprotein compared to control (Control; 4.29 ± 0.3 mg/ml, met-cont; 1.51 ± 0.6 mg/ml p < 0.001, met-delay; 0.7 ± 0.7 mg/ml p < 0.001). MicroCT analysis demonstrated significant decreases in osteolytic lesion number in metformin treated MM-mice (Control; 26 ± 3.6, met-cont; 11.4 ± 0.7 p < 0.001, met-delay; 9 ± 1.5 p < 0.01). Histomorphometry revealed an increase in BV/TV of metformin-mice compared to untreated MM (MM; 2.6 ± 0.41%, met-cont; 4.0 ± 0.43% p < 0.05, met-delay; 4.4 ± 0.46% p < 0.05). Metformin also increased osteoblast number (MM; 1.86/mm ± 0.5, met-delay; 4.7/mm ± 0.7, p < 0.01, met-delay; 6.5/mm ± 1.4 p < 0.01), decreased osteoclast number (MM; 2.86/mm ± 0.4, met-cont; 1.6/mm ± 0.1 p < 0.05, met-delay; 1.2/mm ± 0.2, p < 0.05), as well as increasing trabecular thickness and decreasing trabecular separation. Interestingly, metformin had no significant effect on non-tumour mice, suggesting that the effect of metformin to reduce MM bone disease is indirect, in response to the decrease in tumour burden. Metformin induced a dose-dependent decrease in MM cell viability. Metformin treatment of MM cells induced apoptosis, detected by increased cleaved caspase-3 and PARP. Metformin had no effect on BM stromal cell (BMSC) viability. BMSC-conditioned media (CM) had a protective effect against the anti-MM effects of metformin at 24 h that was lost by 72 h. In contrast, BMSC-CM protected against the anti-MM effects of bortezomib at all time points. Our studies demonstrate strong anti-tumour effects of metformin in the MM-bone microenvironment, suggesting metformin may be effective for the treatment of MM and associated bone disease.
E11 stabilisation controls osteocytogenesis and is disrupted in osteoarthritic subchondral bone thickening

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2. Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, SA, Australia
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The transmembrane glycoprotein E11 is recognised to be critical for early osteocyte commitment. However, its precise role in osteoblast-osteocyte transition (osteocytogenesis) has yet to be established. Here we have explored the regulation and function of E11 expression during osteocytogenesis and bone formation. We have also examined whether these processes are compromised in osteoarthritis in which abnormal subchondral bone remodelling is observed.

We have previously implicated the proteasome in controlling post-translational E11 expression with proteasome-selective inhibitors (ALLN/MG132/lactacystin/Bortezomib/Withaferin-A) dose-dependently increasing E11 expression levels in MLO-A5 and primary osteoblast cells. We have confirmed this proteasomal targeting by immunoprecipitation of all ubiquitinylated proteins which included E11, and by increased levels of ubiquitinylated E11 protein upon addition of the proteasome inhibitors MG132/Bortezomib. Activation of the small GTPase RhoA was observed to be increased concomitant with E11 expression in differentiating MLO-A5 cells. Inhibition of the downstream signalling effector of Rho-A, ROCK (Rho-associated protein kinase), inhibited MLO-A5 cell dendrite formation therefore implicating this signalling pathway as critical in osteocytic dendrite formation. To investigate this further, we generated mice harbouring a conditional deletion of E11 in late osteoblasts (Oc-cre; E11fl/fl) and analysed its bone phenotype through histology, micro-CT scanning and 3-point bending. Immunohistochemistry and western blotting revealed selective deletion of E11 in Oc-cre; E11fl/fl mice, whereas sclerostin expression and localisation to osteocytes were normal. MicroCT analyses of 6-week old Oc-cre; E11fl/fl mice revealed that cortical and trabecular parameters were normal. Similarly, 3-point bending revealed no significant differences in biomechanical properties at this age. To assess whether E11 dysregulation contributes to osteoarthritic pathology, we performed immunohistochemistry on joint sections from a natural model of osteoarthritis, the STR/Ort mouse, and human osteoarthritis patients. Both revealed decreased E11 protein expression in subchondral bone osteocytes in regions of the joint where osteoarthritic pathology was observed.

These data provide a mechanistic basis by which the proteasome regulates E11 stability and osteocyte differentiation. This work adds to our understanding of the factors regulating bone homeostasis, which may lead to future therapeutic approaches for osteoarthritis.
No association between rank signalling pathway variants and bone mineral density in the Aberdeen prospective osteoporosis screening study

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1. Division of Applied Medicine, University of Aberdeen, Aberdeen, UK
2. Division of Population Health Sciences, University of Dundee, Dundee, UK

Post-menopausal osteoporosis is the most common bone disease, associated with low bone mineral density (BMD) and pathological fractures. Targeting therapies to those most likely to develop low BMD is critical for effective fracture prevention. Osteoporosis results when bone resorption and formation become uncoupled. The Receptor Activator of NFκB (RANK) signalling pathway is critical for bone-resorbing osteoclasts, with rare mutations within genes of this pathway causing severe monogenic disorders of osteoclast dysfunction. Common variants near genes encoding RANK and other components of the signalling pathway have been associated with low BMD and fracture risk in genome-wide association studies (GWAS), but the causal variants remain unknown.

We directly sequenced 25 key genes within the RANK signalling pathway in 100 women from the Aberdeen Prospective Osteoporosis Screening Study (APOSS) at extremes of femoral neck and lumbar spine BMD. Groups were matched for age, weight and height, excluding women with self-reported bone or joint problems or on drugs known to affect bone metabolism. Exonic and 1000 bp upstream regions were targeted using Haloplex target enrichment (Agilent) and directly resequenced using Illumina 2 × 100 bp paired-end sequencing at GenePool (Edinburgh). Sequence reads were aligned to GRCh37 and SNPs called and annotated using GATK and Samtools. Previously unreported SNPs were validated by Sanger sequencing. Validated SNPs were genotyped in the entire APOSS cohort (n ~ 3,000) at LGC Genomics.

Two novel missense SNPs identified within NFKB1 were not confirmed by Sanger sequencing. Validated SNPs where the minor allele was overrepresented within the low BMD group (chi-squared $p < 0.01$) were identified in TNFRSF11A, MAPK1 and IKBKB, and genotyped in the entire APOSS cohort. Linear regression with adjustment for covariates indicated that none of these SNPs were associated with BMD in the full APOSS sample ($p_{SNP} \geq 0.2$).

This study was designed to identify causal genetic risk factors for osteoporosis among genes of the RANK signalling pathway. No BMD-associated variants were identified in the coding and 1000 bp upstream regions of the 25 genes examined within the APOSS study population, suggesting that GWAS hits near these genes may act via longer range regulatory mechanisms.

This study was supported by Arthritis Research UK 19531 and MRC G1000435.
Reduced osteoclast TGFβ signaling with age impairs the coupling of bone resorption to bone formation

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2. Department of Medicine, University of Melbourne, Heidelberg, VIC, Australis
3. Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN, USA

Osteoclasts secrete factors that couple bone resorption and formation. These processes are uncoupled with age, resulting in bone loss. TGFβ is abundant in bone and is released and activated by osteoclasts. We have shown that conditioned media from TGFβ-treated osteoclasts stimulates pre-osteoblast migration and differentiation. Because the concentration of TGFβ in bone decreases with age, we hypothesized that reduced osteoclast TGFβ signaling contributes to age-related uncoupling of bone resorption and formation.

Dominant negative TGFβ receptor (Tgfbr)-2 was expressed in osteoclasts using Ctsk promoter Cre (Tgfbr2OclKO). Five month-old animals were assessed by μCT and histomorphometry. Bone marrow-derived osteoclasts were cultured with TGFβ or bone chips to assess TGFβ responses in vitro. Microarray and qPCR were utilized to identify and validate genes induced in TGFβ-treated osteoclasts. Young and old mice (4 and 26 months, respectively) were compared to assess the effect of age on osteoclast TGFβ signaling and gene expression. Immunohistochemistry (IHC) was utilized to assess osteoclast signaling and protein expression in vivo.

MicroCT analysis revealed osteopenia in Tgfbr2OclKO mice with significant reductions in femur and vertebral bone volume. Histomorphometry of the femurs showed no change in osteoclast numbers; however, osteoblast numbers and bone formation rates were reduced 60% in Tgfbr2OclKO mice. Of interest, 2 ng/mL TGFβ induced Wnt1 expression >1000-fold. Wild type osteoclasts cultured on bone also exhibited Wnt1 induction; this response was impaired in Tgfbr2OclKO osteoclasts. IHC of Tgfbr2OclKO femurs revealed a 53% reduction in osteoclast Wnt1 in vivo. Osteoclasts derived from young and old marrow showed similar inductions of Wnt1 by culture on young bone. Consistent with age-related decreases in bone TGFβ, culture of osteoclasts derived from young marrow on old bone resulted in a 72% reduction in TGFβ-induced Wnt1. IHC revealed that osteoclast phospho-SMAD2/3, a marker of Tgfbr signaling, was significantly reduced in old mice in vivo. Osteoclast Wnt1 expression was reduced 64% in old mice.

These data demonstrate that impaired Tgfbr signaling in osteoclasts causes osteopenia by reducing osteoblast numbers and establish a novel paradigm by which TGFβ-stimulated osteoclasts are a source of factors such as Wnt1 that promote osteoblastic bone formation at sites of bone resorption.
Identification of high density mineralised protrusions (HDMPS) in ex-vivo human knee joints

N. P. Thomas¹*, N. Jeffery¹, V. L. Adams¹, J. L. Donnelly¹, L. R. Ranganath¹, R. van ‘t Hof¹, A. Boyde² and J. A. Gallagher¹

¹. Musculoskeletal Biology, University of Liverpool, Liverpool, UK
². Dental Physical Sciences, QMUL, London, UK

High density mineralised protrusions (HDMPS) projecting from the tidemark mineralising front into hyaline articular cartilage (HAC), first reported in several equine studies have recently been identified on the articular surface of femoral heads in people with AKU arthropathy and osteoarthritis (OA). This study investigates the presence and incidence of HDMPS in the human knee.

Nine knees (male = 7, female = 2) were taken from 6 cadavers, age 74–97. Knees were dissected, assigned a Kellgren Lawrence (KL) OA score and studied by MRI, CT and microCT. Dual echo steady state (DESS) MR data were generated isotropically at 0.26 mm resolution. CT images were acquired axially at 1.25 mm intervals (0.36 mm in-plane resolution). Condyles were isolated, cleaned of soft tissue and imaged with microCT (voxel size 19.8 μm).

All knees had signs of OA (KL score ≥1) and HDMPS were observed in several modalities. The contrast against HAC was best represented in DESS scans. DESS data were used to measure protrusions identified as low signal-strength structures extending from the subchondral plate into HAC. HDMPS were found in 9/9 samples. A total of 30 protrusions were identified, ranging from 2 to 10 in a single joint. They are reported equally in tibiae and femora. Morphology was variable and complex: mean depth and width were 1.74 and 1.61 mm, respectively. Seventy-four percent of all protrusions were located in areas central to joint articulation. Protrusions were more common on those with extensive cartilage loss. Initial microCT study suggests reduced trabecular volume deep to HDMPS.

The distribution of protrusions in OA knees suggests they may play a major role in arthropathy. Formation in areas central to articulation is noteworthy; these structures have the potential to fragment under normal loading conditions. Resultant fine particles could abrade HAC. Increased incidence in knees with a greater degree of cartilage degradation may reconcile this and signify association with OA. Variations in protrusion depth, size and shape might suggest these observations are of different stages of a single pathology or may be indicative of physiologically distinct forms. Detectability of HDMPS with clinically available scanning modalities could be useful as a new imaging biomarker for prediction of joint destruction.
Ablation of the androgen receptor in vascular smooth muscle cells demonstrates a role for testosterone in vascular calcification

D. Zhu1*, P. W. F. Hadoke2, A. T. Vesey2, M. R. Dweck2, D. E. Newby2, L. B. Smith3 and V. E. MacRae1

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2. University/BHF Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, UK
3. MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

Background: Vascular calcification powerfully predicts mortality and morbidity from cardiovascular disease. Men have a greater risk of cardiovascular disease, compared to women of a similar age. These gender disparities suggest an influence of sex hormones. Testosterone is the primary and most well-recognized androgen in men. The biological actions of testosterone, and particularly of non-aromatizable dihydrotestosterone (DHT), are predominantly mediated by the Androgen Receptor (AR). Therefore, we addressed the hypothesis that exogenous androgen treatment induces vascular calcification.

Methods: Calcification of murine aortic VSMCs (passage 3) was induced by 3 mM Pi for 7 days. Calcium deposition was determined using a commercial kit. Immunohistochemistry was used to examine AR expression in calcified human femoral arteries. Protein expression was assessed by immunoblotting. Gene expression was analysed by qRT-PCR. Apoptosis was measured by alamar blue and DAPI staining.

Results: Immunohistochemical analysis revealed an up-regulation of AR expression in the calcified media of human femoral artery tissue. Furthermore, in vitro studies revealed increased Pi-induced VSMC calcification following either testosterone (100 nM; 2.1 fold; \( P < 0.05 \)) or DHT treatment (100 nM; 1.6 fold; \( P < 0.05 \)) for 7 days. Testosterone and DHT treatment increased tissue non-specific alkaline phosphatase (Alpl) mRNA expression (\( P < 0.001 \)). No change in the mRNA expression of the osteogenic marker Osterix was observed.

Signal transduction studies in VSMCs revealed that Erk1/2 phosphorylation was increased following testosterone treatment (100 nM) after 10 min (\( P < 0.01 \)) and 30 min (\( P < 0.05 \)). Akt phosphorylation remained unaltered. No effect of testosterone or DHT on nuclei apoptosis was noted.

Unexpectedly VSMC treatment with the AR antagonist flutamide induced calcification, suggestive of non-specific effects. To circumvent this, VSMCs were derived from VSMC-specific AR-ablated (SM-ARKO) mice. Testosterone-induced calcification was blunted in SM-ARKO cells compared to WT (0.52 fold; \( P < 0.05 \)). Consistent with these data, SM-ARKO VSMCs showed a reduction in Osterix mRNA expression (0.84 fold; \( P < 0.001 \)). However, intriguingly, a counter-intuitive increase in Alpl was observed (3.3 fold; \( P < 0.001 \)).

Conclusion: These novel data demonstrate that androgens play a role in inducing vascular calcification through the AR and the Erk1/2 signalling pathway.
Sexual dimorphism in bone shape, rather than mass, is associated with osteoarthritis in Str/ort mice

B. Javaheri*, I. Maric–Mur, M. Hopkinson, A. Aljazzar and A. A. Pitsillides

Comparative Biomedical Sciences, Royal Veterinary College, London, UK

Osteoarthritis (OA) is the most common joint disease, causing pain and limiting mobility. Human OA is only detectable at late stages and, thus, longitudinal assessment of bone pathology across OA development and progression is largely lacking. Male Str/ort mice spontaneously develop OA with known longitudinal trajectory closely resembling human disease, whilst female Str/ort and both genders of parental, control CBA mice show no OA. Herein, we use microCT to examine modifications in tibial mass (trabecular and cortical) and shape (entire cortical shaft) in both genders and genotypes at 10 (pre-OA), 20 (OA onset) and 40+ weeks (advanced OA) to establish relationship between emergent OA and bone mass and shape.

Tibiae from 10, 20 and 40 week-old male/female Str/ort and CBA mice (n = 5/group) scanned using Skyscan 1172, 50 kV, 200 μA, 1600 ms, and 5 μm voxel size. Trabecular analyses performed using CTAn (Skyscan). Whole bone analysis performed using BoneJ.

Analyses of trabecular bone revealed a higher bone mass in female Str/ort mice than in OA-prone males and in CBA mice. Cortical analyses showed the expected gender-related difference in cross-sectional area (CSA) (mass index) in CBA that was surprisingly absent in Str/ort mice, where CSA was greater in female rather than male; Str/ort mice also exhibit generally higher bone mass. Tibial shape analyses of CBA revealed that ellipticity was conserved in males and females (similar shape) and gender-related differences in the predicted resistance to torsion; more likely due to corresponding divergence in shape in bone mass. In contrast, male Str/ort mice showed marked increases in ellipticity at particular locations along the tibia which coincided with OA onset and were exaggerated with progression; no differences in predicted resistance to torsion were observed between male and female Str/ort. These data indicate that the Str/ort strain has a generalised greater bone mass and lacks the gender-related difference wherein males normally predominate. Divergence in ellipticity, however, is seen only in male Str/ort mice and its lack of relatedness to predicted resistance to torsion indicates that modified shape is the unique bone feature related to OA onset suggesting that complex bone: cartilage interplay contributes to OA development in this naturally-occurring mouse model.
Loss of SirT1 dysregulates chondrocytes and leads to an arthritic phenotype in vivo, through decreased control of autophagy

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Objectives: Dysregulated “ageing” mechanisms decrease lifespan and underlie age-related disorders, such as Osteoarthritis (OA). The SirT1 longevity factor controls lifespan and decreases with age. Similarly, the cellular process of autophagy, which identifies, degrades and recycles unwanted proteins and cellular debris, is defective in human ageing and diseases. However, the role of SirT1 in OA and mechanism of how autophagy is regulated in chondrocytes is largely unknown. Here we hypothesize that SirT1 regulates autophagy in chondrocytes, and loss of SirT1 predisposes to OA.

Methods: Novel cartilage specific SirT1 knockout mice were generated by crossing SirT1fl/fl × Aggrecan-CreERT2 (SirT1fl/fl; Agg-CreERT2) and assessed by histomorphometry and microCT. Gene expression profiles were examined in human samples or chondrocyte cell line (HTB-94) treated with the pharmacological inhibitor of SirT1 (EX-527; 100 nM), by siRNA knockdown, or by activation of SirT1 using SRT1720 (500 nM). Electron microscopy, FACs, immunohistochemistry and transgenic LC3-GFP mice were used to quantify autophagy by LC3 conversion.

Results: Human OA samples demonstrate decreased expression of SirT1, whilst pharmacological inhibition and siRNA knockdown of SirT1 decreased gene expression of COL2A1, ACAN and SOX-9 (58 ± 3.0%; p < 0.01). Whilst pharmacological activation of SirT1 stimulated the expression of COL2A1, ACAN and SOX-9 (up to 188 ± 0.9%; p < 0.01). Importantly, pharmacological activation of SirT1 was also shown to positively regulate the gene expression of key autophagy markers (BECN1, ULK-1 and LC3 (up to 601 ± 3.0%; p < 0.01)) and LC3 conversion by western blot and FACs (p < 0.01). SirT1fl/fl; Agg-CreER mice showed an increase in OA disease score compared to control (SirT1fl/fl) animals (18 ± 1.9 vs. 10 ± 1.5; p < 0.01), decreased expression of ACAN and COL2A1 (95 ± 0.003%; p < 0.001) and of BECN1, ULK-1, LC3, ATG5, ATG9, ATG10, and ATG13 (79 ± 0.001%; p < 0.01). In addition, protein expression of LC3 in young cartilaginous hips and the number of autophagosomes in microdissected cartilage both decreased (63 ± 7.9%; p < 0.05) in SirT1fl/fl; Agg-CreER mice compared to controls. A decrease in LC3 immunohistochemical staining was also observed which accompanied the decline in cartilage tissue and epiphyseal volume in SirT1fl/fl; Agg-CreER mice compared to controls.

Conclusion: This data suggests loss of SirT1 leads to an OA-phenotype and dysregulated autophagy in chondrocytes.
Identification of miRNAs involved in osteoblastic differentiation and regulation of sclerostin expression in osteosarcoma cell lines

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MicroRNAs (miRNA) are negative regulators of gene expression through the inhibition of mRNA translation. miRNAs are involved in major cellular functions and have been implicated in various human diseases but their role in metabolic bone diseases is unclear. The aim of this study was to investigate the involvement of miRNA at different stages of human bone development and that are involved in the regulation of sclerostin, a major controller of bone formation. We investigated expression miRNAs and the sclerostin gene in three human osteosarcoma cell lines representing different stages of osteoblast differentiation, MG-63 the least differentiated, TE-85 intermediate and SaOS-2 the most mature. Total RNA was extracted from confluent cell culture. Gene expression was analyzed by RT-PCR and miRNA was analyzed using Affymetrix miRNA 4.0 arrays. We found that SaOS-2 expressed the highest level of sclerostin (0.783 ± 0.02 relative to β-actin) followed by TE85 (0.080 ± 0.01) and MG63 (not detected) (p < 0.001) confirming that SaOS-2 is the most mature while MG63 is the least differentiated. More than 500 miRNAs were differentially expressed between the three cell lines, those with the largest difference in expression were miR-935, miR-143-3p, miR-145-5p, miR-155-5p, miR-3200-3p, miR-584-5p, miR-486-3p, miR-767-5p, and miR-105-5p. The most striking observation to emerge from the data comparison was the expression of miR-155-5p in MG63 was 2842-fold greater than SaoS-2 (p < 0.05) and 1467-fold greater in TE85 than in SaOS-2 (p < 0.05). Online prediction tools were used to predict miRNAs that target sclerostin. Considering the expression pattern of sclerostin in these cell lines, we identified miRNAs which were predicted to inhibit the translation of these proteins, miR-1254, miR-497-5p, miR-195-3p, and miR-195-5p. These were expressed highest in MG63 and lowest in SaoS-2. For example, miR-497-5p was 39.54 times higher in MG63 (290.02 ± 0.02) compared to SaOS-2 (7.31 ± 0.24), (p < 0.05). Our data shows that different stages of osteoblast development are characterised by different sets of highly expressed microRNAs and suggests that these miRNAs could be potential biomarkers in bone development and may provide the basis of new therapeutic approaches to prevent bone loss.
Ethnic differences in the relationship between muscle strength and tibial bone mineral density in ageing UK men

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Declines in muscle strength with age contribute to frailty leading to an increased fracture risk. Global data have shown that fracture risk varies widely between ethnic groups and it is possible that differences in muscle strength between ethnic groups contribute to those differences. The aim of this study was to investigate the associations in muscle strength and tibial bone mineral density (vBMD) between ethnic groups of ageing UK men.

White (W), Black African-Caribbean (B) and South-Asian (SA) men aged 40–85 years were recruited for a study of bone ageing in Manchester, UK. pQCT was performed at the tibia and the outcome measures were – distal 4% site: total vBMD (T.vBMD), trabecular vBMD (Trab.vBMD); 66% site cross-sectional muscle area (CSMA). Muscle strength was assessed by a single 2-leg counter jump to calculate relative muscle force and efficiency (how much force is used to generate sufficient power to jump). Linear regression with a pairwise comparison was used to explore the relationship between muscle strength (force and efficiency; predictors) and bone parameters (outcome) with adjustments for ethnicity, age, weight, height, CSMA, with a muscle (force or efficiency)*ethnicity interaction term. Results are expressed as $\beta$-coefficients (95% confidence intervals) of percent differences between ethnic groups in vBMD per 10% difference in muscle force or efficiency.

In total, 300 men (W:200, B:43, SA:57) were recruited. For a given force, B had greater, negative differences in T.vBMD than W ($-3.8\% [-7.1, -4.8]$, \(p = 0.03\)) and SA ($-4.4\% [0.4, 8.3]$, \(p = 0.03\)). Similarly, B had greater, negative difference for Trab.vBMD than W ($-4.4\% [-8.1, -0.7]$, \(p = 0.02\)) and SA ($-4.8\% [0.4, 9.2]$, \(p = 0.03\)). In contrast, B had greater Trab.vBMD than W ($2.9\% [0.07, 5.7]$, \(p = 0.04\)) as efficiency increased. There were no differences between SA and W.

The negative relationship between force and vBMD in B may be due their bone tissue being distributed over a larger area. Importantly, with increasing muscle efficiency, vBMD increased in B while it did not in W and SA, suggesting a greater response to muscle forces in B compared to W and SA.

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Relationships between DNA methylation and bone mineral content from an epigenome wide association study in the hertfordshire cohort study

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Objectives: Environmental exposures in early life have been shown to affect adult bone health. This may be occurring through epigenetic modifications, such as DNA methylation, playing a role in programming and thus adult disease. The precise mechanisms are currently unknown. We investigated epigenome-wide methylation in the blood of older adults in relation to femoral neck bone mineral content (BMC).

Methods: We studied femoral neck BMC in 49 men and 50 women from the Hertfordshire Cohort Study using dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500). Their DNA methylation was assessed at 481,652 CpG sites (CpGs) in whole blood samples using the Infinium HumanMethylation450 BeadChip (450 K). Relationships were assessed using robust estimate regressions with adjustment for age, sex, plate position, and chip number. Further adjustment for white blood cell composition was then completed.

Results: We found differential DNA methylation at epigenome-wide statistical significance ($p$-value < $1.04 \times 10^{-7}$) for 7 CpGs mapped to 7 genes after adjustment for age, sex, plate position and chip number. These included cg06646682 (DVL1, $p = 8.0 \times 10^{-11}$), cg15891310 (BCAS3, $p = 1.59 \times 10^{-9}$), and cg18529845 (SRD5A2, $p = 3.48 \times 10^{-8}$). DVL1 participates in Wnt signalling by binding to the cytoplasmic C-terminus of frizzled family members and transducing the Wnt signal to downstream effectors. BCAS3 is regulated by and a co-activator of estrogen receptor alpha (ER-α) and is potentially involved in a positive feedback loop leading to ER-α-mediated signal amplification. SRD5A2 converts testosterone into 5-alpha-dihydrotestosterone playing a central role in sexual differentiation and androgen physiology. With the exception of cg18529845, all associations remained significant after additional adjustment for whole blood cell composition.

Conclusion: We identified a set of genes with methylation changes present in late adulthood that were related to femoral neck BMC. Three of these genes have clear links to bone health with relationships to either Wnt signalling or sex steroid metabolism. Our findings implicate epigenetic mechanisms in the pathogenesis of poorer bone health in later life.
**TRIM32 knockout mice develop accelerated osteoarthritis of the knee joint after destabilisation of the medial meniscus (DMM) surgery and upon ageing**

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**Objectives:** The arcOGEN genome wide association study identified the 9q33.1 locus as associated with osteoarthritis in females. *TRIM32* is a candidate gene at this locus encoding a ubiquitin ligase expressed in joints. The study objectives were to investigate knee joint histomorphometry in *TRIM32*-deficient mice in response to surgically-induced and age-induced osteoarthritis.

**Methods:** Joint histomorphometry was assessed by histology and micro computed tomography (microCT) 8-weeks after right knee surgery to destabilise the medial meniscus (DMM; surgery at age 8-weeks) in female wild-type (WT), heterozygous (HET), and *TRIM32*-knockout (KO) mice (*n* = 14/group). Unoperated left knee joints were used as controls. Cartilage degradation of medial and lateral aspects of the tibia and femoral joint surfaces was measured histologically using the Osteoarthritis Research Society International (OARSI) scale; regional scores were collated to determine a total score for each joint. Parameters measured by microCT comprised epiphyseal trabecular volume, thickness, separation, number, and medial and lateral subchondral bone plate thickness of tibial and femoral aspects. Knee joint histomorphometry was also analysed in female WT, HET, and KO mice aged to 10-months (*n* = 12/group). Groups were compared by ANOVA with post-hoc Tukey analyses or Mann–Whitney *U* tests.

**Results:** Joint OARSI scores and microCT parameters were similar in control knees from WT, HET and KO mice after DMM and ageing. Following DMM, joint OARSI scores were more severe in HET and KO mice compared to WT mice (WT vs. KO *p* = 0.014). After DMM, a greater increase in tibial epiphyseal trabecular volume (WT + 10.3% vs. control knee; HET + 11.7%, KO + 23.1%; *p* = 5.0 × 10⁻⁴ WT vs. KO) and number (WT −2.9%, HET −0.6%, KO + 12.0%; *p* = 8.97 × 10⁻⁷ WT vs. KO) occurred in KO than WT mice. In aged mice, tibial medial bone plate thickness (*p* = 0.004), and femoral epiphyseal trabecular volume (*p* = 0.032), thickness (*p* = 0.001), and medial bone plate thickness (*p* = 0.001) were greater in KO compared to WT mice.

**Conclusion:** Increased cartilage degradation and tibial epiphyseal bone changes following DMM, and increased medial knee subchondral bone changes upon ageing, in *TRIM32*-deficient mice support the further study of *TRIM32* in susceptibility to osteoarthritis.

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Regulation of PTH-induced bone loss: a role for monocyte chemoattractant protein-1

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Objectives: Patients with hyperparathyroidism or with acute infusion of hPTH (1–34) show bone loss. Continuous PTH treatment causes cortical bone loss by enhancing endosteal resorption through stimulation of osteoclast formation and activity. Recently, we have shown that PTH’s anabolic effects are abolished in MCP-1−/− mice. In the present study we investigated the role of MCP-1 in PTH-mediated monocyte/macrophage recruitment, osteoclast activity and PTH’s catabolic effects on bone.

Methods: Eighty μg/kg/day of hPTH or saline were delivered for 14 days by osmotic pumps in 8 week old female WT and MCP-1−/− mice. MicroCT was utilized to analyze femurs harvested at death. Subsequently, qPCR was performed using RNAs from distal femurs of hPTH- or saline- infused WT and MCP-1−/− mice. Bone marrow macrophages from MCP-1−/− and WT mice were cultured with M-CSF and RANKL for 7 days for in vitro osteoclast formation.

Results: MicroCT analysis of cortical bone showed that infusion with hPTH induced significant bone loss in WT mice with decreased bone volume/total volume, bone mineral density, mean cross-sectional area and mean polar moment of inertia compared with the saline-treated group. In contrast, hPTH did not cause significant cortical bone loss in MCP-1−/− mice. Immunohistochemistry showed that hPTH increased CD68 positive cells compared with the saline-treated group in bones of WT mice and absence of MCP-1 abolished this effect. Further, BMMs from MCP-1−/− mice showed decreased multinucleated osteoclast formation compared to WT mice and this could be partially rescued with added MCP-1, which also increased osteoclast formation. Messenger RNA analyses of the distal femurs of hPTH-infused mice showed increased expression of NFAT, TRAP, carbonic anhydrase and cathepsin/K in WT mice but no such changes in MCP-1−/− mice.

Conclusion: Here we show that a continuous infusion of hPTH that mimics hyperparathyroidism fails to induce osteoclast formation, bone resorption and cortical bone loss in mice lacking MCP-1. The deletion of MCP-1 blunts the bone catabolic activity of PTH by decreasing recruitment of monocytes and pre-osteoclastic cells and their osteoclastogenic activity. Together, these are the first data to show that MCP-1 is required for the catabolic response of bone to PTH.
High bone mass is associated with bone-forming features of osteoarthritis in non-weight bearing joints and independent of body mass index

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Objectives: Previously we have reported associations between High Bone Mass (HBM) and (a) radiographic knee osteoarthritis (OA), partly mediated by increased body mass index (BMI) in HBM cases and (b) enthesophytes and osteophytes, suggestive of a bone-forming HBM phenotype. We aimed to establish (i) whether HBM is also associated with OA in non weight-bearing joints, (ii) if such OA also demonstrates a bone-forming phenotype, and (iii) whether increased BMI contributes to any identified association.

Methods: HBM cases (identified by screening 335,115 DXA scans; defined by BMD Z-scores ≥+3.2) from the UK-based HBM study were compared with family controls. A single blinded assessor graded AP dominant hand radiographs from cases and controls for features of OA (osteophytes (0–3), joint space narrowing (JSN) (0–3), subchondral sclerosis (0–1)) at the index Distal Interphalangeal Joint (DIPJ) and 1st Carpometacarpal Joint (CMCJ), using an atlas. Analyses used logistic regression, adjusting a priori for age and gender. The mediating role of BMI was explored by further BMI adjustment. A second assessor graded 40 randomly selected radiographs: inter and intra-rater reliability Kappas all ≥0.6.

Results: 315 HBM cases (mean age 61.2 years, 75% female) and 184 controls (54.2 years, 84% female) were included. Osteophytes (grade ≥1) were more common in HBM (DIPJ: 67 vs. 45%, CMCJ: 70 vs. 50%), with adjusted OR [95% CI] 1.78 [1.11, 2.85], p = 0.017 and 1.84 [1.19, 2.85], p = 0.006, respectively; whilst no differences were seen in JSN. Subchondral sclerosis was more common amongst HBM cases than controls, but this was explained by age & gender adjustment. Further adjustment for BMI failed to attenuate the OR for osteophytes in HBM cases vs. controls; DIPJ 1.69 [1.05, 2.74], p = 0.031 and 1st CMCJ 1.71 [1.09, 2.67], p = 0.019.

Conclusion: Our findings support a positive association between HBM and OA in non-weight-bearing joints, which is independent of BMI. This HBM-associated OA is characterised by osteophytes, consistent with a bone forming phenotype, rather than JSN reflecting cartilage loss. It is possible that the same systemic factors (e.g., genetic architecture) which govern HBM may also increase the risk of bone-forming OA.
Increased dentine tubule areal density in a mouse model of osteogenesis imperfecta, oim

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In osteogenesis imperfecta, collagen mutations result in impairment of type-1 collagen production and mineralisation, leading to bone fragility. Dentinogenesis imperfecta – failure to produce dentine with normal mechanical characteristics – is commonly associated with osteogenesis imperfecta. The oim (B6C3f-/-a/-col1a2oim/oim) mouse model of osteogenesis imperfecta displays a dental phenotype including fracture, haemorrhage and dysphagia, which is managed using a special wet food diet. Severe disease requires euthanasia. The dentine tubule is the primary structural unit in dentine, formed by matrix deposition around each odontoblast’s single trailing cell process. Aberrant dental tubule formation might explain oim’s brittle teeth. The aim of this study was to quantitatively investigate dental tubule microstructure of oim teeth in three dimensions. We made 3-dimensional synchrotron X-ray microtomographic images of the left mandibular incisor from 13 wild-type (WT) and 9 oim mice at the I13-2 beamline of the Diamond Light Source, UK. We used a filtered polychromatic “pink” beam (5–35 keV, mean 15 keV), collecting 3001 projections uniformly over 180° at 30–45 ms exposure per projection via a CdWO₄ scintillator, light microscope and pco.edge 5.5 camera (pixel spacing 0.33 μm). Three 200-slice substacks from the tip, mid-portion and neck of each tooth were converted to 16-bit and inverted, so that tubules appeared as bright spots in cross section. Background, artefact and cracks were removed by manual segmentation. TrackMate (v2.7.2) was used to track tubules as they passed through stack slices, forming 3-dimensional track data. Dentine tubule areal density was calculated by dividing number of tracks in a cross-section by dentine area. Dentine area was not significantly different between oim and WT teeth (Mann–Whitney U, p = 0.35), while tubule areal density was significantly greater in oim, with oim having 42% more tubules/mm² than WT (median: WT 7395 tubules/mm², oim 10473 tubules/mm², p < 0.001). We noted occasional terminal dilatation, increased curvature and increased variability in size in oim tubules. The altered 3D dentine tubular porosity in oim incisors may have implications for their mechanical competence. The oim mutant is a promising model for the development of targeted therapies for human dentinogenesis imperfecta.
Osteoporosis and atherosclerosis: higher bone density is associated with greater carotid intima-media thickness in middle-aged women

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Objectives: Osteoporosis and cardiovascular disease (CVD) are common age-related conditions which have frequently been found to be associated. Previous studies have largely focused on older people; the relationship in middle-aged populations is less clear. We examined the relationship between bone characteristics and preclinical atherosclerosis measured by carotid intima-media thickness (cIMT) in mid-aged women.

Methods: Mothers from the Avon Longitudinal Study of Parents and Children, UK population based cohort were examined (n = 2933). Total body (TB) and hip bone mineral density (BMD) along with TB bone area (BA) and bone mineral content (BMC) were measured using DXA scans; bone turnover was assessed by serum beta-carboxyterminal cross linking telopeptide (βCTX) concentrations; cIMT was measured by high-resolution B ultrasound. We examined the association of βCTX and DXA-derived variables with cIMT, using multivariable linear regression, adjusting for a range of confounders. Results are expressed as partial correlation coefficients with 95% confidence intervals.

Results: Mean (SD) participant age was 48 (4) years, BMI 26.2 (5.0) kg/m², and 70% were premenopausal. βCTX was positively associated with cIMT (0.064 [0.027, 0.101] p = 0.001), but attenuated with age adjustment (0.003 [-0.034, 0.040] p = 0.8). Total hip BMD was positively associated with cIMT (0.060 [0.024, 0.097] p = 0.001); this association persisted after adjustment for age, height, lean & fat mass, smoking, education level, estrogen replacement and menopausal status (0.057 [0.017, 0.097] p = 0.005). Equivalent adjusted associations were observed for TB BMD (0.061 [0.017, 0.105] p = 0.006), reflecting associations with both BA (TB BA 0.056 [-0.0001, 0.113] p = 0.050) and volumetric bone density (TB area-adjusted BMC (0.118 [0.009, 0.228] p = 0.034). We found no evidence that associations differed between pre- and post-menopausal women.

Conclusion: Previous published studies, mostly performed in older populations, have suggested low BMD is associated with greater CVD risk. In contrast, we identified weak positive associations between BMD and cIMT, in a predominantly premenopausal female population. Rather than reflecting an association with bone turnover, this appeared to reflect an association with bone size and volumetric density. Our results require further replication and investigation in large prospective studies.

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Conflict of interest: None declared.
Early life motor ability is positively associated with adolescent bone strength

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Objectives: Attainment of early life movement milestones such as walking and running represents the first postnatal exposure of the skeleton to the large muscle and reaction forces associated with locomotion. Accordingly, timing of motor skill acquisition was recently shown to be a primary determinant of bone strength in early childhood. However, effects of early life motor ability on bone in later life are unknown.

Methods: Relationships between gross motor score (GMS, using components of the Denver Developmental Screening Test) at 18 months, and the Avon Longitudinal Study of Parents and Children (ALSPAC) Co-ordination Test (ACT) score at 7 years, and bone outcomes measured at age 17 years, were examined in 2327 ALSPAC participants. Higher scores indicate greater and lower motor ability in GMS and ACT, respectively. Tibia cortical bone mineral content (BMC), periosteal circumference (PC), cortical thickness (CT), cortical bone area (CBA), cortical BMD (BMDc) and cross sectional moment of inertia (CSMI) were assessed by peripheral quantitative computed tomography (pQCT) at 66% distal-proximal tibia length. Dual-energy X-ray absorptiometry (DXA) was used to assess total hip bone mineral density (BMD) and hip CSMI. Data were adjusted for maternal social class, gestation length, birthweight and age at exposure/outcome using multiple linear regression, and are presented as standardised regression coefficients and 95% CI.

Results: GMS and ACT were associated with all bone measures (all \( P < 0.001 \)) with the exception of BMDc (\( P > 0.25 \)). Effects of GMS and ACT on tibia BMC ([0.105 (0.089, 0.121]) and [−0.083 (−0.067, −0.099)]) and hip BMD ([0.086 (0.067, 0.105]) and [−0.089 (−0.07, −0.108)]) were most pronounced. Gender*motor score interactions were observed for most measures, with 41–69% greater regression coefficients in males (all \( P < 0.05 \)). Adjustment for lean and fat mass led to substantial (15–64%) attenuation of regression coefficients, whilst adjustment for physical activity assessed by accelerometry in a cohort subset (350 participants, 144 males) led to minor attenuation (22–23%) in males only.

Conclusion: Early life motor ability may represent a novel risk factor for osteoporosis – particularly in males. These effects are in part mediated by body composition and physical activity. Future studies examining these relationships in older adults would assess effectiveness of motor ability scores in predicting osteoporosis.
The origins of bone and cartilage disease: high throughput bone phenotype screen to identify new genes that determine bone structure and strength

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Bone mineral density is a quantitative trait with 60–90% heritability, 3% of which is accounted for by known genetic variation. The Origins of Bone and Cartilage Disease (OBCD) initiative is an international consortium identifying new genes involved in the pathogenesis of skeletal disease. We developed and validated a rapid-throughput skeletal phenotyping screen based on imaging and biomechanical testing that includes both structural and functional approaches. 1500 International Mouse Phenotyping Consortium (IMPC) knockout mouse lines will be screened over 5 years to identify those with significantly abnormal bone structure and function.

Bone mineral content and bone length are determined by digital X-ray microradiography (Faxitron MX-20), BMD and cortical and trabecular bone 3D microarchitecture by micro-CT (Scanco MicroCT-50), and bone strength by destructive three-point bend testing of femurs and compression testing of vertebrae (Instron-5543 load frame). C57BL6/N strain-specific reference ranges have been established for all parameters using samples from 16 week-old female wild-type mice (n = 94). Thus, samples (left femur, 6–7th caudal vertebrae) from only 2 individual 16 week-old female mice per knockout line are required to identify significant outlier phenotypes with parameters >2SD outside the reference range.

To date we have completed analysis of 233 unselected knockout lines and identified 18 with major abnormalities of both bone structure and strength. One, Sparc

Sparc

knockout mice had previously and independently been generated and are reported to have osteopenic, brittle bones. These findings were replicated in the OBCD screen validating our approach. The other 17 lines target genes not previously associated with skeletal disorders. Six lines have a high bone mass phenotype including Uevld

Uevld

and Usp11

Usp11

both genes involved in the ubiquitination conjugation pathway. The other 11 lines have a low bone mass phenotype, including Daam2

Daam2

and Agap1

Agap1

which is involved in the regulation of endocytosis. These studies demonstrate that skeletal phenotyping of a large series of unselected knockout mice can rapidly identify new genetic determinants of skeletal disease.

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The role of LC3 and autophagy in bone resorption by osteoclasts

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Osteoclasts resorb bone by delivering lysosomes to the highly convoluted ruffled border membrane, where they fuse and release acid and proteolytic enzymes to the bone surface. The autophagy protein LC3 is necessary for bone resorption by osteoclasts, although it has been suggested that this may be through a novel, autophagy-independent process, by promoting lysosomal fusion at the ruffled border. This process would be conceptually similar to LC3-associated phagocytosis (LAP), in which LC3 is acquired by phagosomes through an autophagy-independent process, and controls phagosome maturation by promoting fusion with lysosomes. We have investigated this possibility by using novel mouse models for monitoring LC3 localisation and in which autophagy is selectively ablated. By generating osteoclasts from mCherryGFP-LC3 mice and culturing them on dentine in vitro, we found that LC3 localises within the F-actin ring (i.e., to the ruffled border) in 30% of actively resorbing osteoclasts. Morphological analysis suggests most of these osteoclasts are at an early stage of ruffled border formation and resorptive activity; LC3 localised to nascent actin rings but was absent from mature actin rings associated with extensive resorption pits. By contrast, LC3-positive autphagic vesicles did not accumulate close to the actin ring of osteoclasts. This data is consistent with a potential autophagy-independent role of LC3 in lysosomal fusion. We further investigated this by using an autophagy-deficient mouse model in which FIP200 is conditionally deleted in the myeloid lineage; FIP200 is essential for autophagy, but is not required for LAP. Osteoclasts generated from this model were able to target LC3 to the ruffled border and resorb dentine despite severely impaired autophagy; this indicates that a process similar to LAP, rather than autophagy, controls formation of the ruffled border in osteoclasts. This likely also involves Plekhm1, loss of function mutations in which cause osteopetrosis due to the failure of osteoclasts to form ruffled borders and resorb bone. Plekhm1 is recruited to lysosomes by Rab7, and plays a role in autophagy by mediating the fusion between lysosomes and autophagosomes through binding to LC3 on the autophagosome. In osteoclasts we suspect that the same machinery is utilised for fusion of lysosomes at the ruffled border.
PAR2 response in fluid flow-stimulated chondrocytes

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Osteoarthritis (OA) is a heterogeneous musculoskeletal disease. We have compelling evidence that proteinase-activated receptor-2 (PAR2) is a mediator of OA initiation/progression, integrating matrix deregulation and tissue remodeling/inflammatory damage. PAR2 offers a potentially novel therapeutic target in OA. The onset of OA has mechanical “damage” as a common denominator and we have observed elevated of PAR2 in articular chondrocytes during the OA mouse destabilization of the medial meniscus (DMM) model and in human OA cartilage. PAR2 was also found in osteocytes. Whilst wild type animals exhibit rapid osteophyte formation, PAR2-deficient (PAR2−/−) mice are substantially protected from this process (osteophyte formation = 92.3% in WT, vs. 45.5% in PAR2−/− mice, and the latter were significantly smaller, WT = 2.50 ± 0.27, PAR2−/− = 0.41 ± 0.19 μm³). Osteosclerosis was significant by day 14 in WT (P = 0.023) and remained so at day 28 (P = 0.019) whilst PAR2−/− mice showed no significant osteosclerosis at either day 14 or 28, potentially indicating a slower response to the mechanical changes induced by DMM surgery. This led us to question whether PAR2 may play a role in mechanotransduction. Murine osteocyte-like cells (MLOY4) were stimulated with 7 dyn/cm² of fluid flow shear stress (FSS) and human chondrocyte-like cells (SW1353) with physiological (5 dyn/cm²) and pathophysiological (20 dyn/cm²) FSS for 1 h. During FSS, MLOY4s were administered 10 nM PAR2 activating peptide SLIGRL or control reverse peptide. Immunofluorescence and western blotting showed intrinsic PAR2 presence in MLOY4s, which did not change after FSS stimulation. FSS increased COX2 gene expression 4-fold (P < 0.01). SLIGRL in static conditions induced a 2-fold increase in IL-6 expression, which was reduced to static control levels after FSS (P < 0.01). No other parameters were significant. SW1353s did not contain intrinsic PAR2 under static conditions, yet a marked increase was noted upon stimulation with FSS (mean grey value ± SD: static control, comparable to background, = 92 ± 5.6, 5 dyn = 226 ± 23, 20 dyn = 191 ± 22, P < 0.001, 1way ANOVA). This FSS-induced increase in PAR2 suggests that altered biomechanical loading may initiate PAR2 mediated mechanisms ultimately leading to cartilage degradation, which could have implications for human disease.
Endocrine role of bone: phospho1 a novel regulator of energy metabolism

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Cell-specific gene deletions in mouse and human genetic studies have identified bone as an endocrine organ capable of regulating whole-body glucose metabolism. We now show that the ablation of PHOSPHO1, a bone specific phosphatase, indispensable for bone mineralization, confers a remarkable degree of protection against obesity and diabetes in mice. Phospho1−/− mice (35 day-old) have lower blood glucose (mmol glucose/L), (WT, 9.31 ± 0.31; Phospho1−/−, 7.76 ± 0.39, p < 0.01) and improved glucose and insulin tolerance. Food intake (g/gBW/day: WT, 0.126 ± 0.006; Phospho1−/−, 0.117 ± 0.006, p = 0.29) and energy expenditure are comparable between 35 day-old WT and Phospho1−/− mice fed a control diet. Phospho1−/− mice (120 day-old) resist obesity on a high fat diet (BW: WT, 38.0 ± 1.54 g, Phospho1−/−, 32.4 ± 1.26 g, p < 0.05; visceral fat weight: WT, 13.2 ± 1.34 g; Phospho1−/−, 5.56 ± 1.61 g; p < 0.01). Despite a 20-fold increase in Esp expression (negative regulator of osteocalcin (OCN)), in Phospho1−/− osteoblasts (osb) the serum levels of undercarboxylated OCN were normal, suggesting an OCN-independent mechanism of PHOSPHO1-regulated energy metabolism. A key marker of insulin sensitivity in bone (Akt phosphorylation) was elevated (p < 0.05) in Phospho1−/− mice following a sub-maximal in vivo administration of insulin (1 mU/g), with no changes noted in liver, muscle and fat. Phospho1−/− osb showed an enhanced response to mitochondrial stress parameters in vitro, suggestive of improved respiration and increased basal mitochondrial activity. The latter was associated with elevated GLUT4 expression. Furthermore, Phospho1−/− osb conditioned medium (OCM), but not WT OCM increased basal insulin sensitivity (AKT and GSK phosphorylation) in WT primary osb, decreased insulin-stimulated sensitivity of INS1e cells. Ucp1 expression and acute cold studies suggested a white adipose tissue browning phenotype in Phospho1−/− mice. Mass spectrometry analysis identified > 100 differentially expressed proteins in Phospho1−/− serum associated with the regulation of glycolysis and gluconeogenesis. Mass spectrometry analysis also indicated normal levels of ceramide species in Phospho1−/− mice, whereas Phospho1−/− mice fed 2% choline rich diet displayed a normalisation in insulin sensitivity and fat mass. These data suggest that Phospho1-deficiency improves the metabolic profile of mice in vivo and confers resistance to obesity and diabetes possibly via the alteration of serum/tissue choline levels.
Genome-wide analysis identifies significant predictors of therapeutic response to teriparatide in severe osteoporosis

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Osteoporosis is a common disease associated with low bone mass and an increased risk of fragility fractures. Teriparatide, an anabolic agent, is the preferred treatment for severe osteoporosis, since substantial bone loss has already occurred by the time osteoporosis first presents. Studies have demonstrated superiority to oral bisphosphonates in preventing vertebral fractures, but treatment costs for TPTD are high and the response is variable. Identifying markers to response to TPTD is crucial to target treatment more effectively, but there are no established means of doing this at present. We performed a genome wide association study (GWAS) of 162 patients from UK and Denmark, using an Illumina Omni Express array. Standard quality control tests were applied. Statistical analysis tested the percentage of change in lumbar spine BMD following treatment with TPTD, adjusted by duration of treatment (18–24 months). The data from the two cohorts were analysed separately and results were combined using an inverse-variance meta-analysis. No evidence of inflation was found (l² = 1.001–1.003). Adjusting for gender, age, smoking, alcohol intake, dietary calcium intake, age at menopause and previous bisphosphonate therapy was also performed with similar results to the unadjusted test. We identified a genome wide significant association between changes in spine BMD following TPTD therapy and a SNP on chromosome 3, within the neuroligin 1 gene (p = 8 × 10⁻⁹), with a substantial effect size (beta = 0.654 [95% CI = 0.43–0.87]); heterozygote carriers of the rare A allele (good responders) had a 28% gain in BMD over 24 months compared with a 11.7% gain in GG homozygote’s – a clinically significant difference over 11 standard deviations. Besides, five separate signals in chromosomes 2, 15, 13, 1, and 9 were found suggestively associated to the trait (p = 1 × 10⁻⁷–1 × 10⁻⁶). We combined information from these six hits finding a highly significant association with the number of response alleles carried and change in BMD (24 month change in BMD in those who carried < 2 responder alleles was 8.8% compared with 33.8% for those who carried five or more responder alleles (p = 4.95 × 10⁻¹¹)). Our results will help to gain greater understanding of the genes and pathways responsible for the bone formation. Furthermore, we have established an allelic score in response to TPTD that could be used to improve treatment decisions in clinical practice. An extended study and replication are currently in progress.
Clinical case: clinical and genetic analysis in a unique systemic skeletal disorder characterised by high bone turnover and bone expansion

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Background: Major advances have been made in understanding the molecular basis of rare bone diseases over recent years. We describe clinical and genetic results from a patient with unique bone dysplasia characterised by high bone turnover, radiological features of generalised osteosclerosis and bone expansion alternating with areas of osteolysis, and features that overlap with but are distinct from those in Paget’s disease of bone (PDB).

Case: A 34 year old man presented with generalized weakness, an inability to squat, and lower limb pain on persistent exertion since his mid-teens. He reported limb fractures aged 2, 7, 15, and 21. Sensory-neural hearing loss required hearing aids from aged 28. After delayed primary tooth eruption, he experienced normal puberty, skeletal growth and schooling. He has mildly shortened terminal phalanges, quadriceps wasting and BMI 18 kg/m². He is the eldest of two sons from a non-consanguineous healthy family. Routine biochemistry was normal except raised alkaline phosphatase (134 U/L normal 30–130). Serum βCTX 1.7 μg/L (0.1–0.5) and P1NP 179 μg/L (20–76) were both raised.

Radiographs showed abnormal bone architecture throughout the skeleton, with areas of osteosclerosis alternating with osteolysis, coarse trabecular markings and cortical thickening. Bone density on DXA was high at the spine and hip (T-scores >+4.0). Nerve conduction studies and EMG were normal. Muscle biopsy showed mild non-specific myopathic changes.

Genetic analysis: We performed whole-exome sequencing on the index case and three unaffected family members on an Ion Proton™ Sequencer using standard techniques. On average, each exome generated 42 million mapped reads 90% were covered >20x. We identified 64 damaging variants potentially responsible for the disorder but were unable to narrow the list down further to conclusively define the responsible variant(s). Of note, the exome analysis excluded mutations in known candidate genes for PDB (TNFRSF11A, TNFRSF11B, TNFSF11, SQSTM1, VCP, TM7SF4, CSF1, RIN3, OPTN) and high bone mass syndromes (TCIRG1, CLCN7, PLEKHM1, OSTM1, CA2, IKBKG, ITGB3, LRP5).

Conclusion: We describe a novel skeletal disorder, presumed to be genetic in origin with features overlapping with, but distinct from, PDB which appears to have a novel molecular basis. Further investigations are in progress to define the cause.
Successful use of denosumab to treat osteoporosis in a patient with severe anorexia nervosa

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Osteoporosis in women with anorexia nervosa is almost ubiquitous and difficult to manage in the presence of persistently low body weight. We present a case where a novel approach was taken to improve bone mineral density (BMD). A 29 year old female with a 17 year history of severe enduring anorexia nervosa attended our unit. Osteoporosis was diagnosed aged 24 and she had developed a left calcaneal fracture after minimal trauma 3 weeks prior to presentation. Her BMD at this time confirmed the presence of osteoporosis at the lumbar spine and total hip (T-score −3.3 and −2.9, respectively) and her body mass index (BMI) was low at 15.1 kg/m². She declined therapy previously with oestrogen and bisphosphonate therapy and did not wish daily injections. A decision was made to commence therapy with Denosumab 60 mg by subcutaneous injection every 6 months with monitoring of serum calcium and co-administration of calcium and vitamin D. A further measurement of bone mineral density was made 2 months after completing 3 years of therapy with Denosumab. During the period of treatment the patient did not experience any adverse effects related to the treatment. There was no evidence of hypocalcaemia nor were there further fractures. Her BMI was unchanged and she remained amenorrheic during the treatment course. BMD increased substantially at the lumbar spine (14.8% increase from pre-treatment) and at the left total hip site (1.4% increase from pre-treatment). The measurement at the left femoral neck showed a reduction of −5.7% from its pre-treatment value.

Denosumab is a human monoclonal antibody that binds with high specificity to the receptor activator of nuclear factor-κB ligand (RANKL) and results in decreased bone resorption, decreased bone turnover and reduces vertebral and non-vertebral fractures in postmenopausal women and can be safely administered for at least 6 years. The magnitude of the change in bone mineral density seen in post menopausal women treated with Denosumab is similar to that observed in this case despite the patient’s ongoing low weight and suggests that this agent may be a useful therapy for treating osteoporosis in patients with anorexia nervosa and merits further investigation in the appropriate trial setting.
The role of poly(ADP ribose) in bone mineralization

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Objectives: We have recently shown that poly(ADP ribose) is present in the extracellular matrix at the calcification front of foetal bone growth plate and in the extracellular matrix in an in vitro foetal sheep osteoblast model of developing bone [1]. The objective of the present work is to establish if poly(ADP ribose) is a necessary component of bone mineralization and to understand how it arrives in the extracellular matrix. Our hypothesis is that poly(ADP ribose) acts to spatially locate bone mineralization (either by acting as a scaffold for mineral formation or acting as a signalling molecule to highlight matrix to be mineralized) and that it derives from dying/dead cells.

Methods: Human foetal osteoblasts were used to produce mineralising matrix by the addition of b-glycerol phosphate (2 mM) and dexamethasone (10−7 M) at cell confluence. Poly(ADP ribose) was stained using anti-PADPR antibody (10 H) (Abcam) with a goat anti-mouse-FITC conjugated second antibody and imaged on a Nikon Eclipse Ti microscope. Mineral were imaged using phase contrast on the same samples. Live/dead cell staining was done using calcein AM/ethidium reagents (Invitrogen). To investigate the requirement for poly(ADP ribose) in mineralization poly(ADP ribose) synthesis was inhibited, in some samples, by addition of PARP inhibitors, and mineral/poly(ADP ribose) production examined as above.

Results: Extracellular mineral deposits in the in vitro model showed a high degree of spatial correlation with the presence of poly(ADP ribose) and with dead cells. Preliminary results suggest that inhibiting poly(ADP ribose) synthesis may inhibit mineralization.

Conclusion: Our results, taken together with our previously published results (Chow et al., 2014), suggest that poly(ADP ribose) is an important component of bone matrix mineralization, and that it may act to spatially locate mineral deposits.

Reference

ENPP1 enzyme replacement therapy for generalized arterial calcification of infancy

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Objectives: Generalized Arterial Calcification of Infancy (GACI) is a rare, life-threatening disorder caused by mutations in the gene encoding ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1). The disease is characterized by widespread arterial calcification leading to vascular related complications. ENPP1, a plasma membrane protein, hydrolyzes ATP to produce pyrophosphate (PPi), a key inhibitor of hydroxyapatite formation and vascular calcification. Here, a soluble form of ENPP1 was engineered as a potential replacement therapy using the Enpp1asj and ENPP1−/− mouse models of GACI (1, 2).

Methods: Recombinant protein variants containing the extracellular portion of human ENPP1 fused to human IgG1 Fc (ENPP1-Fc) were created and used in the studies. Enpp1asj/asj mice were placed on acceleration diet (TD.00442) at birth and were treated with ENPP1-Fc, 5 mg/kg subcutaneously (SC), every other day (EOD) beginning at day 14 or 18. Median survival was measured along with serum biomarkers, including FGF23, throughout the 12 weeks study. Additionally, ENPP−/− mice placed on a high phosphate diet were treated EOD with 6 mg/kg NPP1-Fc SC for 3 weeks and aortic calcification was measured.

Results: SC administration of ENPP1-Fc (5 mg/kg) EOD increased the median survival of Enpp1asj/asj mice on the acceleration diet when compared to vehicle treated controls. Body weight was also positively affected in the treated animals. Enpp1asj/asj mice on acceleration diet show progressive elevation of FGF23 levels compared to wild type mice. Treatment with ENPP1-Fc blunted the rise in FGF23 when compared to vehicle treated controls up to the study’s end. Enpp1−/− mice fed a 2% phosphorus diet and receiving SC injection (6 mg/kg) EOD for 18 days had an 86 +/− 5% lower aortic calcium content compared to vehicle-injected mice.

Conclusion: SC administration of a soluble variant of human ENPP1 produced an increased survival, blunted FGF23 elevation, and prevented aortic calcification in mouse models of GACI.
Type 2 diabetes is not associated with increased bone sclerostin expression, despite enhanced serum expression levels at onset of diabetes

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Type 2 Diabetes Mellitus (T2DM) is associated with an impaired skeletal structure and a higher prevalence of bone fractures. Sclerostin is a negative regulator of bone formation produced by osteocytes and there is recent evidence that its expression in serum is elevated in diabetic patients compared to control subjects. In this study, we examined (1) the effect of T2DM on bone architecture and sclerostin levels in a rat model of T2DM (2) the influence of hyperglycemia on sclerostin production by osteoblasts \textit{in vitro}. Bone architecture was measured by microCT in 14 weeks-old Zucker diabetic fatty (ZDF) and Zucker lean male rats that act as controls (\(n = 6\) /group). Serum sclerostin expression was quantified at 9, 11 and 13 weeks-old rats using an ELISA and in femurs of 14 weeks-old rats using qPCR. The number of osteocytic empty lacunae was calculated in tibiae of 14 weeks-old ZDF and lean rats on sections stained with haematoxylin and eosin. Rat osteoblast-like UMR-106 cells were cultured with increasing concentrations of glucose (5, 11, 22, and 44 mM) and sclerostin mRNA expression and protein release determined by qPCR and ELISA, respectively. Our results showed that ZDF rats have lower trabecular bone mineral density and bone mass compared to controls, due to decreases in bone volume and thickness. They also exhibit impaired bone connectivity and cortical bone geometry. Serum sclerostin levels were higher in ZDF compared to lean rats at 9 weeks (+40\%, \(p < 0.01\)), but this difference disappeared at 11 and 13 weeks as the rats get more diabetic. Bone sclerostin mRNA levels were similar in ZDF and lean rats. Similarly, the number of osteocytic empty lacunae in cortical and trabecular bone of ZDF and lean rats were not significantly different. Interestingly, glucose dose-dependently stimulated sclerostin mRNA levels and protein release in UMR106 osteoblastic cells. Altogether, our data suggest that although sclerostin production by mature osteoblasts is increased by hyperglycemia \textit{in vitro}, bone sclerostin levels and number of apoptotic osteocytes are not higher in diabetic bone compared to controls. Further studies are required to determine whether sclerostin could contribute to the deleterious effect of T2DM on bone.
Differences in pain experience between women with and without vertebral fractures: novel independent descriptors identified


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Objectives: Less than one third of osteoporotic vertebral fractures (VFs) come to clinical attention due to a variety of reasons including a lack of evidence about which characteristics of back pain may indicate presence of VF. The aim of this project was to investigate whether the quality or type of back pain in people with VFs is different to those with back pain but no VFs, and if so to develop a simple back pain questionnaire for discrimination.

Methods: A case-control study was undertaken. Inclusion criteria were >60 years, female and thoracic spinal radiograph performed in the previous 3 months. 683 potential participants were approached, and those recruited self-completed a questionnaire including the McGill Pain Questionnaire and the Keele STarT back pain score. Cases were defined at the end of the study as those with a VF identified from spinal radiographs using the ABQ method. Chi-squared tests were used to assess univariable associations and logistic regression to identify independent predictors of VFs. Receiver Operating Characteristic (ROC) curves were used to evaluate the ability of the combined independent predictors to differentiate between women with and without VFs via Area Under Curve (AUC).

Results: 64 cases and 133 controls completed questionnaires. Back pain described as crushing, pain relieved by lying down, pain not spreading down legs, shorter duration of back pain, history of previous fracture, use of walking aids and a diagnosis of polymyalgia rheumatica were independent predictors of VFs. ROC analysis showed the AUC was 0.88 (95% CI 0.82 to 0.94) for these questions.

Conclusion: We identify novel predictors of VFs in older women based on descriptions of back pain. These could be combined to produce a simple questionnaire that has the potential to discriminate between women who are likely to have a VF and should therefore have diagnostic spinal radiographs, and women who have degenerative spinal disease instead.

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Cluster analysis of high resolution peripheral quantitative computed tomography parameters identifies bone phenotypes associated with high rates of prevalent fracture

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Objectives: To identify clusters of bone microarchitecture in older men and women and relate them to fracture prevalence and aBMD.

Methods: We studied 177 men and 159 women, aged 72.1–80.9 years, with HRpQCT (XtremeCT) images (voxel 82 μm) of the distal radius. Standard image analyses were performed for assessment of macrostructure, regional densitometry, cortical porosity and trabecular microarchitecture. K-means partitioning cluster analysis was used to identify 5 clusters in men and 5 in women. Prevalent fracture rates and femoral neck aBMD were determined for each cluster.

Results: Forty four (24.9%) men and 48 (30.2%) women had fractures. Women with fractures were on average 1.7 years older and 3.1 years further from menopause that women who had not fractured (p < 0.05). Although analyses were carried out separately in each sex, two morphologically-similar, high risk clusters were identified in each sex. “Cluster A” contained 20 women (50.0% fractured) and 14 men (35.7% fractured) and showed a phenotype with mean trabecular density and trabecular number both more than 1 SD below the sex-specific cohort mean. “Cluster B” contained 26 women (50.0% fractured) and 30 men (50.0% fractured) and denoted a phenotype with mean cortical thickness and cortical volumetric BMD around 1SD below and, in men, mean total and trabecular area more than 1SD above, the sex-specific cohort mean. Logistic regression showed fracture rates in these clusters to be significantly higher than the lowest fracture risk cluster (E) (p < 0.05). Mean femoral neck aBMD was significantly lower than cluster E in women in cluster A and B (p < 0.001 for both), and in men, in cluster A (p < 0.001) but not B (p = 0.220).

Conclusion: We have identified a cluster (B) that describes a bone phenotype which differs from the conventional view of osteoporosis but with a high proportion of fractures. In men, this phenotype was not associated with lower aBMD measured by DXA and therefore could be missed by current clinical assessments of osteoporosis.
Targeted sequencing for monogenic causes of high bone mass: the clinical severity of the high bone mass phenotype corresponds to different sites of \textit{LRP5} mutation


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Objectives: High bone mass (HBM) can be an incidental clinical finding; however, monogenic HBM disorders (e.g., due to \textit{LRP5} or \textit{SOST} mutations) are rare. We aimed to determine to what extent HBM is explained by mutations in known HBM genes and determine the associated clinical characteristics in a large HBM population.

Methods: 258 unrelated HBM cases were identified from review of 335,115 DXA scans from 13 UK centers. Cases were assessed clinically and underwent sequencing of known anabolic HBM loci: \textit{LRP5} (exons 2, 3, 4), \textit{LRP4} (exons 25, 26), \textit{SOST} (exons 1, 2, and the van Buchem’s disease (VBD) 52 kb intronic deletion 3′). Family members were assessed for HBM segregation with identified variants. Three-dimensional protein models were constructed for identified variants.

Results: Two novel missense \textit{LRP5} HBM mutations ([c.C518T; p.T173M], [c.C796T; p.R266C]) were identified, plus three previously reported missense \textit{LRP5} mutations ([c.A593G; p.N198S], [c.G724A; p.A242T], [c.A266G; p.Q89R]), associated with HBM in 11 adults from seven families. Individuals with \textit{LRP5} HBM (∼population prevalence 5/100,000) displayed a variable phenotype of skeletal dysplasia with increased trabecular BMD and cortical thickness on HRpQCT, and fat mass accumulation in a gynoid distribution on DXA, compared with both non-\textit{LRP5} HBM and controls. One mostly asymptomatic woman, with BMD $Z$-scores L1 $+3.5$, Hip $+1.7$, carried a novel heterozygous nonsense \textit{SOST} mutation (c.C530A; p.S177X) predicted to prematurely truncate sclerostin.

Protein modelling: Protein modelling suggests the severity of the \textit{LRP5}-HBM phenotype corresponds to the degree of protein disruption and the consequent effect on SOST-LRP5 binding. We predict N198S directly disrupts SOST binding and the larger threonine side chain in A242T leads to steric clashes destabilising the SOST binding site; both correspond to severe HBM phenotypes (e.g., BMD $Z$-scores $+3.1$ to $+12.2$, inability to float). Less disruptive structural alterations predicted from p.R266C, p.T173M, p.Q89R associate with less severe phenotypes (e.g., $Z$-scores $+2.4$ to $+6.2$, able to float).

Conclusion: In conclusion, although mutations in known HBM loci may be asymptomatic, they only account for a very small proportion of individuals with HBM, suggesting the great majority are explained by either unknown monogenic causes or polygenic inheritance.
Characterisation of vertical accelerations (g) and rate of change in acceleration (jerk) experienced by older people attending an aerobics class designed to produce high impacts

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Objectives: Aerobics classes may be useful for increasing physical activity (PA) levels in older people. However, we recently found that vertical impacts experienced during a typical class are below the target of around 4 g for bone protection, identified in adolescents and premenopausal women. We aimed (i) to establish the feasibility of using an aerobics class to produce vertical impacts ≥4 g in older individuals as measured by accelerometry; (ii) to determine whether vertical impacts during aerobics classes can be predicted by screening for physical function; (iii) to investigate whether physical function predicts other aspects of muscle performance during aerobics classes, namely jerk.

Methods: Subjects were recruited from attendees at an exercise class for older individuals. Each participant completed an SF-12 questionnaire and short physical performance battery, was fitted with an accelerometer positioned over the hip, and subsequently completed an aerobics class with seven different components, performed at low and high intensity in turn. Maximum g values were identified for each activity. Maximum jerk was derived by dividing the difference between consecutive g values by time elapsed.

Results: 41 participants (seven males), mean 69 years, were included. Mean maximal values approached or exceeded the 4 g threshold for four of the seven high impact components. In multivariate analyses, age (−0.53; −0.77, −0.28), and 4 m walk time (−0.39; −0.63, −0.16) (standardised beta coefficient; 95% CI) were inversely related to maximum g. No association was observed with gender or chair rise time. In contrast, age (−0.28, −0.54, −0.02), walk time (−0.32, −0.57, −0.07) and chair rise time (−0.32, −0.59, −0.05) were all related to aggregated maximum jerk.

Conclusion: Aerobics classes can be used to produce relatively high vertical accelerations in older individuals, although the outcome is strongly dependent on age and physical function. Chair rise time predicted jerk but not g, providing face validity for jerk as a measure of rate of force development which may represent a distinct aspect of muscle function.

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Role of insulin-like growth factor 1 as a potential mediator of the relationship between lumbar muscle mass and bone mineral density in men versus women: a farmers' cohort for agricultural work-related musculoskeletal disorders (farm) study

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Objectives: Insulin-like growth factor 1 (IGF-1) is a growth-promoting polypeptide essential for normal growth and development of muscle and bone. We assessed the potential role of IGF-1 as a mediator between lumbar muscle mass and bone mineral density (BMD) in men and women of the Korean farmers’ cohort for agricultural work-related musculoskeletal disorders (FARM).

Methods: Two hundred sixty-five men, 47 premenopausal women, and 227 postmenopausal women were recruited from Korean farmers. Blood was drawn without fasting, and IGF-1 concentration (mg/dL) was measured using radioimmunoassay. Cross-sectional computed tomography scans were acquired at the mid-L4 vertebral level with a 10-mm slice thickness. Total muscle mass (TMM, cm³) was segmented using standard Hounsfield unit ranges for skeletal muscle (−29 to +150). Spinal back muscle mass (BMM) was computed by manually outlining the psoas and paraspinal muscles. Abdominal wall muscle mass (AMM) was calculated as TMM − BMM. Spinal BMD (g/cm²) was estimated from dual-energy X-ray absorptiometry at the L4 level.

Results: TMM, BMM, and AMM were significantly related to spinal BMD in postmenopausal women (r = 0.61–0.272; P < 0.05, P < 0.001), while only AMM in men (r = 0.185; P < 0.01) and TMM in premenopausal women (r = 0.314; P < 0.05) showed significant associations with spinal BMD. Un-adjusted linear regression showed significant associations in spinal BMD with AMM of men (β = 0.18, P < 0.01), TMM of premenopausal women (β = 0.31, P < 0.05), and TMM, BMM, and AMM of postmenopausal women (β = 0.16–0.27, P < 0.001, P < 0.05). Adjusting for IGF-1 revealed a significant additive positive association between AMM and spinal BMD in premenopausal women (β = 0.29, P < 0.05).

Conclusion: There may exist a sexual dimorphism in the relationship of back muscle mass and IGF-1 to spinal BMD. Additionally, IGF-1 may mediate the complementary association between spinal muscle mass and BMD in men.
Stability of 25-hydroxyvitamin D status from early to late pregnancy

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Objectives: The role of maternal 25-hydroxyvitamin D [25(OH)D] in fetal development is uncertain and findings of observational studies are inconsistent. Most studies have assessed 25(OH)D only once in pregnancy, but the stability of ranking (“tracking”) of an individual’s 25(OH)D during pregnancy is unknown. We therefore determined the tracking of 25(OH)D from early to late pregnancy, and factors which influence this.

Methods: The Southampton Women’s Survey is a prospective mother-offspring birth cohort study. Lifestyle, diet and 25(OH)D status were assessed at 11 and 34 weeks gestation. A Fourier transformation was used to model seasonal variation in 25(OH)D at 11 and 34 weeks gestation. The difference between measured and seasonally modelled 25(OH)D was calculated to generate a season-corrected 25(OH)D for every participant at each gestation. Tracking was assessed using Pearson’s correlation coefficient, and multivariate linear regression used to determine factors associated with change in season-corrected 25(OH)D.

Results: 1753 women were included. Season-corrected 25(OH)D was moderately correlated at 11 and 34 weeks gestation ($r = 0.53$, $p < 0.0001$). Vitamin D supplementation was the strongest predictor of tracking: compared with women who never used supplements, discontinuing supplementation after 11 weeks was associated with a reduction in season-corrected 25(OH)D ($\beta = -7.3$ nmol/l, $p < 0.001$), whereas commencing ($\beta = 12.6$ nmol/l, $p < 0.001$) or continuing ($\beta = 6.6$ nmol/l, $p < 0.001$) supplementation were associated with increases. Higher pregnancy weight gain was associated with reduction in season-corrected 25(OH)D ($\beta = -0.4$ nmol/l/kg, $p = 0.015$), whereas greater physical activity ($\beta = 0.4$ nmol/l/h/week, $p = 0.011$) was associated with increases.

Conclusion: There is moderate tracking of 25(OH)D status through pregnancy. Vitamin D supplementation, weight gain and physical activity are associated with changes in season-corrected 25(OH)D from early to late gestation. These findings have implications for study design and analysis and approaches to intervention studies and clinical care.
Calcium and phosphate synergistically induce the calcification of aortic valve interstitial cells: a role for matrix vesicles?

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Aortic valve calcification (AVC) is characterised by the thickening of the valve leaflets often resulting in stenosis and regurgitation. There is currently no medication to stop its progression, and its cellular mechanisms are not yet fully understood. Ectopic calcification of vascular smooth muscle cells (VSMCs) reportedly share many similarities with osteogenesis, including the secretion of mineralisation-competent nano-vesicular structures, known as matrix vesicles (MVs). However, the role of MVs in AVC is yet to be examined. Therefore, we determined whether elevated calcium (Ca) and/or phosphate (Pi) induced calcification of aortic valve interstitial cells (VICs) via the secretion of MVs.

In vitro studies employing the Sv40T rat VIC cell line revealed that elevated Ca in the culture medium induced calcium deposition at a minimum concentration of 2.7 mM (4.5 fold; \(P < 0.01\)), as determined by. Moreover, Ca treatment above >3.6 mM significantly increased the mRNA expression of the osteogenic markers PiT-1 (2 fold, \(P < 0.001\)), Runx2 (1.2 fold, \(P < 0.05\)) and Msx2 (2 fold, \(P < 0.001\)). While no effect of Pi treatment alone was observed, treatment with 2.7 mM Ca and 2.5 mM Pi synergistically induced calcium deposition (414 fold; \(P < 0.001\)).

Next, MVs were harvested using ultracentrifugation from primary rat VICs that were cultured with control medium or calcifying medium (2.7 mM Ca and 2.5 mM Pi) for 16 h to assess their composition using mass spectrometry. The results revealed that VIC-MVs isolated from the calcifying medium share similar protein content with chondrocyte- and VSMC-derived MVs, including the enrichment of the calcium-binding proteins: annexin (Anx) A2 (4.8 fold), A5 (4.6 fold), and A6 (4.3 fold).

Additionally, immunohistochemical studies showed increased AnxA6 expression in severely calcified human valve tissue compared to the uncalcified control. MV-like structures were also observed by transmission electron microscopy (TEM) in the extracellular matrix (ECM) of heavily calcified human valve samples.

Our data establish calcium as a novel trigger of VIC calcification. These studies are the first to report extracellular vesicles resembling MVs in calcified human aortic valve tissue, suggesting AVC is a cell-mediated process regulated by vesicle release.
Online linkage of FRAX fracture risk assessment to management guidance is used by clinical practitioners. an analysis of access to national osteoporosis guideline group guidance in the UK (July 2013–June 2014)

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In several countries, the online output of FRAX is linked to independent country-specific guidelines (e.g., UK, Lebanon, and Finland) which facilitates treatment decisions according to local assessment guidelines. In the UK, guidance provided by the National Osteoporosis Guideline Group (www.shef.ac.uk/NOGG) was made available in 2008. The aim of this study was to determine the uptake of this facility by exploring website activity using GoogleAnalytics software.

We have undertaken an analysis of FRAX and NOGG website usage for the year between 1st July 2013 and 30th June 2014 (GoogleAnalytic reports 8th August, 2014). During this period, there was a total of 1,774,812 sessions (a user interaction with the website) on the FRAX website with 348,964 of these from UK-based users. Over the same time, 253,530 sessions were recorded on the NOGG website. Of the latter, two-thirds were returning users, one-third new users and the vast majority (208,766,82%) arose from users in the UK. The remainder of users were from other countries with the US comprising approximately half of the non-UK use, demonstrating that some users of FRAX in other countries make use of the NOGG guidance. Of the UK-sourced sessions, the majority were from England, but the session rate (adjusted for population) was highest for Scotland (618 vs. 309/100,000, Scotland vs. England).

Almost all (95.7%) of the UK sessions arose from calculations being passed through from the FRAX tool (www.shef.ac.uk/FRAX) to the NOGG website, comprising FRAX calculations in patients without a BMD measurement (155 K, 74.5%) or FRAX calculations with a BMD result (44 K, 21.2%). A minority of sessions were conducted for other reasons (manual calculations, document downloads, FAQs etc). National Health Service (NHS) sites were identified as the major source of visits to the NOGG website, comprising 64% of the identifiable visiting locations, but this is an underestimate as many sites from within the NHS are not classified as such.

The study shows that the facilitated interaction between web based fracture risk assessment and clinical guidelines is widely accepted by clinical users. The approach could usefully be adopted in all countries for which a FRAX model is available.
High-intensity interval training: a potential novel method for improving bone mass

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Objectives: Exercise plays a key role in improving bone health with, the magnitude of strain placed upon the skeleton playing a crucial role in stimulating osteogenic effects. High-intensity interval training (HIIT) is a form of exercise that utilises repeated high-intensity, short duration exercise efforts, interspersed with recovery intervals, and as such makes it a potential novel method for improving bone mass. This study aimed to look at the effects of a 6-week HIIT programme on bone mass in healthy males.

Methods: Eighteen participants (24.4 ± 6.7 years 1.77 ± 0.09 m 79.0 ± 14.5 kg 25.0 ± 2.8 kg • m\textsuperscript{−2}) were randomly assigned, into either a HIIT or control group. All participants made a single fasted visit to the laboratory, pre- and post-a 6-week, HIIT intervention, where they underwent both a lumbar spine and proximal femur dual-energy X-ray absorptiometry scans (Hologic Discovery A, Hologic Inc, Bedford, MA, USA). HIIT consisted of repeated 30-s bouts on a cycle ergometer (Monark 874E, Monark, Varburg Sweden), interspersed with 4-min of active recovery, with a resistance set at 7.5% of body mass (kg). Participants completed 18 sessions of HIIT over 6-weeks with 4 repeats in the first 2-weeks, 5 in weeks 3 and 4, and 6 in the final 2 weeks of the intervention. Data was analysed using a factorial ANCOVA, with baseline values used as a covariate, and is presented as percentage change from baseline.

Results: HIIT was associated with trends for increased femoral neck bone mineral density (1.75 vs. −1.51% \( p = 0.058 \)), total hip area (4.81 vs. 0.36% \( p = 0.083 \)), and total hip bone mineral content (4.81 vs. 0.05% \( p = 0.059 \)) compared with non-exercising controls.

Conclusion: The current study demonstrates that HIIT can potentially elicit positive bone changes within a short time frame in healthy individuals. Although failing to reach statistical significance this method provides interesting further avenues for investigation to potentially improve bone mass.
Bone mineral content and fracture risk: findings from a UK prospective cohort


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Objective: Poor early growth has been shown to be associated with reduced adult bone mineral content (BMC), but not bone mineral density (BMD). Although low BMD is a well-established risk factor for future fracture, little is known about the performance characteristics of BMC in fracture prediction. We therefore investigated the predictive value of bone area (BA), BMC and BMD for incident fracture in a prospective cohort of UK women.

Methods: 674 women aged 20–80 years, recruited from four GP practices in Southampton, underwent DXA assessment (proximal femur, lumbar spine, total body) between 1991 and 1993. All women were contacted in 1998–1999 with a validated postal questionnaire to collect information on incident fractures and potential confounding factors including medication use, 443 women responded. All fractures were confirmed by assessment of images and radiology reports by a research nurse.

Cox proportional hazards models were used to explore the risk of incident fracture and results are expressed as Gradient of Risk [GR = Hazard Ratio (HR) per 1 SD decrease in the predictor] and 95% CI. Associations were adjusted for age, BMI, alcohol consumption, smoking, HRT, medications and history of fracture.

Results: 55 women (12%) reported a fracture. In fully adjusted models femoral neck BMC and BMD were similarly predictive of incident fracture. Femoral neck BMC: GR = 1.64 (95% CI: 1.19, 2.26; p = 0.002); femoral neck BMD: GR = 1.76 (95% CI: 1.19, 2.60; p = 0.005]). In contrast femoral neck BA was not associated with incident fracture, GR = 1.15 (95% CI: 0.88, 1.50; p = 0.32). Similar results were found with bone indices at the lumbar spine and whole body.

Conclusion: BMC and BMD appear to predict incident fracture with similar gradients of risk, even after adjustment for body size. These findings suggest that factors in early life that are associated with total skeletal mineralisation are likely to have implications for adult fracture risk.
Successful use of denosumab to treat osteoporosis in a patient with severe anorexia nervosa

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Osteoporosis in women with anorexia nervosa is almost ubiquitous and difficult to manage in the presence of persistently low body weight. We present a case where a novel approach was taken to improve bone mineral density (BMD). A 29 year old female with a 17 year history of severe enduring anorexia nervosa attended our unit. Osteoporosis was diagnosed aged 24 and she had developed a left calcaneal fracture after minimal trauma 3 weeks prior to presentation. Her BMD at this time confirmed the presence of osteoporosis at the lumbar spine and total hip (T-score −3.3 and −2.9, respectively) and her body mass index (BMI) was low at 15.1 kg/m². She declined therapy previously with oestrogen and bisphosphonate therapy and did not wish daily injections. A decision was made to commence therapy with Denosumab 60 mg by subcutaneous injection every 6 months with monitoring of serum calcium and co-administration of calcium and vitamin D. A further measurement of bone mineral density was made 2 months after completing 3 years of therapy with Denosumab. During the period of treatment the patient did not experience any adverse effects related to the treatment. There was no evidence of hypocalcaemia nor were there further fractures. Her BMI was unchanged and she remained amenorrheic during the treatment course. BMD increased substantially at the lumbar spine (14.8% increase from pre-treatment) and at the left total hip site (1.4% increase from pre-treatment). The measurement at the left femoral neck showed a reduction of −5.7% from its pre-treatment value.

Denosumab is a human monoclonal antibody that binds with high specificity to the receptor activator of nuclear factor-κB ligand (RANKL) and results in decreased bone resorption, decreased bone turnover and reduces vertebral and non-vertebral fractures in postmenopausal women and can be safely administered for at least 6 years. The magnitude of the change in bone mineral density seen in post menopausal women treated with Denosumab is similar to that observed in this case despite the patient’s ongoing low weight and suggests that this agent may be a useful therapy for treating osteoporosis in patients with anorexia nervosa and merits further investigation in the appropriate trial setting.
Is the human fibula responsive to disuse?

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Objective: The human tibia is a common site for peripheral quantitative computed tomography (pQCT) studies. The tibia commonly experiences combined muscle and reaction forces of several kilonewtons during e.g., walking and running. Removal or attenuation of these forces during bed rest or following spinal cord injury (SCI) results in loss of up to 50% of tibial bone mineral content (BMC). In contrast, the fibula carries only –6% (i.e., it is in tension) to +19% of total shank compressive load dependent on ankle angle. Therefore the fibula may be less influenced by habitual loading, and hence less responsive to disuse.

Methods: To investigate effects of disuse on fibula bone, re-analysis of pQCT scans from two previous studies targeting the tibia were examined. Serial scans at 5% increments from 5 to 95% tibia length in 9 SCI patients aged 39.2 ± 6.2 years, 9–32 years post-injury (representing long-term disuse), and in 9 age, height and mass-matched controls were compared. Also, scans at 4, 14, 38, and 66% distal-proximal tibia length in 9 adult male volunteers aged 32.3 ± 3.7 years undergoing 90 days of 6° head-down tilt bed rest (representing an interventional model of disuse) were examined following 7, 28, and 89 days of bed rest and 14, 90, 180, and 360 days of recovery.

Results: Whilst tibial bone mineral content (BMC) was 22–51% lower in SCI than controls (P < 0.001), no significant group effect was observed in fibula (P = 0.19). Tibia BMC losses of 0.9–6.1% were observed at all sites following bed rest (P < 0.001) – losses were greatest at the epiphyseal 4% site, and at 89 days bed rest or 14 days recovery. Only at 4% tibia length was an effect of bed rest on fibula BMC observed – a 1.4% loss at 14 days recovery (P = 0.01).

Conclusion: Cross-sectional and interventional models suggest that the fibula is less responsive to disuse than the tibia, particularly in diaphyseal regions. Given the dominating influence of loading on tibial bone, more subtle effects of e.g., genetic, nutritional or hormonal factors or pharmacological interventions may be difficult to detect. Hence, the fibula presents a new target for studies investigating non-mechanical influences on bone.
The association between osteoporosis and back pain in a UK population based cohort of older women: a prospective analysis of the cohort for skeletal health in bristol and avon (coshiba)

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Objectives: Traditionally osteoporosis itself is thought to be asymptomatic, with back pain only occurring as a result of vertebral fracture. However, there is a perception among the general public that osteoporosis is painful – perhaps because of confusion with osteoarthritis. We aimed to establish if osteoporosis is associated with back pain in the absence of vertebral fracture: as if so, this has important implications for the management of back pain – an important public health issue. We also examined the independent baseline predictors of future back pain onset.

Methods: Cross-sectional and prospective analysis of a sub-group of women aged 73.8 ± 4.3 years from COSHIBA, a primary-care based cohort of postmenopausal women from the Bristol area. Osteoporosis was diagnosed at baseline from Dual-energy X-ray absorptiometry scans defined as a T score at any site less than −2.5. Self-reported data was collected on osteoporosis clinical risk factors, back pain, falls/mobility risk factors, health care utilisation and socioeconomic position at baseline and 2-year follow up. Vertebral fractures were identified from spinal radiographs at baseline. Chi-squared tests were used to assess univariable associations. Multivariable logistic regression was used to identify independent risk factors for back pain at 2-years.

Results: 244 women had complete data collection. 57 (23.4%) of women had osteoporosis at baseline. At 2 years follow-up 165 (67.6%) self-reported back pain: 37/57(64.9%) of those with osteoporosis and 128/187 (68.6%) of those without osteoporosis. No association was seen between presence of osteoporosis at baseline and back pain at 2 years (OR for back pain of 0.85, 95% CI 0.46–1.60, p = 0.618). Adjustment for potential confounders and exclusion of those with vertebral fractures did not affect results. The use of walking aids and self-reported osteoarthritis at baseline were independent risk factors for back pain at 2 years. Those with back pain had increased healthcare utilization.

Conclusion: Our results confirm that osteoporosis itself is not associated with back pain. This useful and reassuring information needs to be disseminated to the general public.

Funding: COSHIBA recruitment was funded by Arthritis Research UK. This Medical Student project was funded by University of Bristol.
Examining the calcaneus using HR-pQCT: method reproducibility and regional trabecular variation

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Our objectives were to develop a technique to scan the calcaneus using high-resolution peripheral quantitative computer tomography (HR-pQCT) and to assess volumetric bone mineral density (vBMD) and trabecular morphology.

The calcanei of 21 cadaveric specimens (age: 60–101 years) were each scanned twice in situ, with repositioning, using the XtremeCT (SCANCO Medical AG). All specimens were scanned at an integration time of 100 ms and a subset (n = 11) also at 200 ms. Scans were obtained in the transverse plane centred on the midpoint of the calcaneus between the Achilles tendon attachment and flexor digitorum brevis attachment. This central region was divided into 3 equal 110-slice sections (inferior, middle and superior) and underwent a standard evaluation analysis using a 12.25 mm × 12.25 mm × 9.02 mm volume of interest (excluding cortical bone). Parameters included vBMD, trabecular number (Tb.N), trabecular spacing (Tb.Sp) and trabecular thickness (Tb.Th).

All of the measured parameters showed good reproducibility between scans (R² > 0.867, p < 0.001). The vBMD was significantly higher in the superior section compared to the middle (+83%, p = 0.001) and inferior sections (+141%, p < 0.001) and the Tb.Th in the superior section followed a similar trend (vs. middle +110%, p < 0.001; vs. inferior +201%, p < 0.001). There were no differences between the middle and inferior sections for vBMD and Tb.Th. There were no significant differences between the slice sections for Tb.N and Tb.Sp. Increasing the scan integration time from 100 to 200 ms led to an apparent decrease in Tb.N (−19%, p = 0.013) and an increase in Tb.Sp (+34%, p = 0.033), with no effect on vBMD or Tb.Th.

Reproducibility has been demonstrated for measurements of density and trabecular morphology in cadaveric calcanei using HR-pQCT. Examining specific regions of the calcaneus may prove useful in assessing response to treatment and mechanical loading, as well as fracture risk prediction. Future studies will compare our findings with micro-CT and examine reproducibility in vivo.
The origins of bone and cartilage disease: three new imaging techniques for high-throughput joint phenotyping in mice

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Osteoarthritis (OA) is the commonest joint disorder and its incidence is increasing with an aging population. Whilst approximately 50% of an individual risk of developing OA is inherited, thus far, few genetic loci have been associated with the disease. Hence there is an urgent need to identify genes involved in the susceptibility to and progression of OA. The mouse is an essential model for the study of bone and joint disease due to its genetic tractability and wealth of available resources. One such resource is the Sanger Mouse Pipeline (SMP) at the Wellcome Trust Sanger Institute. The SMP is part of the International Mouse Phenotyping Consortium (IMPC), which is deleting each of the >20,000 protein-coding genes in C57/BL6 mice by 2021. The Origins of Bone and Cartilage Disease (OBCD) initiative is an international consortium that is phenotyping mouse mutants generated by the SMP and IMPC to identify genetic determinants of bone and joint disease. In mouse models, joint phenotyping is limited by the need to maintain articular cartilage hydration and by the absolute dimensions of the joints. The current gold standard for quantifying OA in mice is histological analysis, which is destructive, restricted to two dimensions, low-throughput and expensive. We have therefore developed three new techniques to overcome these major limitations. We can now detect reduced articular cartilage thickness and volume using high-resolution contrast-enhanced micro computed tomography and identify subchondral bone sclerosis using point projection X-ray microradiography. Furthermore, direct visualisation of articular cartilage damage can be achieved by (i) generation of a high-resolution joint mould using silicon impression material (ii) production of an acrylic joint replica, and (iii) high-throughput back-scattered electron microscopy imaging of the joint replicas. We have validated these novel techniques using the established destabilisation of the medial meniscus surgical model of OA and gold standard Osteoarthritis Research Society International (OARSI) histological analysis. Combining these three novel and complementary techniques allows for the first time efficient, high-throughput joint phenotyping in mouse models.

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The relationship between circulating pro-angiogenic factors, markers of bone turnover and bone mineral density in post-menopausal women

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Pro-angiogenic factors including vascular endothelial growth factor (VEGF), angiopoietin 1 (ANG-1) and 2 (ANG-2) have been shown, mainly in animal models, to be involved in initiating angiogenesis and in the coupling of bone formation and resorption. ANG-1 promotes the stabilisation of newly formed vessel whilst ANG-2 initiates new vessel formation, allowing better growth factors access and sensitisation to factors such as VEGF. The ratio of ANG1/ANG-2 is often used to demonstrate the balance between the 2 angiopoietins. Little is known about their impact on bone mass and bone turnover in the clinical setting. The aim of our study was to investigate the relationship between circulating concentrations of VEGF, ANG-1, ANG-2 and ANG-1/ANG-2 ratio with bone mineral density (BMD) and bone turnover in post-menopausal women. We studied 393 post-menopausal women aged (mean [SD]) 60 [6] years. BMD was measured at the lumbar spine (LS), femoral neck (FN) and total hip (TH). Fasting blood samples were obtained for the measurement of the pro-angiogenic factors and C-terminal telopeptide of type 1 collagen (CTX, marker of bone resorption) and amino-terminal propeptide of type 1 collagen (P1NP; marker of bone formation). Serum VEGF, ANG-1 and ANG-2 concentrations were measured by ELISA. Eighty eight women had normal BMD, 217 were osteopenic (“T” score < −1 at any site) and 83 had osteoporosis (“T” score < −2.5). Significant correlations were observed between VEGF and ANG-1 (r = 0.23, p < 0.001), ANG-1 and ANG-2 (r = 0.33, p < 0.001) and VEGF and ANG-1/ANG-2 ratio (r = 0.15, p = 0.004). A significant negative association was seen between CTX and ANG-1 (p = 0.042) following multi-linear regression analysis after adjustment for confounders including age, BMI, bisphosphonate use, albumin corrected calcium, eGFR and PTH. In bivariate analysis, a negative correlation was observed between ANG-1/ANG-2 ratio and BMD at the LS only (r = −0.146, p = 0.005). The association remained significant following multilinear regression and adjustment for confounders (p = 0.01). ANG-1/ANG-2 ratio was significantly lower in subjects with normal BMD at the LS compared to those with osteopenia and osteoporosis (ANG-1/ANG-2 ratio: Normal BMD:45.8[30], osteopenia/osteoporosis: 55.1[49], p = 0.018). These data suggest that the angiopoietins may influence bone resorption and bone density.
Vertebral disease in Europe: personal impact

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Objectives: To compare the personal impact of three common vertebral diseases.

Methods: The EVOS-EPOS study was an age-stratified population-based study of radiological vertebral fractures in the lumbar and thoracic spine in over 10,000 men and women aged 50–75. We have new readings for Scheuermann’s disease and lumbar Kellgren-Lawrence score for osteoarthritis. We have now compared the three most common pathologies in the spine for their relative impacts as reflected in the baseline questionnaires. Outcome variables were: back pain in the past year; subjective general health (scale 1–5); activities of daily living (ADLs – 12 at three levels, easily = 2 with difficulty = 1 or not at all = 0 – Lost ADLs were summed ADL points lost); current less past history of sporting and general physical activity. These five outcomes were modelled statistically on disease presence using multivariate regression, adjusting for sex, age, investigational centre, years of education, increase in body mass index and (in the case of back pain, general health and loss of ADLs) the two physical activity variables.

Results: Population prevalences of vertebral fractures (McCloskey-Kanis method), Scheuermann’s and grade 3 + 4 or Grade 4-only lumbar osteoarthritis at baseline were 10.5, 8, 44, and 4%, respectively. There were no significant effects of Scheuermann’s disease on any outcome variable. Increasing K-L score was associated with reduced self-perceived health (p < 0.04) and loss of ADLs (p = 0.001) but not with recent back pain or reduced sporting or general physical activity. The subgroup with Severe K-L Grade 4 OA was associated with faster reduction of sporting activity (p = 0.04) and greater loss of ADLs (p = 0.0007). One or more McCloskey-Kanis fractures was associated with greater loss of ADLs (p < 0.0001), back pain in the past year (p = 0.02) and reduced subjective health (p < 0.0001). In models including all three diagnoses, the effects of a McCloskey-Kanis fracture on loss of ADLs, back pain in the past year and self perceived health remained significant and was larger than that of both K-L score and Scheuermann’s.

Conclusion: The adverse effects on subjective health and reported daily activity of prevalent vertebral fracture at population level exceed those of other common, radiologically diagnosed vertebral diseases.
Development of natural chondrogenic scaffolds from hyaline cartilage for targeted tissue regeneration

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Objectives: There is a current unmet need for biomimetic scaffolds that effectively replicate cartilage architecture with the biological cues needed to direct chondrogenic differentiation. This study aims to develop a novel cell matrix through the decellularization of xenogeneic hyaline cartilage

Methods: Two previously used decellularization techniques were compared and optimized, they included osmotic shock and detergent based protocols. The scaffolds were analysed histologically using hematoxylin and eosin (H&E) staining for cellular content as well as picro-sirus/millers for collagen. Biochemical assessment involved the determination of DNA and GAG content. Differential scanning calorimetry (DSC) was used to assess the integrity of the ECM. Subsequently, the scaffolds were seeded with chondrocytes to determine scaffold toxicity and cell attachment.

Results: Treatment with both protocols yielded a vastly reduced cellular content when examined histologically. DNA content for both the protocols fell below the recommended 50 ng/mg threshold for xenogeneic transplantation, with a 91% (n = 3 p < 0.002) reduction for the osmotic shock protocol and 88% (n = 3 P < 0.002) reduction for the detergent-based protocol. The protocol was optimized with reference to tissue size, which resulted in the production of scaffolds with enhanced GAG content retention [reduction of 16.5% (n = 3 P < 0.0003)]. Thermal analysis using DSC indicated that the osmotic shock protocol had a minimal effect on the collagen denaturation temperature with a reduction of 7.5% (n = 3 P < 0.0002). Examination of the cell seeded scaffolds using confocal microscopy showed that the chondrocytes had not only attached to the scaffolds but migrated into the empty lacunae.

Conclusion: Histological and biochemical assessment indicates that the scaffolds produced were not only extensively decellularized but also retained a sufficient amount of the biological cues for cell attachment and migration. These data demonstrate the possibility of creating biomimetic cartilage scaffolds and highlight their potential use in combination with specific cell types for targeted tissue regeneration.
The influence of the female athlete triad on bone quality in elite endurance runners

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Elite female athletes have exceptionally high physical activity levels. The physiological stresses associated with high exercise can disrupt normal homeostatic processes, altering menstrual cycles and energy balance, leading to a condition known as The Female Athlete Triad. The aim of this study was to examine the bone health of female elite-level endurance runners compared with age-matched controls. Controls (C) (n = 15), eumenorrheic athletes (EA) (n = 10) and amenorrheic athletes (AA) (n = 10) completed dual energy X-ray absorptiometry (DEXA) and peripheral quantitative computed tomography (pQCT) scanning and a 3-day food diary.

The amenorrheic athletes had a greater endochondral circumference of the radial diaphysis than both control and eumenorrheic athletes (16.4% greater than EA and 15.8% greater than C). At the Radial epiphysis the AA demonstrated a 12% significantly greater total area than the C. The C group had 18.2% larger tibia cortical area of the diaphysis than EA athletes. DEXA results (g/cm²) highlighted significant differences between C and AA at non weight bearing sites of head (2.06 ± 0.087 for AA vs. 2.34 ± 0.07 C), ribs (0.62 ± 0.014 vs. 0.68 ± 0.013) and spine (0.9 ± 0.042 vs. 1.05 ± 0.024) ($p \leq 0.05$). Further significant differences existed between the AA and EA group at the trunk (0.8 ± 0.024 vs. 0.9 ± 0.017), pelvis (0.97 ± 0.032 vs. 1.13 ± 0.026), total body (1.1 ± 0.025 vs. 1.2 ± 0.016) and L1-4 (1.0 ± 0.05 vs. 1.16 ± 0.029) ($p \leq 0.05$). Both the AA and EA had significantly lower energy intake per day than controls ($p = 0.016$ and $p \leq 0.001$, respectively), despite the much higher energy expenditure associated with training. Carbohydrate intake over the 3 days was significantly lower in AA and EA than controls ($p = 0.015$ and $p \leq 0.001$, respectively).

These results demonstrate that elite endurance athletes with amenorrhea have lower bone mineral density than controls and eumenorrheic athletes.
Hypocalcaemia in patients receiving denosumab for osteoporosis: audits undertaken in primary and secondary care to assess vigilance of calcium monitoring

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Introduction: Denosumab is a monoclonal antibody which decreases bone resorption by binding to RANK ligands and inhibiting osteoclast formation, activity and survival. It is indicated for use in post-menopausal women with a high risk of osteoporosis who cannot receive bisphosphonates or as secondary prevention in patients who have sustained fractures whilst on bisphosphonates. As hypocalcaemia is a recognised adverse effect of denosumab, the MHRA and CHM advise monitoring of serum calcium and our local guidelines in Dudley (UK) recommend that this is undertaken 2 weeks prior to administration. Audits assessing calcium monitoring were undertaken in primary and secondary care during 2013. Subsequently, a secondary care database which logged each patient receiving denosumab and their calcium levels was created. This was in addition to increasing education regarding calcium monitoring for specialist nurses. A re-audit was undertaken in secondary care during 2014.

Material and methods: The population studied in 2013 came from a pharmacy database of patients who had received denosumab between 20/12/2010-20/11/2013. The group was divided into two: those receiving injections in primary (124) vs. secondary care (82). The serum calcium from the preceding 6 months was verified against the laboratory results database.

Discussion: During the 2013 audit, 91% of patient’s in primary care (83/91) had their serum calcium measured in the previous 6 months and were assumed to be monitored correctly. 9% (8/91) had no measurement. 33 patients were excluded due to incomplete data, death or cessation of therapy. In secondary care, 95% (78/82) of patients had a calcium measurement, with 60% (49/82) being taken within 2 weeks of denosumab administration. Of those patients with a serum calcium result, hypocalcaemia was recorded in 12% (10/83) of patients in primary care and 6% (5/78) in secondary care. Re-audit during 2014 in secondary care showed that 100% (45/45) of patients had a calcium level measured within the previous 6 months. 78% (35/45) of patients had a calcium measurement within the previous 2 weeks and no patients with hypocalcaemia received denosumab.

Conclusion: Improvements in calcium monitoring can be made by increasing education regarding hypocalcaemia and by creation of a denosumab database in secondary care.
Effect of 2 year teriparatide treatment on vertebral strength and trabecular structure in postmenopausal women with osteoporosis assessed by quantitative computed tomography

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Teriparatide (TPTD, PTH 1–34) treatment increases BMD, but the increase is not sufficient to explain the reduction in fracture risk entirely. The aim of this study was to apply a novel patient-specific finite element (FE) model of the disc-vertebra-disc (DVD) unit and image analysis techniques to quantify the effect of TPTD treatment on vertebral strength and trabecular structure.

In an open-label single-centre study, 20 postmenopausal women (age 64.4 ± 15.4 years) with osteoporosis (BMD T score <−2.5 at spine or hip) were treated with TPTD (FORSTEO, 20 micrograms daily) for 104 weeks. We obtained high resolution scans of T12 (0.19 × 0.19 × 0.62 mm³) for trabecular structure and usual resolution scans of L1-3 (0.62 × 0.62 × 0.62 mm³) for volumetric BMD (vBMD) and finite element modelling at baseline, 26, 52, and 104 weeks. The DVD FE models of the L2 vertebral body have transverse-isotropic, elastic-perfectly plastic material properties, the adjacent discs linear-elastic properties for the nucleus pulposus and annulus ground matrix with 4 fibre layers embedded in it to simulate collagen fibres. A pure compressive loading condition was simulated. Vertebral strength was defined using a 0.2% offset method in the load-displacement curve. Trabecular structural variables were calculated based on a 3D adaptation of the parallel plate model (Parfitt et al., 1983) and the mean intercept length method. Variables studied included apparent trabecular bone density (appTbD), bone volume fraction (appBV/TV), trabecular number (appTbN), thickness (appTbTh) and separation (appTbSp).

The L1-4 BMD by DXA and L2 FE-estimated strength increased significantly (P < 0.05) from Week 26 onwards, the L1-3 vBMD by QCT did so from Week 52 onwards, whereas appBV/TV, appTbN and appTbTh at Week 104 only. The mean (±SE) percentage increases at Week 104 from baseline, calculated at individual level, were 11% (±2%) for DXA L1-4 BMD, 22% (±6%) for QCT L1-3 volumetric BMD, 30% (±8%) for the FE estimated strength. Calculated at group level, the mean percentage increases were 60% for appBV/TV, 32% for appTbN and 24% for appTbTh.

In conclusion, 2 years TPTD treatment improves vertebral trabecular structure and FE-estimated strength which may explain its anti-fracture efficacy.

Reference
Reference point micro-indentation (RPI) assesses bone quality to supplement bone mineral density (BMD) and clinical factors (FRAX®) for improved fracture risk assessment at the human femoral neck

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Objectives: Fracture risk assessment centres on BMD alone or combined with clinical factors but does not typically incorporate biomechanical properties. RPI can assess bone quality, and, in vivo tibial measurements have discriminated osteoporotic/fracture patients from controls (Diez-Perez et al., 2010). However, it is unclear what property RPI assesses, how it performs at the femoral neck fracture site and how it can complement existing risk assessment.

Methods: RPI (Biodent HfcTM) was applied to the femoral neck of 46 resected fracture samples (17 male, 83 years IQR 77–87 years) and compared to 16 non-fractured cadaveric controls (7 male; 65 years IQR 61–74 years). BMD was measured in 19 fracture patients (Hologic Discovery) and all controls (cross-calibrated Hologic-mimic using 225 kV X-TEK/Nikon HMX-ST) and single indents (seven samples) were imaged using micro computed-tomography (μCT – Xradia-Versa510). Correlation, multivariate linear-regression and Receiver Operator Characteristic (ROC) analysis were employed to explore relationships between BMD, FRAX and RPI and their ability to discriminate fracture cases from controls.

Results: RPI measured Total Indentation Depth (TID) was greater in fracture (131.3 μm) than control (105.9 μm) cases following age, sex, BMI and height adjustment (p < 0.001). TID was positively associated with FRAX probability (r = 0.31, p = 0.026, n = 53) and inversely with BMD (r = −0.32, p = 0.063, n = 35) but was more strongly correlated with μCT microcrack length (r = 0.79, p = 0.036), imprint depth (r = 0.86, p = 0.013) and porosity (r = 0.68, p = 0.093). A combined score, created by summating TID with BMD or FRAX, improved discriminative ability (ROC = 0.95–0.99 compared to 0.89 for TID alone).

Conclusion: RPI provided assessment of elements of bone quality and permitted discrimination of fracture cases from controls at the femoral neck, independent of BMD and FRAX. Further testing in prospective cohorts is now recommended to establish whether RPI, in its clinical form, could supplement risk assessment.

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Reference
Do subjective memory complaints predict bone outcomes? A two-year prospective cohort study from primary care


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Objective: To assess the relationship between subjective memory complaints (SMCs) and various outcomes over the following years including fractures and health care utilization.

Method: Prospective analysis of the Cohort for Skeletal Health in Bristol and Avon, a primary care based cohort of postmenopausal women. Data from participants were collected by self-completion questionnaires on entry to the study and at 2 years follow-up. SMC was assessed at baseline by asking “Do you, or have you suffered from memory problems in the last 2 years?” Bone outcomes including fractures, measures of frailty including mobility and falls, and healthcare utilisation were assessed 2 years later. A random 5% subsample of self-reported data were validated against GP records. Chi-squared analyses were used to look for simple associations, and logistic regression for independent associations.

Results: 3184 postmenopausal women aged 72.6 ± 4.2 years were included in this analysis. 350 women (11.0%) reported SMC at baseline. They were older (73.3 ± 4.5 years vs. 72.0 ± 4.2 years) and less likely to own their own home (79.0% vs. 87.1%). SMCs at baseline were associated with an increased risk of upper limb fractures over the following 2 years (OR 1.72, 95% CI 1.02 to 2.90, P = 0.039). SMCs was also associated with an increased risk of falls (OR 1.83, 95% CI 1.41 to 2.38, P < 0.001), reduced mobility (OR 2.14, 95% CI 1.62 to 2.82, P < 0.001) and increased healthcare utilisation over the following 2 years (OR for hospital appointments 2.20, 95% CI 1.26 to 3.86, P = 0.005).

Conclusion: We demonstrate that self-reported SMCs may predict an increased risk of falls, bone fractures and healthcare utilisation over the following 2 years. This has important implications – currently all attendees to secondary care are asked about SMCs on admission – our results suggest that this could be an important screening question to allow targeting of interventions to reduce fractures and improve bone health in the postmenopausal population.

Funding: COSHIBA recruitment was funded by Arthritis Research UK.
Perinatal methylation at serotonin transporter SLC6A4 and offspring bone mass: findings from the southampton women’s survey

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Aim: We have previously demonstrated associations between perinatal DNA methylation and offspring bone mass. There is increasing evidence for a role of serotonin in bone health, and we investigated associations between methylation at CpG sites in SLC6A4 (encoding a serotonin transporter) and indices of bone mineral in childhood.

Methods: To identify potentially informative genomic regions, in 24 human umbilical cords from infants in the UK Southampton Women’s Survey (SWS) we used a whole genome methyl-binding domain capture array (Agilent). Following processing to account for CpG density via a Bayesian algorithm (BATMAN), analysis located differentially methylated regions with strong correlations between methylation status and childhood bone size and density assessed by DXA (Hologic Discovery). Based on these findings and biological plausibility, we undertook a more detailed examination at the SLC6A4 locus using pyrosequencing in umbilical cords of 436 children from the SWS assessed by DXA at birth (Lunar DPX-L; whole body) and/or 6 years old (Hologic Discovery; whole body minus head), with appropriate institutional ethics committee approval and parents’ informed consent.

Results: There was wide variation in percentage methylation. After taking into account age and sex, there were associations between methylation at 4 of 5 CpG sites within SLC6A4 and DXA bone indices. Thus, for example, a 1% increase in methylation at CpG site 5 within SLC6A4 was associated with a 0.3 g decrease in bone mineral content [BMC (p = 0.018)] and 0.001 g/cm² decrease in bone mineral density [BMD (p = 0.029)] at birth. At 6 years, a 1% increase in methylation at CpG site 5, was associated with a 2 g decrease in BMC (p = 0.006), and a 0.001 g/cm² decrease in BMD (p = 0.005).

Conclusion: We have demonstrated associations between perinatal DNA methylation at SLC6A4, a gene involved in serotonin metabolism, and offspring bone mineral, which were robust into childhood. With further validation of relationships and functional significance, these findings may inform both potential interventions and identification of novel biomarkers.
The effect of bisphosphonate treatment on bone turnover and bone balance in postmenopausal women with osteoporosis

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Objectives: Postmenopausal osteoporosis is characterised by increased bone turnover, a negative balance (resorption > formation) and increased fracture risk. Bisphosphonates reduce bone turnover and fracture risk but their effect on bone balance is yet to be fully investigated.

Methods: We have compared the effects of bisphosphonates on turnover and balance in postmenopausal women with osteoporosis using a T-score bone marker plot. 165 postmenopausal women (hip and spine BMD T-score ≤ −2.5 or < −1 with a prior fracture) were recruited, mean age 67 years. They were provided with calcium and vitamin D supplements and randomised to receive ibandronate (n = 55, 150 mg/month), alendronate (n = 54, 70 mg/week) or risedronate (n = 56, 35 mg/week). A fasting serum sample was collected at baseline and weeks 1, 2, 4, 12, 13, 48, and 96 on treatment. A control group of 200 healthy premenopausal women received no treatment. PINP and CTX were measured using the iSYS-IDS analyser. Values were log10-transformed and normalised. The T-scores for PINP and CTX value were calculated for each postmenopausal woman using the mean and standard deviation values from the premenopausal group.

Results: By week 96 bisphosphonates reduced mean levels of turnover to -2.1 SD units (95% CI: −2.322, −1.848) below the mean of the premenopausal women $p < 0.001$. Bone balance was positive for all agents in the early phase of treatment. At week 96, mean levels of balance were positive in all treatments combined, (0.3 SD units, 95% CI: 0.145, 0.559), $p < 0.01$ but bone balance was more positive with alendronate only, $p < 0.01$.

Conclusion: In postmenopausal osteoporosis treatment with bisphosphonates improves bone balance by making it more positive and reduces bone turnover, relative to healthy premenopausal women. Bisphosphonates have differing effects on bone turnover and bone balance.
Identification of genetic mutations responsible for odontoclast dysregulation in feline tooth resorption

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Feline tooth resorption (TR) is a common disease causing progressive lesions leading to resorption of the adult dentition due to dysregulation of odontoclasts (ODs). The etiology of TR is still unclear, but studies in man have shown genetic predispositions for root resorption. We hypothesise that similar genetic aberrations causing dysregulation of normal odontoclastic function may be identified in cats.

In vitro feline ODs culture was set up and generated ODs derived from bone marrow showed typical odontoclast characteristics with large multinucleated morphology, positive TRAP activity and resorption activity on mineralised substrates.

Teeth samples were harvested and bone marrow derived ODs were generated from TR affected and TR unaffected cats. Following RNA extraction and assessment of quality, listed genes involved in odontoclastogenesis were tested using PCR. Teeth from TR affected cats showed expression of purinergic receptor 7 (P2RX7), osteoprotegerin (OPG) and interleukin 1 beta (IL1B). Feline ODs differentiated in vitro expressed interleukin 1 beta (IL1B) and vitamin D receptor (VDR). Sanger sequencing of P2RX7, IL1B, OPG and VDR genes showed up to 99% homology with feline genome. Four single nucleotide polymorphisms (SNPs) including two non-synonymous SNPs of VDR were identified.

Future studies: Genome wide approach (RNA and DNA sequencing) and gene silencing of candidate genes in vitro will be performed to elucidate potential genes correlated with TR.
Establishing reference intervals for pyridoxal 5'-phosphate: the national health and nutrition examination survey 2007–2008 data

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Diagnosis of adult-onset Hypophosphatasia (HPP) is important in patients with osteoporosis as treatment with bisphosphonates may result in atypical femur fractures. Pyridoxal 5'-phosphate (PLP), a substrate for alkaline phosphatase (ALP), is elevated in HPP and is a useful measurement in diagnosis. It is important to establish reference intervals for PLP that take into account factors that affect PLP measurement.

We determined factors which influenced PLP results and calculated race and gender specific reference intervals for serum PLP in adults (age 20–80 years) using the National Health and Nutrition Examination Survey (NHANES) 2007–2008 data (n = 4496). Serum PLP was measured by reversed-phase HPLC with post-column derivatization and fluorometric detection.

Factors which influenced PLP results included: inflammation (C-reactive protein > 5.0 mg/L) was associated with lower PLP (median, 44 nmol/L; IQR, 24–77 nmol/L; compared to median, 64 nmol/L; IQR, 37–114 nmol/L; P < 0.001), people with low ALP (< 36.0 U/L) had higher PLP (median, 107 nmol/L; IQR, 59–240 nmol/L) compared to normal (median, 58 nmol/L; IQR, 33–105 nmol/L; P < 0.001), those taking vitamin B6 supplements had higher PLP (median, 73 nmol/L; IQR, 47–123 nmol/L; compared to no supplement, median, 47 nmol/L; IQR, 28–89 nmol/L; P < 0.001). There was no effect of reduced kidney function (eGFR < 60 ml/min) on PLP (median, 62 nmol/L; IQR, 32–120 nmol/L; compared to median, 58 nmol/L; IQR, 34–105 nmol/L; P = 0.68). The Kruskal-Wallis test showed a significant effect of race and gender on PLP (P = 0.014, P = 0.002), but not age (P = 0.49).

The 95% reference intervals were calculated using the Robust method (CLSI Guidelines C28-A3), skewed data was back transformed after logarithmic transformation. Gender-specific reference intervals (with 90% CI) for the three race/ethnicity groups after exclusions are as follows: Mexican American males (n = 68; Geometric Mean, 53; Lower Limit, 13 [10–17]; Upper Limit, 195 [147–253]) and females (n = 88; GM, 45; 12 [10–15] – 147 [119–183]), Non-Hispanic white males (n = 305; GM, 63; 10 [9–12] – 344 [290–402]) and females (n = 361; GM, 55; 8 [7–9] – 310 [264–364]), and Non-Hispanic Black males (n = 73; GM, 55; 9 [6–12] – 336 [248–447]) and females (n = 84; GM, 44; 6 [5–8] – 235 [167–324]).

These reference intervals provide useful information for the diagnosis of adult-onset HPP.
FGF2 promotes osteocyte differentiation through increased E11 expression in vitro

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Objectives: E11 is critical in the early stages of osteocytogenesis and its expression patterns are modified at early stages of osteoarthritis. E11 expression is induced by fibroblast growth factor 2 (FGF2) released from cartilage upon injury both in vitro and in vivo. Here we sought to determine whether FGF2 regulates osteocytogenesis through increased E11 expression, and whether modified osteocyte stability is key in the subchondral bone sclerosis which effectively characterises osteoarthritis.

Methods: MC3T3-E1 clone14 osteoblast-like cells were exposed to different concentrations (0–50 ng/ml) of recFGF2 for 4 and 24 h. E11 mRNA was quantified using RT-qPCR and E11 protein expression was assessed by western blotting. Subsequently, MC3T3 cells were exposed to 10 ng/ml FGF2 for 2, 4, 6, or 24 h and mRNA levels of osteocyte and osteoblast markers were measured. Phenotypic changes were observed with light microscopy.

Results: FGF2 exposure for both 4- and 24-h dose dependently increased E11 mRNA expression (P < 0.05) in MC3T3 cells. At all concentrations, E11 mRNA expression was higher after 4 h exposure of FGF2 than 24 h exposure (P < 0.05), suggesting that E11 is an early osteocyte marker gene. Western blotting data confirmed the RT-qPCR data and showed similar increases in E11 protein in FGF2 treated cells. Further experiments showed that E11 mRNA and protein levels were also elevated at 2, 4 (P < 0.05), 6 (P < 0.01) and 24 (P < 0.05) hours after FGF2 treatment. These FGF2-induced changes in E11 were accompanied by significant (P < 0.05) increases in Phex and Bglap (osteocyte markers) and decreases in Col1a1 and Alpl (osteoblast markers). Visual examination FGF2-treated MC3T3 cells revealed greater adoption of osteocyte characteristics, with increased dendritic formation and length in comparison to control cells.

Conclusion: These data suggest that FGF2 promotes osteocytogenesis through increased E11 expression and further studies will identify if this contributes to the subchondral bone sclerosis observed in osteoarthritis.
Identification of collagen VI in osteocyte lacunae in mature bone

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Collagen VI is a microfibrillar forming protein believed to play an important role in mediating cell-matrix and matrix-matrix interactions. It is ubiquitously distributed throughout connective tissues but its expression and function in bone remains largely unexplored.

In order to gain a better understanding of the role of collagen VI in bone, an immunological survey was conducted on various sections of human bone at different stages of maturity with a monoclonal antibody raised against the α3 chain of type VI collagen. Three osteosarcoma cell lines that represent osteoblast cells at different stages of maturation, MG-63, TE85, and SaOS-2 were also used to investigate collagen VI expression through immunocytochemistry, Western Blot and Real Time PCR.

Collagen VI was found in the pericellular area of osteocytes, osteoblasts but not bone lining cells, abundantly deposited throughout immature osteoid but not mature bone matrix and widespread through the marrow stroma of both immature and mature bone. This pattern of staining remained the same even after decalcification. The degree of differentiation of human cells of the osteoblast lineage can be associated with progressive changes in the expression of collagen VI, with the least differentiated MG63 being positive and the most differentiated SaOS-2 being virtually negative for collagen VI expression.

Results suggest that collagen VI plays a specialised role in the organisation and development of the bone matrix by providing the initial scaffolding, but is subsequently removed as mineralisation proceeds. The restricted location of collagen VI in the surrounding matrix of the osteocytes in mature bone is interesting as this supports the idea that collagen VI plays a pivotal role in the connection between the osteocyte and the surrounding matrix via its cell-matrix and matrix-matrix interactions, possibly alerting the cell to changes in load bearing stress of the surrounding bone.

Collagen VI through its array of cell and matrix molecule binding sites, has a role in stabilising the extracellular matrix during development. Its continued presence in the osteocyte lacunae may be important in the process of alerting cells to areas of bone that need remodelling.
Inter-relationships between leptin, adiponectin, osteocalcin and bone, muscle and fat in post-menopausal women

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There is increasing recognition of complex interrelationships between the endocrine functions of bone and fat. We aimed to determine non-mechanical and mechanical links between metabolic factors, bone, and body composition.

Seventy post-menopausal women were recruited. Bone outcomes were pQCT measures of the distal and diaphyseal tibia; cross-sectional area (CSA), vBMD and cortical CSA. Biomarkers of osteoblast and adipocyte function were leptin, adiponectin, osteocalcin, undercarboxylated osteocalcin (UCOC) and vitamin K1. Body composition measurements were lean mass and percent fat mass which were derived using a four-compartment model. Sequences of regressions, a subclass of Graphical Markov Models, were used to describe the direct (non-mechanical) and indirect (mechanical) interrelationships between metabolic factors and bone, by modelling multiple bone outcomes simultaneously and their relationships with metabolic biomarkers with lean mass (as a surrogate of muscle strength), percent fat mass and height as intermediate explanatory variables.

The graphical Markov models showed both direct and indirect associations linking plasma leptin and adiponectin concentrations with CSA and vBMD. At the distal tibia: lean mass, height, and adiponectin-UCOC interaction were directly explanatory of CSA ($R^2 = 0.45$); at the diaphysis: lean mass, fat mass percent, leptin, osteocalcin, age-adiponectin interaction were directly explanatory of CSA ($R^2 = 0.49$). The regression models best predicting vBMD were much weaker, $R^2 = 0.15$ (distal tibia), 0.18 (diaphyseal tibia). Lean mass and UCOC were associated, the global Markov property of the graph indicated that this association was explained by osteocalcin.

Simultaneous non-mechanical and mechanical effects of leptin and adiponectin on bone, and a potential role for osteocalcin in muscle phenotype, were found. Our results indicate that there may be benefits to targeting both the musculoskeletal and metabolic systems to improve health.

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Bone loss from the skull in response to teriparatide therapy: regional differences in response to anabolic osteoporosis therapy

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Teriparatide is the active fragment (1–34) of human parathyroid hormone and it is one of the few anabolic agents licensed for the treatment of severe postmenopausal osteoporosis. It stimulates both bone formation and bone resorption and so has the potential to cause bone gain or bone loss. Large increases in spine bone mineral density (BMD) have been reported following treatment with teriparatide, but its effects on total body BMD and the BMD of its anatomical sub-regions, for instance in the skull, have yet to be described. The aim of our study was to investigate the effects of teriparatide on total body BMD and the BMD of its anatomical sub-regions.

We conducted a 1 year, open-label, single centre trial. Women (n = 18, age = 65.8 ± 5.1 years) with postmenopausal osteoporosis, defined as a BMD T-score of ≤−2.5 at the hip or lumbar spine by dual energy x-ray absorptiometry (DXA) were recruited. Participants were treated with teriparatide by subcutaneous injection at the licensed dose (Forsteo® 20 mcg daily, Lilly, Basingstoke, UK) for 52 weeks. All women also received calcium and vitamin D supplements (Ad-Cal D3, Prostraken Group plc., Galashiels, UK). We measured BMD of total body, lumbar spine and proximal femur by DXA (Discovery A, Hologic Inc., Bedford, MA, USA) at baseline and then at 12, 26 and 52 weeks. Percentage changes [mean, 95% confidence interval (95% CI)] in BMD were calculated from baseline to weeks 26 and 52. Changes in BMD by anatomical region were examined using repeated measures ANOVA. A p < 0.05 indicated statistical significance.

Fifty-two weeks of teriparatide treatment in women with postmenopausal osteoporosis resulted in a 1.3% decrease (95% CI: −2.6 to −0.1%, p = 0.05) in total body BMD. When considering the anatomical sub-regions of the total body, two sites showed a statistically significant change in BMD. There was a 9.5% increase (95% CI: 6.0 to 12.9%, p < 0.001) in lumbar spine BMD and a 5.2% decrease in BMD of the skull (95% CI: −8.1 to −2.3%, p < 0.01).

We conclude that the BMD response to teriparatide differs by site with an increase at the spine and a decrease at the skull.
**Cathepsin K is a key regulator of sclerostin**

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Objectives: Sclerosteosis is a high bone mass disorder caused by a mutation in the SOST gene encoding sclerostin, a negative regulator of bone remodelling. Sclerostin is secreted by osteocytes to inhibit Wnt signalling in osteoblasts, thus preventing bone formation. Certain stimuli influence sclerostin expression, including mechanical loading, but little is known about the degradation of sclerostin except its internalisation within clathrin-coated vesicles (van Dinther et al., 2013). Our work is focused on understanding the regulation of sclerostin in cells, particularly mechanisms of degradation. An enzyme of interest is cathepsin K, a protease secreted predominately by osteoclasts to degrade extracellular matrix, thus having a role in bone resorption. Additionally, expression has been detected in other cell types and it was previously shown that sclerostin up-regulates osteocytic cathepsin K (Kogawa et al., 2013). We have identified cathepsin K as a potential regulator of sclerostin levels within cells.

Methods: Recombinant His-tagged sclerostin was expressed in *Escherichia coli* and purified using reverse-phase chromatography. Recombinant sclerostin was incubated with cathepsin K before running the digested products on a 16% agarose gel and silver-staining. Quantitative real time-PCR and Western blotting measured mRNA and protein expression after knockdown of cathepsin K using siRNA in periodontal ligament cells for 48H.

Results: Recombinant sclerostin identity and functionality were confirmed by mass spectrometry and a β-catenin assay, respectively. After incubating with 10–50 nM cathepsin K, 250 nM sclerostin was degraded into two fragments of approximately 13 and 9 kDa. Silencing by siRNA decreased cathepsin K mRNA expression by 80–90% and significantly increased sclerostin protein expression as assessed by Western blot densitometry.

Conclusion: Cathepsin K is a regulator in the degradation of sclerostin. Current research is establishing how this could work mechanistically *in vivo*, as well as identifying other factors which influence sclerostin production.

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**References**


The role of osteocytes in targeted remodelling of third metacarpal bone in the TB racehorse

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Objectives: The aetiopathogenesis of microdamage induced long bone fractures in horses remains unknown. These fractures are likely the result of inadequate bone remodeling in response to damage. Osteocytes regulate bone formation through an apoptosis-mediated mechanism, however, preliminary equine data suggests a non-apoptotic role in microdamage regulation. This study aims to identify an association of osteocyte apoptosis and/or osteocytic osteolysis with the presence of microdamage in the third metacarpal bone (Mc-III) in thoroughbred (TB) racehorses.

Methods: 30 Mc-III bones were obtained; 10 from bones fractured during racing (Group F), 10 from the contralateral limb (CL) and 10 from unraced controls (C). Each Mc-III bone was divided into fracture site, condyle, condylar groove and sagittal ridge. Blocks were bulk-stained in 1% basic fuchsin (JT Baker® Basic Fuchsin, SureChem Products Ltd), embedded in polymethylmethacrylate and sectioned. Microdamage (microcracks and diffuse microdamage) was quantified. Apoptotic osteocytes were detected using The DeadEnd™ Fluorometric TUNEL System (Promega) in cryosections.

Results: Group F microdamage was elevated 38.9 ± 2.6% compared to Groups CL and C. There was no difference in osteocyte number and % apoptotic cells between CL and C bones. However, there were significantly less apoptotic cells in Group F compared to CL (p < 0.002). The difference was greatest on the sagittal ridge where the rate of apoptotic cells was 22.2 ± 11.0% in Group F and 47.0 ± 19.6% in Group CL samples.

Conclusion: There is increased microdamage and less osteocyte apoptosis in Mc-III bones that have fractured during racing. This implies that an apoptosis-mediated mechanism of bone remodeling is unlikely to be involved in the fractures seen in these animals. Further studies investigating the role of osteocytic osteolysis are ongoing.
Variation of osteoblast function at different skeletal sites

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Objectives: To establish if skeletal site variation exists in osteoblast function. To determine if different components in total hip replacement (THR) or total knee replacements (TKR) are more likely to loosen owing to underlying differences in osteoblast cell function.

Methods: Six patients were included into each study arm; average age in those undergoing THR was 78 (range 71–89 years, four males, two females) and 68.3 in those receiving TKR (Range 61–74 years, three males, three females). Samples from the femoral head, neck and acetabulum and the femur and tibia were obtained in those undergoing THR and TKR, respectively, and osteoblasts were harvested using sequential enzymatic digestion. Released cells were then cultured to passage three and seeded into corresponding well plates. Assays were performed to measure cell proliferation, collagen production, alkaline phosphatase (ALP) expression and cell mineralisation of osteoblasts at the different skeletal sites.

Results: In the hip three regions, differences were seen in cell proliferation ($p < 0.001$), cumulative collagen release ($p = 0.0081$), ALP expression ($p = 0.0007$) but not mineralisation ($p > 0.05$). In cells harvested from the tibia and femur of the knee, statistically significant patterns of anatomic variability were also demonstrated for example when looking at ALP expression ($p = 0.046$).

Conclusion: When compared to the femoral component, the higher long-term failure rate of the acetabular component used in THR has been well established, some have attempted to establish mechanical fatigue failure as a potential aetiology (Zant et al., 2008). The same is true of the tibial component in regards to TKR with some sighting cementing technique as the route to aseptic loosening (Cawley et al., 2013). This study provides preliminary data suggesting variable cell function may account for a greater propensity for acetabular and tibial components used in total joint arthroplasty to undergo aseptic loosening. Understanding the underlying basis for these results may lead to targeting treatments or modification of operative techniques in order to decrease the requirement for revision procedures.

References


An anatomical investigation of a suspected case of ollier’s disease

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Ollier’s disease is a rare condition with a prevalence of 1/100,000. It is characterised by multiple enchondromas (benign cartilaginous neoplasms) manifesting asymmetrically in areas close to the metaphysis. This idiopathic disease results in skeletal dysplasia with the potential for malignant change. A male donor diagnosed with Ollier’s disease was bequeathed for anatomical examination and provided a unique opportunity to comprehensively investigate for the first time the morphology and pathology of the condition with radiographs, CT, MRI as well as dissection.

The radiographs displayed asymmetric involvement of the limbs with mineralised tissue emerging from the long bones. The right side presented larger and more numerous calcified growths than the left. Furthermore, a number of abnormalities were also identified, including distal fusion of the right tibia and fibula, lateral bending of the long bones, enlargement of the right lateral ventricle of the brain and a shortened cranial base.

The musculature was also abnormal. For instance, a large neoplasm was discovered on the right radial head raising flexor carpi radialis and displacing the brachioradialis muscle. The deep branch of the radial nerve runs superficial to the mass placing stress on the nerve. The neoplasm was indented when pressed, suggesting a cartilaginous cap superficial to the calcified structure. Interestingly in the right foot, a tendon of flexor digitorum longus could be seen inserting into a growth of the 2nd metatarsal bone, suggesting this particular mass was formed as the result of a traction epiphysis.

The presence of multiple neoplasms has resulted in many surgeries and caused chronic pain in life due to its involvement on musculature and nerves. It should be noted that the tumours closely resemble hereditary multiple exostoses on the radiographs rather than Ollier’s disease. This alternative interpretation will be examined further through dissection, histology and microCT scans. This work represents an ongoing intensive investigation into the donor’s condition using macro and micro techniques to provide a better understanding of the aetiology and progression of the disease.
Differential effects of PTH on key regulators of osteoblast mineralisation

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The anabolic effects of intermittent PTH on bone are attributed to various mechanisms including inhibition of the Wnt signalling antagonist sclerostin. Nevertheless, there exists a shortage of knowledge surrounding the effects of PTH on key regulators of mineralisation. It is well characterised that the ablation of Alpl, Phospho1 or Smpd3 results in pronounced skeletal hypomineralisation with combined ablation of Alpl and Phospho1 completely abolishing the initiation and progression of skeletal mineralisation.

This study examined the effects of bovine (b)PTH 1–34 on Alpl, Phospho1 and Smpd3 expression. MC3T3-E1 (clone-14) osteoblast-like cells display temporal increases in Phospho1, Alpl and Smpd3 expression (150, 60 and 60-fold, respectively, by day 10; p < 0.001). At day 10, Phospho1 mRNA was significantly reduced after a 15 min exposure to bPTH (50 nM; 80% decrease; p < 0.001). This inhibition was enhanced after 1 and 6 h bPTH exposures (96 and 93% decrease, respectively; p < 0.001) and persisted to 24 h (48% decrease; p < 0.05). Smpd3 expression was similarly reduced after 6 and 24 h exposures (97 and 91%, respectively; p < 0.001). A reduction in PHOSPHO1 and SMPD3 protein was observed after 24 and 48 h bPTH treatment. In contrast, Alpl mRNA levels increased after 1 (2.8-fold; p < 0.05) and 6 h (3.6-fold; p < 0.001) bPTH exposure which was consistent with increased TNAP protein after 24 and 48 h bPTH treatment. Phospho1, Smpd3 and Alpl showed dose dependent (0.05 nM–50 nM) responses to 24 h bPTH treatment. Indeed, a 50% reduction (p < 0.001) in Phospho1 and Smpd3 expression was achieved by the addition of 0.5 nM bPTH with comparable changes at the protein level. Induction of Alpl mRNA and protein was achieved with 5 nM bPTH. Further analyses revealed that the cAMP activator forskolin induced a suppression of Phospho1 comparable to the effects of bPTH (93 and 96%, respectively). Forskolin stimulated Alpl expression (2.4-fold; p < 0.05). The suppression of Phospho1 expression by bPTH was partially obstructed by the PKA-inhibitor PKI 5–24 (45% reduction compared to 75% observed in bPTH treated only cultures). In summary, bPTH shows potent effects on the expression of PHOSPHO1, SMPD3 and TNAP during osteoblast mineralisation. Initial studies implicate the cAMP/PKA signalling pathway as the mediator of these effects.
**Effect of *Rubus Coreanus* vinegar supplementation on bone bio-parameters in growing rats**

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Objectives: The purpose of the study was to investigate the effects of *Rubus coreanus* (RC) vinegar on bone length in growing rats.

Methods: A total 32 three-week-old female Sprague-Dawley rats (40–50 g) were fed AIN-93G and were given water *ad libitum* for experimental period. Animals were randomized into the four groups: control (CON : gavage fed distilled water (DI), \( n = 8 \)), calcium group (Ca : gavage fed calcium carbonate 1.2% in DI, \( n = 8 \)), Casein phosphopeptide (CPP : gavage fed CPP 1 mg/1 ml in DI, \( n = 8 \)) and *Rubus coreanus* vinegar group (RCV : gavage fed the 5.2% vinegar 5 mg/kg B.W/day, \( n = 8 \)). After sacrificed, tissue was removed from each femur and tibia, and the bone was placed in ethanol until analysis. The left femur and tibia length were measured using by a digimatic caliper.

Results: Final body weights, weight gain, food intake and caloric intake of the CPP group were the highest \((P < 0.05)\) among the four groups. Kidney weights of the CPP groups were significantly higher than those of the other groups. Femur length did not differ among the experimental groups. However, tibia length of the RCV groups were significantly highest \((P < 0.05)\) among the four groups.

Conclusion: Functions of vinegar regarding bone health has not been suggested previously. From the result of this study, 5.2% of RC vinegar supplementation for 8 weeks showed positive effects on the tibia length in young rats.

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ009826)” Rural Development Administration, Republic of Korea.
Mechanosensing in osteocytes following exposure to clinically relevant concentrations of metal ions after HIP replacement


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Background: Elevated levels of cobalt (Co) and chromium (Cr) in patients following metal-on-metal hip replacements and modular total hip arthroplasty directly affect osteoclast and osteoblast cell viability and function in-vitro (Andrews et al., 2011), with implications for patient bone health. In this study, we provide evidence that Co and Cr compound these direct effects by altering osteocytes’ regulation of bone remodelling following mechanical stimuli.

Methods: Fluid shear-stress induced changes in intracellular Ca^{2+} were observed in real-time in murine osteocyte cell-line (MLO-Y4) following 30 min or 24 h exposure to 50 or 500 μg/L combinations of Co^{2+} and Cr^{3+}. Time-lapse fluorescence images were analysed using ImageJ and the data expressed relative to control as area under the curve (AUC) and peak intensity. The effect of fluid shear-stress on osteocyte gene expression (RANKL, Dkk-1, CX43 and Gp38) in absence and presence of metal ions was assessed using real time RT-PCR.

Results: Following 30 min and 24 h exposure to Co and Cr, a reduction in cellular response (AUC) to mechanical stimuli was observed for 50 μg/L (p < 0.0001) and 500 μg/L (p < 0.0001) compared to untreated controls. A reduction in peak response was also observed for both 50 μg/L (p < 0.0001) and 500 μg/L (p < 0.0001) at both time-points. MLO-Y4 cells robustly expressed all the genes with the rank order of expression Cx43 > Gp38 > RANKL > Dkk-1. Application of fluid shear-stress upregulated Gp38 and RANKL expression and reduced Dkk-1 expression. Cells exposed to Co and Cr prior to loading had a blunted increase in Gp38 and RANKL expression following fluid shear-stress, with the effect being dose-dependent, whilst the reduction in Dkk-1 expression was unaffected. CX43 gene expression was unaffected by either loading alone or loading following Co and Cr treatment.

Conclusion: The data suggests that Co and Cr at concentrations observed in patient serum and hip aspirate following hip replacement may impair osteocyte response to mechanical stimuli and reduce the intensity of their response. Alterations in osteocyte-mediated regulation of bone remodelling in response to mechanical loading by metal ions will have significant detrimental effects on bone health.

Reference

Human osteoblasts differentiating to osteocytes in 3D culture secrete FGF-23 and sclerostin and respond to mechanical loading

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Osteocytes are master regulators of bone homeostasis and are integral to the anabolic response of bone to mechanical loading. We have developed and characterised novel, 3D methodologies to (i) differentiate mouse osteoblasts to osteocytes and (ii) apply physiological mechanical loading to 3D cultures. Here we apply our methods to human osteoblasts (hOBs). Specifically we investigated whether (i) mature osteocyte markers, FGF-23 and sclerostin, are secreted during osteocytogenesis, and (ii) the differentiated cells respond to mechanical loading.

hOBs, obtained from surgical waste, were maintained in type I collagen gels in αMEM containing 10% human serum for 15 days. FGF-23 and sclerostin secretion was measured at days 3, 7, 11 and 15 (ELISA; n = 2 samples). For the loading experiments (n = 2 samples), cells/gels in αMEM containing 10% FCS were loaded (2.5 N, 10 Hz, 5 min) on day 10 (unloaded gels as controls) and VEGF and IL-6 (ELISA) secretion measured 0.5 and 24 h post-loading.

FGF-23 was secreted at each time-point in each differentiation assay but concentrations differed significantly between samples (e.g., day 3, 1,093.32 and 217.24 pg/ml in samples 1 and 2, respectively). When corrected for cell number a significant reduction in FGF-23 was observed over time in both samples (e.g., 3,253.92 day 3/1,953.7 day 15/sample 1; 215.0 day 3/49.73 day 15/sample 2 – all pg/ml/2 × 10⁴ cells). Interestingly, FGF-23 was not secreted in cultures maintained in αMEM containing FCS. Sclerostin was also secreted in hOB cultures with a much lower concentration at day 3 when compared to day 15 (9.56 and 121.57 pg/ml, respectively). Mechanical loading of cultures showed that one sample did not respond, whereas the other increased (p < 0.01) VEGF and IL-6 secretion after 24 h (e.g., control 38.0 vs. loaded 95.6 pg/ml/10⁴ cells).

hOB cells maintained in 3D cultures secrete FGF-23 at each time-point, and although concentrations differ between samples, within each sample concentrations decreased with time. These osteoblast/osteocyte cultures also secrete varying concentrations of sclerostin, which increased with time in culture. These cultures responded to mechanical loading by modulating VEGF and IL-6 secretion. This work supports the use of these methods for further studies of human osteocytogenesis and osteocyte responses to loading.
Progression of osteoarthropathy in alkaptonuria patients monitored by \(^{18}\text{F} – \text{NaF pet}

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Alkaptonuria (AKU) arises from a genetic deficiency of HGD, an enzyme involved in tyrosine metabolism. AKU is characterised by high circulating homogentisic acid some of which is deposited as ochronotic pigment in connective tissues, leading to multisystemic damage dominated by premature severe osteoarthropathy. In cartilage, ochronosis leads to a disturbance of mechanical loading resulting in aberrant remodelling of the subchondral bone.

Pathological changes in joints as a result of pigmentation can be imaged throughout the skeleton using fluorine-18 labelled sodium fluoride positron emissions tomography (\(^{18}\text{F}-\text{NaF PET}\)) which allows quantitative assessment of focal bone remodelling.

\(^{18}\text{F} PET\) scans of 41 patients from the National Alkaptonuria Centre were analysed using Image J (1.49j). Uptake of \(^{18}\text{F}\) was scored anatomically in bone and cartilage. The incidence of hotspots in bone was highest in the hip (93%), lumbar vertebrae (78%) and thoracic vertebrae (73%). We propose that pigmentation of cartilage causes stiffening which result in aberrant transmission of mechanical loading to subchondral bone. This leads to altered bone remodelling which shows up as hotspots on the \(^{18}\text{F}-\text{NaF PET}\) scans. Secondary to this and with advancing disease progression, the cartilage becomes calcified. The incidence of hotspots in the cartilage was found to be highest in the knee (65%) followed by the hip (52%), and the foot (52%). The cubic centimetre yield of signal per unit of body mass was plotted against age. The trend of this data is hypothesised to depict a process of growth and remodelling in the younger patients, followed by osteogenic maturity around the age of 40, followed by disease pathology in the older patients. The difference between the 19–30 and 42–52 age groups was statistically significant (\(P = 0.01\)). We conclude that the uptake of \(^{18}\text{F}\) into subchondral bone can be used to assess the progression of joint involvement in AKU patients.
Structure model index is defeated by concave surfaces normally present in trabecular bone

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Since 1997, the standard method for measuring the rod- and plate-like nature of trabecular bone has been the Structure Model Index (SMI) (Hildebrand and Rüegsegger, 1997). In SMI, a mesh of triangles is fitted to the bone surface. The mesh is then dilated very slightly away from the surface and the change in surface area relative to bone volume gives a signature for plate-like (SMI = 0), rod-like (SMI = 3), and spherical (SMI = 4) regions. Unfortunately, SMI thus formulated does not account for concave curvature, which is present in normal trabecular bone in the form of saddle curves, furrows and bowl-shaped depressions. SMI summarises both concave and convex surfaces’ negative and positive contributions. BoneJ calculates SMI according to Hildebrand and Rüegsegger’s original description, and reports the concave fraction of the surface by measuring the area of the triangles that shrunk during mesh dilation. BoneJ also reports the positive and negative components of the SMI sum. Ellipsoid factor (Doube, 2015) fits ellipsoids inside trabeculae and is designed to be robust to surface curvature. We applied BoneJ’s (v1.4.1) SMI and ellipsoid factor algorithms to 114 X-ray microtomographic images of trabecular bone from the femoral head and condyle of a wide range of mammals and birds and found strong, significant correlations between concave fraction and SMI ($R^2 = 0.828, p < 0.0001$), and between bone volume fraction (BV/TV) and the negative component of SMI ($R^2 = 0.533, p < 0.0001$). Ellipsoid factor was only very weakly related to BV/TV ($R^2 = 0.102, p = 0.0005$) and concave fraction ($R^2 = 0.066, p = 0.006$). Concave fraction varies widely (0.22–0.69, mean 0.50). SMI is not fit for its stated purpose of measuring plates and rods in trabecular bone because it is strongly distorted by naturally occurring concavities that occupy a large fraction of the bone surface. We propose the ellipsoid factor as a robust alternative to SMI to measure rod and plate geometry in trabecular bone.

References

Osteopetrosis-distinct morphological changes in a rare skeletal disease

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Introduction: Osteopetrosis is a heterogeneous group of rare inherited bone diseases characterised by increased bone mass. Although important progress has been made in understanding the molecular mechanisms underlying development of osteopetrosis, histopathological analyses of human bone tissues with osteopetrosis are considerably rare. Herein, we present our experience with four cases of osteopetrosis.

Methods: All bone biopsies were embedded in methyl-methacrylate without decalcification and stained with Goldner trichrome, toluidine blue, Prussian blue, and tartrate-resistant acid phosphatase staining method. Results of genetic analyses were provided by cooperating clinicians.

Results: Mutation analyses detected CLCN7 mutation in one case, TCIRG1 mutation in two patients and KINDLIN-III mutation in one case. The case with CLCN7 mutation showed excessive thickened bone trabeculae with broad remnants of mineralised cartilage covered by enlarged fl at osteoclasts. TCIRG1-mutated cases can be best described as osteopetromalacia (osteopetrorickets) due to a substantial increased volume of non-mineralised bone. The osteoclasts were predominantly relatively tall cells and built cellular seams on the trabecular surface. the last case with KINDLIN-III mutation showed increased bone mass and severely enlarged osteoclasts which were partly detached from the trabecular surface.

Conclusion: Results of recent animal experiments and improved molecular diagnostics importantly increased our knowledge about bone metabolism and hereditary bone diseases. Distinct morphological changes of osteoclasts can be explained by causative genetic defects in the development, differentiation and function of these highly specialised multinucleated cells. While histopathological analyses of human disease tissues are rare, they can shed a light on understanding of distinct morphological changes linked with specific mutations found in the osteopetrosis patients.
Ultrastructure of bone revealed by serial block face imaging scanning electron microscopy

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Serial block face imaging SEM is a relatively new technique by which 3-dimensional data are acquired. An ultramicrotome is placed within the vacuum chamber of the SEM and the blockface of an appropriately prepared sample is scanned by the electron beam. Backscattered electrons are detected from the blockface after each section. This method can generate large, high-resolution, data sets which are then available for 3D reconstruction. The method has not been used before to image bone tissue and here we present our first data obtained from mouse bone. We prepared trabecular bone from mouse tibia by fixation in glutaraldehyde followed by demineralisation in EDTA and postfixation in heavy metals (osmium ferrocyanide, thiocarbohydrazide-osmium liganding and uranyl acetate and lead aspartate en-block staining) to make the tissue more conductive and achieve high membrane contrast. Samples were embedded in epoxy resin and relevant sections trimmed. Section thinness of around 30 nm was achieved for some samples. Data sets were analysed using Amira software and reconstructions of osteocyte networks and osteocyte connections with other cells made. While there is a lot of “missing information” in such datasets, due to the section thickness (this material is lost to the imaging process), these reconstructions are revealing new information, such as showing direct contacts between osteocyte processes and endothelial cells. This imaging modality offers a more direct appreciation of osteocyte networks and interactions with a variety of cells than the “indirect” method of resin-etching SEM, that shows the spaces in which osteocytes and other cells reside, rather than image the cells directly. We suggest that both methods are used in parallel to better understand the complexity of bone cell networks in 3D and are complemented by transmission EM (either in regular 2D, or by TEM tomography) to achieve the highest resolution for selected areas. We propose that the nanoanatomy of bone remains relatively unknown and encourage further studies using these novel methodologies.
Regulation of articular cartilage homeostasis by the N-end rule Ubiquitin-protein ligase UBR5


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Our work has revealed the N-end Rule Ubiquitin-protein ligase UBR5 as a potent suppressor of Hedgehog signalling and osteoarthritis-associated changes in murine articular cartilage (AC). Using Prx1-Cre combined with a floxed UBR5 mutant allele we deleted UBR5 function in the developing murine limb buds. Homozygous UBR5 mutant (UBR5 mt) embryonic limbs appeared morphologically normal, but 6-week old UBR5 mt limbs exhibited a number of dramatic osteoarthritis-associated changes to the AC that included: chondrocyte clustering, increased numbers of hypertrophic-like chondrocytes, osteophytes, vascular invasion and cartilage fibrillation. By 12 weeks of age, UBR5 mt animals exhibited dramatic AC loss down to the subchondral bone.

Supporting the histological observations, immunohistochemistry revealed dramatically irregular expression patterns for markers hypertrophic and “resting” chondrocytes markers. Based on observation made with UBR5’s Drosophila homolog, we hypothesised that these cellular and molecular changes may have been due to changes in the Hedgehog signalling landscape of the adult AC. IHH-mediated signalling plays a central role in governing stem/progenitor cell function in various tissues, including juvenile and adult bone. In agreement, Indian Hedgehog ligand, its receptor Patched and the product of one of the pathway’s target genes, Gli1, were all upregulated in UBR5 mt AC.

Work in other murine tissues also supports UBR5’s role as an important regulator of stem/progenitor cell function. We now propose that UBR5, through influencing Hedgehog signalling, governs stem/progenitor-mediated control of AC homeostasis. We are addressing this hypothesis by using Hedgehog pathway gain-and loss-of-function alleles to modify the UBR5 mt AC phenotype and investigating isolated Ubr5 mt stem/progenitor chondrocyte function.
UBR5, an E3 Ubiquitin-protein ligase, governs hedgehog-regulated heterotopic tendon ossification

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Our studies into the N-end Rule Ubiquitin-protein ligase UBR5 revealed its role in controlling heterotopic tendon ossification in the mouse limb and hypothesise that UBR5 regulates stem/progenitor behaviour to control tendon homeostasis.

Spatiotemporal bone development is tightly regulated to maintain a functioning skeleton. However, in certain diseases heterotopic ossification (HO) can occur in soft tissue. Using Prx1-Cre combined with a floxed UBR5 mutant allele (UBR5 mt) we deleted UBR5 function in the developing murine embryonic limb bud. Micro-CT analysis revealed tendon/ligament HO in homozygous UBR5 mt adult animals. HO was progressive, occurred at multiple sites and was first detected at 6 weeks of age. Histological analysis identified numerous chondrocytes within the Achilles tendon midbody, suggesting a potential role for UBR5 in suppressing chondrogenesis.

Our work in other tissues indicates UBR5 as an important regulator of stem/progenitor cell function. Furthermore, through studies in Drosophila and the murine articular cartilage, we revealed that UBR5 regulates Hedgehog (HH) ligand production and signal transduction. Indian Hedgehog signalling plays a central role in controlling stem/progenitor cell function in various tissues including the adult skeleton. UBR5 mt animals treated with the HH pathway antagonist cyclopamine resulted in an enhanced level of HO. Cyclopamine treatment in a wild-type background did not promote HO, but did promote increased ossification of normal sessamoid bones. Therefore, it appears that Hedgehog pathway inhibition alone is necessary, but not sufficient for HO. In conclusion, it appears that UBR5 and HH signalling normally act to suppress heterotopic tendon ossification.
A new paradigm for bone formation in remarkable endosteal bone appositional rates in rat distal femur

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Objectives: To study details of bone growth rates and mechanisms in the distal femur of young rat using multiple combined 3D imaging methods.

Methods: Fluorescent mineralisation front labels – calcein was given at 12 days and tetracycline 3 days before termination. X-ray microtomography (microCT 8 μm voxels) of right femurs was conducted before cutting the femurs longitudinally in the midline. The lateral halves were PMMA embedded for block face microscopy, using confocal LM for histology and to read labels. 20 kV BSE SEM was used to image mineral content; more BSE imaging after iodine staining was also used for histology. Finally, all bone was dissolved to produce 3D casts of marrow and capillary blood vessel canal space. The medial halves were macerated for 3D BSE SEM. After analysis of the right-side data, we studied left femurs with 6 μm microCT, then cut them transversely to create thick “rings” at defined distances from the distal condyles. The end faces were polished and used for imaging labels with confocal LM and the samples were then macerated for 3D SEM. All these types of image were cross correlated to produce composites which can be best understood by dynamic, sequential, repetitive display techniques.

Results and Conclusions: Remarkably high values for endosteal apposition were measured, with nearly matching high periosteal resorption rates to be assumed. In places, almost the entire 400 μm thickness of the shaft was translocated in 15 days. This rapid endosteal bone growth is associated with the inclusion of capillary blood vessels which penetrate the osteoblastic layer at near normal incidence to the formative surface at ~50 micron spacing. This is a previously undescribed mode of compact cortical lamellar bone formation.

Trabeculae drift centrally in parallel with cortical surfaces. Thus their double label intervals must be understood in a full 3D context. MicroCT imaging “loses” small trabeculae which are physically retained even in macerated SEM samples. Real loss of trabeculae occurs by their burial in compact bone at endosteal growth surfaces as well as through resorption. The “gold standard” must be held to be the combination several imaging methods.
Blood flow regulates bone angiogenesis and osteogenesis through notch signaling

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Angiogenesis and osteogenesis are tightly linked during the development of the mammalian skeletal system. Distinct capillary subtypes have been shown to play an important role in coupling of angiogenesis and osteogenesis. Growing blood vessels in the metaphysis of long bones show the presence of specialized structures like distal arches and buds, but their precise role in bone angiogenesis is not known. Here, by using a novel intravital imaging technique, we describe the mechanism of blood vessel growth in murine long bone, which involves the extension and fusion of buds. The organisation of arteries and veins in long bone generates a peculiar flow pattern, which, as experimental manipulations reveal, controls bone angiogenesis and Notch activity in endothelial cells. In aged mice, which have been reported to exhibit reduced blood flow in bone, distal arterioles and endothelial Notch activity were significantly reduced. Restoring endothelial Notch signalling in aged mice reactivated bone angiogenesis and enhanced osteogenesis. We therefore propose that blood flow acts upstream of endothelial Notch signalling to coordinate the growth of vessels and bone formation.
Modulation of ectopic ossification in tissue engineered skeletal muscle by an inflammatory environment

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Objectives: If the mechanisms governing ectopic bone formation are understood they have the potential to be exploited for the development novel regenerative therapies for fracture prevention and repair. The present study utilised a novel in vitro model of skeletal muscle to define the relationship between post-traumatic inflammation and ectopic bone formation.

Methods: A skeletal muscle model was developed by seeding C2C12 muscle precursor cells (MPCs) within a type-I collagen gel (Sharples et al., 2012). Uniaxial tension was produced by anchoring the gel at opposing ends of a well plate using polyethylene flotation bars. The model was cultured with high glucose DMEM and 20% FBS for 4 days, after which the FBS was replaced with 2% horse serum to facilitate myoblast fusion along the axis of tension. After myotube formation the model was exposed to combinations of osteogenic factors and inflammatory cytokines previously identified in serum/effluent following trauma (Evans et al., 2012) Cells were released from the model by digestion with 0.1% collagenase. Phenotypic analysis was carried out using flow cytometry. Cells released from constructs were plated at 10x10^5 per cm^2 and cultured in growth medium. After 24 h ossification was determined using alizarin red (AR) staining and MicroCT.

Results: Following culture in a 3D environment unfused MPCs were released by enzymatic digestion. AR staining showed that models treated independently with BMP2, TGF-β, or PDGF ossified following release from the 3D environment after just 24 h. Exposure to PDGF led to significantly (P < 0.05) more mineral deposition than exposure to BMP-2, the most ubiquitously applied osteoinductive factor. Using flow cytometry we showed that ossification was accompanied by a change in MPC phenotype to a novel Sca-1+ /CD73+ cell type. Finally, we identified that ectopic ossification could be prevented in the presence of an inflammatory environment.

Conclusion: This study identified a novel osteogenic/non-myogenic population of Sca-1+/CD73+ cells in skeletal muscle. We showed for the first time that ossification can be enhanced by PDGF, but that the presence of an inflammatory environment may act to modulate this osteogenic response.

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References
Could a recently identified isoform of SQSTM1 be the culprit for the development of Paget’s disease of bone?

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Paget’s disease of bone (PDB) is caused by mutations in a ubiquitously-expressed gene, Sequestosome-1 (SQSTM1). Two protein isoforms of SQSTM1 are produced, and both are affected by PDB-causing mutations: SQSTM1/p62 is well-described whereas little is known of the expression, functional role or contribution to PDB of the shorter SQSTM1-55. We have described previously that SQSTM1-55 is the predominant isoform in human osteoclast-like cells and the isoforms are encoded by at least four transcripts. Here we study the expression, localisation and function of both SQSTM1 isoforms (wild-type (WT) and mutated) to decipher the disease mechanism of PDB.

Transcript expression was assessed using dual-luciferase promoter-reporter assays for the ~1.5 kb region in front of each transcript, transfected into Human Embryonic Kidney (HEK293) and HeLa cells. Intracellular localisation of WT and mutant (P392L, E396X, G425R) SQSTM1 for both isoforms was investigated using fluorescently-tagged expression vectors in HEK293 and HeLa cells. Activation of NFκB was assessed using NFκB dual-luciferase reporter assays in HEK293-derived cell lines stably transfected with WT or mutated 55 kDa- or 62 kDa-SQSTM1.

Promoter-reporter assays revealed that the SQSTM1 genomic location uses at least two distinct promoters. Cap Analysis Gene Expression data from various human cells and tissues support this, and demonstrate cell/tissue-specific promoter usage. The effects of PDB-causing mutations appear to depend on the isoform in which they are expressed. Transfection of fluorescently-tagged SQSTM1 expression vectors revealed differences in cellular localisation dependent on the isoform and the mutation. Similarly, NFκB activation in response to TNFa is altered by SQSTM1 mutations in an isoform- and mutation-specific manner: NFκB was activated similarly in stable cell lines expressing wild-type SQSTM1-55 or SQSTM1/p62, while PDB-causing mutations activated or inhibited NFκB activity dependent on the isoform in which they were expressed (two-way ANOVA interaction \(p < 0.0001\)). For example, NFκB activity was reduced in 62-P392L relative to WT while 55-P392L increased activity.

The results described here begin to explain how mutations in a ubiquitously-expressed gene can produce the osteoclast-specific phenotype observed in PDB.

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Ochronotic pigment deposition in the spine of AKU mice and its relationship to joint-loading

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Alkaptonuria (AKU) is an ultra-rare disorder characterised by ochronotic pigment deposition, primarily in the cartilage of load-bearing joints resulting in an early onset, severe osteoarthropathy. Previously we have focussed on ochronotic pigment distribution in the tibio-femoral joints of AKU mice. We wanted to determine if the spine, which is the initial site of pigment deposition in humans, is pigmented in AKU mice and whether there was a link between the amount of pigment deposited and joint-loading.

The L1 and L2 vertebrae were harvested from BALB/c AKU mice aged between 15 and 66 weeks and processed for histology. Schmorl’s stain was used to quantify the number of pigmented chondrons in the intervertebral discs (IVD).

Using Schmorl’s stain we identified the earliest deposition of ochronotic pigment in the IVD of AKU mice at 30 weeks, and an increase in the number of pigmented chondrons representing the progressive pathogenesis of AKU across the lifespan. The pattern of pigmentation was similar to that we have previously observed in the tibio-femoral joints with pigment deposition increasing with age, something which also occurs in man. Interestingly the earliest deposition of pigmentation in the IVD was much later than in the tibio-femoral joint, when it occurred at 15 weeks.

The appearance of pigmentation in the spine of AKU mice later than pigmentation in the knee was somewhat unexpected, particularly as in man the earliest pigmentation is seen in the spine and then progresses to the knee and hip. It is likely that joint-loading plays a role in this as humans, who are bipedal, experience increased force on the spine in comparison to a mouse. This may explain why the spine becomes pigmented later in the lifespan of AKU mice, as their hind and forelimbs are likely to experience increased loading compared to the spine resulting in micro trauma and initial pigment deposition. Subsequent abnormal force distribution across the hind and forelimbs due to accumulation of ochronotic pigment may then translate into abnormal loading of the spine leading to pigment deposition in the IVD.

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Exploring the 15Q24.1 chromosomal locus containing promyelocytic leukaemia (PML) gene for susceptibility to paget’s disease of bone

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Paget’s disease of bone (PDB) is a common skeletal disorder characterised by focal abnormalities in bone turnover. Genetic factors are important in PDB and GWAS identified a susceptibility locus for PDB on chromosome 15q24. The strongest association within this locus was with SNP rs5742915 (p.Phe645Leu) within coding region of PML. The aim of this study was to fine map this locus to identify functional genetic variants predisposing to PDB using targeted next generation sequencing approach.

The region contains six genes but the strongest association signal was located within PML, a tumour suppressor gene that is involved in multiple cellular functions including cell growth, senescence, apoptosis, protein degradation and antiviral responses but has never been implicated in bone metabolism. It may be involved by controlling maturation of myeloid cells, proliferation and osteogenic differentiation of human mesenchymal stem cells and/or through its involvement in bone remodelling pathways such as TGF-β, IFN-γ and Wnt signalling.

Targeted DNA sequencing of a 200 kb region surrounding the rs5742915 SNP in 138 PDB cases and 50 controls identified several variants including three novel protein coding and seven novel possible regulatory variants. The GWAS SNP rs5742915 along with three protein coding and two SNPs in the regulatory region showed significant association with PDB. Few SNPs identified were located in regions enriched with histone modification marks and localised to important bone related transcription factor binding sites.

We next investigated PML gene expression in bone cells and found that PML was expressed in RAW 264.7 osteoclast-like cells as well as bone marrow derived macrophages and at all stages during their differentiation into osteoclasts. PML was also expressed at all stages of osteoblast differentiation in cultured cells derived from mouse calvaria.

In conclusion, several protein coding and potential regulatory variants have been found at the 15q24 locus which are being validated in a larger cohort of PDB cases and unaffected controls. PML has been found to be expressed in bone cells and further studies are ongoing including knocking out and overexpressing PML in these cells to establish its role in bone metabolism and PDB pathogenesis.
The link module from human TSG-6 (Link_TSG6) inhibits bone resorption in the OVX mouse

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Objectives: We reported previously that TSG-6 acts as an autocrine regulator of osteoclast activity and inhibits bone resorption \textit{in vitro} (Mahoney et al., 2008; 2011). Therefore, this protein has the potential to be developed as a novel treatment for bone loss (Patents: EP2001499 B1, JP_5346216_B2, US 9,066,908). The objectives here were: (i) to evaluate the efficacy of the recombinant Link module from human TSG-6 (Link_TSG6) in the ovariectomised (OVX) mouse model of osteoporosis and (ii) to investigate the mechanism underlying the anti-resorptive activity of Link_TSG6.

Methods: OVX mice (8–10/group) were treated with Link_TSG6 for 4 weeks, where treatment was initiated either at the time of ovariectomy or 2 weeks later. Efficacy was assessed by quantification of CTX-I and PINP in sera and by micro-CT and histomorphometry of long bones. Osteoclast precursors, derived from murine bone marrow or human PBMCs, were cultured on dentine slices with M-CSF/RANKL ± Link_TSG6 prior to quantification of TRAcP-positive cell differentiation, lacunar resorption and F-actin ring formation.

Results: Link_TSG6-treated OVX mice had significantly reduced CTX-I levels compared to vehicle-treated controls, but (unlike Zoledronate) Link_TSG6 caused no reduction in PINP. Micro-CT and histomorphometry showed that trabecular bone loss was suppressed in Link_TSG6-treated animals, with the number, size and activity of osteoclasts at the bone surface all being reduced whilst osteoblast function was not impaired. \textit{In vitro}, Link_TSG6 potently inhibited osteoclastic resorption and F-actin ring formation and also reduced osteoclast numbers.

Conclusion: Inhibition of bone resorption, but not formation, by Link_TSG6 \textit{in vivo} suggests potential advantages over existing anti-resorptive treatments for osteoporosis that significantly impair the bone-remodelling unit. Through a combination of \textit{in vitro} and \textit{in vivo} data we are developing an understanding of the mechanisms via which Link_TSG6 mediates protective effects in bone tissue.

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References
Does the origin matter? mesenchymal stem cells derived from adipose tissue vs. bone marrow. osteogenic differentiation and extracellular matrix formation

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Mesenchymal stem cells (MSCs) may be isolated from various tissues. In the context of bone tissue engineering the most appropriate source of MSCs is adipose tissue (AT) and bone marrow (BM). The aim of this study was to compare two types of human MSCs and test the effect of medium composition on osteogenic differentiation and extracellular matrix formation.

Cells were isolated from three donors who provided informed consent. MSCs were cultured in three types of media, that differed by the presence of ascorbic acid, β-glycerophosphate and dexamethasone. Cells were seeded on the nanofiber polycaprolactone scaffold created by electrospinning.

AT-MSCs proliferated faster, exhibited better ability to form extracellular collagen network and expressed rather early markers of osteogenesis. BM-MSCs expressed more late markers of osteogenesis, suggesting that AT-MSCs are less specialized and compared to BM-MSCs more “immature” in terms of osteogenic differentiation. Composition of the culture medium also affected cell proliferation, extracellular matrix formation and expression of osteogenic genes.

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High-resolution imaging to address the genetic underpinnings of canine skull morphology and disease

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Objectives: Perhaps the most striking morphological feature of dogs is their craniofacial diversity. A major limitation to understanding the biology behind canine skull morphology and its oft-related morbidities is the difficulty of collecting skeletal phenotypes, diagnosis, and genotypes from dogs at large.

Methods: In order to explore the biological underpinnings of the morphological space occupied by canine skulls, we analyzing high-resolution computed tomography (CT) scans of canine patients: pedigree dogs representing 90+ morphologically diverse breeds (median = 2, range 1–22) and 74+ mixed breed dogs of varying sizes and skull morphologies. Using geometric morphometrics, we have analyzed three-dimensional skull reconstructions to categorize skull shapes, sizes and anatomical substructures (e.g., face, cranial vault, and mandible). In parallel, patients enlisted in our study were genotyped by SNP arrays and representatives of morphological extremes were whole genome sequenced. Together, we are positioned to conduct GWAS and fine mapping to uncover causal variants, as well as model phenotypic effect sizes of the variants we uncover.

Results: Our pilot genome wide association study (n = ~350) detected skull quantitative trait loci (QTL) that influence rostrum length in dogs. Among the QTL we identified, we fine mapped a previously described QTL that corresponds to a selective sweep on chromosome 1. Analysis of this region indicates SPARC related modular calcium binding 2 (SMOC2) is a major determinant of brachycephalic skull conformation. Interestingly, mutation of SMOC2 was previously associated with Type I dentin dysplasia. Our results suggest that the dental defects associated with this gene could extend to the craniofacial structures of the viscerocranium.

Conclusion: These efforts lay the foundation for future work whose overarching goals are to leverage clinic-based, high-resolution imaging of canine patients to address morbidities that result from aberrant skull morphology.

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Temporal trends in UK rates of knee arthroscopy in the over 60S do not reflect evidence, but do reflect the model of healthcare delivery

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Objectives: Multiple RCTs and meta-analyses demonstrate the poor efficacy of knee arthroscopy in older patients. Though it is difficult to define the “correct rate” of a “discretionary” procedure, surgical rates should reflect evidence for practice. We assessed whether UK temporal trends of knee arthroscopy were influenced by changing evidence over the last decade.

Methods: We assessed published data from the Hospital Episode Statistics (HES) dataset for NHS England and obtained directly comparative data for NHS Scotland from a nationally held dataset. Data was for the time period 01 April 2000 to 31 March 2014 where procedure codes W82.2, W85.1, W85.2, and W85.8 were recorded.

Results: In England, there has been a substantial rise in overall knee arthroscopy volume, from 185 procedures per 100,000 population to 267 per 100,000 an increase of 69%. In Scotland, overall rates rose from 111 per 100,000 to 115 per 100,000, an overall increase of 4%.

In both countries, rates of arthroscopic irrigations have fallen markedly, however, this reduction has been dwarfed by increases in the number of meniscal resections performed.

Conclusion: Despite continued high-level evidence of poor efficacy, rates of knee arthroscopy in the over 60s paid for by the NHS have increased over the last decade. Rates of arthroscopic irrigation have fallen in line with changing guidelines, but there are no clear physiological reasons why need for meniscal resection should have increased – or differ so widely between Scotland and England. When incidence, infrastructure, surgeon training and patient demographics are the same, variation in surgical rates can reflect provider priorities as opposed to patient need.

The fragmented system of musculoskeletal service delivery in England with private companies rewarded by procedure volume (as opposed to patient outcomes) differs markedly to the situation in Scotland where clinician responsibility from initial referral through surgery and outcome has been maintained. Rates of arthroscopy in NHS England now reflect those of private healthcare insurance systems such as the US and Australia. This may not be sustainable.
Polycaprolactone nanofibers and thrombocytes rich solution functionalization of 3D B- tricalcium phosphate scaffold accelerated large bone defect regeneration

in vivo

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Objectives: Nowadays, the most promising approach for bone tissue regeneration is to develop an osteoconductive and osteoinductive scaffold recruiting of mesenchymal stem cells migration, proliferation and differentiation. Nanofibers were already used as a carrier for adhesion of bioactive molecules for improving of proliferation and differentiation of cells in vitro and in vivo (Filova et al., 2013). Platelet rich plasma (PRP), as a natural source of growth factors, is clinically well characterized, and is the most promising source of growth factors for tissue engineering applications. Incorporation of the nanofiber drug delivery system into a 3-dimensional scaffold from osteoconductive and osteoinductive materials is a new approach in bone regeneration. Commercially used 3D matrices from β- Tricalcium phosphate for bone regeneration in dentistry was functionalized by Polycaprolactone nanofibers as a drug delivery system and Thrombocyte rich solution as a source of natural growth factor and tested in vivo on rabbit model.

Methods: Microparticles from Polycaprolactone nanofibers (PCL) were used as a template for adhesion of thrombocyte rich solution (TRS) as a source of natural bioactive molecules, mixed with commercial 3D matrices and implanted into femur condyle of rabbits as a spongy bone defects model. The commercial 3D matrices without any functionalization and defect without any treatment were used as a control. After 45 and 90 days a qualitative and quantitative histological analysis was performed.

Results: New formed bone tissue was observed at both groups of scaffolds. After 90 days a higher volume of collagen type I was shown at PCL and TRS functionalized scaffold in comparison with non-functionalized scaffold group.

Conclusion: The functionalization by PCL nanofibers as a drug delivery system and TRS accelerated bone tissue regeneration in vivo.

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Reference

ATP-binding cassette transporter A8 (ABCA8) is required for osteoblastic functions of SAOS-2 cells

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Extracellular ATP regulates cellular activities via cell surface P2 receptors. Osteoblasts constitutively release ATP and this release is increased in response to mechanical stimuli. The mechanisms of ATP release are not yet fully understood but may involve transmembrane ATP binding cassette (ABC) proteins. Members of this super family of proteins are required for a diverse range of functions that include lipid transport, regulation of ion channels, and efflux of chemotherapeutic drugs in addition to ATP release.

SaOS-2 cells were subjected to mechanical loading by fluid flow. ATP was measured by luciferase assay with lactate dehydrogenase (LDH) quantified as a control for cell death. ABC mRNA expression was investigated by low density array. ABCA8 expression in SaOS-2 cells was knocked down by siRNA transfection and this knockdown was confirmed by immunocytochemistry. Proliferation was assessed by MTS assay. Enzymatic activity of alkaline phosphatase (ALP) was measured and normalised to DNA with the picogreen assay.

Mechanical loading of SaOS-2 cells induced immediate ATP release (basal 2.94 nM, load 6.46 nM, \( P < 0.01 \)). Loaded SaOS-2 cells had increased mRNA expression of ABCA8 (563 fold cf basal), ABCB6 (13 fold cf basal) and ABCA11 (11 fold cf basal). ABCA8 siRNA transfected cells showed reduced staining for ABCA8 protein (58% lower cf mock transfected cells, \( P < 0.05 \)). ATP release was unaffected by ABCA8 siRNA transfection. However, MTS activity was decreased (20% lower cf mock transfected, \( P < 0.001 \)) as was ALP activity (60% lower cf mock transfected, \( P < 0.01 \)).

This study focused on the functional role of ABCA8 in SaOS-2 osteoblastic cells. Mechanical stimulation engendered both ATP release and ABCA8 expression, however, it appears that ABCA8 is not required for ATP release. This functional study identified novel underlying cellular roles for ABCA8 in maintaining osteoblast proliferation and mineralisation. These findings may help to explain a recently identified correlation between ABCA8 gene expression and bone mineral density in post-menopausal women.