7th Danish Bone Research Workshop

March 8–10, 2024

Early Career Investigator (ECI) pre-program March 7–8

Danish Bone Society (DKS) spring meeting March 8

Program & Abstracts

Sponsored by

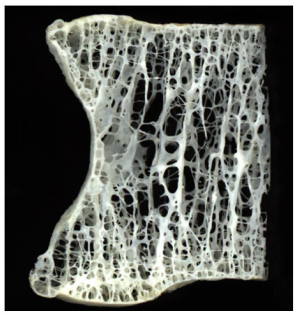
Organizing committee	
Thomas Levin Andersen, OUH, SDU & AU	Thomas.Levin.Andersen@rsyd.dk
Jesper Skovhus Thomsen, AU	JST@biomed.au.dk
Tanja Sikjær, AUH	Tanja.Sikjaer@gmail.com
Pernille Hermann, OUH	Pernille.Hermann@rsyd.dk
Morten Frost Nielsen, OUH	FrostNielsen@yahoo.com
Torben Harsløf, AUH	torbhars@rm.dk
Christina Møller Andreasen, SDU	cmandreasen@health.sdu.dk

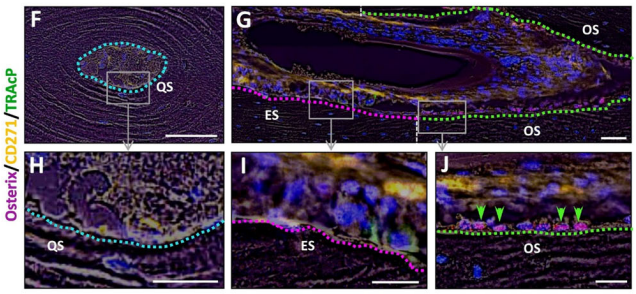
Early Career Investigator (ECI) pre-program sub-committee	
Frederik Duch Bromer, AU	Anastasiia Sadetskaia, AU
Helena Margaretha Bardenfleth, AUH	Maja Østergaard, AU

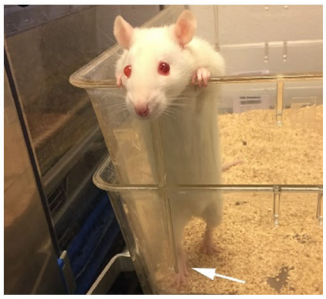
Early Career Investigator (ECI) award committee	
Jesper Skovhus Thomsen, AU	Frederik Duch Bromer, AU
Morten Frost Nielsen, OUH	Marta Diaz-del Castillo, AU
	Christina Møller Andreasen, SDU

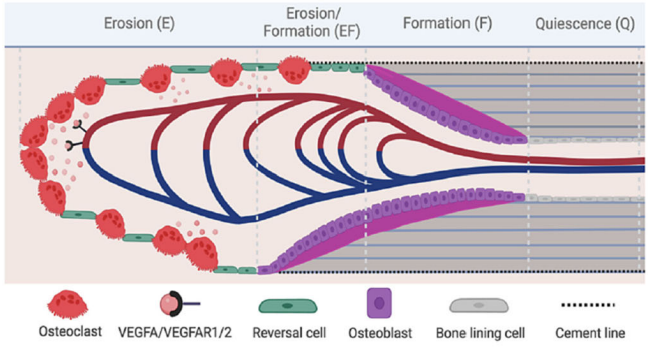
PhD course in Bone Biology

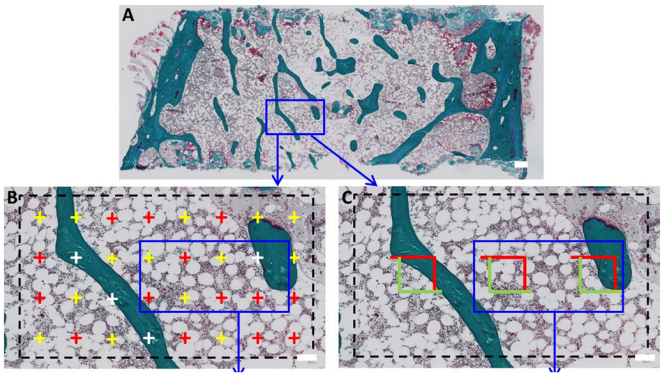
In the fall 2024, the Graduate School of Health, Aarhus University, will offer its well-established PhD course in Bone Biology at **Sandbjerg Estate**.











Criteria for participation: University degree in medicine, dentistry, nursing, or master's degree in other fields and/or postgraduate research fellows (PhD students and research-year medical students).

Requirements for participation: As an introductory course, no previous experience in bone biology is required.

Aim: To provide the participants with an overview of bone biology under normal, physiological, and pathophysiological conditions, bone therapies, and basic and advanced methodologies in bone research.

Workload: The course has an expected workload of 87 hours.

Instructors: The most knowledgeable bone scientists in Denmark.

Participation in the course is without cost for:

- PhD students, Health Research Year students from Aarhus University
- PhD students enrolled at *partner universities of the Nordoc collaboration*
- PhD students from other institutions in the open market agreement for PhD (courses https://www.nordochealth.net/courses)

Course leaders: Thomas Levin Andersen and Jesper Skovhus Thomsen

Symposium on Metabolic Bone Disease

Clinical and Basic Perspectives

30 April – 3 May 2024

Comwell HC Andersen Odense / ODEON

Join us at our symposium on metabolic bone disease to gain a deeper and wider understanding of state-of-the-art metabolic bone research and take the opportunity to extend your network and enhance the potential for collaboration within and beyond national borders.

You will delve into cutting-edge findings in both clinical and basic research and clinical practice – ranging from treating osteoporosis and managing rare bone diseases to the importance of exercise, anabolic signalling, calories and cellular metabolism.

SPEAKERS

Alexander Robling, Professor
Indiana University School of Medicine (US)

Bente Langdahl, Professor
Aarhus University (DK)

Natasha Appelman-Dijkstra, Internist-Endocrinologist
Leiden University Medical Center (NL)

Nathalie Sims, Professor
University of Melbourne (AU)

Lars Rejnmark, Professor
Aarhus University Hospital (DK)

Pouneh Fazelli, Associate Professor
University of Pittsburgh School of Medicine (US)

Richard Eastrell, Professor
University of Sheffield Medical School (UK)

Ryan Chai, Research Officer
University of New South Wales Sydney (AU)

Steve Stegen, Assistant Professor
Catholic University of Leuven (BE)

Wolfgang Kemmler, Professor
Friedrich-Alexander University Erlangen-Nürnberg (DE)

READ MORE AND REGISTER AT THE DDEA WEBSITE

Registration deadline: 17 March 2024

*If you are a PhD student you can also sign up for the **pre-symposium PhD course** on our website.*

ddeacademy.dk

Organised by
Danish Diabetes and Endocrine Academy

Danish Diabetes and Endocrine Academy

Organising committee
Morten Frost, Professor, University of Southern Denmark (DK), Bente Langdahl, Professor, Aarhus University (DK), Alexander Rauch, Associate Professor, University of Southern Denmark (DK), Ryan Riddle, Professor, University of Maryland (US)

Thursday, March 7

17:00 –	**Registration opens**
18:00 – 19:00	***DINNER*** *(MAGASINET)*
19:00 – 20:30	**ECI SESSION 1** *(STALDEN)* ***BASIC BONE KNOWLEDGE AND TECHNICAL SKILLS*** Lecturer: Thomas L. Andersen and Jesper Skovhus Thomsen
20:30 –	**TEAM BUILDING ACTIVITIES** *(STALDEN)*

Friday, March 8

7:15 – 8:00	**Morning swim and Sauna** *(LOBBY)*
8:00 – 9:00	**BREAKFAST AND NETWORKING** *(MAGASINET)*
9:00 – 12:30	**ECI SESSION 2** *(STALDEN)* **WORKSHOP – IMPROVING YOUR PRESENTATION** Lecturer: Sven Ole Schmidt
12:30 – 13:30	**LUNCH AND NETWORKING** *(MAGASINET)*
13:30 – 14:30	**ECI WALK-AND-TALK**
14:30 – 15:00	**BREAK** *(STALDEN)*
15:00 – 16:00	**DKS SESSION 1** *(STALDEN)*
15:00 – 15:15	Velkomst *Formand for DKS, Janni Dahl Hald*
15:15 – 16:00	Hvordan kan vi bruge de danske sundhedsregistre til at undersøge naturhistorien af Osteogenesis Imperfecta? *Overlæge Lars Folkestad, Endokrinologisk Afdeling, OUH*
16:00 – 16:20	**BREAK** *(STALDEN)*
16:20 – 18:00	**DKS SESSION 2** *(STALDEN)*
16:20 – 17:05	Knoglens mikro- og nanostruktur i Osteogenesis imperfecta *Professor Henrik Birkedal, Interdisciplinært Nanoscience Center, AU*
17:05 – 17:15	**Kort pause**
17:15 – 18:00	PTH behandling af hypoparathyroidisme *Professor, overlæge Lars Rejnmark, Hormon- og Knoglesygdomme, AUH*

Early Career Investigator (ECI) pre-meeting

Danish Bone Society (DKS) spring meeting

Friday, March 8

18:00 – 19:10 ***DINNER*** *(MAGASINET)*

19:10 – 20:10 **SESSION 1** *(STALDEN)*

MOSEKILDE AWARD RECIPIENT KEYNOTE LECTURE
Moderators: *Thomas Levin Andersen*

19:10 – 19:55 Osteoporosis treatment: Treat to Target and Sequential Therapy
Prof. PhD Bente Langdahl, Aarhus University, Denmark

20:10 – 21:00 **SESSION 2** *(STALDEN)*

SHORT ORALS OF POSTERS
Moderators: *Morten Frost Nielsen & Anders Nordholm*

20:10 – 20:15 **Poster 1 ▪** TALLYHO/JngJ as a model for type 2 diabetes-induced bone disease
Lejla Emini, Juliane Salbach-Hirsch, Johannes Krug, Katharina Jähn-Rickert, Björn Busse, Martina Rauner, Lorenz C. Hofbauer

20:15 – 20:20 **Poster 3 ▪** Fibrous Dysplasia Bone Pain – Peripheral and Central Pathology
Chelsea Hopkins, Julie Benthin, Ruth Elena Martínez Mendoza, Juan Antonio Vazquez Mora, Luis Fernandez De Castro Diaz, Marta Diaz del Castillo, Alison Boyce, Michael T. Collins, Juan Miguel Jimenez Andrade, Anne-Marie Heegaard

20:20 – 20:25 **Poster 5 ▪** Newly formed bone and how to find it: Mineral growth enclosed by high-zinc-content borders
Maja Østergaard, Kristine H. Neldeborg, Thorbjørn E.K. Christensen, Tanja T. Sikjær, Lars Rejnmark, Henrik Birkedal

20:25 – 20:30 **Poster 7 ▪** Zoledronic acid prevents spine and hip bone loss after bariatric surgery – A randomized, placebo controlled study
Søren Gam, Simon Lysdahlsgaard, Bibi Gram, Anne Pernille Hermann, Martin Weber Kusk, Claus Bogh Juhl, Stinus Gadegaard Hansen

20:30 – 20:35 **Poster 9 ▪** Characterization of Key Cells in the Bone Microenvironment of Metastatic Breast Cancer Patients
Melisa K. Uguz, Emma K.E. Bengtsson, Malene H. Nielsen, Bilal El-Masri, Thomas L. Andersen, Anne Marie B. Jylling, Christina M. Andreasen

20:35 – 20:40 **Poster 11 ▪** The direct healthcare costs of fracture treatment in T1D
Annika Vestergaard Kvist, Morten Frost, Andrea M. Burden, Peter Vestergaard, Troels Kristensen

20:40 – 20:45 **Poster 13 ▪** Perilacunar bone – same but different
Anne Marie Møller Faaborg, Jonas Palle, Thorbjørn Erik Køppen Christensen, Nina Kølln Wittig, Annemarie Brüel, Thomas Levin Andersen, Tilman A. Grüneawald, Henrik Birkedal

20:45 – 20:50 **Poster 15 ▪** Real-Time Live Imaging of Osteoclast Activation via Cathepsin K Activity in Bone Diseases
Eun Jung Lee, Seyoung Koo, Da Hyeon Yun, Jong Seung Kim, Serk In Park

20:50 – 20:55 **Poster 17 ▪** Innervation in multiple myeloma: a spatially resolved histological study of human bone
Martin S. J. Christensen, Rebecca E. Andrews, Aritri Mandal, Andrew D. Chantry, Anne-Marie Heegaard, Thomas L Andersen, Marta Diaz-del Castillo

20:55 – 21:00 **Poster 19 ▪** Mystery of cement lines in bone structure: spatial distribution of Zn and changes in surrounding matrix mapped by synchrotron-radiation experiments.
Anastasiia Sadetskaia, Jonas Palle, Nina Kølln Wittig, Carsten Pedersen, Annemarie Brüel, Maik Kahnt, Manuel Guizar-Sicairos, Henrik Birkedal

21:00 – 22:00 **POSTERS** *(STALDEN)*

POSTER VIEWING, ODD POSTER NUMBERS ONLY

Saturday, March 9

8:00 – 9:00	**BREAKFAST AND NETWORKING** *(MAGASINET)*
9:00 – 10:00	**SESSION 3** *(STALDEN)*
	BONE AND CANCER Moderators: *Marta Díaz del Castillo & Eva Maria Wölfel*
9:00 – 9:30	Osteoblast-dependent regulation of anti-tumoral immunity *Prof. PhD Serkin Park, Korea University College of Medicine, Seoul, Korea*
9:30 – 10:00	New insights into pathogenesis of myeloma bone disease *Assoc. Prof. PhD Abbas Jafari, Dept. of Cellular and Molecular Medicine, Copenhagen University, Denmark*
10:00 – 10:30	**BREAK** *(STALDEN)*
10:30 – 11:30	**SESSION 4** *(STALDEN)*
	FROM NUCLEOBASE TO LONG-READ: CURRENT AND NOVEL METHODS FOR MOLECULAR GENETIC ANALYSES IN RESEARCH Moderators: *Anja L. Frederiksen + Ching-Hsin Huang*
10:30 – 11:00	DNA variant analyses to long-read *Prof. PhD Inge Søkilde Pedersen, Dept. of Molecular Genetics, Aalborg University Hospital, Denmark*
11:00 – 11:30	Gene expressioin and single cell analysis *Prof. PhD Mads Thomassen, Clinical GENOME center, Odense University Hospital, Denmark*
11:30 – 13:30	**LUNCH AND NETWORKING** *(MAGASINET)*
13:30 – 14:30	**SESSION 5** *(STALDEN)*
	ANTI-RESORPTIVE ADJUVANT TREATMENT IN ORTHOPEDICS Moderators: *Birgitte Villadsen & Anne Sophie Sølling*
13:30 – 14:00	Local intraoperative zoledronate and postoperative denosumab decrease migration of cement-less total knee arthroplasty by suppression of bone resorption: A randomised, double-blinded radiostereometric study of 82 patients with 5-year follow-up *MD, PhD-student Karina Nørgaard Linde, Dept. of Orthopaedics, Aarhus University Hospital, Denmark*
14:00 – 14:30	Treatment of bone tumors – cases *MD, PhD-student Andrea René Jørgensen, Dept. of Orthopaedics, Aarhus University Hospital, Denmark*
14:30 – 16:00	**GROUP PICTURE & WALK AND TALK** *(IN FRONT OF THE MANOR HOUSE)*
16:00 – 17:00	**SESSION 6** *(STALDEN)*
	DIABETES AND BONE Moderators: *Jens-Erik Bech Jensen & Line Underbjerg*
16:00 – 16:30	Changes to bone structure in humans with type 2 diabetes *Prof. PhD Petar Milovanovic, Inst. of Anatomy, University of Belgrade, Serbia*
16:30 – 17:00	Incretin hormones and bone health in diabetes *Prof. PhD Morten Frost, University of Southern Denmark, Denmark*
17:00 – 17:30	**BREAK** *(STALDEN)*

17:30 – 18:45	**SESSION 7** *(STALDEN)*
	SHORT ORALS OF POSTERS Moderators: *Hanne Skou Jørgensen & Christina Møller Andreasen*
17:30 – 17:35	**Poster 2 ▪** Fracture Characteristics of Human Cortical Bone Influenced by the Duration of in vitro Glycation *Mei-Chun Lin, Praveer Sihota, Sofie Dragoun Kolibová, Imke A.K. Fiedler, Johannes Krug, Eva M. Wölfel, Benjamin Ondruschka, Mustafa Citak, Felix Klebig, Felix. N. Schmidt, Katharina Jähn-Rickert, Mahan Qwamizadeh, Björn Busse*
17:35 – 17:40	**Poster 4 ▪** An activin type II receptor ligand trap prevented loss of cortical bone strength and cancellous bone mass in a mouse model of severe disuse osteopenia *M.F. Poulsen, F. Fisher, J. Lachey, J. Seehra, C. Andersen, M. Eijken, J.S. Thomsen, A. Brüel, A. Lodberg*
17:40 – 17:45	**Poster 6 ▪** Prevalence of osteoporosis among type 1 diabetes patients *Jakob Starup-Linde, Julie Støy, Bente Langdahl, Torben Harsløf*
17:45 – 17:50	**Poster 8 ▪** Do osteolytic fractures in multiple myeloma heal? Preliminary findings from a study of 22 bone biopsies from vertebral fractures of multiple myeloma patients. *Mette Bøegh Levring, Marta Diaz-delCastillo, Line Adsbøll Wickstroem, Ida Bruun Kristensen, Charlotte Guldborg Nyvold, Moustapha Kassem, Mikkel Østerheden Andersen, Thomas Levin Andersen, Niels Abildgaard*
17:50 – 17:55	**Poster 10 ▪** Intratrabecular tunneling is an overlooked mode of remodeling, which is overactivated by intermittent parathyroid hormone treatment in both humans and rabbits *Lisbeth K Thomsen, Kaja S Laursen, Ali R Rizvi, Kim Harrison, Lindsay Loundagin, Xuan Wei, Tanja Sikjaer, Jesper S Thomsen, Christina M Andreasen, Lars Rejnmark, David Cooper, Thomas L Andersen*
17:55 – 18:00	**Poster 12 ▪** Characterizing the myeloma bone disease in Vk*MYC mouse model of multiple myeloma *Clarissa Schmal, Marija Simic, Ya Xiao, Alicia Armero Catalá, Peter Croucher, Michelle McDonald, Abbas Jafari*
18:00 – 18:05	**Poster 14 ▪** Acute effects of GLP-1 and GIP on human bone turnover *Nina Wærling Hansen, Morten Svarer Hansen, Bolette Hartmann, Morten Frost*
18:05 – 18:10	**Poster 16 ▪** Accumulation of Advanced Glycation End products and Microarchitecture of Bone Tissue in Subjects with or without Type 2 Diabetes Mellitus *H. Bardenfleth, J. Starup-Linde, K. Hygum, T. Harsløf, A. Sadetskaia, H. Birkedal, B. Langdahl*
18:10 – 18:15	**Poster 18 ▪** Mapping RANKL- and OPG-expressing cells in bone tissue: The bone surface cells as activators of bone resorption and promotors of the denosumab rebound effect *Bilal M. El-Masri, Christina M. Andreasen, Kaja S. Laursen, Viktoria B. Kofod, Xenia G. Borggaard, Malene H. Nielsen, Jesper S. Thomsen, Annemarie Brüel, Mads S. Sørensen, Lars J. Hansen, Albert S. Kim, Michelle M. McDonald, Julia F. Charles, Jean-Marie Delaisse, Thomas L. Andersen*
18:15 – 18:20	**Poster 20 ▪** Hypercalciuria in Patients with Post-Surgical Hypoparathyroidism *Sarah Thornhøj, Line Underbjerg, Lene Ring Madsen*
18:20 – 18:25	**Poster 22 ▪** ISWI-mediated chromatin remodeling directing stromal cell differentiation *Kaja Madsen, Alexander Rauch*
18:25 – 18:30	**Poster 24 ▪** The ear: a physiological model of Rankl-Opg mediate osteoclast regulation *Viktoria B. Kofod, Bilal M. El-Masri, Faranak Aboutalebi, Lars J. Hansen, Malene H. Nielsen, Jesper S. Thomsen, Christina M. Andreasen, Thomas L. Andersen*
18:30 – 18:35	**Poster 26 ▪** Investigation of the temporal changes during anti-sclerostin antibody treatment in mice using time-lapse µCT Frederik Duch Bromer, Annemarie Brüel, Jesper Skovhus Thomsen
18:35 – 18:40	**Poster 28 ▪** Balanced basic multicellular unit activity in cortical bone of ovariohysterectomized rabbits *Lindsay L. Loundagin, Kim D. Harrison, David M.L. Cooper*
18:40 – 18:45	**Poster 30 ▪** Preparation of cylindrical bone samples for high-resolution X-ray computed tomography *N.K. Wittig, C. Pedersen, J. Palle, M. Østergaard, T.E.K. Christensen, M. Kahnt, A. Sadetskaia, J.S. Thomsen, A. Brüel, H. Birkedal*

18:45 – 18:55	**DANISH DIABETES ACADEMY** *(STALDEN)*
18:45 – 18:55	*Tore Sønne B Christiansen*
19:00 – 21:00	**GALA DINNER** *(MAGASINET)*
21:00 – 22:00	**POSTERS** *(STALDEN)*
	POSTER VIEWING, *EVEN* POSTER NUMBERS ONLY

Sunday, March 10

8:00 – 9:00	**BREAKFAST AND NETWORKING** *(MAGASINET)*
9:15 – 10:15	**SESSION 8** *(STALDEN)*
	CLINICAL AND TRANSLATIONAL BONE METABOLISM Moderators: *Jens Bollerslev & Chelsea Hopkins*
9:15 – 9:35	SIPH- A Scandinavian Fairytale MD *PhD Mikkel Pretorius, Oslo University & Oslo University Hospital, Norway*
9:35 – 9:55	Case-series of adults with X-linked hypophosphatemia suffering from end stage renal disease (ESRD) Assoc. Prof. *PhD Trine E. Finnes, Oslo University Hospital and University of Oslo, Norway*
9:55 – 10:15	Akromegali: Trabecular Bone Score and Hip Structural Analyses in patients during treatment of Acromegaly *MD PhD Ansgar Heck, Oslo University Hospital, Norway*
10:15 – 10:45	**BREAK** *(STALDEN)*
10:45 – 11:45	**SESSION 9** *(STALDEN)*
	CORTICAL BONE REMODELING Moderators: *Nina Wærling Hansen & Catherine Williams*
10:45 – 11:15	Time-lapsed 4D Tracking of Cortical Bone Remodeling in the Rabbit: What Have We Learned so Far? *Prof. PhD David Cooper, University of Saskatchewan, Canada*
11:15 – 11:45	Decoding Cortical Bone Remodeling: The Importance of Osteoprogenitors and Vascular Networks *Assoc. Prof. PhD Christina M. Andreasen, University of Southern Denmark*
11:45 – 12:00	**CONCLUDING REMARKS** *(STALDEN)*
	Associate Prof. PhD Thomas L. Andersen (President) *Aarhus University, University of Southern Denmark & Odense University Hospital* ***THE AWARD FOR EARLY CAREER INVESTIGATORS***
12:00 – 13:00	**LUNCH AND FAREWELL** *(MAGASINET)*

Osteoporosis treatment: Treat to Target and Sequential Therapy

Bente Langdahl

Dept. of Endocrinology, Aarhus University Hospital and Dept. of Clinical Medicine, Aarhus University

The overarching goal of osteoporosis management is to prevent fractures. A goal-directed approach to long-term management of fracture risk helps ensure that the most appropriate initial treatment and treatment sequence is selected for individual patients. Goal-directed treatment decisions require assessment of clinical fracture history, vertebral fracture identification (using vertebral imaging as appropriate), measurement of bone mineral density (BMD) and consideration of other major clinical risk factors. Treatment targets should be tailored to each patient's individual risk profile and based on the specific indication for beginning treatment, including recency of fracture, number and severity of prior fractures, and BMD levels at the total hip, femoral neck, and lumbar spine. Instead of first line bisphosphonate treatment for all patients, selection of initial treatment should focus on reducing fracture risk rapidly for patients at very high near-term risk, such as in those with recent fractures. Initial treatment selection should also consider the probability that a BMD treatment target can be attained within a reasonable period of time and the differential impact on BMD and magnitude of fracture risk reduction with osteoanabolic and antiresorptive therapy.

Data from trials with antiresorptive and osteoanabolic agents show an inverse relationship between the BMD level achieved in patients on treatment and the subsequent risk of fracture. New evidence shows that total hip BMD appears to be the most useful treatment target because it consistently predicts the risk of both vertebral and nonvertebral fractures. In addition, new evidence provides guidance for identifying a BMD target. Other studies have confirmed the benefit of treating with an osteoanabolic before an antiresorptive drug vs. the reverse order to maximize BMD gain, particularly in the hip, and the probability of achieving different BMD treatment targets beginning with osteoanabolic vs antiresorptive agents at varying starting BMD levels.

Most guidelines suggest prescribing an oral bisphosphonate as initial treatment for all patients with osteoporosis. In contrast, goal-directed treatment individualizes treatment decisions based on an individual's fracture risk, current BMD and individualized treatment target. Based on these considerations, some patients will benefit most from osteoanabolic therapy as initial treatment.

Goal-directed therapy is a strategy for the long-term management of patients receiving treatment for osteoporosis. Achieving treatment targets might require intensification of therapy if a fracture occurs or the patient remains far from a BMD target despite osteoporosis treatment. This intensification could include replacing a bisphosphonate with denosumab, replacing a bisphosphonate or denosumab with an osteoanabolic agent or adding an osteoanabolic agents to ongoing treatment with a bisphosphonate or denosumab. It might also include a repeat course of osteoanabolic medication. It must be acknowledged, however, that the BMD effects of switching from antiresorptive to osteoanabolic agents are not as robust as those seen when initiating treatment with an osteoanabolic agent. Furthermore, the evidence supporting the safety and efficacy of repeat courses of osteoanabolic agents is extremely limited.

Mosekilde Award winners	
2021	Jean-Marie Delaisse
2023	Bente Lomholdt Langdahl, AUH

Young Investigator Award winners	
2018	Christina Møller Andreasen, SDU
2019	Marta Díaz del Castillo, KU
2020	Mikkel Bo Brent, AU
2021	Xenia Goldberg Borggaard, SDU
2022	Frederik Duch Bromer, AU
2023	Oliver Lassen Slavensky, AU

TALLYHO/JngJ as a model for type 2 diabetes-induced bone disease

Lejla Emini,[1] Juliane Salbach-Hirsch,[1] Johannes Krug,[2] Katharina Jähn-Rickert,[2,3] Björn Busse,[2,3] Martina Rauner,[1] Lorenz C. Hofbauer[1]

[1]Department of Medicine III and Center for Healthy Aging, Technische Universität Dresden Medical Center, Dresden, Germany, [2]Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [3]Mildred Scheel Cancer Career Center Hamburg, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Background & aim: T2DM increases fracture risk due to bone micro-structural and material deficits, though the mechanisms remain unclear. Preclinical models mimicking diabetic bone disease are required to further understand its pathogenesis. The TALLYHO/JngJ (TH) mouse is a polygenic model recapitulating T2DM in humans. Due to incomplete penetrance of the phenotype, ~25 % of male TH mice never develop hyperglycemia, providing a strain-matched nondiabetic control. A comprehensive characterization of the metabolic and skeletal phenotype of diabetic TH mice (high-TH) and compared them to either their nondiabetic TH controls (low-TH) or the recommended SWR/J controls to evaluate their suitability to study diabetic bone disease in humans.

Methods: A metabolic characterization was done for 12-week-old TALLYHO/JngJ and SWR/J mice. Further, µCT and histomorphometry were applied to determine the skeletal phenotype. ELISA's were performed to determine bone turnover markers. Finally, silver nitrate staining and high-resolution-µCT were utilized to assess the osteocyte lacunar network.

Results: Blood glucose level was higher in high-TH compared to both controls, respectively (+69%, $p<0.001$ vs. SWR/J and +58% vs. low-TH, $p<0.001$). High-TH had a higher body weight compared to SWR/J (+40%, $p<0.001$) and low-TH (+10%, $p=0.004$). ipGTT and ipITT revealed impaired glucose tolerance and insulin resistance in high-TH compared to controls (+85%, $p<0.001$ vs. SWR/J and +46%, $p<0.001$ vs. low-TH). The skeletal phenotype in the femora exhibited increased cortical BV/TV (+2.3%, $p=0.005$), BMD (+7.12%, $p<0.001$), and thickness (+9.49%, $p<0.001$) compared with SWR/J. In contrast, at the trabecular site of the femur, high-TH exhibited low BV/TV (-70%, $p<0.001$), low Tb.N (-37%, $p<0.001$) while Tb.Sp was higher (+36%, $p<0.001$) than SWR/J. This was also evident in the fourth lumbar vertebrae. Histomorphometry of the vertebrae revealed no changes in Ob.N/B.Pm despite increased P1NP in SWR/J compared with both TH groups (+57%, $p<0.001$ vs. low-TH and +76%, $p<0.001$ vs. high-TH). Oc.N/B.Pm was increased in SWR/J compared with high-TH (+69% $p=0.001$) along with TRAcP5B (+58%, $p<0.001$ vs. low-TH and +40%, $p=0.002$ vs. high-TH). BFR/BS and MAR remained unchanged in all three groups except MS/BS was higher in SWR/J compared to TH groups (+31.8%, $p=0.007$ vs. low-TH and +25.9%, $p=0.031$ vs. high-TH). Silver nitrate staining showed augmented osteocyte number and dendrites in SWR/J (+57%, $p=0.008$) compared with high-TH. 3D assessment of the femur exhibited higher lacunar volume and density in SWR/J compared with both TH subgroups (-19% $p<0.0001$ vs. low-TH, and -24% $p=<0.0001$ vs. high-TH). Osteocyte morphology showed increased sphericity in SWR/J compared with TH subgroups (-5.5% $p<0.0001$ vs. low-TH and -3.7% $p<0.0001$ vs. high-TH).

Conclusion: In summary, our study highlights the utility of the TH mouse to study T2DM, but not necessarily T2DM-induced bone disease.

Fibrous Dysplasia Bone Pain – Peripheral and Central Pathology

Chelsea Hopkins,[1] Julie Benthin,[1] Ruth Elena Martínez Mendoza,[2] Juan Antonio Vazquez Mora,[2] Luis Fernandez De Castro Diaz,[3] Marta Diaz del Castillo,[1,4] Alison Boyce,[5] Michael T. Collins,[3] Juan Miguel Jimenez Andrade,[2] Anne-Marie Heegaard[1]

[1]University of Copenhagen, Denmark, [2]Reynosa Aztláan Universidad Autónoma de Tamaulipas Reynosa, Mexico, [3]National Institute of Dental and Craniofacial Research, NIH, USA, [4]University of Aarhus, Denmark, [5]Metabolic Bone Disorders Unit, NIH, USA

Background: Fibrous dysplasia (FD) is a rare mosaic bone disease arising from embryogenic gain-of-function mutations in GNAS and characterized by areas of abnormal fibro-osseous tissue that results in bone deformities, fractures, and pain, which can be debilitating. Clinical studies suggest the presence of both nociceptive- and neuropathic-like components of the pain phenotype in FD patients (Spencer, 2022; Golden, 2023). Despite this, there is limited knowledge of the underlying mechanisms and pain treatment is ineffective.

Aim: Identify peripheral and central nociceptive mechanisms in a translationally relevant mouse model of FD.

Methods: A mosaic-like, doxycycline-inducible transgenic mouse model (Zhao, 2018) was used in this study. A Cre-LoxP system under the Prrx1 promoter (bone marrow stromal cells) allows the expression of the causative GαsR201C mutation of FD through a tetracycline-inducible system. Male and female mice (FD and control) were given doxycycline for two and three weeks, respectively. Longitudinal x-ray imaging and end-stage histological staining of the bone confirmed FD development. Nociceptive behavior was assessed by grid hanging, burrowing, wheel running, and cage activity. Morphine (10mg/kg) and ibuprofen (30mg/kg) were given to female mice before grid hanging and burrowing, respectively. Using immunohistochemistry, bones were analyzed for changes in innervation and vascularization, dorsal root ganglions (DRGs) for evidence of nerve damage and the spinal cord (SC) for changes in neuropeptide expression and glial cells.

Results: X-ray imaging confirmed FD model development and FD mice displayed deficits in grid hanging, burrowing, wheel running, and cage activity. Mice treated with ibuprofen maintained their burrowing, significantly better than vehicle-treated controls. FD morphine-treated mice performed significantly better in grid hanging than vehicle-treated mice on day 11, but not day 18. FD mice ran significantly less than the control mice and were significantly less active in their cages. FD lesions in the tibio-femoral region were highly innervated (sensory and sympathetic nerve fibers) and vascularized in female and male mice. In DRGs from male mice (but not female), increased activating transcription factor-3 (ATF3) and tyrosine hydroxylase (TH) staining was observed. There was no difference in SC glial cell presence and neuropeptide expression between FD and control mice.

Conclusion: This study is the first to characterize a nociceptive phenotype in a model of FD. Analgesics improved nociceptive behaviors and within FD lesions, sensory and sympathetic nerve fibers and blood vessels were present, which may contribute to nociception. ATF3 and TH expression in DRGs has been associated with nerve damage – a feature of peripheral neuropathy. No significant changes were observed in the SC, but further studies are necessary. This study demonstrated that nociceptive mechanisms occur in this FD model, which may contribute to FD pain. This model has the potential to be used to determine other mechanisms and test novel therapeutics for FD pain.

Newly formed bone and how to find it: Mineral growth enclosed by high-zinc-content borders

Maja Østergaard,[1] Kristine H. Neldeborg,[1] Thorbjørn E.K. Christensen,[1] Tanja T. Sikjær,[2,3] Lars Rejnmark,[2] Henrik Birkedal[1]

[1]Dept. of Chemistry and iNANO, Aarhus University (AU), [2]Department of Endocrinology and Internal Medicine, Aarhus University Hospital (AUH), [3]Institute of Clinical Medicine, Aarhus University (AU)

Background: Bone is formed and regulated under tight biological control. Disorders in these control mechanisms can have serious consequences for bone health. Patients suffering from hypoparathyroidism (hypoPT) have reduced parathyroid gland capacity and hence impaired ability to make parathyroid hormone (PTH), which is essential for maintaining bone. Consequently, this causes a reduction in bone turnover and as a result, the patients are unable to regulate a part of the mineralization process [1-5]. This in turn provides an opportunity for investigating this process when studying materials from patients given PTH-replacement therapy.

Aim: This scenario of having the mineralization process turned "off" and "on" again provides the perfect circumstances to gain insight into the normal-case mineralization mechanism and its impact on the mineral properties and elemental distribution on the micro-and nanoscale in human bone.

Methods: Iliac crest biopsies from hypoPT patients treated with PTH(1-84) for 6 months (*N*=4) followed by 24 months of conventional treatment (*N*=1) were cut and polished to dimensions of app. 1 mm×500 µm×25 µm and measured with 2D scanning X-ray fluorescence (XRF) and X-ray diffraction (XRD) at the P06 beamline at PETRA-III, DESY. The samples were mapped with isotropic steps of 10 µm and regions-of-interest (ROI) with isotropic steps of 0.5 µm. From this, elemental distributions and mineral property maps were qualitatively assessed (for now).

Results: Newly formed bone areas are identified in both the absorption signal and amount of crystalline material (scale factor), figure 1. These areas of bone in different maturation stages can be correlated to the different mineral properties, in figure 1 exemplified by the width of the mineral nanocrystals (ACS ⊥ c) that is a measure of mineral maturation and/or growth. Notably, the areas of newly formed bone are bordered by mineralization fronts, which are high in zinc, like also observed in the growth plate [6] or the cement lines [7].

Conclusion: Mineral properties and elemental distributions change between areas of new and more mature bone. Such results can shed a light on the detailed mineralization process.

[1] Rejnmark, L. et al., Osteoporos Int, **24**, 1529-1536, doi:10.1007/s00198-012-2230-4 (2013).
[2] Rejnmark, L. et al., Endocrinol Metab **30**, 436-442, doi:10.3803/EnM.2015.30.4.436 (2015).
[3] Sikjaer, T. et al., JBMR, **26**, 2358-2370, doi:10.1002/jbmr.470 (2011).
[4] Sikjaer, T. et al., JBMR, **28**, 2232-2243, doi:10.1002/jbmr.1964 (2013).
[5] Christiansen, P. van D. & Sikjær, T. et al., JBMR Plus, **7**: e10829, doi: 10.1002/jbm4.10829 (2023).
[6] Barreto, I.S. et al., Adv. Sci., **7**, 2002524, doi: 10.1002/advs.202002524 (2020).
[7] Pemmer, B. et al., Bone, **57**, 184-193, doi:10.1016/j.bone.2013.07.038 (2013).

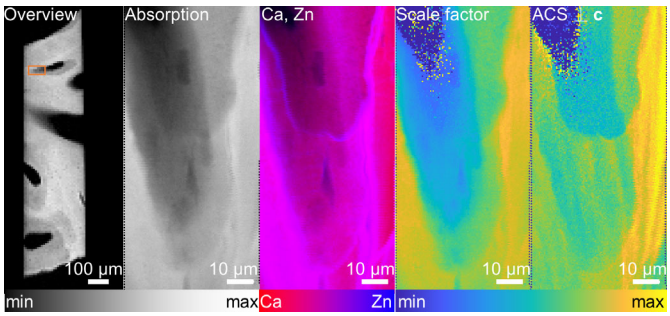


Figure 1. Mineral formation in PTH(1-84) treated bone. Overview of X-ray absorption and high resolution maps of absorption (grey), Ca (red) and Zn (blue) content, scale factor, and crystal width.

Zoledronic acid prevents spine and hip bone loss after bariatric surgery – A randomized, placebo controlled study

Søren Gam,[1,5,6] Simon Lysdahlsgaard,[4,6] Bibi Gram,[2,6] Anne Pernille Hermann,[3] Martin Weber Kusk,[4,6] Claus Bogh Juhl,[1,5,6] Stinus Gadegaard Hansen[1,6]

[1]Dept. of Endocrinology, Esbjerg Hospital, University Hospital of Southern Denmark, Denmark, [2]Research Unit of Endocrinology: Bariatrics and Diabetes, University Hospital of Southern Denmark, Esbjerg, Denmark, [3]Dept. of Endocrinology, Odense University Hospital, Odense, Denmark, [4]Imaging Research Initiative Southwest (IRIS), Hospital of South West Jutland, University Hospital of Southern Denmark, Esbjerg, Denmark, [5]Steno Diabetes Center, Odense, Denmark, [6]Dept. of Regional Health Research, University of Southern Denmark, Odense, Denmark.

Background: Bariatric surgery leads to significant bone loss and increases the risk of fracture. Small pilot studies indicate that bisphosphonates (e.g. zoledronic acid (ZOL)) may prevent or limit bone loss in patients undergoing bariatric surgery, but this needs to be clarified in sufficiently powered studies.

Aim: The aim of this study was to investigate the effects of ZOL for the prevention of bone loss after bariatric surgery.

Methods: In this randomized, double-blinded, placebo-controlled study, we enrolled fifty-nine patients eligible for bariatric surgery (age: 49.6 ± 6.6, weight: 123.7 ± 18.0 kg, BMI: 42.3 ± 5.3, female/male: 42/17 (mean ± SD)). Patients were randomized to a single intravenous dose of either ZOL 5 mg or placebo (PLA) before surgery and were assessed at baseline and 12-month postsurgery. Bone turnover markers (P1NP & CTX-1) were determined, and further, we assessed volumetric bone mineral density (vBMD) and areal BMD (aBMD) in the spine, total hip, and femoral neck by Quantitative Computer Tomography (QCT) and dual-energy X-ray absorptiometry (DXA). We determined safety by the numbers of adverse events (AE), serious AE (SAE), adverse reactions (AR), and serious AR (SAR). We used a mixed-effect model with repeated measures adjusted for age, gender, and surgery to determine the treatment effect of ZOL.

Results: There were no differences in baseline characteristics and bone outcomes at baseline ($p > 0.05$). The mean weight loss was similar between the groups (ZOL = 32 ± 8 vs PLA = 31 ± 12 kg, $p = 0.90$). For the spine, hip, and femoral neck BMD, we observed a significant difference between groups in favor of ZOL ($p < 0.01$). For the spine and femoral neck, ZOL had no changes whereas PLA had declines in the spine (vBMD: -4.1%, aBMD -3.1%, $p < 0.001$) and femoral neck (vBMD: -4.1%, aBMD: -6.3%, $p < 0.001$). For the total hip, the losses of BMD were blunted in ZOL compared to PLA (vBMD: -1.6% vs -4.9%, aBMD: -4.0% and -8.0%, $p < 0.01$). P1NP did not change for ZOL, while in PLA it increased by 76.6% compared to baseline ($p < 0.001$). For CTX, ZOL had a blunted increase compared to PLA (101.0% vs 172.0%, $p < 0.001$). The number of AE and SAE were similar between groups. ($P > 0.05$). However, significantly more AR were reported in ZOL compared to PLA ($P = 0.02$), with no SAR reported.

Conclusion: Zoledronic acid prevented or blunted bone loss in the spine, and hip after bariatric surgery compared to placebo. In a clinical setting, ZOL may be used to prevent bone loss in selected patients with an increased risk of fracture.

Characterization of Key Cells in the Bone Microenvironment of Metastatic Breast Cancer Patients

Melisa K. Uguz,[1,*] Emma K.E. Bengtsson,[1] Malene H. Nielsen,[1] Bilal El-Masri,[1] Thomas L. Andersen,[1,2,3] Anne Marie B. Jylling,[3] Christina M. Andreasen[1,3]

[1]Molecular Bone Histology lab, Research Unit of Pathology, University of Southern Denmark, Denmark, [2]Dept. of Forensic Medicine, Aarhus University, Denmark, [3]Dept. of Pathology, Odense University Hospital, Denmark

Background: Bone is one of the major targets for breast cancer metastases and the interaction between cancer cells and stromal cells is crucial for establishment of the tumor supportive microenvironment.

Aim: To quantify the density of key cells and blood vessels near the bone surface in biopsies from metastatic breast cancer (MBC) patients in relation to remodeling activity.

Methods: Bone marrow biopsies from 10 MBC-treated (MBC-T: radiation + chemotherapy, zoledronic acid, age 51–92 years) and 10 MBC-untreated (MBC-U: newly diagnosed or cancer free >2 years, age 38–87 years) were included. Bone remodeling activity was assessed on Masson Trichrome stained sections, while neighboring sections were multiplex immunofluorescent stained for CK7/19, TRAcP, perilipin, CD34, and DAPI to identify cancer cells, osteoclasts, adipocytes, blood vessels, and nuclei, respectively. Densities were quantified in a zone of 0–100 µm from the bone surface using HALO software.

Results: Untreated MBC patients had higher bone volume ($p < 0.025$), but fewer cancer cells ($p < 0.047$) than treated MBC patients. Both groups had more eroded than quiescent bone surfaces (MBC-T: $p < 0.02$; MBC-U: $p < 0.002$), with no evidence of bone formation. Treated MBC patients had more cancer cells above eroded surfaces than untreated MBC patients ($p < 0.005$), mainly in the 25–100 µm marrow zone. Blood vessel density was higher in untreated MBC patients near the bone surface (0–25 µm), but similar in the 25–100 µm zone irrespectively of remodeling stage. Untreated MBC-patients tended to have more adipocytes, however not significantly different from treated patients. Both groups had osteoclasts at the bone surface (0–25 µm), with no significant difference. Notably, some patients had a high osteoclast density in the marrow distant from bone surfaces.

Conclusion: Despite anti-resorptive therapy, treated MBC patients had more eroded surfaces and cancer cells than untreated MBC patients. Furthermore, ongoing bone formation was absent in all patients. Interestingly, a subset of the treated MBC patients displayed a significant number of osteoclasts, even distant from bone surfaces.

The direct healthcare costs of fracture treatment in T1D

Annika Vestergaard Kvist,[1,2,3,4] Morten Frost,[1,2,5] Andrea M. Burden,[3] Peter Vestergaard,[5,6,7] Troels Kristensen[8,9]

[1]Department of Endocrinology and Metabolism, Molecular Endocrinology Stem Cell Research Unit (KMEB), Odense University Hospital (OUH), Odense, Denmark,[2]Department of Clinical Research, University of Southern Denmark (SDU), Denmark,[3]Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH-Zurich, Zurich, Switzerland,[4]Steno Diabetes Center North Denmark, Aalborg, Denmark,[5]Steno Diabetes Center Odense, OUH, Denmark,[6]Department of Endocrinology, Aalborg University Hospital (AAUH), Aalborg, Denmark,[7]Department of Clinical Medicine, AAUH, Denmark,[8]Research Unit of General Practice, Institute of Public Health, SDU, Denmark,[9]DaCHE, Faculty of Health Sciences, Department of Public Health, SDU, Denmark

Background: Patients with type 1 diabetes (T1D) have an increased risk of sustaining a fracture, and risks of nonunion, poor wound healing, infections, and complications are higher in T1D after a fracture compared to persons without diabetes. Nevertheless, it is largely unknown how bone health and fracture-related complications affects the cost of fractures in T1D.

Aim: This study aims to estimate the direct healthcare cost of fractures in T1D compared to controls. We hypothesized that cost of fractures in patients with T1D are higher than in the non-diabetic background population. As a secondary aim, potential cost drivers of fractures were analyzed.

Methods: This study included patients aged 18 years or above in Denmark with a hip, humerus, forearm, foot, or ankle fracture from 2011 to 2015. The treatment group of patients with T1D were identified using ICD-10 codes and prescription medication. Next, this group was matched with non-diabetic individuals based on fracture site, sex, age, and CCI using coarsened exact matching. The direct healthcare costs were calculated within one year after the fracture. Random forest models were applied to assess drivers of fracture treatment costs.

Results: In this study 302, 203, 346, 290, and 149 cases of hip, humerus, forearm, foot, and ankle fractures were matched with controls. The direct healthcare cost within one year after fracture was significantly higher among patients with T1D compared to controls for humerus (€1,689 vs. €810), forearm (€842 vs. €575), foot (€402 vs. €320), and ankle fractures (€1,395 vs. €806). By contrast, the direct healthcare cost of hip fractures was similar in patients with T1D and controls (€10,483 vs. €10,795). Regardless of fracture type, the primary cost drivers for fractures, among all patients (both cases and controls) were longer duration of hospitalization, followed by surgical interventions.

Conclusion: The direct healthcare cost of humerus, forearm, foot, and ankle fracture treatment was higher among individuals with T1D, although no difference was identified for hip fracture treatment between T1D and controls. The primary cost driver of fracture treatment was longer durations of hospitalization followed by surgical interventions. Understanding the differences in healthcare costs of fracture treatment among T1D and controls are necessary to address optimization of care and prevention of complications, which could lead to lower treatment costs.

Perilacunar bone – same but different

Anne Marie Møller Faaborg,[1] Jonas Palle,[1] Thorbjørn Erik Køppen Christensen,[1] Nina Kølln Wittig,[1] Annemarie Brüel,[2] Thomas Levin Andersen,[3,4] Tilman A. Grüneawald,[5] Henrik Birkedal[1,6]

[1]Interdisciplinary Nanoscience Center, Aarhus University (AU), Aarhus, Denmark, [2]Department of Biomedicine, AU, [3]Department of Clinical Research, University of Southern Denmark (SDU), Odense, Denmark, [4]Department of Forensic Medicine, AU, [5]Institute Fresnel, Aix Marseille University, Marseille, France, [6]Department of Chemistry, AU

Background: The importance of the osteocyte in bone biology is well established both in the local bone matrix and on an organism level and includes vital functions such as signaling in bone mineralization and phosphate homeostasis, strain detection, and calcium mobilization [1]. This means that the perilacunar region is an essential interface for the osteocyte to exert its regulatory functions [1-3]. There are suggestions that the perilacunar region differs from the surrounding bone matrix, e.g. by the presence of demineralized perilacunar halos in the Hyp mouse, which is a model of XLH [4]. A better understanding of the structure of the perilacunar matrix may therefore improve the understanding of this key interface.

Aim: To investigate the distribution of oligo-elements and hydroxyapatite mineral properties in the perilacunar region as a function of distance from the osteocyte lacunae.

Methods: Healthy bone samples from 5 individuals (ages 12-76) were investigated using 2D scanning nanobeam X-ray diffraction and fluorescence at three different synchrotron facilities. Beam sizes were 75-400 nm in diameter. X-ray diffraction gives information on hydroxyapatite nanocrystals in bone while X-ray fluorescence probes the distribution of heavy elements. The obtained diffraction data were azimuthally integrated, and Rietveld refined to find the hydroxyapatite crystallite properties while the fluorescence spectra have been fitted to obtain maps of the relative calcium and zinc content. The obtained properties are evaluated as a function of distance the osteocyte lacunae.

Results: Zinc is found to be upconcentrated towards the osteocyte lacunae surface. The hydroxyapatite crystallite parameters obtained by orientational averaging are independent of distance from the lacunar surface. This indicates that zinc is unlikely to be included into the hydroxyapatite crystals. Further, the data suggest that orientational averaging may obscure possible local orientation-dependent variations, which indicates that a more advanced orientation dependent analysis may be needed.

Conclusion: Investigation of the perilacunar bone mineral matrix reveal a distance dependance in the zinc content from the osteocyte lacunae surface. The results are promising for a further analysis of the hydroxyapatite crystallite properties in the perilacunar region.

1. Robling, A.G. and L.F. Bonewald, *The Osteocyte: New Insights.* Annual Review of Physiology, 2020. **82**(1): p. 485-506.
2. Wittig, N.K., et al., *Bone Biomineral Properties Vary across Human Osteonal Bone.* ACS Nano, 2019. **13**(11): p. 12949-12956.
3. Reznikov, N., et al., *Biological stenciling of mineralization in the skeleton: Local enzymatic removal of inhibitors in the extracellular matrix.* Bone, 2020. **138**: p. 115447.
4. Hoac, B., et al., *Genetic Ablation of Osteopontin in Osteomalacic Hyp Mice Partially Rescues the Deficient Mineralization Without Correcting Hypophosphatemia.* Journal of Bone and Mineral Research, 2020. **35**(10): p. 2032-2048.

Real-Time Live Imaging of Osteoclast Activation via Cathepsin K Activity in Bone Diseases

Eun Jung Lee[1], Seyoung Koo[2], Da Hyeon Yun[1], Jong Seung Kim[2], Serk In Park[1]

[1]Department of Biochemistry and Molecular Biology, Korea University College of Medicine, [2]Department of Chemistry, Korea University, Seoul, Korea

Background: Osteoclasts are crucial in bone resorption, and their abnormal activities are linked to various bone diseases. Understanding their behavior in vivo is key to developing effective treatments. Cathepsin K (CatK), a lysosomal cysteine protease essential for osteoclasts' bone-resorbing function, is a critical indicator for monitoring their activation.

Aim: To develop a tool for real-time intravital imaging of osteoclast activity, utilizing Cathepsin K enzymatic activity.

Methods: A peptide substrate specific to CatK was conjugated with a TAMRA fluorophore and a BHQ-2 quencher, developing a fluorescence resonance energy transfer (FRET)-based fluorogenic probe (**CatKP1**) that enables fluorescence signaling upon CatK's enzymatic cleavage. Osteoclasts, cultured in vitro from murine bone marrow monocytes, were observed with confocal microscopy to determine CatK's subcellular location. For intravital imaging, we employed three mouse models: osteoporosis induced by ovariectomy, bone loss from RANKL injection, and bone metastasis via intra-tibial breast tumor cells injection. The CatK probe was administered by tail-vein injection (50 μmol), and facilitated live real-time images in the proximal tibia with a small skin incision under anesthesia.

Results: CatKP1 exhibited higher sensitivity than previously reported probes (CatKP2 and CatKP3), and showed distinct specificity by reducing the fluorescent signals in the presence of odanacatib (ODN, a CatK specific inhibitor). These fluorescent signals did not show in other cells, including breast tumor cells, and were only observed in the lysosomes within osteoclasts. Additionally, strong fluorescent signals were evident at the ruffled border, indicating precise subcellular localization in bone-resorptive lesions via confocal imaging. Two-photon intravital microscopy revealed a notable increase in fluorescence signals from the probe in all disease models compared to the control group, providing longitudinal real-time images of osteoclasts on the endosteal surface or trabecular bone in live mice. The fluorescence signals were specific to bone, not detectable in other tissues, and significantly diminished in mice treated with zoledronic acid or ODN, supporting that CatKP1 can be proposed as a valuable probe for monitoring osteoclast activation.

Conclusion: We developed CatKP1, a novel probe for the real-time visualization of CatK, enabling the monitoring of functional osteoclasts in live mice. CatKP1 is potentially valuable for bone biology research and the development of therapeutics targeting CatK or osteoclasts.

Innervation in multiple myeloma: a spatially resolved histological study of human bone

Martin S. J. Christensen,[1] Rebecca E. Andrews,[2, 3] Aritri Mandal,[3] Andrew D. Chantry,[2,3] Anne-Marie Heegaard,[4] Thomas L Andersen,[1,5,6,7] Marta Diaz-delCastillo[1,2,3,7]

[1]Department of Forensic Medicine, Aarhus University, Aarhus, Denmark. [2]Sheffield Myeloma Research Team, Department of Oncology and Metabolism, Mellanby Centre for Bone Research, University of Sheffield Medical School, Sheffield, UK. [3]Department of Haematology, Sheffield Teaching Hospitals NHS Foundation Trust, Royal Hallamshire Hospital, Sheffield, UK. [4]Department of Drug Design and Pharmacology, University of Copenhagen, Copenhagen, Denmark. [5]Department of Clinical Research, University of Southern Denmark, Odense, Denmark. [6]Department of Clinical Pathology, Odense University Hospital, Odense, Denmark. [7]The Danish Spatial Imaging Consortium (DanSIC)

Background: Multiple myeloma (MM) is a painful bone plasma cell cancer that is always preceded by the asymptomatic conditions monoclonal gammopathy of undetermined significance (MGUS) and smouldering multiple myeloma (SMM). Pain and fatigue are the main symptoms of patients with MM; however, its underlying mechanisms remain unknown and largely understudied.

Overall aim: To investigate the density and spatial relationship of plasma cells, blood vessels and nerve profiles in the bone marrow of MGUS/SMM, or newly diagnosed MM (NDMM).

Methods: Formalin-fixed, paraffin-embedded diagnostic iliac crest bone biopsies from 37 NDMM or MGUS/SMM patients were triple immunostained for CD138 (plasma cells), CD34 (blood vessels) and PGP9.5 (nerve fibres). AI-assisted histology (HALO, v3.5) was used to recognize and spatially evaluate the relation between nerves, MM cells and blood vessels.

Results: Histomorphometric analyses of biopsies from precursor and NDMM patients showed unchanged bone volume per total volume (BV/TV). As expected, the density of $CD138^+$ malignant plasma cells was increased in NDMM biopsies, but no differences in $CD34^+$ blood vessel nor $PGP9.5^+$ nerve profile density were observed between groups. Detailed spatial characterization revealed increased $CD34^+$ blood vessel density away from compared to proximate to the bone surface in both groups, as well as higher $PGP9.5^+$ nerve profile density within close proximity to $CD34^+$ blood vessels (<50 μm) in all patients, as expected. Interestingly, the density of $PGP9.5^+$ nerve profiles proximate to $CD138^+$ MM cells (<25 μm) was increased away from compared to proximate to the bone surface in both precursor and NDMM patients, suggesting a possible effect of CD138+ malignant plasma cells in neurogenesis. Importantly, the density of $CD138^+$ cells was significantly correlated to $PGP9.5^+$ nerve profile patients in precursor patients but not in NDMM, suggesting that nerve density does not continue to increase past a certain threshold of $CD138^+$ MM density.

Conclusion: This is the first clinical evaluation of bone innervation in MGUS/SMM and MM. Our results indicate that malignant MM cells do not induce systemic angiogenesis or nerve sprouting, but may release neurotrophic factors that support the close proximity between $CD138^+$ malignant plasma and nerves in the deep marrow. We observed a significant correlation between MM cell density cells and nerve profiles, which is lost upon certain $CD138^+$ cell threshold. This is in line with our published mouse data and suggests that accumulation of cancer cells creates a neurotoxic microenvironment, perhaps through resource depletion, that that impairs bone innervation.

Further perspective: Further studies are ongoing to evaluate nerve profile subtypes and their correlation to patient-reported pains scores.

Mystery of cement lines in bone structure: spatial distribution of Zn and changes in surrounding matrix mapped by synchrotron-radiation experiments.

Anastasiia Sadetskaia,[1] Jonas Palle,[1] Nina Kølln Wittig,[1] Carsten Pedersen,[1] Annemarie Brüel,[2] Maik Kahnt,[3] Manuel Guizar-Sicairos,[4] Henrik Birkedal[1]

[1]Dept. of Chemistry and iNANO, Aarhus University (AU), Aarhus, Denmark, [2]Dept. of Biomedicine, AU, Denmark, [3]NanoMAX, MAX IV, Lund, Sweden, [4]Paul Scherrer Institute (PSI), Villigen, Switzerland

Background: Cement lines are one of the most mysterious bone organisation units involved in the remodelling process. They are ~5 um thick densely mineralized borderlines emerging between the newly laid and old bone, e.g., as a part of osteon formation. Their nanostructure, relation to the surrounding matrix, role, and function in biomineralization and remodelling are still poorly understood. Recent studies suggest that cement lines are enriched in Zn [1] and that the associated mineral matrix differs from the rest of bone [2]. However, *why* Zn accumulates in the cement lines remains unclear. This question might be linked to the form in which Zn is stored – in mineral or as part of a protein – and a first step to answering this question lies in sub-micron spatial mapping of Zn around cement lines.

Aim: We aimed to map 3D spatial distribution of Zn in cement lines and surrounding matrix, including the osteocyte lacunar region, with the use of synchrotron-radiation X-Ray Fluorescence (XRF) with a sub-micron step size. In addition, the results were supported by X-Ray diffraction (XRD) measurements of the same region to jointly correlate the mineral properties with the Zn distribution.

Methods: Sections of human femoral bone (male, age=84) were cut by hand under a microscope, mounted on pins and milled into ~20 μm diameter cylinders using a custom-built lathe. Between cutting and milling stages, in-house 3D X-Ray microscopy was used to map and verify the placement of cement line and osteocyte lacunae within the 3D sample volume. The 3D XRF/XRD data were collected at NanoMAX beamline, MAX IV, Sweden, with X-Ray energy of 12 keV and step size down to 80 nm. They allowed to map Zn and Ca content, as well as mineral properties, in 3D. Custom-written MATLAB code was used for analysis.

Results: To the best of our knowledge, this is the first high resolution 3D mapping of Zn distribution near cement lines. The reconstructed XRF maps provide outstanding information on the Zn spatial distribution in the cement line regions as well as the osteocyte lacuna-canalicular network. Both are enriched with Zn. Interestingly, the newly formed mineral right at the border of cement line seems to be lacking the Zn content compared to the older matrix. Collected XRD patterns suggest the orientational effects of the mineral around cement line.

Conclusion: The excellent sub-micron resolution allowed for more precise mapping and open the possibility of correlating the elemental distribution data with other techniques. This gives a strong basis for understanding the role of accumulated levels of Zn in the cement lines and in turn the structure of cement lines.

[1] Pemmer B, et al. Bone. 2013; 57(1):184-93. doi: 10.1016/j.bone.2013.07.038

[2] Grünewald TA, et al., IUCrJ. 2023; 10(Pt 2):189-198. doi: 10.1107/S2052252523000866

Consequences of Persistent Hyperparathyroidism in Kidney Transplant Recipients

Ina Karstoft Ystrøm,[1] Hanne Skou Jørgensen[1,2,3], Christian Fynbo Christiansen[4], Ditte Hansen[5], Pieter Evenepoel,[6] Per Ivarsen,[1,3]

[1]Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark, [2]Department of Renal Medicine, Aalborg University Hospital, Aalborg, Denmark, [3]Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark, [4]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark, [5]Department of Nephrology, Herlev Hospital, University of Copenhagen, Herlev, Denmark, [6]Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Belgium.

Background: Kidney transplant recipients have a markedly increased risk of long-term complications, including fractures, compared to the background population. Ongoing disturbances of mineral metabolism is common post-transplant, including persistent hyperparathyroidism reported in 70% of patients more than 1 year after kidney transplantation. These disturbances may convey negative effects on long-term skeletal health. However, the effect of these disturbances remains uncertain and guidelines on treatment options as well as optimal treatment targets are lacking, leaving many patients untreated.

Aim: To investigate the consequences of disturbed mineral metabolism on skeletal health in kidney transplant recipients and bring insight into risk factors of bone loss in the post-transplant period.

Methods: We will utilize the comprehensive Danish health-registries to build a large, well-characterized cohort of kidney transplant recipients across 20 years, to investigate long-term skeletal outcomes. Second, we will collaborate with a kidney transplantation center in Leuven, Belgium, utilizing a dataset of kidney transplant recipients with detailed bone phenotyping, including dual-energy x-ray absorptiometry, peripheral quantitative computed tomography, transiliac bone biopsies, and mineral and bone turnover markers, to investigate risk factors of bone loss post-transplant.

Perspectives: With the increasing age and life expectancy of kidney transplant recipients, the prevention of long-term complications such as osteoporosis becomes ever more important. This project aims to improve skeletal health after kidney transplantation. Deliverables include: 1) Increased knowledge of long-term consequences of disturbed mineral metabolism – that should contribute to better-defined treatment targets and treatment decisions, and 2) Insight into risk factors of bone loss in the post-transplant period – that should contribute to improved risk stratification, with the ultimate goal of targeted interventions to prevent bone loss and minimize fracture risk.

Specialized Spatial Molecular Imaging Platforms for Bone Research

Ching-Hsin Huang,[1,2] Thomas L. Andersen[1,2]

[1]*Danish Spatial Imaging Consortium (DanSIC), University of Southern Denmark (SDU) (https://www.dansic.dk)*
[2]*Research Unit of Pathology, Department of Clinical Research, University of Southern Denmark, Odense, Denmark*

Background: Spatial analysis stands as a pivotal technological frontier propelling the scientific community's comprehension of biological inquiries. The Danish Spatial Imaging Consortium (DanSIC) was established to provide state-of-the-art 2D and 3D technologies, specializing in exploring spatial transcriptomics information within bone specimens.

Aim: This presentation aims to elucidate the potential of specialized spatial molecular imaging technologies at DanSIC in advancing bone research.

Methods: DanSIC offers four imaging platforms: (1) Olympus multi-slide scanners, (2) a GeoMx Digital Spatial Profiler (DSP) platform, (3) a Light Sheet microscope, (4) a Xenium single-cell RNA analyzer. All four platforms have been used in various tissues including bones.

Results: Our Olympus slide scanner supports various imaging techniques such as brightfield, dark-field, polarized light, and 7-plex fluorescence, with the capability to scan at multiple z plan within the sections (Fig1A). Only pixels in focus within the z-stack are selected, forming an EPI plan that facilitates the analysis of uneven sections which is a classical problem in bone histology. The GeoMx DSP is a robust spatial multi-omic platform. It non-destructively profiles RNA (up to 20,000 mRNA) and protein expression in distinct tissue compartments and cell populations (Fig1B-C). The UltraMicroscope Blaze Light Sheet microscope enables automated 3D imaging of optically cleared whole tissues, revealing any tissue structure and the spatial distribution of different cell types and their gene expressions in 3D coordinates (Fig1D). The Xenium In Situ Analyzer integrates high-resolution imaging and onboard data analysis, enabling the analysis of 400 mm² of fresh-frozen or FFPE tissues section and target 100 to 1000 of RNAs in individual cells across a tissue sample (Fig1E-F).

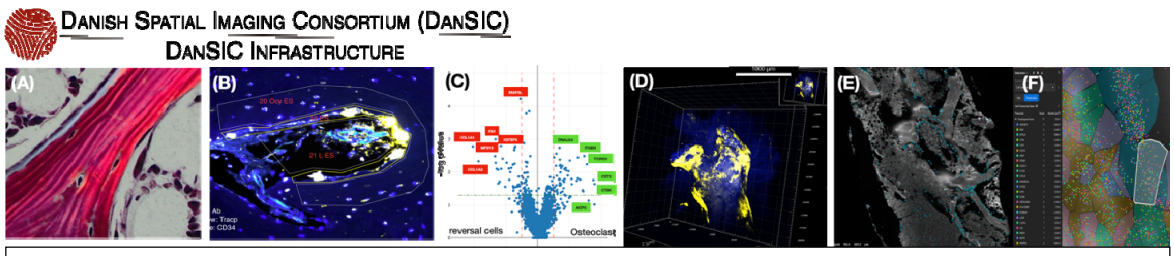


Fig 1. Spatial technologies in bone research at DanSIC. A: Bone histology & histomophometry; B-C: GeoMx DSP for RNA profiling in regions of interest; D: 3D image of mouse femur showing bone forming cells (yellow); E-F: Xenium on patient bone biopsy showing expressed genes in single osteoblast.

Conclusion: Cutting-edge spatial imaging technologies at DanSIC provide detailed insights into transcript expression and protein levels at a single to subcellular level, offering spatial information across 2D or 3D tissues. These advancements are poised to play an essential role in unraveling the complexities of bone-related diseases, bone remodeling process, aging, and beyond.

ALEXIS – Osteoporosis: Deciphering Mechanistic and Epigenetic Clues for Predicting Alendronate Treatment Discontinuation Outcomes

Simone SR Nielsen,[1,2,3] Jacob B Olesen,[1,2] Pernille Hermann,[4] Ryan Ross,[5] Thomas LG Andersen,[2,3] Kent Søe[1,2]

[1]Clinical Cell Biology, Dept. of Pathology, Odense University Hospital, Odense, Denmark; [2]Dept. of Clinical Research, University of Southern Denmark, Odense, Denmark;[3] Molecular Bone Histology, Dept. of Pathology, Odense University Hospital, Odense, Denmark; [4] Dept. of Endocrinology, Odense University Hospital, Odense, Denmark; [5] Dept. of Anatomy & Cell Biology, Rush University Medical Center, Chicago, Illinois, USA.

Background: Osteoporosis is a systemic bone disease characterized by a reduction of bone mass, deteriorated bone microarchitecture, and fragility fractures. It is caused by an imbalance between osteoclastic bone resorption and osteoblastic bone formation. To prevent osteoporotic fractures patients are treated with anti-resorptive agents such as bisphosphonates. An example is alendronate (ALN). Long-term use of ALN has been associated with an increased risk of atypical femoral fractures (AFFs). It is therefore of interest to investigate the outcomes of ALN discontinuation.

Hypotheses: 1. Osteoclast (OC) activity and sensitivity to ALN *in vitro* are linked to the pre-clinical and clinical potency of ALN. 2. An epigenetic profile of peripheral blood mononuclear cells can predict changes in BTMs *in vivo* and the behavior/sensitivity of osteoclasts *in vitro* between the continuation or discontinuation group.

Methods: For the PhD study 70 postmenopausal osteoporotic women treated with ALN (>3 years and no fracture or >5 years and latest low-energy fracture >3 years ago) are included. Patients are randomly distributed into two groups: continuing or discontinuing treatment and followed for 3 years. Clinical and demographic information is collected at baseline, furthermore, full blood is collected for DNA methylation analysis. BTMs (CTX and PINP) are measured at 0, 6, and 12 months. DXA scans are performed at 0 and 12 months. At 0 and 12 months, OCs are generated *in vitro* from blood donations, and mature multinucleated OCs are reseeded onto bone slices coated with ALN and left to resorb bone for three days. Their sensitivity will be determined by identifying the IC50-values.
The pathophysiology of AFFs is investigated using a lactation/low Ca^{2+} rodent model. During lactation dams are switched to a low Ca^{2+} diet to induce bone resorption, and at weaning dams are switched back to a normal Ca^{2+} diet to allow bone formation. During this period, dams are injected subcutaneously twice a week with saline or 28 µg/kg ALN. After 28 days, dams are bred and a second round of bone remodeling is induced. At the second weaning dams are sacrificed. Rodent OCs will be generated from bone marrow *in vitro* and like the human cultures the resorption activity and sensitivity to ALN will be investigated. Femurs will be fixed in 4% paraformaldehyde for 24 hours and later used for histomorphometry to determine the effects of ALN.

Future perspectives: We hope that our combined *in vitro,* pre-clinical, and clinical analyses will aid in better understanding and predicting the risks and benefits of discontinuing ALN treatment.

Combined cryo and paraffin bone histomorphometry: a quick and novel approach opening new opportunities

Malene H. Nielsen,[1,2,5] Jeanette K. Petersen,[2] Ditte Hansen,[3] Thomas L. Andersen[1,2,4,5]

[1]Molecular Bone Histology (MBH) lab, Department of Clinical Research, University of Southern Denmark, Denmark, [2]Department of Pathology, Odense University Hospital, Odense, Denmark, [3]Department of Nephrology, Copenhagen University Hospital, Herlev and Gentofte, Denmark, [4]Molecular Bone Histology (MBH) lab, Department of Forensic Medicine, Aarhus University, Aarhus, Denmark, [5]Danish Spatial Imaging Consortium (DanSIC)

Background: Histomorphometry have for decades been performed on sections from undecalcified methylmethacrylate embedded bone biopsies. However, this procedure is slow and prevent that the bone biopsies can be easily used for immunostainings, and exclude any *in situ* RNA and DNA studies. This study focus on a novel bone histomorphometric procedure combining the analysis of undecalcified cryo-sections and decalcified paraffin sections from the same biopsy. This provide a faster bone histomorphometry on human bone specimens, while making it possible to perform advanced *in situ* protein, RNA and DNA analysis.

Aim: To provide a faster bone histomorphometry on human bone specimens, while making it possible to perform advanced *in situ* protein, RNA and DNA analysis.

Methods: The methodology have been applied to 3 mm Jamshidi transiliac human bone biopsies. The dynamics of the mineralization are labelled using tetracycline that are taken at day −17, −16, −15 and −6, −5, −4 (2 × 500 mg per day). The biopsies are fixed for 24 hours in 10% neutral buffered formalin (NBF) using pulses of vacuum to improve penetration. Upon arrival, the biopsy is pretreated in 30% sucrose in PBS before it is undecalcified cryo-embedded in a cryo-embedding compound (Milestone). Six µm cryo-sections are obtained using the Kawamoto tape method, and either unstained fluorescence microscopic scanned to analyze tetracycline labels (mineralization), or von Kossa stained and light microscopic scanned to analyze osteoid surfaces. Upon approval of the cryo-sections, the bone biopsy is thawed, decalcified in formic acid for 6 hours at 37°C and embedded in paraffin. Three and a half µm paraffin sections are Masson trichrome stained to analyze erosion, bone structure and cortical pore histomorphometry, as well as immunohistochemistry stained for TRAcP to analyze osteoclasts, and if needed H&E stained to analyze bone marrow morphology. All scans are performed on an Olympus VS200 fluorescence slide scanner and all analysis are performed digitally using VS200 Desktop software.

Results: From the arrival of the bone specimen, the cryo- and paraffin-sections are stained and ready for digital analysis within 5 workdays, which is 10–12 days shorter than with the classical MMA-method. The quality of the cryo-sections is challenging, but suitable for the analysis mineralization and osteoid, while the cellular analysis should only be performed in the paraffin sections. As the tetracycline was more visible in the cryo-sections, we expect to reduce the labelling from three to one day per time point. Furthermore, this novel methodology renders the biopsies suitable for advanced immunostaining and *in situ* RNA/DNA analysis, including novel spatially resolved transcriptomics.

Conclusion: This combined cryo and paraffin bone histomorphometry method provide a faster result, and opens up to a completely new world of opportunities.

SHORT-term effects of GLucagon-like peptide One on BonE (SHORT-GLOBE)

Julie Bjerrelund,[1,2] Morten S. Hansen,[1,2], Nina Hansen,[1,2] Lærke Gasbjerg,[3] Alexander Rauch,[1,2,4] Morten Frost[1,2,4]

[1]Department of Endocrinology, Odense University Hospital, Denmark, [2]Clinical Institute, Faculty of Health Sciences, University of Southern Denmark, [3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, [4]Steno Diabetes Centre Odense, Odense University Hospital, Denmark

Background: Bone is constantly remodeled by a coupled process of resorption by osteoclasts and formation of by osteoblasts. Glucagon-like peptide 1 (GLP-1), secreted postprandially, has emerged as a potential regulator of bone remodeling. Recently, Nina Hansen from our group showed that short-term GLP-1 exposure (two hours) *in vivo* led to an 80% reduction in the bone resorption marker Collagen I, cross-linked C-terminal telopeptide (CTX) in bone marrow serum from healthy young males. In contrast, preliminary data from our group have shown that three-days exposure to GLP-1 *in-vitro* increases the activity of both osteoclasts and osteoblasts. The *in vitro* findings have not been corroborated by physiological studies. As such, the understanding of the relevance of GLP-1 to human bone physiology currently only covers acute effects. Furthermore, a clinical trial conducted on non-diabetic individuals undergoing long-term treatment with the GLP-1 receptor agonist (GLP-1RA), semaglutide, demonstrated an increase in the bone resorption marker CTX. The discrepancy between immediate effects observed *in vivo* and longer-term effects seen in the clinical trial underscores the need to clarify the effect of extended GLP-1 exposure *in vivo.*

Aim: The ambition of this study is to outline the effect of native GLP-1 in the skeleton. Specifically, this study will demonstrate the impact of four days exposure to native GLP-1 on bone turnover in healthy individuals.

Methods: This is a randomized, single-blinded, crossover study involving 12 healthy individuals aged 18 to 40 years with a BMI between 18.5 and 24.9 kg/m^2. The study compares the effects of four days of continuous infusion of native GLP-1 or a matching volume of saline on bone formation, assessed by bone formation marker procollagen type I N-terminal propeptide (P1NP), and bone resorption, assessed by the resorption marker CTX. PINP and CTX will be measured in peripheral blood and bone marrow serum. This study will also investigate the effect of long-term GLP-1 exposure on cell composition in the bone marrow, using single-cell RNA sequencing. Furthermore, we will investigate the effect of GLP-1 on expression of proteins in bone marrow tissue using digital spatial profiling.

Conclusion: This study will elucidate the sub-acute impact of GLP-1 on human bone formation and resorption uncovering the relevance of GLP-1 to human bone physiology. If GLP-1 increases bone formation and enhances overall bone turnover, GLP-1 based pharmaceuticals may benefit patients with low bone turnover, such as patients with type 2 diabetes. The emergence of pharmaceuticals based on GLP-1 that are commonly used as treatments of type 2 diabetes and obesity underline the relevance of research into the impact of GLP-1 to human bone physiology.

The potential of using keel bone fracture in laying hens to investigate bone pain in a high mineral turnover, late mineralizing bone – a research plan.

C.J.A. Williams,[1] H. Lauridsen,[2] K. Hansen,[3] H. Birkedal,[4] A.B. Riber[1]

[1]Dept. of Animal and Veterinary Sciences, AU Viborg, Aarhus University , [2]Dept of Clinical Medicine, AU, [3]Dept. of Forensic Medicine, AU[4],Dept. of Chemistry and iNANO, AU

Background: >80% of Danish laying hens have evidence of previous fractures to the keel bone (*carina*) by their point of slaughter. The keel bone is thin, complex, and mineralises late. It forms the attachment point for flight muscles, overlays the muscular gizzard and respiratory air sacs and appears in these birds to be subject to at least three types of pathology – i) keel bone deformation, ii) rarer cranial fractures associated with external trauma, and iii) very common caudal fractures which may be associated with internal forces e.g. egg deposition. The high Danish fracture burden falls within a high and variable internationally reported fracture range and has been associated with the early start of lay, high mineral turnover and high egg production of modern laying hens. The modern laying hen turns over more than 1.2 g of calcium every 24 h corresponding to app. 10% total body calcium stores and on average produces 350 eggs per year. This enormous mineral turnover is supported by mobilization of calcium by the medullary bone in laying birds, as well as dietary input. Currently, keel bone fractures have been associated with i) decreases in performance of highly motivated behaviours which can been ameliorated with some analgesics and ii) decreases in adult hippocampal neurogenesis consistent with chronic stress, but the pathway from bone to brain for nociceptive information is not studied.

Aim: To investigate the microstructure of and nociceptive pathway from the keel bone in growing and naturally fractured hens.

Methods: *Ex vivo:* Micro CT on mid and end of lay hens, to extend the current imaging based understanding of keel bone fracture, in the context of bone mineral density and fracture description. Histological investigation of the innervation of the keel bone with the aim of differentiating nociceptive from autonomic neuronal contribution. *In vivo:* Behavioural observation, respirometry, ECG and activity during husbandry from 24–56 weeks (covering the period of maximum fracture acquisition, and peak lay). Thermal and mechanical nociceptive threshold measurement at skin overlying keel bone and on distant site (*tarsus*).

In situ: Electrophysiology of the keel bone

Input from established mammalian bone knowledge is welcomed, as are collaborative opportunities.

Metal Emissions from Traffic Pollution as a Risk Factor for Osteoporosis

Joudi Altaleb,[1,2,3,4,5,6,8] Ian Mudway, [1,2,3,4,5] Richard Abel,[6] Ulrich Hansen[7,8]

[1]Environmental Research Group at Imperial College London (ICL), [2]School of Public Health (SPH) at Imperial, [3]MRC Centre for Environment and Health, [4]Medical Research Council Centre for Environment and Health (MRC-CEH), [5]NIHR-PHE Health Protection Research Units in Environmental Exposures and Health and Chemical and Radiation Threats and Hazards, [6]Musculoskeletal laboratory (MSk) in the Faculty of Medicine at ICL, [7]Medical Engineering Group within the Mechanical Engineering Department at ICL, [8]Biomechanical Engineering Department at ICL

Background: Osteoporosis poses a significant global health challenge. In England and Wales, approximately 180,000 fractures occur annually due to osteoporosis, incurring an annual cost of £4.7 billion for the NHS. Epidemiological connections have been established between PM2.5 pollution and osteoporosis, along with low mineral bone content. Irrespective of potential confounders, a notable correlation has been found between men over 65 years old residing in areas with elevated PM2.5 levels and increased rates of hospital admissions for osteoporosis-related fractures. Although outdoor air pollution has been identified as an independent risk factor for osteoporosis and fractures due to bone fragility, little is known about the mechanisms underlying its association with bone damage.

Aim: To identify the mechanisms linking exogenous heavy metals from traffic-related air pollution to the predisposition to osteoporotic fragility fractures.

Methods: An FEA model, cell culture studies and investigation of bone cell reactions when mechanically loaded within a bioreactor. This study is developing and performing computational models including both the effects of mechanical and biological effects on bone cell behaviour. The model is based on micro-CT images of bone fragments exposed to mechanical loading and particulate pollution in a bioreactor to better inform the biological mechanisms that can be used to better understand and develop improved algorithms of the bone remodelling process. With the established physiological computational model long-term effects (beyond that which can be investigated using in-vitro cell culture studies and bioreactor experiments) will be carried out to determine the long-term effects of heavy metal on bone conditions such as osteoporotic fragility fractures.

Results: TBD

Conclusions: TBD

Osteoblast-dependent regulation of anti-tumoral immunity

Serkin Park

Korea University College of Medicine, South Korea

New insights into pathogenesis of myeloma bone disease

Abbas Jafari

Dept. of Cellular and Molecular Medicine, Copenhagen University, Denmark

DNA variant analyses to long-read

Inge Søkilde Pedersen

Dept. of Molecular Genetics, Aalborg University Hospital, Denmark

Gene expressioin and single cell analysis

Mads Thomassen

Clinical GENOME center, Odense University Hospital, Denmark

Local intraoperative zoledronate and postoperative denosumab decrease migration of cementless total knee arthroplasty by suppression of bone resorption: A randomised, double-blinded radiostereometric study of 82 patients with 5-year follow-up

Karina Nørgaard Linde,[1,2] Søren Rytter,[1,2] Bente Lomholt Langdahl,[2,3] Frank Madsen,[1] Maiken Stilling[1,2]

[1]Dept. of Orthopaedics, Aarhus University Hospital (AUH)l, Aarhus Denmark, [2]Dept. of Clinical Medicine, Aarhus University, Aarhus, Denmark, [3]Dept. of Endocrinology and Internal Medicine, AUH, Aarhus Denmark

Background: Cementless tibial implants migrate initially until osseointegration. Antiresorptives suppress bone resorption and may improve the fixation of cementless tibial implants.

Aim: To investigate if adjuvant treatment with antiresorptives (zoledronate and denosumab) can improve fixation and preserve the periprosthetic bone of cementless total knee arthroplasty.

Method: A 3-way double-blinded, placebo-controlled randomised clinical study with 82 patients. The patients received a cementless total knee arthroplasty (Regenerex tibial implant) and were randomised to either placebo (NaCl) (n=27), intraoperative local zoledronate on the cut tibial bone (n=28) or postoperative subcutaneous denosumab (n=27) on the first or second day after surgery and again after 6 months. Primary outcome was tibial implant migration measured by radiostereometric analysis (RSA) with 5 years follow-up. Migration was evaluated by y-translation (subsidence) and maximum total point motion (MTPM). Secondary outcomes were periprosthetic bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DXA), biochemical bone turnover markers (CTX, P1NP, osteocalcin, BSAP), and patient-reported outcomes.

Results: The intervention groups had statistically significantly less subsidence of the tibial implant than the placebo group at 5-year follow-up: mean difference for zoledronate of 0.50 mm (95% CI 0.23 to 0.78, p<0.001) and for denosumab of 0.30 mm (95%CI 0.03 to 0.58, p=0.027) compared to placebo (Figure 1). CTX was lower in the zoledronate group than in the placebo group at the 2- and 6-week follow-up; however, in the denosumab group, CTX was lower at follow-up at 2 and 6 weeks, 6 months, and at 1 year. Biochemical bone formation markers were similar in the placebo and the zoledronate group, but lower in the denosumab group. Periprosthetic BMD was higher in the intervention groups, with the highest periprosthetic BMD in the zoledronate group at the 5-year follow-up with a difference in the lateral region of 7.62% (95%CI 0.62 to 14.61, p=0.03) compared to the placebo group.

Conclusion: Local zoledronate and systemic denosumab decrease migration of cementless tibial implants by reducing periprosthetic bone resorption. Our findings support implementation of local intraoperative zoledronate during knee arthroplasty surgery to improve implant fixation and preserve tibial bone, which may increase the survival of cementless tibial implants.

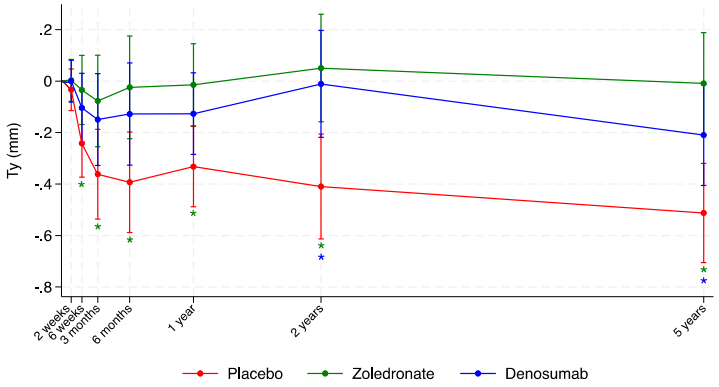


Figure 1: Plotted estimated Mean Ty (mm) with 95% CI comparing the intervention groups with the placebo group, *p<0.05

Treatment of bone tumors – cases

Andrea René Jørgensen

Dept. of Orthopaedics, Aarhus University Hospital, Denmark

Changes to bone structure in humans with type 2 diabetes

Petar Milovanovic

Inst. of Anatomy, University of Belgrade, Serbia

Incretin hormones and bone health in diabetes

Morten Frost

University of Southern Denmark, Denmark

Fracture Characteristics of Human Cortical Bone Influenced by the Duration of in vitro Glycation

Mei-Chun Lin,[1] Praveer Sihota,[1] Sofie Dragoun Kolibová,[1] Imke A.K. Fiedler,[1,2] Johannes Krug,[1] Eva M. Wölfel,[1] Benjamin Ondruschka,[3] Mustafa Citak,[4] Felix Klebig,[4] Felix. N. Schmidt,[1] Katharina Jähn-Rickert,[1,5] Mahan Qwamizadeh,[1,2] Björn Busse[1,2]

[1]Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany[2]Interdisciplinary Comeptence Center for Interface Research (ICCIR), University Medical Center Hamburg-Eppendorf, Hamburg, Germany[3]Institute of Legal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany[4]Helios ENDO-Klinik Hamburg, Hamburg, Germany[5]Mildred Scheel Cancer Career Center Hamburg, University Cancer Center Hamburg, University Medical Center Hamburg, 22529 Hamburg

Background: Advanced glycation end products (AGEs) accumulate in various tissues, including bone, due to aging and other pathological states, such as diabetes mellitus.

Aim: For assessing the direct effects of AGEs on bone material quality and biomechanical properties, a comprehensive controlled *in vitro* study was undertaken across multiple length scales.

Methods: Human tibial bone from an organ donor was sectioned into 90 beams following autopsy, randomly assigned to three groups (n = 5 per group) of ribose (c=0.6 M) treatment – glycated for 7, 14, and 21 days, and three control groups (n=5 per group). The microstructure of bone samples was determined using microCT, while fluorometric analysis assessed total fluorescent AGE (fAGE) levels, and Fourier-transform infrared and Raman spectroscopy bone matrix composition. To determine the fracture characteristics, different mechanical testing methods including tissue-level nanoindentation, and three-point bending of non-notched and notched beam-shaped specimens were performed.

Results: Total fAGEs levels elevated after 7, 14, and 21 days of incubation compared to controls ($p=0.019$, $p=0.035$, $p=0.017$; respectively), and increased sugar-to-matrix ratio after 21-day glycation compared to controls ($p=0.013$). Analysis of bone matrix composition showed elevated carbonate-to-phosphate ratios in the interstitial bone after 14-day and 21-day glycation compared to controls ($p=0.014$ and $p=0.028$; respectively). Mechanical testing of notched specimens exhibited a increased yield force, pre-yield toughness, and growth toughness at 14-day glycation compared to controls, and to both 7-day and 21-day glycation ($p<0.05$).

Conclusion: Our experimental study on varying glycation periods underscored the dynamic connection between glycation and mechanical characteristics. Specifically, the duration of glycation affects the fAGE accumulation and thus the energy required to initiate and propagate fractures. In a clinical setting, this could contribute to the reduction of mechanical performance in diabetes mellitus and otherwise affected patients.

An activin type II receptor ligand trap prevented loss of cortical bone strength and cancellous bone mass in a mouse model of severe disuse osteopenia

M.F. Poulsen,[1] F. Fisher,[2] J. Lachey,[2] J. Seehra,[2] C. Andersen,[1] M. Eijken,[3] J.S. Thomsen,[1] A. Brüel[1], A. Lodberg[1]

[1]Department of Biomedicine, Aarhus University, Aarhus, Denmark. [2]Keros Therapeutics, Lexington, USA. [3]Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark.

Background: Muscular disuse is a powerful catabolic stimulus for bone. The use of TGFβ superfamily antagonists has a compelling rationale in the setting of disuse since activin and myostatin inhibition is known to stimulate both bone and muscle anabolism.

RKER-065 is a modified activin type II receptor that is designed to neutralize activin A and B, GDF11, and myostatin signaling with reduced inhibition of BMP9 signaling.

Aim: In a mouse model of severe disuse induced by botulinum toxin A (Btx), we tested a research version of the investigational ligand trap KER-065 (RKER-065) for its ability to preserve bone strength and architecture.

Methods: 16-week-old B6 mice were randomized into Baseline (n = 10), Ambulating+Vehicle (Amb+Veh, n = 12), Amb+RKER-065 (n = 12), Btx+Veh (n = 12), and Btx+RKER-065 (n = 12). On study day 1, Btx was administered to the right quadriceps and calf muscles and Veh or RKER-065 (10 mg/kg) was administered ip twice weekly. Bone properties were evaluated using DXA, μCT, and mechanical testing.

Results: Compared to the Amb+Veh group, Btx+Veh had a lower rectus femoris muscle mass (–45%) and gastrocnemius muscle mass (–55%), demonstrating atrophy as a result of disuse. At the same time, Btx+Veh had a lower whole-femur BMD (–19%), cortical thickness (–12%), cortical strength (–17%), cancellous bone density (BV/TV, –53%), and femoral neck strength (–45%) compared to Amb+Veh. The deterioration in cortical and cancellous structural and mechanical parameters was countered by RKER-065. Compared to Btx+Veh, Btx+RKER-065 showed significantly greater whole-femur BMD (+15%) and cortical thickness (+14%) and metaphyseal BV/TV (+141%) – maintaining these parameters at the level of Amb+Veh. Administration of RKER-065 completely prevented loss of diaphyseal fracture strength (no difference vs Amb+Veh) and loss of femoral neck strength was significantly mitigated (+41% vs Btx+Veh).

Conclusion: Taken together, these data support the potential of KER-065 to offset osteopenia induced by muscle disuse.

Prevalence of osteoporosis among type 1 diabetes patients

Jakob Starup-Linde[1], Julie Støy[2], Bente Langdahl[1], Torben Harsløf[1]

[1]Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark, [2]Steno Diabetes Center Aarhus, Aarhus University Hospital, Aarhus, Denmark

Background: Type 1 diabetes (T1D) is associated with an increased fracture risk. Recent American Diabetes Association (ADA) guidelines recommend a treatment threshold of bone mineral density (BMD) T-score ≤-2.0.

Purpose: In this study we aim to investigate the prevalence of osteoporosis (DXA) in T1D ≥50 years.

Methods: In this cross-sectional database study we collected data from routine clinical DXA which was implemented in the period 2020-2022 and offered to men and postmenopausal women with T1D ≥50 years seen in the diabetes outpatient clinic.

DXA of the lumbar spine and hip were performed by cross-calibrated Hologic Horizon scanners.

Information on biochemical measures and osteoporosis risk factors were collected from questionnaires and electronic health records. Adherence to prescribed alendronate was audited using the prescription database. Permission for data collection was received from Region Central Jutland, Denmark.

Results: In total 640 T1D individuals were investigated. Of these 17.8 % had osteoporosis based on a T-score ≤-2.5 and 28.6 had osteoporosis based on a T-score ≤-2.0. In multivariate analysis, increasing age (1 year) (OR=1.3, 95 % CI :1.0;1.7) and a family history of osteoporosis (OR=1.9, 95 % CI :1.2;3.0) were associated with an increased risk of osteoporosis, whereas increase in BMI (1 kg/m^2) were associated with a decreased risk of osteoporosis (OR=0.87, 95 % CI: 0.82;0.92). HbA1c levels and diabetes duration were not related to the presence of osteoporosis. Among those treated with alendronate (n=76) for osteoporosis the compliance rate was 92.7 %.

Conclusion: In conclusion, the present study finds an osteoporosis prevalence of 17.8 % in postmenopausal women or men ≥ 50 years of age with T1D and using a T-score ≤-2.0 28.6 % has osteoporosis. These findings illustrate that a high proportion of T1D has osteoporosis using ADA guidelines.

Do osteolytic fractures in multiple myeloma heal? Preliminary findings from a study of 22 bone biopsies from vertebral fractures of multiple myeloma patients.

Mette Bøegh Levring,[1,2,5] Marta Diaz-delCastillo,[3] Line Adsbøll Wickstroem,[1,4] Ida Bruun Kristensen,[2] Charlotte Guldborg Nyvold,[5] Moustapha Kassem,[6] Mikkel Østerheden Andersen,[1,4] Thomas Levin Andersen[1,7], Niels Abildgaard[1,2]

[1] Dept. of Clinical Research, University of Southern Denmark (SDU), Odense, Denmark, [2]Dept. of Hematology, Odense University Hospital (OUH), [3]Dept. of Forensic Medicine, Aarhus University, Aarhus, Denmark, [4]Center for Spine Surgery and Research, Lillebaelt Hospital, Middelfart, Denmark, [5]Haematology-Pathology Research Laboratory, Research Unit for Haematology and Research Unit for Pathology, SDU and OUH, [6]Dept of Endocrinology and Metabolism, Molecular Endocrinology & Stem Cell Research Unit (KMEB), OUH and SDU [7]Dept. of Pathology, OUH

Background: In Multiple myeloma (MM), an incurable cancer complicated by myeloma bone disease (MBD), MM cells disrupt the bone remodeling homeostasis, increasing bone resorption and decreasing formation, causing high fracture risk, pain, and disability for patients. The traditional perception of MBD is that lesions do not heal even in cancer remission. Studies applying skeletal computer tomography (CT) have shown healing signs such as sclerosis in up to 70% of patients, but in limited lesions per patient. Previous studies of MBD on biopsies examine standard iliac crest biopsies; however, little is known of what is happening locally at the lytic lesion or fracture.

Aim: To evaluate the mechanisms of MBD development and bone lesion healing directly in osteolytic fracture sites of MM patients.

Methods: From 21 MM patients, 22 trephine bone biopsies were collected during vertebroplasty treatment of a vertebral body collapse. Formalin-fixed, paraffin-embedded biopsies were sectioned and Masson's Trichrome stained. Whole-tissue slidescans (Olympus VS200, Japan) were histomorphometrically analyzed with the OlyVIA software (Olympus, Japan). Clinical characteristics (e.g. age, sex, BMI, diagnosis date, myeloma subtype, anti-myeloma and anti-resorptive treatments, biochemical assays, skeletal surveys and reported pain) were collected from medical charts. Fracture date was estimated based on patient reported pain onset and results of skeletal scans.

Results: Patient mean age was 73.4 years and 17/21 (81%) were male; 13/21 were newly diagnosed, 7/21 had become symptomatic from smoldering myeloma, and one had relapsed MM. One patient was untreated, 19/21 were on first treatment line, and 1/21 on the 7th line. Median time from diagnosis was 88 days (IQR: 1030 days) and median estimated fracture age was 79 days (IQR: 66 days). New bone formation was seen in 16/21 patients (76%), but the percentage of newly formed bone volume per total bone volume (TBV) was highly heterogeneous between patients (median: 33%, IQR: 55%). Newly formed bone volume was positively correlated with TBV but independent of fracture age. Moreover, patients with no measurable new bone formations experienced fractures up to 113 days prior to procedure.

Conclusion and future perspective: *Our preliminary data suggest that bone formation is present in most MM osteolytic fractures, though with a large degree of heterogeneity and independent of fracture age. In a subset of patients, the newly formed bone volume was surprisingly large and warrants further investigation* of the quality of vertebral fracture site healing in MM. Further studies will evaluate whether bone formation at osteolytic sites in MM is dictated by underlying clinical characteristics through evaluation of paired trephine iliac crest bone biopsies.

Intratrabecular tunneling is an overlooked mode of remodeling, which is overactivated by intermittent parathyroid hormone treatment in both humans and rabbits

Lisbeth K Thomsen,[1,2] Kaja S Laursen,[3] Ali R Rizvi,[4] Kim Harrison,[4] Lindsay Loundagin,[4] Xuan Wei,[4] Tanja Sikjaer,[5] Jesper S Thomsen,[6] Christina M Andreasen,[1,2] Lars Rejnmark,[5] David Cooper,[4] Thomas L Andersen[1,2,3]

[1]Molecular Bone Histology-lab, Research Unit of Pathology, Dept. of Clinical Research, University of Southern Denmark, Denmark, [2]Dept. of Pathology, Odense University Hospital, Denmark, [3]Dept. of Forensic Medicine, Aarhus University, Denmark, [4]Dept. of Anatomy Physiology and Pharmacology, College of Medicine, University of Saskatchewan, Saskatchewan, Canada, [5]Dept. of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark, [5]Dept. of Biomedicine, Aarhus University, Denmark

Background: Intratrabecular tunneling – resorption of bone within the trabeculae – was first observed in patients with *hyper*parathyroidism or renal osteodystrophy. Later, when once-a-day injections of parathyroid hormone (rhPTH) was introduced to patients with *hypo*parathyroidism (hypoPT) or osteoporosis, intratrabecular tunneling was again observed. However, this phenomenon is rarely systematically quantified and may reflect an overlooked mode of trabecular remodeling.

Aim: To determine the extent and classification of intratrabecular tunneling in patients with hypoPT treated with rhPTH and investigate PTH-treated rabbits as a potential pre-clinical model.

Methods: 68 iliac crest bone biopsies collected from 50 patients with hypoPT randomized to receive either placebo (PLB6; n=25) or 100 µg/day rhPTH(1–84) s.c. (PTH6; n=25) for 6 months as an add-on to conventional treatment. 18 participants continued open-labeled treatment with either i) conventional treatment (CON30; n=5), ii) continued rhPTH-treatment (PTH30; n=5), or iii) were withdrawn from rhPTH treatment (PTHw30; n=8) for an additional 24 months before a second bone biopsy was collected. Each biopsy underwent µCT and was sectioned for histomorphometry of the intratrabecular tunneling. As a comparative, intratrabecular tunneling was investigated with µCT in the calcanei of ovariohysterectomized rabbits (n=4) treated once-a-day with PTH(1–34) for 4 weeks.

Results: Trabecular porosity originating from intratrabecular tunneling increased 18-fold in PTH6 compared with PLB6 (p=0.0001), and while reduced in PTH30 the intratrabecular porosity remained significantly higher than in CON30 (p=0.0384). Trabecular pore density increased 2.5-fold in PTH6 relative to PLB6 (p=0.0009), and 1.8-fold in PTH30 relative to PTH6 (p=0.0549). In PTH30, the intratrabecular tunneling of existing pores increased 10-fold compared with PTH6 (p=0.0052), and its contribution to intratrabecular porosity was significantly increased at PTH30 (30-fold relative to PTH6, p=0.0466; 190-fold relative to CON30, p=0.0372). The significantly higher porosity in PTH6 and PTH30 was derived from non-quiescent pores compared to PLB6 (7.6-fold)/CON30 (95.4-fold), while non-quiescent porosity in PTHw30 was significantly lower than PTH6 (p=0.0008). Intratrabecular tunneling was evident in rabbit calcanei in as little as 4 weeks of PTH(1–34) treatment. Preliminary observations suggest similar remodeling patterns as that seen in the human biopsies.

Conclusion: Intratrabecular tunneling is an overlooked mode of trabecular remodeling and is significantly increased with rhPTH treatment and accumulates with treatment-time. Rabbits treated with PTH provide a suitable pre-clinical model for investigating the generation of intratrabecular tunnels. By use of time-lapsed imaging, the dynamics of intratrabecular remodeling can be further elucidated using this rabbit model.

Characterizing the myeloma bone disease in Vk*MYC mouse model of multiple myeloma

Clarissa Schmal,[1] Marija Simic,[2] Ya Xiao,[2] Alicia Armero Catalá,[1] Peter Croucher,[2] Michelle McDonald,[2] Abbas Jafari[1,3]

[1]Department of Cellular and Molecular Medicine, University of Copenhagen. [2]Skeletal Diseases Program, The Garvan Institute of Medical Research, Darlinghurst, Australia. [3]Laboratory of Molecular Endocrinology (KMEB), Odense University Hospital, Denmark.

Background: Multiple myeloma (MM) is caused by neoplastic expansion of clonal plasma cells and represents the second-most frequent hematologic cancer. Up to 80% of MM patients develop myeloma bone disease (MBD), a hallmark of which is the development of osteolytic lesions that can lead to skeletal related events such as fractures, spinal cord compression and bone pain, and increased risk of death. The Vk*MYC mouse model of MM is based on activating the expression of the MYC gene in the germinal centers, leading to formation of malignant plasma cells and development of major symptoms of MM, including MBD. However, the extent of skeletal changes in the Vk*MYC model is not well characterized.

Aims: To investigate manifestation of MBD in the femur of Vk*MYC mouse model of MM and determine the effect of skeletal maturity on MBD development in this model.

Methods: Female C57BL/6 mice were intravenously injected with saline or 250.000 Vk11451-GFP cells via the tail vein at the age of 6 or 16 weeks. Mice were terminated 8-9 weeks after cell injection. Tumor burden was assessed using flowcytometry analysis of GFP positive cells in the spleen. MicroCT analysis was used to assess the formation of osteolytic lesions in the femur and to determine the structural bone parameters in the proximal femur. ELISA analysis was used to determine the levels of P1NP and CTX in the serum.

Results: Flowcytometry analysis showed successful establishment of MM. We found significant deterioration of the trabecular bone structured in Vk*MYC as compared to saline-injected (control) mice, evidenced by decreased bone volume fraction (-49.2%±13, $P<0.05$), bone surface area (-50.3%±12, $P<0.05$), bone surface to bone volume (-22.3%±2, $P<0.001$), trabecular number (-46.3%±3, $P<0.0001$) and connectivity density (-79.5%±6, $P<0.05$), together with increased trabecular separation (+86.3%±12, $P<0.0001$) and thickness (+20.3%±2, $P<0.001$). Analysis of the cortical bone parameters indicated increased cortical porosity (+164.5%±25, $P<0.0001$) and decreased cortical thickness (-26.9%±1, $P<0.001$) in the Vk*MYC mice as compared to controls. Importantly, a significant level of osteolytic lesions was observed in Vk*MYC mice. Interestingly, we observed that the extent of bone loss and deterioration of the bone structure was significantly higher in the recipient mice at the age of 6 weeks, as compared to 16 weeks. In addition, we found an increased level of bone resorption marker (serum CTX) in the 6-weeks Vk*MYC mice as compared to control mice, whereas bone formation marker (serum P1NP) was not altered. However, P1NP levels were significantly decreased in the 16-weeks Vk*MYC mice as compared to control mice, whereas CTX levels were similar to the control mice.

Conclusions: Our studies indicate a significant age-dependent manifestation of myeloma bone disease in the femur of the Vk*MYC mouse model of MM.

Acute effects of GLP-1 and GIP on human bone turnover

Nina Wærling Hansen,[1,2] Morten Svarer Hansen,[1,2] Bolette Hartmann,[3] Morten Frost[1,2,4]

[1]Department of Molecular Endocrinology, KMEB, Odense University Hospital, Denmark, [2]Clinical Institute, Faculty of Health Sciences, University of Southern Denmark, [3]Department of Biomedical science, University of Copenhagen, Denmark, [4]Steno Diabetes Centre Odense, Odense University Hospital, Denmark

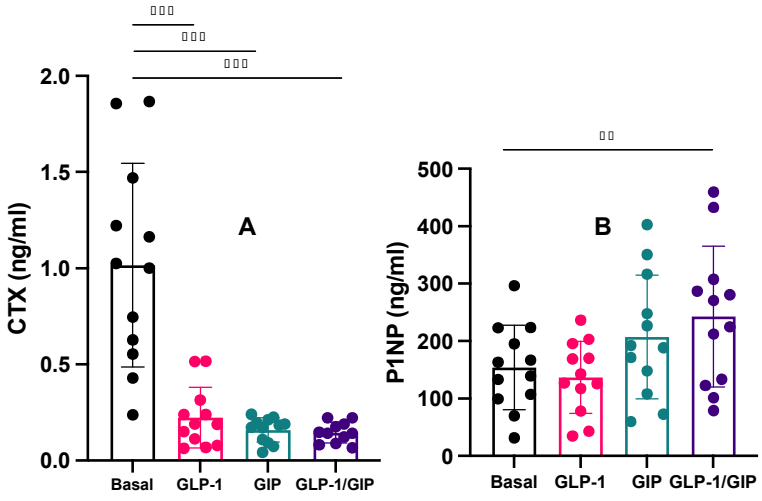
Background: Incretin hormones are widely and increasingly used to treat obesity and T2D, which are both associated with changes in fracture pattern and fracture risk. While short-term infusion of GLP-1 and GIP decreases biochemical markers of bone resorption in peripheral blood (PB), GIP is suggested to transiently increase bone formation markers whereas GLP-1 has neutral effects on bone formation markers. However, the effect of GIP and GLP-1 in bone marrow (BM) is unknown.

Purpose: We hypothesized that a short-term infusion of GIP and GLP-1 a) would demonstrate that changes in bone resorption measured in plasma from PB reflects changes in the BM, b) would show differential effects on bone formation markers, without c) affecting differentiation capacity in vitro.

Methods: Following ethical approval 12 healthy male subjects (22–43 years) underwent 2-hour infusions of either GIP, GLP-1 or the combination on three separate study days. Glucose was infused to mimic a postprandial glucose level of 8 mM. PB samples were collected every 30 minutes (–30, 0, 30, 60, 90, 120 min.). Iliac crest BM-aspirates were collected at baseline and post-intervention. Bone turnover markers, P1NP and CTX were measured in BM-plasma and in PB. Skeletal stem cells were isolated and differentiated to osteoblast and adipocytes to study differentiation capacity.

Results: GIP and GLP-1 potently decreased CTX both in PB (38% and 34%, respectively (not shown)) and BM (84% and 78%, respectively (Fig. A)). P1NP increased (10%) transiently after 30 minutes in the PB (not shown) and was (34%) higher after 2 hours in the BM (Fig. B). GLP-1 exerted no effect on P1NP. Neither GIP nor GLP-1 affected cellular differentiation measured by Alp-activity and by lipid accumulation (not shown).

Conclusion: Incretin hormones have differential effects on bone turnover markers. Both exert acute anti-resorptive effects, while GIP demonstrate a transient effect on bone formation. Mechanisms behind differential effects of incretin hormones on bone turnover remain to be established.



Accumulation of Advanced Glycation End products and Microarchitecture of Bone Tissue in Subjects with or without Type 2 Diabetes Mellitus

H. Bardenfleth,[1,2] J. Starup-Linde,[1,2] K. Hygum,[1,2] T. Harsløf,[1,2] A. Sadetskaia,[3,4] H. Birkedal,[3,4] B. Langdahl[1,2]

[1]Dept. of Endocrinology, Aarhus University Hospital, Aarhus, Denmark, [2]Dept. of Clinical Medicine, Aarhus University, Aarhus, Denmark, Department of Chemistry, Aarhus University, Aarhus, Denmark, [4] The Interdisciplinary Nanoscience Center INANO, Aarhus University, Aarhus, Denmark

Background: Despite comparable or increased bone mineral density (BMD) in patients with type 2 diabetes mellitus (T2DM), T2DM is associated with an increased risk of fragility fractures, suggesting impairment of bone microarchitecture. Advanced glycation end products (AGEs) are proteins or lipids that accumulate in the organic matrix of bone, and are increasingly present in T2DM, strongly driven by disease duration and poor glycemic control. This study aims to examine levels of AGEs in T2DM and healthy controls and evaluate the effect of disease on trabecular bone thickness.

Methods: Randomly selected eligible males (>40years, BMI<35kg/m2) were invited to participate. Disease duration≥5 years, HbA1c≥48mmol/mol throughout the last 24months. Controls with lifelong HbA1c<45mmol/mol. Users of anti-osteoporotic drugs and systemic glucocorticoids were excluded. Skin AGE (SAF-AGE) accumulation was quantified using autofluorescence (AGE Reader™). Regional and whole-body BMD was measured using DXA. Iliac crest biopsies were obtained using Jimshidi™ and trabecular bone thickness (Tr.Th.) was measured with X-ray microtomography (μCT) and Dragonfly processing software.

Results: Twenty-six male T2DM (HbA1c 60.2mmol/mol [57.4-62.9]) and 26 sex-, age- (67.1yrs±6.7 vs. 67.4yrs±7.3), BMI- (28.7kg/m^2±4.6 vs. 28.2kg/m^2±3.5), and fat%- (from DXA;33.0%±4.8 vs. 33.0%±4.4) comparable nondiabetic controls were included. Mean diabetes duration was 15.4±8.0yrs. BMD showed no significant difference in regional and whole-body T-scores. T2DM had significantly higher SAF-AGE values (2.9±0.5AU vs. 2.3±0.5AU,$p<0.05$). No significant association was found between SAF-AGE and HbA1c or between SAF-AGE and age, diabetes duration, BMD, whole body fat percentage, BMI, and Tr. Th., respectively. No significant difference in Tr.Th. (150.1μm±45.3 vs 139.0μm±38.5).

Conclusion: Elevated AGEs in T2DM may explain the increased risk of fragility fractures, independent of BMD and bone structure, that is found in patient with T2DM. Skin AGE was not associated with HbA1c and may represent longstanding glycemic excursions or a longer duration of hyperglycemia than two years.

Keywords: Type 2 diabetes; Bone quality; Fragility fractures; Advanced Glycation End-products; Bone mineral density; Skin autofluorescence; Trabecular thickness

Mapping RANKL- and OPG-expressing cells in bone tissue: The bone surface cells as activators of bone resorption and promotors of the denosumab rebound effect

Bilal M. El-Masri,[1,2,3] Christina M. Andreasen,[1,2,3] Kaja S. Laursen,[4] Viktoria B. Kofod,[1,2,3] Xenia G. Borggaard,[1,2,3] Malene H. Nielsen,[1,2,3] Jesper S. Thomsen,[5] Annemarie Brüel,[5] Mads S. Sørensen,[6] Lars J. Hansen,[6] Albert S. Kim,[7] Michelle M. McDonald,[7,8] Julia F. Charles,[9] Jean-Marie Delaisse,[1,2] Thomas L. Andersen [1,2,3,4]

[1]Dept. of Pathology, Odense University Hospital, Odense, Denmark, [2]Dept. of Clinical Research, University of Southern Denmark, Odense, Denmark, [3]Danish Spatial Imaging Consortium, University of Southern Denmark, Odense, Denmark, [4]Dept. of Forensic Medicine, Aarhus University, Aarhus, Denmark, [5]Dept. of Biomedicine, Aarhus University, Aarhus, Denmark, [6]Dept. of Otorhinolaryngology – Head and Neck Surgery and Audiology, University Hospital of Copenhagen, Rigshospitalet, Copenhagen, Denmark, [7]Skeletal Diseases Program, Garvan Institute of Medical Research, Sydney, NSW, Australia, [8]Cancer Theme, School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney [9]Dept. of Orthopedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA USA

Background: Denosumab is an anti-RANKL antibody that inhibits bone resorption, leading to increased bone mineral density, and reduced fracture risk. However, discontinuation of denosumab causes rebound resorption, increasing the risk of multiple vertebral fractures in osteoporotic patients.

Aim: To investigate the cellular mechanisms activating osteoclastic bone resorption and leading to rebound resorption after denosumab discontinuation.

Methods: We utilized *in situ* hybridization (ISH) on human and murine bone sections as a direct approach to identify key cells that orchestrate the activation of osteoclastic bone resorption through the RANK/RANKL/OPG-pathway. ISH was performed across species, skeletal sites, and during recombinant osteoprotegerin (OPG:Fc) treatment in mice.

Results: OPG:Fc treatment in mice induced an increased expression of *Tnfsf11* (encoding RANKL) in trabecular, but not endocortical bone surface cells. Additionally, a decreased expression of *Tnfrsf11b* (encoding OPG) was detected in bone surface cells and osteocytes near the bone surface in both compartments. After treatment withdrawal, osteoclasts were primarily located on trabecular bone surfaces, and to a lesser extent on endocortical bone surfaces. Under physiological conditions of human/mouse bone, *TNFSF11*/*Tnfsf11* is mainly expressed by osteoprogenitors proximate to osteoclasts, while *TNFRSF11B/Tnfrsf11b* is predominantly expressed by osteocytes and osteoblasts. Finally, we found high expression of *Tnfrsf11b* and limited expression of *Tnfsf11* in chondrocytes of the epiphyseal growth plate, articular cartilage, and in fibrocytes lining the bone of the inner ear, reflecting a matrix protective mechanism at these sites.

Conclusion: Under physiological conditions, osteoprogenitors, which include bone lining cells, reversal cells, lumen cells, and canopy cells, constitute the main source of RANKL, while osteocytes, osteoblasts, fibrocytes lining the inner ear, and chondrocytes of the growth plate and articular cartilage are major sources of OPG. OPG:Fc treatment in mice (mimicing denosumab treatment in humans) resulted in accumulation of osteoclast activation sites with increased RANKL-expressing bone surface cells on trabecular surfaces, but not on endocortical bone surfaces. Moreover, OPG:Fc treatment resulted in a decreased expression of OPG in bone surface cells and osteocytes near the bone surface, causing a local shift in the RANKL/OPG ratio, setting the stage for rebound resorption after treatment discontinuation.

Hypercalciuria in Patients with Post-Surgical Hypoparathyroidism

Sarah Thornhøj,[1] Line Underbjerg,[1] Lene Ring Madsen[2,3]

[1]Dept. of Endocrinology and Internal Medicine, Aarhus University Hospital, [2]Dept. of Internal Medicine, Gødstrup Hospital, [3]Steno Diabetes Center Aarhus

Background: Hypoparathyroidism (HypoPT) is a rare disorder characterized by hypocalcemia with low levels of parathyroid hormone. The most common cause of HypoPT is following neck surgery whereas about 25% are due to non-surgical causes. Patients with HypoPT have an increased risk of hypercalciuria, impaired renal function, nephrocalcinosis, and kidney stones. In otherwise healthy individuals hypercalciuria is associated with renal stone formation, therefore guidelines recommend that hypercalciuria in patients with HypoPT should be avoided by screening 24-hour urine calcium every second year. In case of hypercalciuria reduction in calcium supplements or thiazide diuretics is standard treatment.

Aim: To implement systematic screening for hypercalciuria in patients with Post-Surgical Hypoparathyroidism (PS-HypoPT) according to guidelines.

Methods: Quality insurance project. All patients with chronic HypoPT, treated in Clinic of Diabetes and Hormone diseases, Gødstrup Hospital, was identified via ICD-codes on diagnoses and/or via clinic lists. Patients, who has not collected a 24-hour urine sample within the last 2 years was contacted by letter with an offer of investigation.

Results: 51 patients were identified. 43 patients (36 women, 7 men) wished to participate in the investigation. Mean duration of disease was 13 years. Mean age at time of 24-urine collection was 62 years. Only 14% of the identified patients had completed a 24-hour urine collection within 2 years. Median urine calcium was 5.9 mmol/24-hour. 17 (39%) of the participants had a 24-hour urine calcium above gender specific reference level. Hypercalciuria led to changes in medicine in 11 participants, where nine patients have collected a follow-up 24-hour-urine sample. In patients (n=4) reduced in calcium supplements mean 24-hour urine calcium decreased from 12.4 mmol/24-hour to 11.8 mmol/24-hour (p=0.8). If patients (n=5) were treated with Thiazides mean 24-hour urine calcium decreased from 14.5 mmol/24-hour to 11.4 mmol/24-hour (p=0.07).

Conclusion: 24-hour urine samples have not been applied as a routine screening procedure. We hope with this project to draw attention to screen and treat hypercalciuria and treatment with thiazides seems to be effective.

ISWI-mediated chromatin remodeling directing stromal cell differentiation

Kaja Madsen,[1] Alexander Rauch[1]

[1]*University of Southern Denmark, KMEB, Denmark.*

Background and aim: Linage determination of stromal progenitor cells is a complex process involving activation of gene programs that drive differentiation and repression of genes maintaining multipotency. Rewiring of lineage specific gene expression is controlled by multiple mechanisms such as chromatin accessibility. The latter is modulated by ATP-dependent chromatin remodeling complexes. Some chromatin remodeling complexes have shown to be important for determining stromal progenitor fate, with SWI/SNF being reported to be pro-adipogenic and INO80 to be pro-osteogenic. However, the effect of ISWI on stromal progenitor fate has not been described, why I in this study aim to elucidate the function of ISWI during osteogenic and adipogenic differentiation.

Material and methods: I compared *in vitro* differentiation potential of human stromal progenitors exposed to pharmacologic or genetic inhibition of SMARCA5, the ATPase subunit of ISWI, using ED2-AD101, siRNA mediated knockdown, and stable CRISPR-Cas9 knockout. *De novo* bone formation of control cells and CRISPR-Cas9 mediated SMARCA5 knockout cells was tested in ectopic bone formation assays. Furthermore, RNA-seq, DNase-seq and ChIP-seq of ISWI, H3K27ac and MED1 were performed to test genome wide association of ISWI with lineage-selective change in gene expression and enhancer activity.

Results and conclusion: *In vitro* experiments showed decreased osteogenic potential for stromal progenitor cells upon CRISPR-Cas9 mediated knockout (*FoldChange: 0.7±0.1, p=0.003),* transient siRNA knockdown and pharmacological inhibition of SMARCA5. Furthermore, a correlation between change in ISWI binding and accessibility of chromatin (DNase-seq) during differentiation was found. Enrichment analysis showed that gene induction during osteoblast differentiation as well as adipocyte differentiation was associated with nearby gain in ISWI binding (+/- 50,000 bp from TSS).

The ear: a physiological model of *Rankl-Opg* mediate osteoclast regulation

Viktoria B. Kofod[1,2], Bilal M. El-Masri[1,2], Faranak Aboutalebi[1,2], Lars J. Hansen[3], Malene H. Nielsen[1,2], Jesper S. Thomsen[4], Christina M. Andreasen[1,2,5], Thomas L. Andersen[1,2,5]

[1]Molecular Bone Histology Lab, Dept. of Pathology, Odense University Hospital, Odense, Denmark, [2]Dept. of Clinical Research, University of Southern Denmark, Odense, Denmark, [3]Dept. of Otorhinolaryngology - Head and Neck Surgery and Audiology, Rigshospitalet, Copenhagen, Denmark, [4]Dept. of Biomedicine, Aarhus University, Aarhus, Denmark, [5]Dept. of Forensic Medicine, Aarhus University, Aarhus, Denmark

Background: Bone remodeling is an ongoing process that replaces old bone tissue with new bone tissue. Bone remodeling consists of four phases; activation, initial resorption, reversal-resorption, and formation. Although much is known about the resorption and formation phases, the activation phase, which initiates initial osteoclastic bone resorption, remains poorly understood. Activation of osteoclasts is regulated by osteoblast lineage cells through the expression of receptor activator of NF-κB ligand (RANKL) and its decoy receptor osteoprotegerin (OPG). Throughout life, the perilymphatic space containing the inner ear structures is protected from bone resorption and thus bone turnover, which in rodents (and other species) has been linked to a high local expression of O*pg*. Focal loss of this protection may result in pathological remodeling, as seen in otosclerosis. Therefore, the temporal bone of the inner and middle ear provides a unique model to study the events that activate osteoclastic bone resorption.

Aim: To investigate the spatial in situ expression of *Rankl* and *Opg* within the rat temporal bone of the inner and middle ear with variable regulation of osteoclast activation.

Methods: The study was conducted on rats aged 10 days, 19 weeks, and 8 months (n=5/age). The temporal bones were fixed, decalcified, paraffin-embedded, and sectioned. The sections were stained with fluorescence *in situ* hybridization (FISH) with probe pairs targeting *Opg* and *Rankl* mRNA. Undecalcified bone from the middle and inner ear was investigated using microcomputed tomography.

Results: The microcomputed tomography images revealed the highly complex structure of the ear. In the inner ear, the study confirmed that fibrocytes within the spiral ligament of cochlear had an extremely high local expression of *Opg.* Interestingly, the osteocytes embedded in the surrounding bony wall of the cochlear had minimal *Opg* expression. *Rankl* was expressed by the bone surface cells within the bulla wall of the middle ear at day 10 and was absent at later time points. A high expression of *Opg* was found surrounding the tympanic membrane (eardrum). Again, *Opg* was not present in the osteocytes, and in general, *Rankl* was absent. In the bulla wall of the middle ear, the inner resorptive side had higher *Rankl* expression and lower *Opg* expression. In comparison, the outer formative side had low *Rankl* expression and higher *Opg* expression during modeling drift/growth of the bulla wall.

Conclusion: The study is still ongoing and demonstrates a distinctive protective mechanism against osteoclastic bone resorption in the inner ear and around the tympanic membrane, characterized by remarkably high local *Opg* expression. In contrast, the bulla wall displays elevated *Rankl* expression and reduced *Opg* expression at the inner resorptive side allowing for bone drift of this bone. As a future perspective, lactating rats would be studied to test the hypothesis that this unique protection would still be maintained.

Investigation of the temporal changes during anti-sclerostin antibody treatment in mice using time-lapse µCT

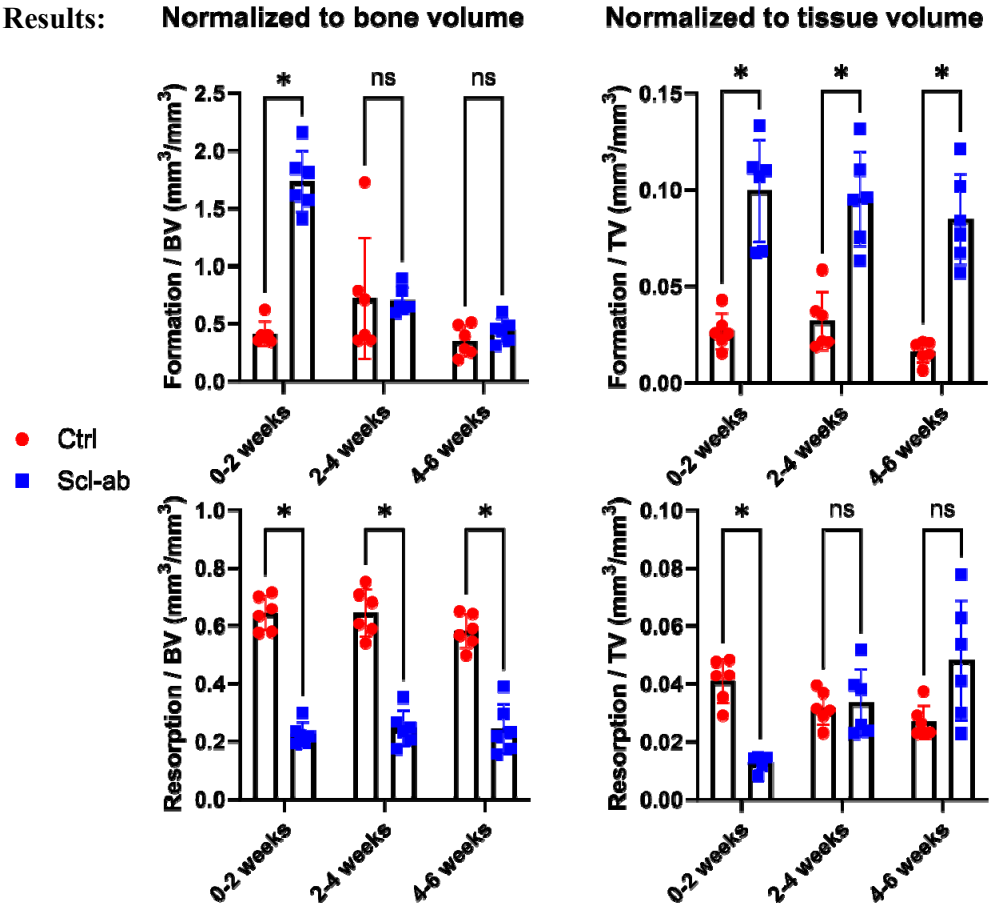
Frederik D. Bromer,[1] Annemarie Brüel,[1] Jesper S. Thomsen[1]

[1]*Dept. of Biomedicine, Aarhus University, Aarhus, Denmark*

Background: Anti-sclerostin antibody (Scl-ab) treatment is the newest type of anti-osteoporosis therapy approved for clinical use. Recent studies have indicated that the effect of treatment tends to decrease over time. Therefore, we wanted to investigate the temporal changes in bone in mice treated with anti-sclerostin antibody.

Methods: Twelve C57BL/6JBomTac female mice were injected subcutaneously with either Scl-ab (25 mg/kg) or vehicle (Ctrl) twice weekly for 6 weeks. The distal femur underwent in-vivo µCT (voxel size = 10.4 μm^3) every two weeks starting from day 0. Bone formation and resorption were determined at the metaphyseal trabeculae from superimposed µCT images at succeeding time points.

Results:



Conclusion: Scl-ab induced a constant and significant increase in bone formation relative to tissue volume (TV) during the 6 weeks. However, when normalized to existing bone volume (BV), the effect of Scl-ab treatment appeared to diminish due to the massive increase in BV after the first two weeks. As bone volume increases, a constant absolute increase will have a relatively smaller impact on the overall amount of bone. Furthermore, Scl-ab induced a constant and significant decrease in bone resorption when normalized to BV, whereas this was not the case when normalized to TV. As the absolute amount of bone increased in Scl-ab mice with time, a constant relative loss of bone will result in an increase in the absolute bone loss.

Balanced basic multicellular unit activity in cortical bone of ovariohysterectomized rabbits

Lindsay L. Loundagin,[1] Kim D. Harrison,[1] David M.L. Cooper[1]

[1]*Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, Saskatoon, Canada*

Background: The characteristic bone loss in postmenopausal osteoporosis is often attributed to an elevated remodeling rate at the organ-level and imbalanced remodeling activity at the level of basic multicellular units (BMU). The latter is considered negative BMU balance, in which the volume of bone resorption is greater than that formed per remodeling event. Indeed, recent work with an ovariohysterectomy (OVH) rabbit model of osteoporosis demonstrated a respective 2.4- and 6.4-fold increase in cortical porosity and activation frequency, but it is unclear if a negative BMU balance also contributes to OVH-induced elevated porosity. Furthermore, it is unknown how remodeling dynamics (e.g. rate of resorption and formation) may be altered to either maintain or disrupt BMU balance.

Aim: To investigate the spatial and temporal balance between bone resorption and formation within intracortical BMU-related remodeling spaces in an OVH rabbit model of osteoporosis.

Methods: Ten New Zealand white rabbits (six-months old) were randomly assigned to a treatment or control group which received OVH or SHAM surgery, followed by a 10-week recovery period. To track individual remodeling events, the distal right tibia from each rabbit was first imaged *in vivo* at the Canadian Light Source using in-line phase contrast microCT (voxel size=13 μm), then again *ex vivo* post-mortem two weeks later using desktop microCT (voxel size=5 μm). The 3D datasets were co-registered and longitudinal erosion rate (LER) was calculated as the distance traversed by BMU cutting-cones in the 14-day interval between scans. The radial profile of the remodeling space was derived from its 3D morphology and automatically partitioned into a resorption and formation zone based on the maximum radius. The balance between resorption and formation was assessed by maximum radius, canal radius and wall thickness of the remodeling space. The length of each zone and LER were used to calculate the duration of resorption and formation, as well as the radial infill rate.

Results: In OVH rabbits, 130 remodeling spaces were randomly selected for evaluation while only 27 remodeling spaces in total were present for evaluation in SHAM rabbits. The maximum radius of remodeling spaces in OVH rabbits was larger than that in SHAM (52.72 μm vs 36.86 μm; $p \leq 0.001$) while canal radius was similar between groups (13.35 μm vs 13.38 μm; $p=0.971$). This resulted in a higher wall thickness for OVH (39.19 μm vs 26.21 μm; $p \leq 0.001$). Progression of the cutting cone did not differ as the median LER was 41.3 and 40.1 μm/day in OVH and SHAM and radial resorption rate was 11.2 and 10.6 μm/day ($p=0.418$), respectively. The time for the resorption phase to pass through a cross section was 4.2 and 3.5 days for OVH and SHAM, respectively ($p=0.098$). Formation duration was 50% longer in OVH than SHAM (21.0 vs 13.2days; $p \leq 0.001$) but the radial infill rate was nearly identical between groups (OVH=2.1 μm/day; SHAM=2.0 μm/day; $p=0.971$).

Conclusion: Although remodeling spaces were larger in OVH rabbits, the increased resorptive activity was accompanied by increased formation such that BMUs remained balanced to an extent similar to SHAM BMUs. Maintaining BMU balance came at the cost of a longer formation phase as the rate of radial infill was unchanged. These results suggest that the increased porosity observed in this OVH rabbit model is predominately due to increased activation of remodeling events rather than negative BMU balance. This is in contrast to the negative BMU balance observed in human osteoporotic bone and may be due to the young age or earlier disease stage of these rabbits.

Preparation of cylindrical bone samples for high-resolution X-ray computed tomography

N.K. Wittig,[1] C. Pedersen,[1] J. Palle,[1] M. Østergaard,[1] T.E.K. Christensen,[1] M. Kahnt,[2] A. Sadetskaia,[1] J.S. Thomsen,[3] A. Brüel,[3] H. Birkedal[1]

[1]Dept. of Chemistry and iNANO, Aarhus University, Aarhus C, Denmark, [2]MAX IV Laboratory, Lund University, Lund, Sweden, [3]Dept. of Biomedicine, Aarhus University, Aarhus C, Denmark

Background: X-ray computed tomography (CT) encompasses a powerful set of techniques for studying bone structure. However, they depend on preparation of suitable samples – an especially demanding task for high-resolution studies using sub micro-computed tomography (µ-CT). As the resolution is increased, the FOV decreases, making it crucial to develop ways to prepare tiny samples. Moreover, an ideal sample is cylindrical with a diameter smaller than the field of view (FOV) of the experiment, and the specific research questions being addressed may require that the samples are extracted from precise locations within the whole-bone context. In practice this has been a steep challenge to achieve.

Aim: Development of protocols for preparation of small cylindrical bone samples for sub µ-CT.

Methods: A combination of subtractive sample preparation methods (diamond-wheel cutting, micro-lathe (µ-lathe) milling and focused ion beam milling) and inspection techniques (optical microscopy and laboratory µ-CT).

Results: Two µ-lathe based sample preparation workflows were developed to prepare bone samples for two different types of CT experiments in terms of setup, research question, and sample requirements (see Figure 1). The first experiment investigates the effect of secondary hyperparathyroidism on the lacuno-canalicular network with high-throughput synchrotron µ-CT, requiring preparation and scanning of equivalent samples from many specimens. This was achieved through a sequence of cutting and embedding steps finalized by lathe milling to give samples with homogeneity in bone site, shape, and size – matched to the FOV of the experiment. This improved the success rate of sample preparation as well as the quality of the synchrotron µ-CT data. The second experiment, investigating the interface between osteonal and interstitial bone with combined nano-CT (ptychography) and X-ray fluorescence nano-CT, required preparation of a few-micrometer-sized sample from a localized position within the whole bone. This was achieved by incorporation of laboratory CT measurements at critical points along the preparation pathway, which included both lathe milling and focused ion beam milling, to keep track of the feature of interest and guide the process.

Conclusion: The two workflows provide examples of preparation pathways that can be standardized and strongly increase the throughput, quality, and success rate of CT experiments.

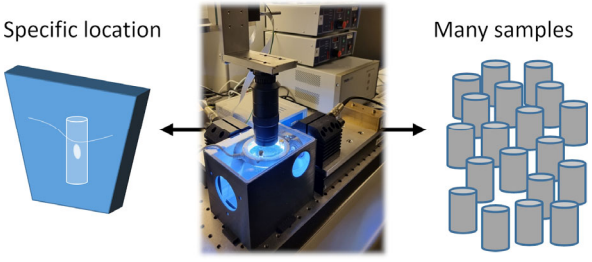


Figure 1. A custom built µ-lathe is incorporated in two separate workflows to prepare cylindrical bone samples from specific locations within the whole-bone context (left) and for consistent high-throughput sampling of many specimens for comparative studies (right).

SIPH- A Scandinavian Fairytale

Mikkel Pretorius

Oslo University & Oslo University Hospital, Norway

Case-series of adults with X-linked hypophosphatemia suffering from end stage renal disease (ESRD)

Trine E. Finnes,[1,2] Ansgar Heck[1,2]

[1]Oslo University Hospital, [2]University of Oslo

X-linked hypophosphatemia (XLH) has an incidence of 1 in 20 000 births. Presently, > 460 mutations related to XLH have been stated in the literature. The genotype-phenotype correlation is weak, and it is thus challenging to predict prognosis and outcome. Although the clinical appearance and symptoms are dominated by musculoskeletal alterations and pain, chronic kidney disease (CKD) is a feared complication. The development of nephrocalcinosis and CKD is associated with the conventional treatment of the disease. However, fibroblast growth factor 23 (FGF 23), the key protein in the pathogenesis of the disease is described to be associated with increased risk of cardiovascular outcomes in CKD, independent of calcium, phosphate, and parathyroid levels. In contrast to CKD in non-XLH-patients, the FGF-23 levels are already high at early age in XLH. It is unclear to which degree the high levels of FGF-23 contributes to cardiovascular disease and thereby increases the risk of CKD secondary to cardiovascular disease. We here describe time course, treatment, results from kidney biopsies and cardiovascular co-morbidity in six adult patients with XLH and ESRD identified in a register study in eastern Norway.

Akromegali: Trabecular Bone Score and Hip Structural Analyses in patients during treatment of Acromegaly

Ansgar Heck

Oslo University Hospital, Norway

Time-lapsed 4D Tracking of Cortical Bone Remodeling in the Rabbit: What Have We Learned so Far?

D.M.L. Cooper,[1] K. Harrison,[1] L.L. Loundagin,[1] Xuan Wei[1]

[1]*Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, Saskatoon, Canada*

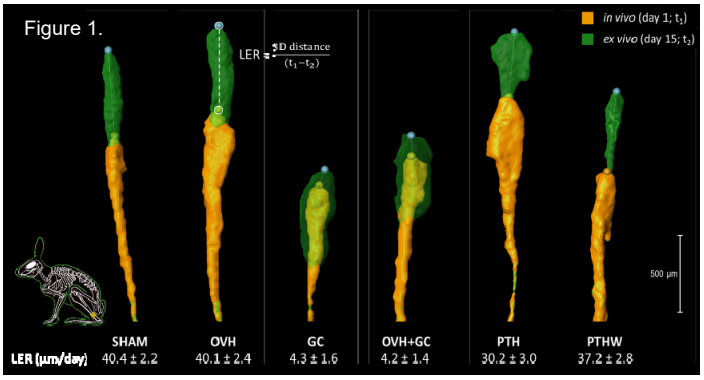
Background: Histomorphometry based upon fluorochrome labelling has long provided a means of tracking dynamic bone formation within the discrete basic multicellular units (BMUs) which carry out remodeling. Conversely, a methodology for tracking bone resorption at this level has been lacking. Although four-dimensional (4D) time-lapsed *in vivo* micro-CT imaging of trabecular bone was first reported nearly 20 years ago, applications of this approach remain rare and applications to cortical bone porosity even more so. The central challenge presented by micro-CT is the non-linear relation between resolution and radiation dose. To overcome this, our team developed a synchrotron-based in-line phase contrast micro-CT approach to maximize detection of cortical pores, including BMU-related remodeling spaces, in rabbits while minimizing radiation dose (~2 Gy).

Aim: This presentation provides a summary of our recent 4D analyses of BMU longitudinal erosion rate (LER) in several rabbit models of elevated cortical bone remodeling/porosity.

Methods: The common experimental platform utilized 6-month-old female New Zealand White rabbits which were imaged: 1) *in vivo* at the BioMedical Imaging and Therapy facility of the Canadian Light Source synchrotron (t_1 voxel size=13 µm); 2) *ex vivo* laboratory micro-CT (t_2 voxel size=5 µm) two weeks later. LER (µm/day) was assessed by manual landmarking of cutting cone tips in the co-registered datasets (see Figure 1 below). Experimental groups have included ovariohysterectomy (OVH), sham OVH (SHAM), glucocorticoid-dosed (GC), OVH+GC, parathyroid hormone (PTH) and PTH withdrawal (PTHW).

Results: Representative time-lapsed 4D renderings of remodeling spaces are provided in Figure 1 with the *in vivo* scan depicted in orange and the *ex vivo* scan in green. Mean LER +/- standard deviations are also presented. LER values for SHAM and OVH animals matched the 40 µm/day value inferred from the advance of the BMU closing cones in classic studies of LER in canines. Active daily PTH dosing reduced LER, whereas LER values increased to nearly SHAM levels in PTHW animals in the two weeks immediately following PTH withdrawal. Glucocorticoids, alone or in combination with OVH, disrupted the advance of BMUs, resulting in radially expanding remodeling spaces.

Figure 1.



Conclusion: These findings demonstrate the utility of the 4D time-lapsed approach for the study of BMU dynamics, particularly spatio-temporal behaviors that have been largely inaccessible. We believe this approach, combined with advanced histological techniques and spatial-omics, hold great potential for further unravelling the regulation of BMU activity and combatting related diseases.

[a]*Harrison, KD, et al. (2022) JBMR 37(11), 2244-2258.* [b]*Cooper, DML, et al. (2023) Bone. 176, 116864.* [c]*Loundagin, LL, et al. (2023) JBMR 38(Suppl 1).https://tinyurl.com/Loundagin-ASBMR2023*

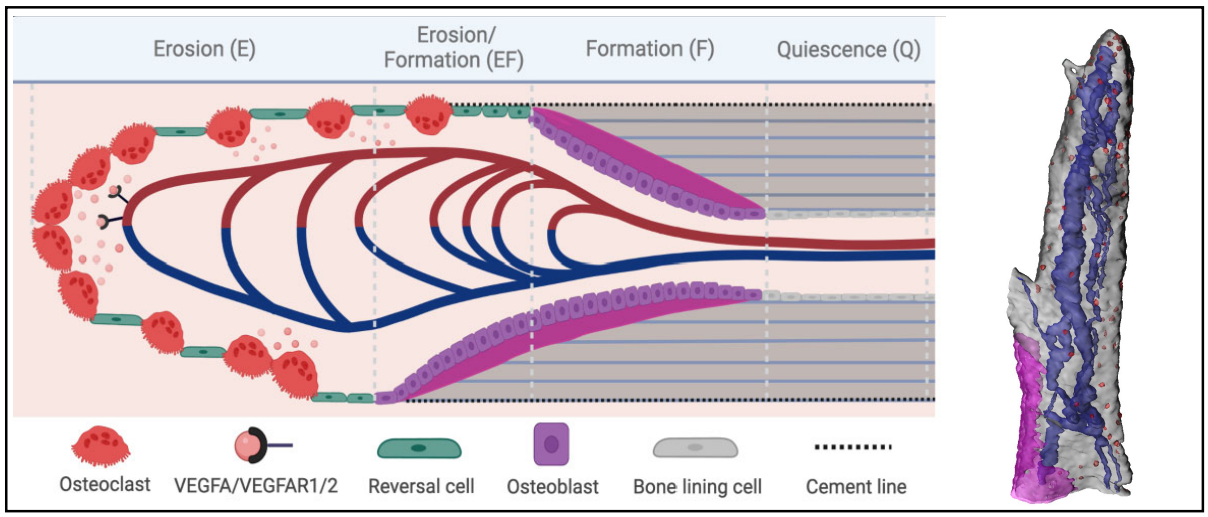
Decoding Cortical Bone Remodeling: The Importance of Osteoprogenitors and Vascular Networks

Christina M. Andreasen[1,2]

[1]*Molecular Bone Histology lab, Research Unit of Pathology, University of Southern Denmark, Denmark,* [2]*Dept. of Pathology, Odense University Hospital, Denmark*

Bone remodeling is a dynamic process that maintains skeletal health by replacing old bone tissue with new. During this process, osteoprogenitors play a pivotal role in initiating bone formation. Osteoprogenitors are spatiotemporal cells found within the bone microenvironment. They are recruited during the reversal-resorption phase, where osteoclasts resorb old bone. These osteoprogenitors differentiate into osteoblastic reversal cells, which serve as precursors for bone-forming osteoblasts. The initiation of bone formation depends on achieving a critical density of these osteoblastic reversal cells. Proliferation and recruitment of osteoprogenitors contribute to reaching this threshold. Naturally, blood vessels are integral to bone remodeling as the vascular network within the bone acts as a conduit, facilitating the migration and colonization of osteoprogenitors during this dynamic process.

Collectively, cortical bone, characterized by its compact structure and isolated haversian canals, provides an optimal setting for studying osteoprogenitor recruitment and exploring the significance of the vascular network.



Andreasen C et al. Bone 173:116787, 2023

Danish Diabetes and Endocrine Academy

Funded by the Novo Nordisk Foundation

Are you an early-career researcher within the research fields of **diabetes**, **metabolism** and/or **classical endocrinology**? Are you seeking funding for your research, excellent education activities or new collaborations? Then take a look at some of Danish Diabetes and Endocrine Academy's activities in 2024.

30 April-3 May *Odense, DK*	PhD course and Symposium on Metabolic Bone Disease
6-8 May *Aalborg, DK*	Intermediate Course on Reproducible Research in R
30-31 May *Copenhagen, DK*	Symposium on Neuroendocrine Control of Energy Metabolism
June	Call for DDEA PhD Scholarships & Postdoc Fellowships
26-29 August *Ebberup, DK*	DDEA PhD Summer School on Diabetes, Metabolism & Endocrinology
23-27 September	DDEA Postdoc Summit
October *Copenhagen, DK*	Neuroendocrine Tumours – What's Hot and Important
21-24 October *Nyborg, DK*	PhD course on Basic Cardiometabolic Research
December *Odense, DK*	Advanced Course on Reproducible Research in R

We have many more symposia, networking events and workshops with renowned experts that we encourage PhD students and Postdocs to participate in.

Read more and sign up at www.ddeacademy.dk. We hope to see you!

Participants

Name	Affiliation	E-mail
Rasmus Aabling	Dept. of Clinical Medicine, Comparative Med Lab, AUH	rasaab@clin.au.dk
Joudi Altaleb	Imperial College London, United Kingdom	joudi.altaleb22@imperial.ac.uk
Signe Marie Andersen	DanSIC, SDU	sandersen@health.sdu.dk
Thomas Levin Andersen	Molecular Bone Histology (MBH) lab, SDU/OUH/AU	Thomas.levin.andersen@rsyd.dk
Christina Møller Andreasen	Molecular Bone Histology (MBH) lab, SDU	cmandreasen@health.sdu.dk
Amel Batouche		
Helena M. Bardenfleth	Department of Endocrinology & Internal Medicine, AUH	hebard@rm.dk
Henrik Birkedal	iNANO and Department of Chemistry, AU	hbirkedal@chem.au.dk
Julie Bjerrelund	Department of Molecular Endocrinology, KMEB, OUH	jbjerrelund@health.sdu.dk
Jens Bollerslev	Faculty of medicine, University of Oslo, Oslo, Norway	jens.bollerslev@medisin.uio.no
Ulla Bollerslev	Oslo, Norway	
Frederik Duch Bromer	Department of Biomedicine, AU	fd@biomed.au.dk
Marta Díaz del Castillo	MHB, Department of Forensic Medicine, AU	marta@forens.au.dk
Tore Christiansen	Danish Diabetes and Endocrine Academy	tore.christiansen@rsyd.dk
David Cooper	University of Saskatchewan, Canada	dml.cooper@usask.ca
Iman Diab	Molecular Bone Histology Lab (MBH-Lab), SDU	imdia18@student.sdu.dk
Sofie Elkjær	Department of Molecular Endocrinology, KMEB, OUH	selkjaer@health.sdu.dk
Bilal Mohamad El-Masri	Molecular Bone Histology Lab (MBH-Lab), SDU	elmasri@health.sdu.dk
Lejla Emini		
Florence Figeac	Department of Molecular Endocrinology, KMEB, OUH	ffigeac@health.sdu.dk
Trine Elisabeth Finnes	Oslo University Hospital & University of Oslo, Norway	b16330@ous-hf.no
Anja Lisbeth Frederiksen	Dept. of Clinical Genetics OUH/AAUH	anja.lisbeth.frederiksen@rn.dk
Anne Marie Møller Faaborg	iNANO and Department of Chemistry, AU	amf@inano.au.dk
Søren Gam	Overvægt, Diabetes og Hormonsygd., Esbjerg Sygehus	soren.gam@rsyd.dk
Jannie Dahl Hald	Department of Endocrinology & Internal Medicine, AUH	jannie.hald@midt.rm.dk
Nina Wærling Hansen	Department of Molecular Endocrinology, KMEB, OUH	nwhansen@health.sdu.dk
Torben Harsløf	Department of Endocrinology & Internal Medicine, AUH	torbhars@rm.dk
Ansgar Heck	Rikshospitalet, Oslo University Hospital, Norway	ansgar.heck@medisin.uio.no
Anne-Marie Heegaard	Department of Drug Design and Pharmacology, KU	amhe@sund.ku.dk
Chelsea Hopkins	Department of Drug Design and Pharmacology, KU	chelsea.hopkins@sund.ku.dk
Ching-Hsin Huang		
Abbas Jafari	Department of Cellular and Molecular Medicine KU	ajafari@sund.ku.dk
Jens-Erik Beck Jensen	Hvidovre University Hospital, Endocrine Department	jens-erik.beck.jensen@regionh.dk
Andrea René Jørgensen	Department of Clinical Medicine, Orthopedic Surgery, AUH	anjo@clin.au.dk
Hanne Skou Jørgensen	Institute of Clinical Medicine, AU & AAUH	hsjorgensen@clin.au.dk
Fatima Khassouk	Molecular Bone Histology (MBH) lab, SDU & AU	fatimakhassouk@outlook.com
Viktoria Kofod	Molecular Bone Histology Lab (MBH-Lab), SDU	vikof19@student.sdu.dk
Annika Vestergaard Kvist	SDU & Steno Diabetes Center Nordjylland	annika.k@rn.dk
Bente Langdahl	Endocrinology, Aarhus University Hospital	bente.langdahl@aarhus.rm.dk
Kaja Søndergaard Laursen	Department of Forensic Medicine, AU	kasl@forens.au.dk
Natasha Laursen	Department of Endocrinology & Internal Medicine, AUH	natalu@rm.dk
Eun Jung Lee	Korea University College of Medicine, Seoul, Korea	ejlee3365@korea.ac.kr
Mette Bøegh Levring	SDU and Department of Hematology, OUH	mette.boegh.levring@rsyd.dk
Mei-Chun Lin	University Medical Centre Hamburg-Eppendorf, Germany	m.lin@uke.de
Jakob Starup Linde	Department of Endocrinology & Internal Medicine, AUH	Jakob.Linde@auh.rm.dk
Karina Nørgaard Linde	Department of Clinical Medicine, Orthopedic Surgery, AUH	karina.linde@clin.au.dk
Lindsay Loundagin	University of Saskatchewan, Canada	ll.loundagin@usask.ca
Maria Lerche Mace	Department of Nephrology, Rigshospitalet, Copenhagen	maria.lerche.mace@regionh.dk
Kaja Madsen	Department of Molecular Endocrinology, KMEB, OUH	Kajamadsen@health.sdu.dk
Vivi Mäkinen	Department of Endocrinology & Internal Medicine, AUH	VIVMAA@rm.dk
Petar Milovanovic	Center of Bone Biology, University of Belgrade, Serbia	drpmilovanovic@gmail.com
Malene H Nielsen	Molecular Bone Histology Lab (MBH-Lab), SDU	malene.hykkelbjerg.nielsen@rsyd.dk
Morten Frost Nielsen	Department of Endocrinology / KMEB LAB, OUH	morten.munk.frost.nielsen@rsyd.dk
Pernille Yde Ø Nielsen	Ossiform ApS, Odense	po@ossiform.com
Simone Snitgård R Nielsen	Clinical Cell Biology, Pathology Research Unit, SDU, OUH	Simone.Snitgard.Rosendal.Nielsen@rsyd.dk
Anders Nordholm	Department of Nephrology, Rigshospitalet, Copenhagen	anders.nordholm@regionh.dk
Mia Nymark	Molecular Bone Histology Lab (MBH-Lab), SDU	minym21@student.sdu.dk
Serkin Park	Korea University College of Medicine, Seoul, Korea	serkin@korea.edu
Inge Søkilde Pedersen	Clinical Laboratory Geneticist, Aalborg University Hospital	isp@rn.dk
Michael Pedersen	Dept. of Clinical Medicine, Comparative Med Lab, AUH	michael@clin.au.dk
Nichole Pedersen	Molecular Bone Histology Lab (MBH-Lab), SDU	nichp19@student.sdu.dk
Mathias Flensted Poulsen	Department of Biomedicine, AU	mfp@biomed.au.dk
Mikkel Pretorius	Oslo University Hospital, Dep for Endocrinology, Norway	mikkel.pretorius@gmail.com
Alexander Rauch	Department of Molecular Endocrinology, KMEB, OUH	arauch@health.sdu.dk
Lars Rejnmark	Department of Endocrinology & Internal Medicine, AUH	lars.rejnmark@rm.dk
Amal Roble		
Anastasiia Sadetskaia	iNANO and Department of Chemistry, AU	asad@inano.au.dk
Clarissa Schmal	Department of Cellular and Molecular Medicine, KU	cschmal@sund.ku.dk
Tanja Sikjær	Department of Endocrinology and Internal Medicine, AUH	Tanja.sikjaer@gmail.com
Sabine Lykke Skjøtt	Molecular Bone Histology Lab (MBH-Lab), SDU	saskj21@student.sdu.dk
Kent Søe	Clinical Cell Biology, Pathology Research Unit, SDU, OUH	kent.soee@rsyd.dk
Anne Sophie Sølling	Department of Endocrinology & Internal Medicine, AUH	annesoel@rm.dk
Martin Sønderbøl	Molecular Bone Histology Lab (MBH-Lab), SDU	msjc@forens.au.dk
Maiken Stilling	Department of Clinical Medicine, Orthopedic Surgery, AUH	maiken.stilling@clin.au.dk
Mads Thomassen	Research unit of Clinical Genetics, OUH	Mads.Thomassen@rsyd.dk
Jesper Skovhus Thomsen	Dept. of Biomedicine, Aarhus University	jst@biomed.au.dk
Lisbeth Koch Thomsen	Molecular Bone Histology Lab (MBH-Lab), SDU	lkochthomsen@health.sdu.dk
Sarah Thornhøj	Department of Endocrinology & Internal Medicine, AUH	sarthorn@rm.dk

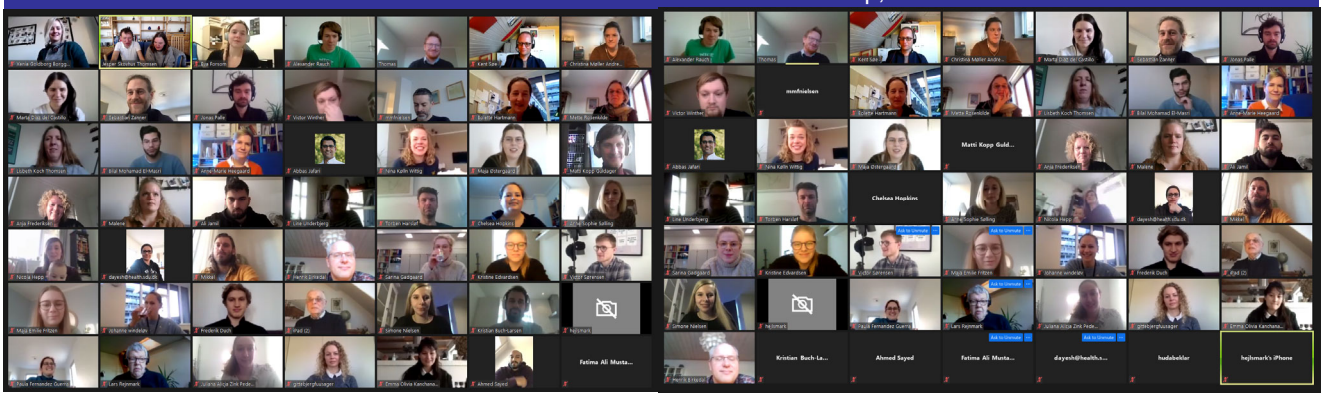
Louise Tjelum	Nordsjællands Hospital, Endo. & Nefrologisk afdeling	louise.tjelum@regionh.dk
Melisa Kalkan Uguz	Molecular Bone Histology Lab (MBH-Lab), SDU	mekal18@student.sdu.dk
Line Underbjerg	Department of Endocrinology & Internal Medicine, AUH	linund@rm.dk
Birgitte Villadsen	Molecular Bone Histology Lab (MBH-Lab), SDU	bvilladsen@health.sdu.dk
Cecilie Mosevang Vinten	Clinical Cell Biology, Pathology Research Unit, SDU, OUH	cevin21@student.sdu.dk
Catherine Williams	Department of Animal & Veterinary Sciences, AU	catherine.williams@anivet.au.dk
Nina Kølln Wittig	iNANO and Department of Chemistry, AU	nkw@inano.au.dk
Eva Maria Wölfel	Department of Molecular Endocrinology, KMEB, OUH	ewolfel@health.sdu.dk
Wafaa Ahmed Yassin	Molecular Bone Histology Lab (MBH-Lab), SDU	wayas21@student.sdu.dk
Ina Karstoft Ystrøm	Department of Renal Medicine, AUH	inakje@rm.dk
Maja Østergaard	iNANO and Department of Chemistry, AU	majaoester@inano.au.dk

1st Danish Bone Research Workshop at Sandbjerg, 2018

2nd Danish Bone Research Workshop at Sandbjerg, 2019

3rd Danish Bone Research Workshop at Sandbjerg, 2020

4th virtual Danish Bone Research Workshop, 2021



5th Danish Bone Research Workshop at Sandbjerg, 2022

6th Danish Bone Research Workshop at Sandbjerg, 2023