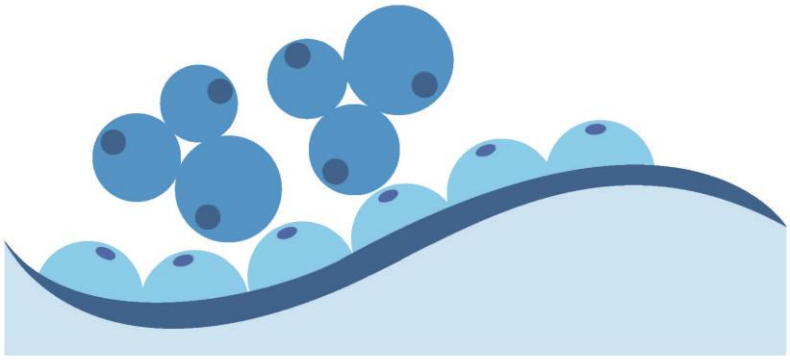
2ND BONE MARROW ADIPOSITY MEETING

Rotterdam, the Netherlands
August 25 + 26, 2016



BMA 2016

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DEPARTMENT OF INTERNAL MEDICINE, ERASMUS MC, ROTTERDAM

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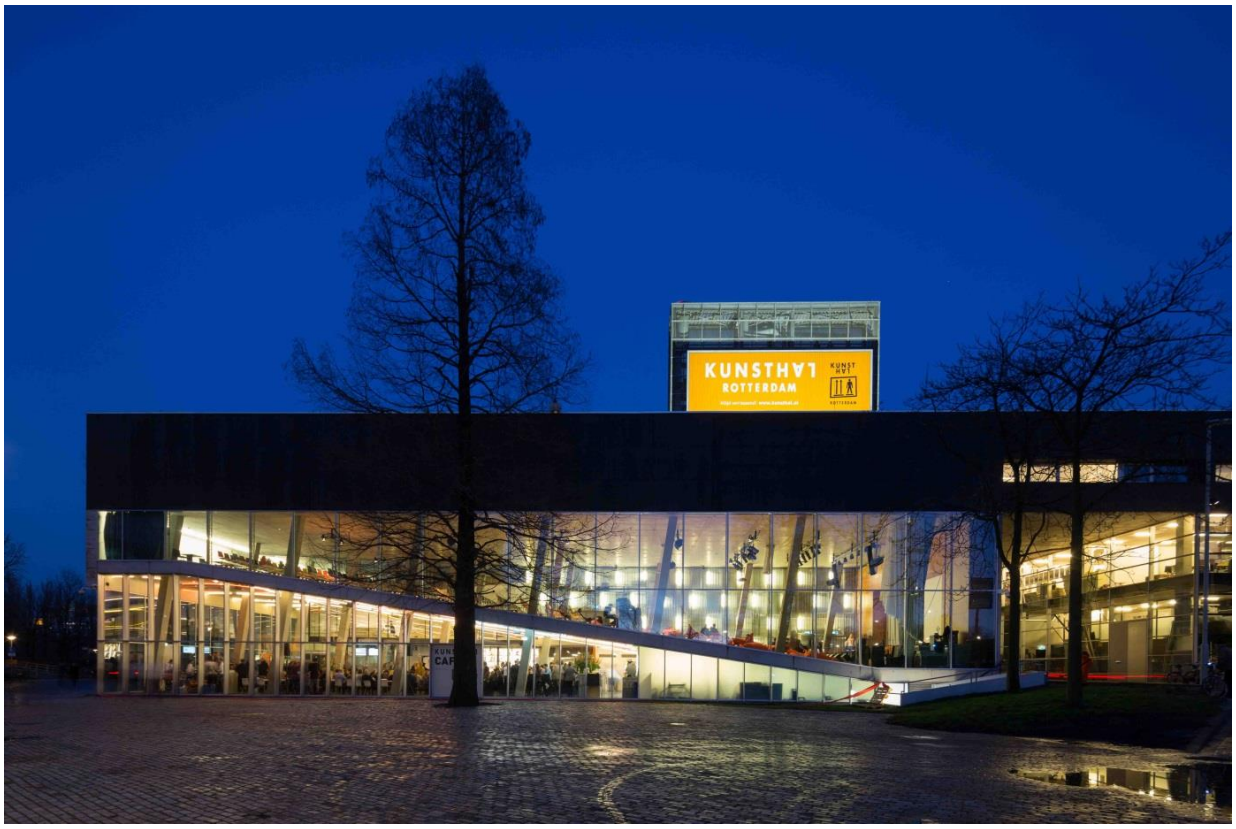

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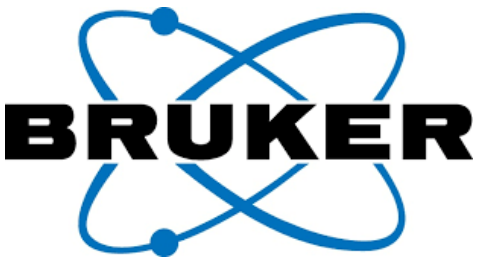

PROGRAM

Thursday, August 25th, 2016

7.30-08.30 ***REGISTRATION***

8.30-08.45 ***Welcome and introduction:*** **Hans van Leeuwen**

SESSION I:
Is there a link between bone marrow fat and peripheral fat?

Chairs: Christophe Chauveau and Patric Delhanty

08.45-09.15 ***Invited speaker 1:*** **Will Cawthorn** *p.11*
Bone marrow adipose tissue: starving for attention?

09.15-09.45 ***Invited speaker 2:*** **Peter Arner** *p.12*
Is there a link between bone marrow fat and peripheral fat?

09.45-10.00 ***Short oral 1:*** **Urszula Iwaniec** *p.23*
Housing growing mice at thermoneutral temperature (32°C) increases cancellous bone volume fraction and marrow adiposity.

10.00-10.15 ***Short oral 2:*** **Eudes-Brian Mvoula** *p.24*
Down regulation of Sirtuin type 1 (Sirt1) expression increases marrow adiposity by inducing lipid storage and glucocorticoid metabolism and by acetylation of Runx2 and FOXO1 in bone marrow of anorexia nervosa mouse model.

10.15-10.30 ***Short oral 3:*** **Erica Scheller** *p.25*
Leptin-induced loss of marrow adipose tissue is mediated by sympathetic and sensory neurotransmission.

10.30-11.15 ***NETWORKING BREAK: ODD POSTERS***

SESSION II:
What happens during differentiation of an adipocyte in bone marrow?

Chairs: Aline Clabaut and Jeroen van de Peppel

11.15-11.45 ***Invited speaker 3:*** **Mara Riminucci (in honour of Paolo Bianco)** *p.13*
Marrow adipocytes and fibrous dysplasia of bone.

11.45-12.15 ***Invited speaker 4:*** **Pamela Robey** *p.14*
Regulation of the fate of skeletal stem cells, the common precursors of bone, stroma and marrow adipocytes.

12.15-12.45 ***Invited speaker 5:*** **Saverio Cinti** *p.15*
The Adipose Organ.

12.45-13.00 ***Short oral 4:*** **Michaela Reagan** *p.26*
Bone Marrow Adiposity is Modulated by Osteocyte-Derived Molecules including Sclerostin.

13.00-13.15 ***Short oral 5:*** **Phil Salmon** *p.27*
Calcified and noncalcified biological tissue imaging and analysis by MicroCT, and the case of bone marrow.

13.15-14.00 ***LUNCH***

SESSION III:
Are all bone marrow adipocytes equal?
Chairs: Pierre Hardouin and Andre van Wijnen

14.00-14.30 ***Invited speaker 6:*** **Dimitrios Karampinos** *p.16*
MRI of bone marrow adiposity.

14.30-15.00 ***Invited speaker 7:*** **Jeanine Prompers** *p.17*
Functional imaging of bone marrow adiposity.

15.00-15.30 ***Invited speaker 8:*** **Clifford Rosen** *p.18*
Site Specific Differences in Bone Marrow Adipocytes:
Are all marrow adipocytes equal?

15.30-15.45 ***Short oral 6:*** **Alessandra Bierwagen** *p.28*
Measurement of Red and Yellow Bone Marrow Fat Composition using MRI and MRS.

15.45-16.30 ***NETWORKING BREAK: ODD POSTERS***

SESSION IV:
Short Orals
Chairs: Olaia Naveiras and Hans van Leeuwen

16.30-16.45 ***Short oral 7:*** **Meghan McGee** *p.29*
Dietary Kynurenine, the oxidized metabolite of tryptophan, suppresses osteoprogenitor expression of Hdac3 resulting in increased marrow adiposity and age-related bone loss.

16.45-17.00 ***Short oral 8:*** **Jason Horton** *p.30*
Limited field radiotherapy induces local and abscopal effects on bone structure and marrow adiposity.

17.00-17.15 ***Short oral 9:*** **Gina Woods** *p.31*
Are Sex Hormones Negatively Associated with Vertebral Bone Marrow Fat?

17.15-17.30 ***Short oral 10:*** **John Diedrich** *p.32*
Bone Marrow Adipocytes Alter the Metabolic Phenotype of Metastatic Prostate Cancer Cells Through Activation of HIF-1α.

17.30-17.45 ***Short oral 11:*** **Celine Gillet** *p.33*
Bone marrow derived-MSC from non-traumatic osteonecrotic patients display a critical susceptibility to lipotoxicity.

17.45-18.00 ***Short oral 12:*** **Andre van Wijnen** *p.34*
Molecular differences in Mesenchymal Stromal Cells from Adipose versus Bone Marrow Tissue

18:00 ***GROUP PHOTO***

19.00-22.00 ***NETWORKING DINNER IN HOTEL NEW YORK***

Friday, August 26th, 2016

SESSION V:
Is there a role for bone marrow adipocytes and hematopoietic regeneration and cancer metastasis?

Chairs: Claire Edwards and Marjolein van Driel

08:30-9.00	**Invited speaker 9: Olaia Naveiras** Bone marrow adiposity in regenerative hematopoiesis.	*p.19*
09.00-09.30	**Invited speaker 10: Izabela Podgorski** Bone marrow adipocytes and bone metastasis.	*p.20*
09.30-09.45	***Short oral 13:*** **Adrien Guerard** The medullary adipocytes contribute to the bone metastasis of prostate cancer and this effect is regulated by obesity.	*p.35*
09.45-10.00	***Short oral 14:*** **Manar Shafat** Leukemic bone marrow adipocytes preserves and regulates proliferative leukemic blasts via FAPB4 and CPT1.	*p.36*
10.00-10.15	***Short oral 15:*** **Emma Morris** Multiple myeloma regulates bone marrow adipocyte number, localisation and adipokine secretion.	*p.37*
10.15-11.00	***NETWORKING BREAK: ALL POSTERS***	

SESSION VI:
Do bone marrow adipocytes influence skeletal health?

Chairs: Mark Horowitz and Bram van der Eerden

11.00-11.30	***Invited speaker 11:*** **Guillaume Penel** Do bone marrow adipocytes influence skeletal health: A lipid story?	*p.21*
11.30-12.00	***Invited speaker 12:*** **Peter Bisschop** Marrow adipose tissue and human bone health.	*p.22*
12.00-12.15	***Short oral 16:*** **Eleni Douni** Analysis of bone marrow adiposity in human RANKL-expressing transgenic mouse models of osteoporosis.	*p.38*
12.15-12.30	***Short oral 17:*** **Beate Lanske** PTH1R deficient mesenchymal stem cells favor adipogenesis over osteogenesis in vivo.	*p.39*
12.30-12.45	***Short oral 18:*** **Mara Riminucci** Marrow adipocytes and fibrous dysplasia of bone.	*p.40*
12.45-13.15	***STATUS OF BONEAHEAD:*** **Pierre Hardouin**	
	CONCLUDING REMARKS: **Hans van Leeuwen and Olaia Naveiras**	
13:15	***LUNCH / ADJOURN***	

INVITED SPEAKERS

Will Cawthorn

Peter Arner

Mara Riminucci

Pamela Robey

Saverio Cinti

Dimitrios Karampinos

Jeanine Prompers

Clifford Rosen

Olaia Naveiras

Izabela Podgorski

Guillaume Penel

Peter Bisschop

ABSTRACTS

INV = invited speakers (pages (11-22)

O = oral presentations (pages 23-40)

P = poster presentations (page 41-57)

Will Cawthorn

Bone marrow adipose tissue: starving for attention?

The University of Edinburgh, Edinburgh, UK

Bone marrow adipose tissue (MAT) comprises over 10% of total adipose mass in lean, healthy humans, suggesting that it plays a role in normal physiology. MAT further increases with ageing and in diverse clinical conditions, including osteoporosis, oestrogen deficiency, type 1 diabetes, radiotherapy, and in response to pharmacological agents such as thiazolididnediones or glucocorticoids. These observations suggest that MAT may directly impact human health and disease; however, the formation and function of MAT remains poorly understood.

Perhaps most notably, and in striking contrast to other adipose depots, MAT also increases during states of caloric restriction (CR). Many changes that occur during CR are physiological adaptations that have evolved to improve the ability to survive and/or recover from starvation. Moreover, CR is associated with decreased risk of cancer and cardiometabolic diseases, and is therefore currently of great interest as a strategy to improve human health. Thus, understanding why MAT increases during CR may shed further light on its physiological and pathological functions. We previously revealed that, during CR, MAT contributes to increased circulating levels of adiponectin, an adipocyte-derived hormone that can promote improved cardiometabolic health. This finding suggests that MAT is an endocrine organ that can exert systemic effects. However, many questions remain unanswered. For example, what are the mechanisms underlying MAT expansion during CR? Does MAT contribute to the beneficial effects of CR on cardiometabolic health? And do the endocrine properties of MAT extend beyond CR, to other physiological or pathological conditions?

In this presentation I discuss some of our lab's recent and ongoing studies that have begun to address these questions. By improving our understanding of why MAT increases during CR, we aim to reveal new knowledge of the formation and function of bone marrow adipocytes, and ultimately to understand how these cells impact health and disease.

Peter Arner

Is there a link between bone marrow fat and peripheral fat?

Karolinska Institute, Stockholm, Sweden

Human adipose tissue is in a highly dynamic state with rapid turnover of fat cells and the generation rate of new fat cells is doubled in obese as compared to non-obese subjects. This high turnover necessitates a renewable source of adipogenesis (making of fat cells). It is still controversial if different types of peripheral fat cells (white, brown, and beige) originate from the same or specific precursor cells. Whether they just reside in the adipose tissue or also originate from other sources such as bone marrow is also a matter of debate and rodent data are conflicting. In humans, however, it is clear from independent studies on bone marrow transplanted patients that precursor cells in bone marrow are important contributors to the adipogenesis in white subcutaneous adipose tissue. Circulating stem cells are capable of entering the human white adipose tissue and proliferate/differentiate into fully mature fat cells. This process is ongoing throughout the entire human lifespan and is markedly accelerated among obese subjects. Bone marrow precursor cells stand for at least 20% of total adipogenesis in subcutaneous white adipose tissue among the very obese. Following bone marrow transplantation the pool of donor derived fat cells becomes saturated much faster among obese than lean patients. Using a panel of cells surface markers we recently identified a population of human subcutaneous stem cells with similar characteristics as circulating stem cells which further supports the important role of bone marrow for the generation of fat cells in humans.

Mara Riminucci

Marrow adipocytes and fibrous dysplasia of bone

Cristina Remoli1, Rossella Labella1, Biagio Palmisano1, Samantha Donsante1, Alessandro Corsi1, Isabella Saggio2, Kenn Holmbeck3, Pamela Gehron Robey3, Mara Riminucci1 and Paolo Bianco1

1 Department of Molecular Medicine, Sapienza University of Rome, Italy; 2 Department of Biology and Biotechnology “C. Darwin”, Sapienza University of Rome, Italy; 3CSDB, NIDCR, NIH, Department of Health and Human Services, USA.

The development of marrow adipose tissue (MAT) is a post-natal event that occurs at specific times and sites in the growing skeleton. Fibrous dysplasia of bone is a genetic disease, caused by activating mutations of the Gsα gene (R201C, R201H), that appears in post-natal life and often progresses across the skeleton with a spatial and temporal pattern that overlaps with that of MAT. We have generated the first transgenic models of FD in which the GsαR201C sequence is expressed under the control of ubiquitous and constitutive promoters. The analysis of these mice has revealed that the development of the elementary tissue changes of FD, including osteomalacia, osteolysis and marrow fibrosis, associates with the emergence of a BAT-like phenotype in marrow adipocytes. This phenotype can be ascribed to the increased intracellular cAMP caused by the Gsα mutation, precedes the appearance of the abnormal FD osteogenic tissue (which expresses the inhibitor of bone matrix mineralization, MGP, and contributes to formation of osteomalacic bone), and accompanies the inappropriate bone resorption typically observed within FD lesions. To better understand the role of MAT in FD, we have recently established additional murine transgenic lines by crossing R26-LSL-GsαR201C mice with mice expressing Cre recombinase under the control of different adipocyte-specific promoters, such as Fabp4 and AdipoQ. The preliminary analysis of the R26-LSL-GsαR201C;Fabp4-Cre mice has demonstrated the appearance of a skeletal phenotype in the tail vertebrae with typical radiographic and histological features of FD. The R26-LSL-GsαR201C;AdipoQ-Cre transgenic mice are currently under investigation. Altogether, these different transgenic lines are expected to provide conclusive evidence as to the role of adipocytes in FD, specifically in the tissue changes (e.g., osteomalacia) that are the major determinants of skeletal morbidity caused by the disease.

Pamela Robey

Regulation of the fate of skeletal stem cells, the common precursors of bone, stroma and marrow adipocytes

Paolo Bianco1, Mara Riminucci1, Sukanya Suresh2, Constance T. Noguchi2, Luis Fernandez de Castro Diaz3, Jason A. Horton3, Andrew Pak3, Joanne Shi3, Kenn Holmbeck3, Arun Balakumaran3, Pamela G. Robey3

1Sapienza Università di Roma, Rome, Italy; 2National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD USA; 3National Institute of Dental and Craniofacial Research, Bethesda, MD USA;

During early embryonic development, mesenchymal condensations are specified to differentiate into provisional cartilage templates, which are subsequently replaced by bone (and importantly, including marrow) through an endochondral process. The developmental sequence by which the bone/marrow organ is established, is characterized by the initial formation of bone, with subsequent vascular invasion whereby committed osteogenic cells are recruited to form pericytes on the abluminal surface of blood vessels. These pericytes elaborate the hematopoiesis-supportive stroma, and lastly, differentiate into marrow adipocytes. It is now known that skeletal stem cells (SSCs), a subset of bone marrow stromal cells (BMSCs) are pericytes, and that they control skeletal homeostasis based on tight regulation of their ability to form bone, stroma and adipocytes. The balance of the phenotypes emanating from SSCs is mediated by both extrinsic changes in the bone marrow microenvironment, and intrinsic changes due to genetic mutations that manifest as skeletal diseases, as illustrated by molecular modifications, generating mouse models of disease. A number of examples of the waning and waxing of marrow adipogenesis during post-natal life include: 1) increased (abnormal) bone formation at the expense of stroma and marrow adipocytes, induced by activating mutations in the PTH/PTHrP receptor and components of its downstream signaling pathway, 2) increased formation of hematopoiesis-supportive stroma accompanied by a decrease in bone and marrow adipocytes based on overproduction of erythropoietin in the bone marrow microenvironment, 3) increased adipogenesis due to loss of MT1-MMP in mature osteogenic cells leading to inability to cleave Dlk1 from their surfaces, which potentially acts as a negative regulator of adipogenesis by SSCs, and 4) displacement of hematopoietic marrow by adipogenic marrow in dyskeratosis congenital, a disease of premature aging caused by mutations in genes that control telomere length. Taken together, the factors that affect marrow adiposity are varied, and controlling the fate of SSCs appears to be central to the maintenance of an appropriate level of marrow adiposity in post-natal life.

This research was supported by Telethon grant # GGP09227 (PB, MR), NIAMS 1K99AR066737 (JAH), and the DIR, NIDDK (SS, CTN) and DIR, NIDCR (LFdCD, AP, JS, KH, AB, PGR), both a part of the IRP, NIH, DHHS.

Saverio Cinti

The Adipose Organ

University Of Ancona (Politecnica Delle Marche), Ancona, italy

In mammals, the adipocyte is a lipid-laden cell forming the parenchyma of a multi-depot organ, the adipose organ. The white adipocyte stores lipids to release them, in the form of free fatty acids, during fasting, while the brown adipocyte burns glucose and lipids to perform thermogenesis. A recently characterized, third type of adipocyte does appear in the subcutaneous depot of the adipose organ of female mice during pregnancy and lactation: the pink adipocyte. The pink adipocytes are mammary gland alveolar epithelial cells with the essential role of producing and secreting milk for pup nourishment. Emerging evidence suggest that they derive from the transdifferentiation of the subcutaneous white adipocytes. Different metabolic and environmental challenges highlight the extraordinary plasticity of the mammalian adipose organ. Cold exposure leads to an increase of the “brown” component of the adipose organ to warrant thermal homeostasis. Under positive energy balance, the “white” component enlarges to a some extent to allow storage of the excess of nutrients. Finally, during pregnancy the “pink” component develops in the subcutaneous depots to satisfy pup nutritional needs. At cellular level, the plasticity of the adipose organ appears to occur not only through proliferation and differentiation of stem cells but, distinctively, via a direct transformation of fully-differentiated adipocytes that under proper stimuli by reprogramming their genetic expression change phenotype and, consequently, function. Understanding the transdiffererentiation properties of the adipocytes is expected to offer not only new biological insights, but also possible therapeutic strategies to combat the metabolic syndrome (“browning”) and the breast cancer (“pinking”).

Giordano et al. Convertible visceral fat as a therapeutic target to curb obesity.
Nat Rev Drug Discov. 2016 Jun;15(6):405-24.

Dimitrios Karampinos

MRI of bone marrow adiposity

Technical University of Munich, Munich, Germany

Bone marrow fat imaging is emerging as a useful tool in characterizing treatment-induced bone marrow damage in cancer patients and in understanding the relationship between bone health and bone marrow adiposity. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are ideal imaging techniques for non-invasively investigating the properties of bone marrow fat. The most important MRI and MRS methods for assessing properties of bone marrow fat will be first reviewed, including T1-weighted imaging, single-voxel magnetic resonance spectroscopy (MRS), and chemical shift encoding-based water-fat imaging. The basic principles for measuring bone marrow fat fraction and bone marrow fatty acid composition parameters with the above methodologies will be presented. Special attention will be given to approaches for removing confounding effects in the measurement of the bone marrow fat fraction and the challenges associated with measuring bone marrow fat unsaturation. Applications of bone marrow fat MRI and MRS in a variety of clinical settings will be then presented. Specifically, the use of MRI to address bone marrow composition changes after cancer treatment will be reviewed. Previous MRI and MRS studies measuring bone marrow fat fraction and fat unsaturation in the context of aging and osteoporosis will be presented. Finally, previous studies investigating the relationship between bone marrow fat, other fat depots and bone health in patients with obesity and type 2 diabetes will be discussed.

Jeanine Prompers

Functional imaging of bone marrow adiposity

Eindhoven University of Technology and UMC Utrecht, Eindhoven, the Netherlands

It has been recognized that bone marrow fat (BMF) may be an indirect marker of bone health. Magnetic resonance imaging (MRI) and spectroscopy (MRS) allow for non-invasive quantification of BMF and have greatly contributed to recent investigations of the relationship between BMF and bone density and strength, showing that BMF is elevated in conditions associated with reduced bone density, such as osteoporosis. Next to the amount of BMF, BMF composition and especially the degree of BMF unsaturation may also affect bone health. In the ^{1}H MR spectrum, the smaller lipid peaks contain information about lipid composition. However, in vivo ^{1}H MR spectra of bone marrow typically display broad signal linewidths and at 1.5 and 3 Tesla MR scanners resolution of the smaller lipid peaks is difficult. Several more advanced MRS methods have been proposed to overcome this limitation, such as two-dimensional ^{1}H MRS and ^{1}H-decoupled ^{13}C MRS. However, these techniques cannot be routinely used because of long scan times and the need for special hardware. Recently it was shown that ^{1}H MRS of bone marrow at 7 Tesla provides excellent spectral resolution and that from 4 lipid peaks in a relatively narrow spectral bandwidth (methylene protons at 1.30 ppm, allylic methylene protons at 2.03 ppm, diallylic methylene protons at 2.77 ppm, and α-methylene protons at 2.25 ppm as reference) the fraction of saturated, mono-unsaturated and poly-unsaturated BMF can be determined. In this lecture, the application of ^{1}H MRS to measure BMF composition will be discussed together with its limitations.

Clifford Rosen

Site Specific Differences in Bone Marrow Adipocytes: Are all marrow adipocytes equal

Clifford J Rosen, Mark Horowitz, Ormond MacDougald and Beate Lanske

Maine Medical Center Research Institute, Scarborough, USA

Marrow adipocytes occur in both physiologic and pathologic states. In humans, the appendicular marrow converts from hematopoietic elements to adipogenesis around the time of peak bone acquisition. In contrast, in the axial skeleton, bone marrow adipocytes begin to appear after puberty and peak after the age of 50, converting more than 50% of the hematopoietic marrow to an adipose depot. All told, about 10% of adult fat is found in the bone marrow. In rodents, marrow adipocytes in the distal tibia and distal vertebrae are present at or around the same time that other fat depots are developing. In contrast, marrow adipose tissue appears much later during aging or after exposure to nutrient or pharmacologic exposure in the proximal and mid shaft of the femur and tibia. The more antral vertebrae are the last sites to show marrow replacement with adipocytes. We have previously labeled these latter sites as regulated MAT, and the former as constitutive. Although we have shown these sites indeed have different lipid composition in rats, it is less clear in the mouse whether there are major distinctions in function as well as composition. Moreover, in the absence of the PTH1R, we demonstrated that the distal region of constitutive MAT was markedly enhanced despite its location. Interestingly, in older humans, the 'regulated' MAT in the vertebrae appears to be more saturated and associated with fractures. However, we also showed recently that in hip fracture patients, unsaturated lipids are more predominant in the marrow. This would suggest there may be differential use of fatty acids that determine the type of marrow adipocyte that we characterize. Whether these cells can switch characteristics from regulated to constitutive remains to be determined as we develop novel methods for isolating marrow adipocytes from different regions in mice.

Olaia Naveiras

Bone marrow adiposity in regenerative hematopiesis

Josefine Tratwal, Vasco Campos, Yannick Yersin, Shanti Rojas-Sutterlin, Nicola Vannini and Olaia Naveiras

Laboratory of Regenerative Hematopoiesis, Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland

In 1882 E. Neumann first noted the inverse relationship between adipogenesis and hematopoiesis within the human bone marrow. Specifically, he documented the predominance of yellow adipocytic marrow in distal bones in homeostatic conditions, and the infiltration of distal sites by red hematopoietic marrow in disorders of inefficient or malignant hematopoiesis including pernicious anemia, thalassemia and leukemia. During the 1970's, M. Tavassoli and collaborators first proposed the term regulated and constitutive marrow adipocytes (rMAT vs. cMAT) based on differences in fatty acid saturation as revealed by performic acid Schiff (PFAS) staining, and revealed the plasticity of the red-to-yellow and yellow-to-red transitions through seminal studies on ectopically transplanted marrow.

We are interested in studying the effect of bone marrow adipogenesis in hematopoiesis. It is now well known that red-to-yellow marrow transitions occur in all scenarios of hematopoietic insult, whether due to hematopoietic toxicity (i.e. irradiation, chemotherapy, benzene exposure) or hematopoietic stem cell (HSC) insufficiency (i.e. Fanconi anemia). In fact, the inverse relationship between bone marrow adipogenesis and hematopoiesis is so predictive that human pathologists determine hematopoietic activity or "cellularity" as the ratio of hematopoietic cells to adipocytes within the bone marrow, with aplasia usually defined as a cellularity lower than 10%.

In 2009 we determined a net negative effect of bone marrow adipocytes in hematopoiesis both in homeostasis (mostly reflecting the effect of cMAT) and upon stress hematopoiesis following irradiation-mediated aplasia (mostly reflecting the effect of rMAT). Others have since validated the potent effect of PPARg inhibitors in accelerating hematopoietic recovery. To further dissect this phenomenon, we have developed an in vitro system to model the red-to-yellow-to-red marrow transition, and an image-recognition tool that allows for unbiased quantification of BM adipocytes in vivo (MarrowQuant Plug-In). Data compatible with the hypothesis that preadipocytes support hematopoiesis while fully mature adipocytes inhibit rapid HSC proliferation will be presented.

Izabela Podgorski

Bone marrow adipocytes and bone metastasis

Wayne State University School Of Medicine, detroit, Michigan, USA

Metastatic growth in bone is a complex process involving reciprocal interactions between the tumor cells and the host bone microenvironment. As vital and active components of bone marrow stroma, adipocytes are starting to emerge as key regulators of tumor cell adaptation, survival and progression in bone. A number of solid tumors, including cancers of the prostate, breast, lung, bladder and melanoma, have a propensity to colonize and grow in skeletal sites, but mechanisms behind their ability to thrive in the harsh bone marrow niche are not well-understood. We have shown previously that marrow adiposity accelerates growth and aggressiveness of experimental prostate tumors in the bone. Specifically, our studies revealed that interaction and lipid transfer between bone marrow adipocytes and tumor cells leads to upregulation of lipid transporters, such as FABP4 and CD36, and has growth and invasion stimulatory effects on the tumor cell. Our recent studies also demonstrate that adipocytes are involved in shaping of the tumor metabolism through HIF-1α activation and switch to a glycolytic phenotype. Our overarching hypothesis is that paracrine tumor cell-adipocyte interactions in the marrow are key drivers of tumor metabolic adaptation and survival in bone. Through a series of complementary experimental approaches from biochemical tools through in vitro adipocyte-tumor cells co-cultures to in vivo models, our findings to date reveal the functional relationship between adipocyte-supplied lipids, ER stress, and activation of pro-survival pathways in metastatic tumor cells. Our studies point to bone marrow adipocytes as critical regulators of tumor behavior in bone. Our long-term objective is to identify the mechanisms responsible for adaptation and chemoresistance of bone-metastatic cancers and reveal new targets for therapeutic intervention.

Guillaume Penel

Do bone marrow adipocytes influence skeletal health: A lipid story?

Guillaume Penel, Alexandrine During

University of Lille − ULCO, PMOI, EA 4490, 59000 Lille, France

Description of quantitative modifications of adipose tissue within the bone marrow (BM) compartment have reported during aging (marrow conversion). The implication of bone marrow adiposity during skeletal pathologies like osteoporosis or femoral head osteonecrosis have been also evocated for many years. But the limited understanding of the involved mechanism and the relevance of these information minor the impact of these descriptions. Based on new technical approach, there is now growing clinical and experimental evidences of the crucial role of bone marrow adiposity (BMA) and of lipids in early stages of the physiology and pathologies of bone. In particular, the local impact of the lipids content of BMA is now better understood. Skeletal lipids content can be divided in two compartment, i. Lipids content of mineralized matrix and, ii. Lipids content of the BMA tissue. The first ones vary with species whereas the second varies with species, anatomical site, diet and age. The role of lipids have to be considered at different level. During biomineralization processes, phospholipids are recognized as key factors. In particular, phosphatidylserine and sphingomyelin act as major actors during nucleation and bone mineralization respectively. During bone remodeling lipids content impact the bone mass. Fatty acids could have direct or indirect impact on bone cells structure and membrane especially and, therefore modulate cell functions and viability. The role of cholesterol as catabolism agent have been demonstrated in vitro and in vivo via an action on osteoblastic and osteoclastic lineages. Finally, lipids must be now considered as one of the signaling molecules in bone metabolism. Recently lipids mediators like ceramide or endocannabinoids have been proposed as local hormones playing complex and sometime ubiquitous role on bone physiology. A better understanding of their action on bone appear crucial and should give raise to new and promising strategies for skeletal resorptive diseases.

Peter Bisschop

Marrow adipose tissue and human bone health

Academic Medical center and VU Medical Center, Amsterdam, the Netherlands

Marrow adipose tissue (MAT) is a unique fat depot that is functionally different from white and brown adipose tissue. Although the existence of MAT has been described decades ago, our understanding of its function has remained limited compared to other fat depots. In children MAT is only present in the most distal parts of the skeleton, but with ageing red marrow, i.e. hematopoietic tissue, is gradually replaced by yellow marrow as MAT extends to more proximal sites of the skeleton.
Several conditions associated with high MAT are also associated with low bone mass including estrogen deficiency, immobilisation, starvation, alcoholism, and the use of glucocorticoids and PPARgamma agonists. Human studies further indicate that increased MAT correlates with low bone formation, low bone mineral density and osteoporosis. These correlations have led to the paradigm that MAT is a negative regulator of bone mass. The increase in MAT and loss of bone mass that occur after ovariectomy in experimental studies and in women after menopause are highly consistent and suggest an important and well conserved role for the gonadal axis in the regulation of marrow fat.
Identification of the mechanisms by which bone marrow adipocytes interact with bone metabolism could provide a perspective for novel targets to maintain bone health.

Urszula Iwaniec

Housing Growing Mice at Thermoneutral Temperature (32°C) Increases Cancellous Bone Volume Fraction and Marrow Adiposity

Urszula T. Iwaniec1, Kenneth A. Philbrick1, Carmen P. Wong1, Dawn A. Olson1, Adam J. Branscum2, and Russell T. Turner1

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A reciprocal relationship is often noted between cancellous bone volume fraction and bone marrow adiposity in long bones of mice, although causality has yet to be established. Studies evaluating this relationship have been typically performed in mice housed at room temperature. However, cancellous bone loss in weight-bearing bones in mice housed at room temperature normally begins during growth (at approximately 2 months of age), even as the bones continue to elongate and cortical bone continues to be accrued. We have recently shown that the premature bone loss can be prevented by housing mice within the thermoneutral range (32°C, temperature where basal rate of energy production is at equilibrium with heat loss), supporting the hypothesis that mild cold stress associated with room temperature housing has a negative impact on cancellous bone in growing mice. The impact of housing temperature on bone marrow adiposity is unknown. We therefore evaluated marrow adipose tissue (MAT), cancellous bone volume fraction, and bone formation in distal femur and expression of genes associated with adipogenesis and osteogenesis in tibia in female B6 mice (n=10/group) housed at room temperature (22°C) or thermoneutral (32°C) from 2 to 4 months of age. Mice housed at 32°C consumed 40% less food than mice housed at 22°C. Although significant differences in body weight were not detected, mice housed at the higher temperature exhibited higher abdominal white adipose tissue weight (+54%) and higher leptin levels (+130%). Furthermore, these mice maintained higher cancellous bone volume fraction (+220%) in the distal femur metaphysis; the sparing effect of thermoneutral housing on cancellous bone was associated with higher indices of bone formation, lower indices of bone resorption, and higher bone marrow adiposity (adipocyte area/tissue area, +142%; adipocyte number/tissue area, +94%). At the gene level, mice housed at 32°C had significantly higher mRNA expression for bone matrix proteins (e.g., osteocalcin and type 1 collagen), adipokines (e.g., leptin and adiponectin), intracellular mediators of insulin singling (e.g., Insr and Irs2) and fat accumulation (e.g., Ppard and Acacb), and had differential expression of growth factors that regulate bone and fat metabolism (e.g., BMPs, BMPRs, Smads, Vdr, Wnts and Wnt antagonists). In summary, compared to room temperature, mice housed at thermoneutral have higher cancellous bone volume fraction, higher MAT, and higher expression of genes associated with osteoblastogenesis and adipogenesis. These findings question the widely held view that increased marrow adiposity occurs at the expense of osteoblast differentiation and suggest that environmental factors, including adaptation to mild cold stress induced by room temperature housing, may alter the relationship between bone and MAT.

Eudes-Brian Mvoula

Down regulation of Sirtuin type 1 (Sirt1) expression increases marrow adiposity by inducing lipid storage and glucocorticoid metabolism and by acetylation of Runx2 and FOXO1 in bone marrow of anorexia nervosa mouse model

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Background: Sirt1, a histone deacetylase, is implicated in the regulation of osteoblast/adipocyte differentiation. Its activation by resveratrol increases osteoblastogenesis and reduces adipogenesis. At present, no study focused on the link between Sirt1 and osteoporosis related to anorexia nervosa. In a separation-based anorexia mouse model (SBA) we developed, Sirt1 expression is reduced in bone marrow culture when compared to culture from control mice. In vitro, this decrease of Sirt1 is associated with a rise in adipogenesis and a decrease in osteoblastogenesis.
Objective: To explore the mechanism by which decrease in Sirt1 expression is responsible for the strong adipocyte differentiation of bone marrow stromal cells (BMSCs) in our model.
Methods and results: BMSCs from control and SBA mice were differentiated to osteoblasts and adipocytes in a codifferentiation medium with or without resveratrol and sirtinol, as activator and inhibitor of Sirt1 respectively. BMSCs from SBA mice expressed high levels of genes related to lipid storage (Fasn, Cidec, and Plin1) and glucocorticoid metabolism (HSD 11b). Decrease in Sirt1 expression by sirtinol enhanced mRNA expression of all these genes. However, the expression of these genes was reduced by resveratrol- induced Sirt1 expression. Interestingly, low expression of Sirt1 in BMSCs of SBA mice or its inhibition by sirtinol induced a higher acetylation of Runx2 and FOXO1, as assessed by immunoprecipitation and western blot respectively. Moreover, activation of Sirt1 by resveratrol induced deacetylation of Runx2 and FOXO1. Organotypic cultures of tibias from SBA mice displayed an increase in marrow adipocyte number (histology) and a decrease in cortical and trabecular parameters (micro tomography) compared to control mice.
Conclusion: These finding suggest that SBA protocol induces a sustainable down regulation of Sirt1 leading to an increased adipogenesis and lower osteogenesis by up regulating genes related to lipid storage and glucocorticoid metabolism through the acetylation of both Runx2 and FOXO1.

Erica Scheller

Leptin-induced loss of marrow adipose tissue is mediated by sympathetic and sensory neurotransmission

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Background. Marrow adipose tissue (MAT) has the potential to exert both local and systemic effects on metabolic homeostasis, skeletal remodeling, and hematopoiesis - yet the mechanisms underlying MAT turnover and expansion remain largely unknown. We have previously reported that prolonged cold-exposure, a model of enhanced sympathetic tone, leads to 70% loss of regulated marrow adipose tissue (rMAT) in mice. By contrast and in the same model, constitutive MAT (cMAT) in the distal tibia remains unchanged.
Objective. We hypothesized that rMAT is acutely regulated by norepinephrine through the β3-adrenergic receptor, furthermore, that differences in sympathetic drive contribute to site-specific regulation of rMAT, but not cMAT.
Methods. Intracerebroventricular (ICV) cannulae were implanted in 13-week-old male C3H/HeJ mice. After 7-days, mice received 1.5 µg ICV-leptin or vehicle control in combination with subcutaneous saline, β3-adrenergic antagonist, or a combination of sensory neurotransmitter antagonists. Three injection cycles were performed over 24-hours (9am, 6pm, and 6am) followed by euthanasia at 9am on the second day.
Results and Conclusion. Leptin decreased body mass and food intake and caused a 43% decrease in rMAT volume at the proximal tibial metaphysis while cMAT in the distal tibia and tail remained unchanged. Inguinal and gonadal white adipose tissue mass (iWAT, gWAT) was decreased, by 28% and 36% respectively, due to diminished adipocyte cell size. Peripheral antagonists did not change leptin-mediated decreases in body mass or food intake. However, the β3-adrenergic antagonist completely rescued ICV-leptin induced loss of rMAT volume and iWAT/gWAT cell size. Unexpectedly, inhibition of sensory neurotransmission also blocked loss of rMAT, with partial rescue of iWAT and gWAT adipocyte size. These findings provide novel insight into the site-specific regulation of MAT by the nervous system and its relationship to peripheral WAT, and suggest that coordination of both sympathetic and sensory neurotransmission is necessary for acute regulation of rMAT.

Michaeal Reagan

Bone Marrow Adiposity is Modulated by Osteocyte-Derived Molecules including Sclerostin

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Background: The bone marrow (BM) adipose depot is a dynamic, complex collection of adipocytes regulated by both external signals and cell-autonomous factors. It is highly likely that bone-derived factors modulate bone marrow adipose tissue (BMAT).
Objective: Our goal was to explore the regulation of BMAT by skeletal cells. We hypothesized that osteocyte-derived factors may inhibit or induce adipogenic differentiation.
Methods and Results: We found that osteocyte conditioned media induced adipogenic differentiation of 3T3-L1 cells by >2 fold ($p<0.05$) in vitro. We identified sclerostin, a Wnt-inhibitor secreted from osteocytes, as a protein able to induce adipogenesis in 3T3-L1 cells (1.5 fold) and BM-MSCs from both mouse (1.5-fold) and human (3-fold). Moreover, sclerostin induction of BMAT formation in vitro was in part due to its antagonistic effects on the adipogenic-inhibitory Wnt1 signaling pathway. However, in vivo, we observed significantly increased BMAT in osteocalcin-Cre/iDTR (inducible diphtheria toxin (DT) receptor) mice, both immediately and 3 weeks after stopping a 2 week, daily DT injection treatment. These mice have low osteocyte and osteoblast counts, and significantly decreased circulating sclerostin (assessed by ELISA, days 1, 2 and 7), suggesting that other factors from skeletal cells, such as osteocalcin, normally limit BMAT. To create a more physiologically relevant BMAT model, we developed the first 3-D BMAT model from silk scaffolds and BM-MSCs differentiated into adipocytes. This model, imaged with confocal microscopy, recapitulates bone-adipose interactions more realistically than 2-D and demonstrates the feasibility of utilizing 3-D models to investigate relationships between cells in the BM niche.
Conclusion: In sum, this work demonstrates that there are both pro-BMAT (sclerostin) and anti-BMAT (under investigation) factors produced by skeletal cells that coordinately regulate BMAT and represent novel targets for modulating BMAT and bone. This work contributes to knowledge of BMAT function and origination in the context of healthy and diseased BM.

Phil Salmon

Calcified and noncalcified biological tissue imaging and analysis by MicroCT, and the case of bone marrow

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MicroCT is a 3D nondestructive imaging technique providing a map of xray attenuation over isotropic volumes over several mm-cm distance. It is therefore unrivalled as a method for analysing morphology and composition of objects including biological tissue. It is however limited by the need for contrasting xray absorption in imaged material. For this reason the first and largest adopter of microCT in biological sciences were the bone and dental related fields. The presence of calcium phosphate minerals in these tissues confers strong xray contrast. Lung and fat also possess xray absorption contrast allowing microCT imaging without staining. Other soft tissues have been more challenging for microCT due to minimal contrast between tissues mostly consisting of water and low-Z elements. Contrast can however be added by staining, where a high-Z element is selectively bound to certain tissues. Effective microCT stains include phosphotungstic acid, which has affinity for collagen and fibrin, and Lugol's iodine, a solution of iodine and potassium iodide in water and a source of free elemental iodine. More such stains are being developed. MicroCT stains are more effective after any buffer present in stored tissue is washed out with water [1]. Recently Kerkhofs [2] has demonstrated that adipocytes and blood vessels within rodent bone marrow can be visualised and segmented by microCT following staining with a new contrast agent, Hafnium metal-substituted polyoxotungstate (Hf-POT), followed by high resolution microCT imaging. This makes possible 3D architectural analysis of these structures within marrow. MicroCT analysis of bone marrow requires an image analysis technique for separation of the medulla from cortical bone. A new method for this based on iterative morphological operations is also presented.

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Alessandra Bierwagen

Measurement of Red and Yellow Bone Marrow Fat Composition using MRI and MRS

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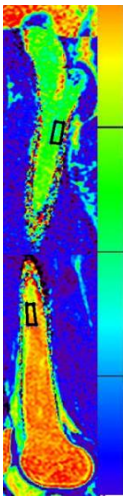
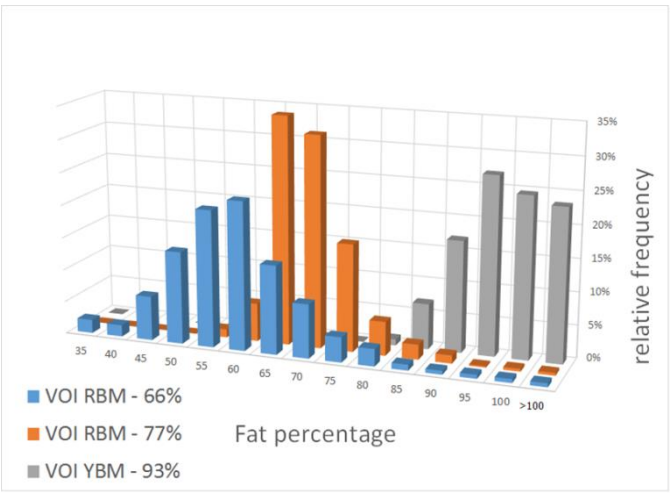
Background: Fat content and composition of human bone marrow have been non-invasively assessed using magnetic resonance imaging (MRI) and spectroscopy (MRS). Previous MRI and MRS studies measured fat content and composition of hematopoietic red (RBM) in the spine and fatty yellow bone marrow (YBM) in the femur or tibia. However, recent rodent studies have shown that fat composition is also dependent on the measured region, i.e. central vs peripheral bone marrow. This complicates comparison of RBM and YBM measured in different bones.
Objective: This study aimed to apply a method that allows to clearly identify and quantify red and yellow bone marrow in the same bone, here the human femur, and to compare fat unsaturation in different bones.
Methods: 6 healthy volunteers were studied on a 3-T MR scanner. RBM and YBM were identified based on fat fraction. ^{1}H spectra were acquired in RBM and YBM of the diaphysis in the right femur and the tibia [PRESS, TR=4s, TE=200ms, NSA=64, VOI=2×0.7×0.7cm³]. The unsaturation index was calculated as (=CH/(CH2+CH3)), fat fractions as (CH2+CH3)/(CH2+CH3+H2O). Femoral (F/IP/OP/W) images were acquired via a 3D T1 fast-field-gradient echo pulse sequence (Flip Angle:5°, TR/TE1/TE2=5.0/1.2/2.5ms, resolution:2x2x2mm). VOIs were segmented automatically using the fat-only images. The color-coded fat fraction (fat/(water+fat) was evaluated pixel-by-pixel and plotted into a histogram using the mDixon color scale.
Results and Conclusion: Histograms of VOI fat content show that it is feasible to measure VOIs in the human femur with clearly separated fat composition which can be assigned to RBM and YBM. The unsaturation index in YBM of the human tibia is higher than that obtained from YBM of the femur (tibia: 9.77±0.38%, femur: 7.73±1.94%, p=0.03). In conclusion the comparison of bone marrow composition in varying bones is delusive and not necessary as RBM and YBM can be distinguished within the same bone by fat-water imaging.





Meghan McGee

Dietary Kynurenine, the oxidized metabolite of tryptophan, suppresses osteoprogenitor expression of Hdac3 resulting in increased marrow adiposity and age-related bone loss

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Background: Aging skeletons develop osteoporosis and increased marrow fat although the mechanism remains poorly understood. Our previous studies support Hdac3 as a regulator of this process, as osteoprogenitor Hdac3 expression is decreased in aged vs. young mice, and decreased expression of Hdac3 causes an osteopenic, high marrow fat phenotype and lipid storage in osteoprogenitors. However, causative factors for decreased Hdac3 expression with aging are unknown. Our group has shown that aromatic amino acids (tyrosine, phenylalanine, tryptophan) function as antioxidants with a protective role in bone, whereas their oxidized byproducts antagonize this effect and promote bone loss. Circulating levels of kynurenine, the oxidized product of tryptophan, are increased in aged (24mo) vs. mature (12mo) mice, and we recently observed that kynurenine-treated mice have high marrow fat and low bone mass, mimicking the phenotype of Hdac3-deficient and aged animals. This raises the possibility that kynurenine could be a mechanistic link between aging and bone loss.
Objective: We explored intersecting roles of kynurenine and osteoprogenitor Hdac3 in marrow adiposity and osteoprogenitor lipid storage.
Methods and Results: Mature (12 months) male C57BL6 mice were fed 18% protein (normal diet), 8% protein+50μM kynurenine or 8% protein+100μM kynurenine for 8 weeks. We also injected young male CD-1 mice with kynurenine (2 or 20 mg/day) for 10 days to study kynurenine's acute effects. Kynurenine caused bone loss through increased osteoclast activity and decreased osteoblast activity. Bone marrow adiposity was dose-dependently increased in kynurenine-fed mice, and osteogenic BMSC cultures from kynurenine-treated mice showed decreased expression of Hdac3 and co-factor NCoR1, increased expression of lipid storage genes Cidec and Plin1, and no changes in expression of Pparγ2 or Fasn. These results mimic expression patterns in osteoprogenitors from Hdac3-insufficient and aged mice.
Conclusion: Aging-induced increases in kynurenine may suppress Hdac3 which impairs osteoblast function and increases marrow fat with age.

Jason Horton

Limited field radiotherapy induces local and abscopal effects on bone structure and marrow adiposity

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Background: Exposure of skeletal anatomy to ionizing radiation can induce imbalances in local bone metabolism that result in striking bone pathology that may predispose to atraumatic fracture. Interestingly, irradiated bones show a striking expansion of marrow adipose tissue (MAT) that displaces hematopoietic marrow. Whether this expansion of MAT is linked to the deleterious changes in the metabolic, structural and mechanical properties of the bone following radiation exposure is unclear.
Objective: Examine the temporal relationship of MAT expansion and skeletal pathology induced by limited field radiotherapy.
Methods and results: With endorsement of the local IACUC, 12wko female c57BL/6J mice were randomized into groups of n=35, and the right hind-limb was exposed to either sham, single 5Gy or a series of four daily 5Gy fractions of 250kVp x-rays. Lead shielding and beam collimation were used to protect the remainder of the body. Bilateral hind limbs were harvested at baseline and 0.5, 1, 2, 4, 8 and 16 weeks post-irradiation. Femora were examined by µCT bone morphometry, followed by decalcification and osmium-enhanced µCT-derived MAT morphometry. Radiation-exposure resulted in dose-dependent, progressive alteration of bone structure, relative to sham irradiated mice. Whereas bones receiving fractionated radiation rapidly accumulated MAT, single-dose exposed bones accumulated MAT more gradually, achieving statistically significant difference from sham-irradiated bones only at later time points. Interestingly, significant loss of trabecular bone was also observed in the contralateral limb of mice receiving fractionated radiation. However, only minor changes were observed with respect to marrow adiposity in the contralateral limb of these mice.
Conclusion: The observation of abscopal effects of radiation on bone structure and marrow adiposity further complicates our already limited understanding of the factors governing the bone-marrow microenvironment response to radiation. Ongoing studies are focused on identifying mechanistic factors that may link radiation-induced bone disease and MAT expansion.

Gina Woods

Marrow Fat, Trabecular and Cortical Bone, and Prevalent Vertebral Fracture in Older Adults

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Background: Bone marrow fat (BMF) increases with age and may influence the development of osteoporosis. We previously reported that among 257 older persons from the AGES-Reykjavik study BMF and trabecular vBMD at the spine and hip were inversely correlated in women. It remains to be clarified if BMF is associated with cortical bone.
Objective: To assess the relationship between vertebral BMF, QCT measures of BMD, and prevalent vertebral fracture (PVF) in an expanded cohort of 257 men and 238 women from the AGES-Reykjavik study.
Methods/Results: Participants were age 71+, not using bone active medications. In 2011, 257 participants were assessed and in 2015, 238 participants. BMD was assessed by QCT. PVF was assessed by DXA VFA. BMF (ratio of fat to water + fat, %) was measured with a 1.5-T MR scanner. Multivariable linear models were run with mean BMF (L1-L4) and either PVF or log-transformed QCT BMD values.
Participants were 78.3 (SD 3.7) years, with a mean BMI of 27.3 (SD 3.9) kg/m^2, mean BMF of 54.4% (SD 8.4%), and 104 (21.1%) had PVF. Average BMF, adjusted for age, sex, trabecular spine BMD and visit date, was higher in those with vs. without PVF (56.06% vs. 53.84%, p = 0.02). Per SD increase in BMF, spine trabecular BMD was 12.1% lower (p<0.0001), integral BMD was 3.4% lower (p=0.0001), as was vertebral compressive strength (12.8%, p<0.0001). There was no evidence of interaction by sex, with the exception of vertebral compressive strength (-7.9% difference in men and -18.4% in women, p for interaction 0.03). Total hip values including trabecular, cortical and integral BMD were also lower in persons with higher BMF.
Conclusions: Older men and women with higher BMF have lower trabecular and cortical bone density and are more likely to have PVF.

Table 1: % Difference in QCT Bone Outcome for each 1-SD Increase in Marrow Fat *

(n=495)	% Difference	95% CI	p-value
Spine			
Trabecular BMD	-12.11	(-15.95, -8.09)	<0.0001
Integral BMD	-3.41	(-5.08, -1.71)	0.0001
Vertebral Compressive Strength	-12.81	(-17.55, -7.79)	<0.0001
Femoral Neck			
Trabecular BMD	-2.04	(-5.01, 1.03)	0.19
Cortical BMD	-0.72	(-1.48, 0.05)	0.07
Integral BMD	-2.00	(-3.63, -0.34)	0.02
Total Hip			
Trabecular BMD	-2.23	(-4.26, -0.16)	0.03
Cortical BMD	-0.64	(-1.25, -0.02)	0.04
Integral BMD	-1.78	(-3.36, -0.18)	0.03

*Adjusted for age, sex, BMI and visit date

O10

John Diedrich

Bone Marrow Adipocytes Alter the Metabolic Phenotype of Metastatic Prostate Cancer Cells Through Activation of HIF-1α

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Background: Bone is a preferential site of metastasis from prostate cancer (PCa). Age and obesity, conditions that increase adipocyte numbers in bone marrow, are risk factors for skeletal metastases from PCa. Research in our laboratory focuses on understanding the interactions between adipocytes and tumor cells that have infiltrated the bone marrow.

Objective: Specifically, we are examining how the secretion, transport, and uptake of adipocyte-supplied factors promote metastatic progression in bone.

Methods and Results: We have previously shown that exposure of PCa cells to marrow adipocytes in vitro leads to a lipid transfer between the two cell types. We also demonstrated that the resulting lipid droplet accumulation in tumor cells is associated with increased expression of major lipid transporters and altered tumor metabolism. Using in vivo models of marrow adiposity and in vitro co-culture systems we demonstrate that bi-directional interactions between marrow fat cells and tumor cells are responsible for activating hypoxia inducible factor-1 alpha (HIF-1α) signaling and driving Warburg metabolism in metastatic PCa cells. By employing RT PCR, immunoblotting analyses and functional metabolic assays we show, that adipocyte-activated HIF-1α signaling in PCa cells leads to significant increases in expression and activity of glycolytic enzymes, augments lactate production, and reduces mitochondrial oxidative phosphorylation. We also show that these metabolic effects are reversed by siRNA-mediated downregulation of HIF-1α. Additional data stemming from the lipidomic analyses of adipocytes and PCa cells cultured alone or in co-culture reveal 60 candidate lipids that are differentially secreted upon adipocyte-PCa cell interactions. Among the identified lipids, several have the potential to affect metabolism of neighboring cells through the activation of HIF-1α signaling.

Conclusion: Our overarching objective is to reveal specific mechanisms behind bone marrow adipocyte involvement in progression, aggressiveness and chemoresistance of metastatic PCa in the bone marrow niche and to identify novel targets for therapeutic intervention.

Celine Gillet

Bone marrow derived-MSC from non-traumatic osteonecrotic patients display a critical susceptibility to lipotoxicity.

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Background: Non-traumatic osteonecrosis (ON) is a multifactorial bone disease characterized by osteomedullary necrosis which can evolve to subchondral fracture and bone collapse. Different pathogenic mechanisms have been proposed, among them an increase of bone marrow (BM) fat resulting from adipocytes accumulation. BM adipocytes affect the BM cells microenvironment by releasing cytokines, adipokines and free fatty acids (FFAs). Saturated or unsaturated FFAs inversely influence the viability and function of bone cells.
Objective: To explore the impact of palmitate and oleate, two FFAs predominant in the human organism and diet, on function and survival of mesenchymal stem cells (MSC) isolated from osteonecrotic patients (ON_{MSC}) and healthy volunteers (HV_{MSC}) and to analyze the FFAs present in their BM fluid and serum.
Methods: MSC were isolated from BM aspirated from the iliac crest and cultured in standard or adipogenic medium. The BM fluid FFAs profile was analyzed by GC. Gene and protein expression were determined by RT-qPCR and western blot, respectively; cytokine secretion was quantified by ELISA.
Results: Exposure to the saturated FFA palmitate favored MSC differentiation through the adipogenic lineage at the expense of the osteoblastic cells. Moreover, adipogenesis is intensified in ON_{MSC}. The susceptibility to palmitate toxicity was aggravated in ON_{MSC} and associated with dysregulation of the mechanisms (ERK activation, SCD1 and CPT1 expression) implicated in the protection against lipotoxicity. Palm-induced IL-6 and IL-8 secretion was also exacerbated in ON_{MSC}. Finally, the FFAs profile was modified in the BM fluid, but not in the serum, of osteonecrotic patients compared to healthy subjects.
Conclusion: The greater susceptibility to lipotoxicity of ON_{MSC} could be related to dysregulation of molecular protective mechanisms against lipotoxicity. Palmitate-induced cytokines secretion could favor osteoclastic activity. Altogether, we suggest that marrow adipocytes enlargement could be deleterious for bone forming cells and could play a role in the pathogenesis of ON.

Andre van Wijnen

Molecular differences in Mesenchymal Stromal Cells from Adipose versus Bone Marrow Tissue

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Human mesenchymal stromal/stem cells (MSCs) from bone marrow are more potent than those sourced from adipose-tissue to commit to an osteogenic cell fate. However, because adipose tissue is a much more convenient and abundant source for human MSCs than painful bone marrow aspirates from the iliac crest, it would be advantageous to convert adipose-tissue derived MSCs (AMSCs) into bone marrow derived MSCs (BMSCs). Therefore, we addressed the molecular basis of the biological difference between AMSCs and BMSCs from multiple human donors by RNA-seq analysis. Comparison of the transcriptomes of both cell types defined a small set of transcription factors (TFs) and RNA binding proteins that are highly upregulated (>10 fold), as well as robustly expressed (>1 normalized reads per kilobasepair per million mapped reads) in adipose- but not bone marrow-derived MSCs. One of these factors is ZNF467, a Zn finger transcription factor previously shown to be involved in adipogenesis. Indeed, siRNA knock-down of ZNF467 in AMSCs prevents adipogenic differentiation, while not altering osteogenic differentiation ex vivo. Furthermore, siRNA depletion of the RNA binding protein PCBP3 resulted in the same outcome. Thus, key regulatory factors may be elevated in AMSCs to prime these cells for differentiation into the fat cell lineage. As second approach, we compared the effects of epigenetic and cytoskeletal drugs that recently have been shown to control MSC lineage commitment. We first tested the inhibitor GSK126, which reduces condensed facultative heterochromatin by blocking the H3K27me3 transferase inhibitor EZH2, a powerful epigenetic regulator (EpiReg). Treatment of AMSCs with GSK126 promotes osteogenesis, while inhibiting adipogenic differentiation with appropriate differentiation stimuli. For comparison, Cytochalasin D, a fungal metabolite that blocks actin polymerization, promotes osteogenic differentiation even in the absence of osteogenic induction cocktail, and does not perturb adipogenesis. Taken together, our results suggest that TFs, RNA binding proteins, EpiRegs and actin organization can be leveraged to control the adipose versus osteoblastic cell fates of MSCs.

Adrien Guerard

The medullary adipocytes contribute to the bone metastasis of prostate cancer and this effect is regulated by obesity

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Background: We have recently demonstrated that mature adipocytes of the periprostatic adipose tissue act as a driving force for the local dissemination of prostate cancer (PCa) through the secretion of the CCL7 chemokine, and that this effect was amplified by obesity. Then, PCa cells metastasize to distant site such as bone. During this dissemination, PCa cells interact with bone marrow where the main components are medullary adipocytes (MedAd)
Objective: We investigated the role of the MedAd secretions in the bone metastasis process of PCa. We also explored the amplification of this effect in obesity and aging, two known risk factor for bone metastasis in PCa.
Methods and results: Using a series of 35 samples from patients, we first showed in vitro (Boyden chamber assay) that conditioned mediums from human MedAd (MedAd-CM) were able to chemoattract PCa cells (by contrast to paired conditioned medium obtained from subcutaneaous adipocytes) with a strong amplification by obesity or aging. The chemoattractive potential of medAd-CM was mediated by the chemokine CCL7 which interact with one of its receptor CCR3 on tumor cells, as shown using pharmacological inhibitors, blocking antibodies and gene repression strategies. To validate this effect in vivo, we used the murine cell line RM1-BM able to localize to the bone after intra-cardiac injection. We observed that the loss of CCR3 in tumor cells abrogates their bone metastatic homing.
Conclusions: This study show for the first time a mechanism that could explain the increased bone metastatic dissemination of prostate cancer linked to obesity and aging. These data highlight the fact that medullary adipocytes, using the CCR3/CCL7 axis, are able to control the distant dissemination of PCa cells to the bone. In a context of obesity or aging, medullary adipocytes show a different phenotype leading to an increased secretion of CCL7 and enhanced dissemination.

Manar Shafat

Leukemic bone marrow adipocytes preserves and regulates proliferative leukemic blasts via FAPB4 and CPT1

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Background: Acute myeloid leukemia (AML) is an age-associated disease with poor survival outcomes in the >70 age group. The leukemic bone marrow microenvironment is a key contributor to AML progression. Given that percentage of marrow adipose tissue (MAT) increases with age, we investigated the relationship between marrow adipocytes and leukemic blasts.
Objective: In this study, we explored the role of MAT in regulating the survival and proliferation of AML. We hypothesize that MAT-derived FABP4 transporter protein regulates the transfer of free fatty acids (FFA) to AML blasts, and blast-derived CPT1 regulates FFA mitochondrial metabolism.
Methods and results: We used primary AML blasts and in vitro-differentiated adipocytes from BM-derived mesenchymal stem cells. Lentiviral-knockdown (KD) of FABP4 in MAT inhibited FFA transfer to blasts and its subsequent proliferation in AML/MAT co-culture. Moreover KD of CPT1 in AML showed decreased survival of blasts when cultured with MAT. Chemical Inhibitors of FABP4 (BMS309403) and CPT1 (Etomoxir) also decreased blast proliferation when cultured with MAT. Next we used the Seahorse XFp-analyzer to measure oxygen consumption rate (OCR) in primary AML compared to primary AML cultured with MAT. Results show that AMLs have increased OCR and fatty acid oxidation when cultured with MAT, moreover this was inhibited by Etomoxir. Finally, we used an in-vivo xenograft model to determine the significance of CPT1 in AML survival. NSG (NOS/SCIDgamma) mice were injected with primary AML with CPT1-KD knockdown and AML control-KD. Mice with CPT1-KD AML blasts had significantly increased survival compared with AML control-KD.
Conclusion: FABP4 KD in the adipocytes decreases lipid transport to the AML blasts thereby reducing proliferation. CPT1 is vital to FFA metabolism in the AML blasts with in vivo CPT1 knockdown animals showing increased survival times. These data identify new therapeutic targets for the treatment of AML.

Emma Morris

Multiple myeloma regulates bone marrow adipocyte number, localisation and adipokine secretion

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Background: Multiple myeloma (MM) is a fatal hematologic malignancy where tumour growth and bone disease are dependent upon cellular interactions within the bone marrow. Bone marrow adipocytes (BMAs) have an emerging role in bone physiology, however their contribution to MM pathogenesis is poorly understood. BMAs are a major source of adiponectin, an adipokine negatively associated with MM, suggesting a novel mechanism by which BMAs may regulate myeloma growth within the bone microenvironment.
Objective: Our aim was to elucidate the reciprocal relationship between MM cells, adiponectin and BMAs in vitro and in vivo.
Methods and results: To determine the effects of MM cells on BMAs in vivo, we used the 5TGM1-MM model. The number and location of BMAs were quantitated at weekly intervals following MM inoculation. A significant negative correlation between tumour burden and BMA number was demonstrated. Further analysis demonstrated an increase in BMAs closely associated with tumour, and a reduction in BMAs in areas of non-tumour bone marrow, suggesting a differential response of BMAs within the myeloma-bone microenvironment. Co-culture of MM cells with BMAs or BMSCs increased MM cell viability and decreased apoptosis, with a greater effect of BMAs to promote viability as compared to BMSCs. A significant increase in adiponectin mRNA and protein was detected in BMAs, as compared to BMSCs. An adiponectin receptor agonist induced MM apoptosis, however co-culture of MM cells with BMAs significantly decreased adiponectin mRNA and protein, suggesting that MM cells down-regulate adiponectin and avoid the tumour-suppressive effect of this adipokine.
Conclusion: Despite the overall decrease in BMAs with MM progression, BMAs are increased when closely associated with MM cells in vivo. Our studies suggest a supportive effect of BMAs on MM growth and survival, mediated in part by a reduction in adiponectin. Targeting BMAs and adiponectin could be a promising therapeutic in the treatment of MM.

Eleni Douni

Analysis of bone marrow adiposity in human RANKL-expressing transgenic mouse models of osteoporosis

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Background: Osteoporosis is characterized by increased bone resorption, reduced osteoblastogenesis and excessive bone marrow adipogenesis. However, it is not clear which factors drive the differentiation of mesenchymal stem cells towards adipocytes instead of osteoblasts and whether there is a direct or indirect effect of bone resorption in bone marrow adiposity (BMA). Receptor activator of nuclear factor-κB ligand (RANKL) is a central regulator of bone remodeling by mediating osteoclast-induced bone resorption. We have recently generated novel osteoporosis mouse models by expression of human RANKL in transgenic mice (TghuRANKL)[1]. Tg5516 line expressing RANKL at low levels develop mild trabecular bone loss while a more severe osteoporotic phenotype was identified in the Tg5519 line overexpressing RANKL with features of severe trabecular bone loss and cortical porosity. A common characteristic in both transgenic lines is the development of BMA in parallel with bone resorption.
Objective: In this study we investigated the progression of BMA in each TghuRANKL osteoporosis model and explored the expression profile of adipogenic genes.
Methods and results: Histological analysis demonstrated that TghuRANKL transgenic mice develop progressive bone marrow adiposity, which was quantified by micro-computed tomography upon staining of lipids with osmium tetroxide. More specifically, 4-mo-old Tg5516 mice developed BMA at distal femural metaphysis, a site of active bone resorption, with significant increase of marrow adiposity volume compared to WT littermates. More extended BMA was identified at the severe osteoporosis model Tg5519, expanding at distal femural epiphysis, metaphysis but also to diaphysis at sites close to cortical porosity. Expression analysis with pPCR of adipocyte markers and transcription factors favoring adipocyte differentiation demonstrated increased expression in C/EBPa, PPARγ2, FABP4, adiponectin, and leptin in femur extracts from TghuRANKL mice.
Conclusion: TghuRANKL mice constitute novel genetic mouse models for investigating the mechanisms that lead to marrow adiposity in conditions supporting osteoclastogenesis and bone resorption.

1. Rinotas V, Niti A, Dacquin R, Bonnet N, Stolina M, Han CY, Kostenuik P, Jurdic P, Ferrari S, Douni E. (2014). Novel genetic models of osteoporosis by overexpression of human RANKL in transgenic mice. Journal of Bone and Mineral Research; 29(5):1158-69.

Beate Lanske

PTH1R deficient mesenchymal stem cells favor adipogenesis over osteogenesis in vivo

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Background: Age-related osteoporosis is characterized by enhanced skeletal fragility, low bone mass, increased marrow-adiposity and high bone resorption. Recent studies have shown that bone-marrow-pre-adipocytes produce receptor-activator-of-nuclear-factor-kB-ligand (Rankl) and support differentiation and function of osteoclasts-like cells *in vitro*. PTH1R signaling has been shown to promote osteoblastogenesis while suppressing adipogenesis in mesenchymal stem cells (MSCs), however the mechanism is still unclear.
Objective: The aim of this study is to investigate the role of PTH1R signaling in regulating MSC fate and coordinating bone resorption.
Methods and Results: Mice with conditional deletion of PTH1R in MSCs (*Prx1cre;PTH1R$^{fl/fl}$*) showed a substantial increase in marrow-adipose-tissue (MAT) by 3-weeks of age quantified by osmium-tetroxide-staining. Isolated MAT from these mice showed high expression of adipose-related genes such as *Pparγ, Cebp-α,β,Δ, Fabp4* and *Adiponectin* compared to *PTH1R$^{fl/fl}$* controls. Bone-marrow-stromal-cells (BMSCs) under adipogenic culture from *Prx1cre;PTH1R$^{fl/fl}$* mice showed augmented adipogenic differentiation *in vitro*. Moreover, PTH(1-34) significantly inhibited *in vivo* and *in vitro* adipogenesis in control mice and BMSCs but not in PTH1R-ablated mice or cells. Lineage tracing experiments using BMSCs from *Prx1Cre;PTH1R$^{fl/fl}$/Tomato$^{fl/+}$* mice suggested that adiponectin-positive adipocytes derived from Tomato-positive-Prx1 lineage cells. Interestingly, the enhanced adipogenesis in mutants was accompanied by increased bone resorption, low bone mass and high *Rankl* mRNA expression in MAT and bone-marrow as well as high RANKL protein levels in serum and supernatant from spun marrow. Furthermore, no *Rankl* mRNA was detected in peripheral adipose tissue suggesting RANKL originates from MAT. Flow-cytometry analysis of BMSCs from *Prx1Cre;PTH1R$^{fl/fl}$/Tomato$^{fl/+}$* mice showed increased pre-adipocytes (Pref-1) producing RANKL, leading to the associated up-regulation of RANKL.
Conclusion: The data indicates that loss of PTH1R from MSCs favors adipogenesis, with augmented RANKL expression and enhanced osteoclastogenesis accompanied by increased bone resorption suggesting a critical role for PTH in determining cell-fate in the marrow niche, and that marrow adipocytes can mediate bone resorption.

Mara Riminucci

Marrow adipocytes and fibrous dysplasia of bone

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The development of marrow adipose tissue (MAT) is a post-natal event that occurs at specific times and sites in the growing skeleton. Fibrous dysplasia of bone is a genetic disease, caused by activating mutations of the Gsα gene (R201C, R201H), that appears in post-natal life and often progresses across the skeleton with a spatial and temporal pattern that overlaps with that of MAT. We have generated the first transgenic models of FD in which the Gsα^{R201C} sequence is expressed under the control of ubiquitous and constitutive promoters. The analysis of these mice has revealed that the development of the elementary tissue changes of FD, including osteomalacia, osteolysis and marrow fibrosis, associates with the emergence of a BAT-like phenotype in marrow adipocytes. This phenotype can be ascribed to the increased intracellular cAMP caused by the Gsα mutation, precedes the appearance of the abnormal FD osteogenic tissue (which expresses the inhibitor of bone matrix mineralization, MGP, and contributes to formation of osteomalacic bone), and accompanies the inappropriate bone resorption typically observed within FD lesions. To better understand the role of MAT in FD, we have recently established additional murine transgenic lines by crossing R26-LSL-Gsα^{R201C} mice with mice expressing Cre recombinase under the control of different adipocyte-specific promoters, such as Fabp4 and AdipoQ. The preliminary analysis of the R26-LSL-Gsα^{R201C};Fabp4-Cre mice has demonstrated the appearance of a skeletal phenotype in the tail vertebrae with typical radiographic and histological features of FD. The R26-LSL-Gsα^{R201C};AdipoQ-Cre transgenic mice are currently under investigation. Altogether, these different transgenic lines are expected to provide conclusive evidence as to the role of adipocytes in FD, specifically in the tissue changes (e.g., osteomalacia) that are the major determinants of skeletal morbidity caused by the disease.

Kerensa Beekman

Quantification of bone marrow adipose tissue using gradient-echo MR imaging.

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Background: Quantitative chemical shift imaging (QCSI) is the gold standard for in vivo quantification of bone marrow adipose tissue (MAT). However, this technique is not widely available and is limited to marrow fat quantification in small regions due to long scanning time. Therefore we aimed to quantify marrow adipose tissue in the spine using quantitative gradient-echo (Q-GRE) MR imaging.
Objective: to determine the feasibility of quantitative gradient-echo (Q-GRE) MR imaging for MAT quantification.
Methods: MAT was quantified in L3-5 of 11 Gaucher's disease patients using both Q-GRE (scan time approximately 1 minute) and Dixon QCSI (spin-echo sequence; scan time 21 minutes) with the same 1.5 Tesla MRI-scanner, measured on the same day. Regions-of-interest were drawn in the vertebral bodies and the marrow fat fraction as a percentage of total signal was obtained. Subsequently, fat fractions were measured in the total spine (C1-L5) of 40 healthy subjects (median age 51 years; range: 23-76, 26 males; 14 females) using Q-GRE.
Results: In Gaucher's disease patients L3-5 fat fractions, measured with QCSI and Q-GRE showed similar results (ICC of L3-5 was 0.80 (95% CI 0.32-0.95, p<0.001). In healthy subjects mean Q-GRE fat fraction (± SD) was 36.06% (±9.87), 37.93% (±8.05) and 43.39% (±9,51) for the cervical, thoracic and lumbar spine respectively (LMM: p<0.001). Women below 50 years of age had a lower fat fraction compared to men of the same age (mean ± SD: 32.05% (±2.27) vs. 37.01% (±7.84) Mann-Whitney U: p=0.254), whereas women over 50 had a higher fat fraction compared to men of the same age (mean ± SD: 48.12% (±6.55) vs. 42.28% (±7.68); Mann-Whitney U: p=0.51).
Conclusion: Q-GRE imaging is a fast, accurate and widely available alternative for QCSI for the quantification of MAT and allows simultaneous quantification of MAT in large parts of the skeleton.

Vasco Campos

Accelerating the yellow to red bone marrow transition during hematopoietic stem cell transplantation

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Background: Worldwide, more than 50.000 Hematopoietic Stem Cell Transplantations (HSCT) are performed annually, although the mortality rate still is close to 50% within the first three years after allogeneic transplantation. Forty percent of these fatalities relate to the patients being severely immune compromised during the post-ablation period, before the graft has fully reconstituted the hematopoietic system. Reducing the time of engraftment is therefore critical to increasing the chance of survival in these patients. Preventing bone marrow adipocyte formation in the post-transplant period has been demonstrated to accelerate hematopoietic stem cell (HSC) engraftment and subsequent hematopoietic recovery in mice.
Objective: We are interested in finding new ways to reduce bone marrow adipocyte formation during HSCT and thereby accelerating the highly plastic transition between yellow (adipocytic) and red (hematopoietic) bone marrow.
Methods and Results: In order to uncover novel promoters of the yellow to red bone marrow transition, we performed a high-throughput label-free 2D in vitro screening on the bone marrow-derived mesenchymal stromal cell (MSC) line, OP9. This cell line was demonstrated to be both a useful model to efficiently differentiate into adipocytes as well as a support hematopoiesis in vitro. Using Digital Holographic Microscopy, we screened the Prestwick library of FDA-approved drugs and natural compounds for inhibitors of adipocytic differentiation based on real-time lipid accumulation. Enhancement of hematopoiesis by these anti-adipogenic candidates is then tested via newly developed 2D and 3D in vitro HSC/MSC co-culture systems using OP9s and primary murine hematopoietic stem and progenitor cells. Finally, marrow adipocyte formation can be measured in vivo after HSCT and correlated with the kinetics of hematopoietic recovery.
Conclusion: All current clinical approaches to enhance hematopoiesis target the HSC itself. Here we propose targeting marrow adipogenesis as an alternative strategy to accelerate the yellow to red bone marrow transition thereby improving post transplant survival.

Aline Clabaut

Transdifferentiation of MSC-derived osteoblasts following coculture with MSC-derived adipocytes

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Background: In osteoporosis, bone loss is accompanied by an increase of adiposity in bone marrow. A dialogue between adipocytes and osteoblasts is one of the ways occurring in the competition between Mesenchymal Stem Cells (MSCs) lineage commitments, supporting adipocyte differentiation at the expense of osteoblast differentiation. Using an in vitro coculture model based on human primary MSCs, we previously shown that MSC-derived adipocytes induce MSC-derived osteoblasts to differentiate towards an adipocyte-like phenotype. Indeed, upon coculture, MSC-derived osteoblasts showed appearance of adipocyte and decrease of late osteogenic mRNA markers.
Objective: To confirm transdifferentiation of osteoblast to adipocyte under the influence of secreted products released by MSC-derived adipocyte.
Methods and results: Double immunofluorescence microscopic analyses were performed to show the co-localization of adipogenic and osteoblastic proteins on a single cell level. These results clearly showed that at less 12% of the osteoblastic cells expressed the adipogenic marker PPARy2, when osteoblasts cells are incubated during only 48h with adipocyte conditioned medium. On molecular level, such conversion was confirmed by upregulated expression of reprogramming gene (OCT4). Moreover, whole genome methylation analyses showed that levels of *5*-methylcytosine (5-mc) was strongly decreased in osteoblastic cells after co-culture accompanied by changes in the expression profile of enzymes implicated in methylation.
Conclusion: These complementary data strengthen our hypothesis of osteoblastic cells transdifferentiation towards an adipocyte phenotype and suggest that this phenotypic commitment could be controlled by epigenetic mechanisms.

Xavier Coutel

Assessment of bone marrow adiposity in the mandible of adult ovariectomized rats

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Backgrounds: More and more data argue for an involvement of bone marrow adiposity (BMA) in bone pathophysiology. BMA alterations have been reported to be age, gender and site specific. Mandibular bone is composed of specific functional areas (alveolar bone surrounding teeth) and has a high bone turnover compared to other skeletal sites. However, BMA distribution is unknown on this particular bone site.
Objective: The aim of this study is to investigate bone microarchitecture and BMA alteration in the mandible (tooth-bearing / non tooth-bearing areas) and tibiae of ovariectomized rats.
Methods: Female Sprague-Dawley rats were OVX or SHAM operated at 6 months old. 9 Hemi-mandibles and tibiae were harvested in each group 4 months after surgery. 9 SHAM animals were euthanized at baseline. Ex vivo µCT analyses were performed with the same acquisition parameters using a Skyscan 1172 (Bruker, Kontich, Belgium) at $10\mu m^3$ voxel size. Bone histomorphometry and BMA parameters were assessed in the trabecular bone of the condyle, the alveolar bone of the first molar, and the proximal tibia. BMA imaging and measurements were obtained after bone decalcification and osmium staining.
Results: At Baseline, trabecular bone volume, number and thickness were higher in both alveolar process and condyle compared to tibiae. Interestingly, medullar volume was inferior in the alveolar and the condylar bone compared to tibiae. BMA volume was higher in the tibiae compared to the mandibles. However, the AV/MV ratio was higher in the alveolar bone and lower in the condylar bone compared to tibia. 4 months post-surgery, a decrease in bone microstructure parameters is observed and is associated with an increase of the adipose compartment compared to baseline.
Conclusion: Mandibular bone shows a denser trabecular network compared to tibia. Present data suggest specificities in the microarchitecture and the BMA distribution in mandibular tooth-bearing and non tooth-bearing bone sites.

Antoine Dalla Valla

Induction of stearoyl-CoA desaturase-1 expression protects human mesenchymal stem cells against palmitate-induced lipotoxicity

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Background: Accumulation of adipocytes in the bone marrow (BM) niche is a phenomenon observed in osteoporotic and osteonecrotic patients. The impact of free fatty acids (FFAs) release by BM adipocytes on bone cells viability and function is still debated.
We previously showed that saturated FFAs (SFAs) like palmitate trigger endoplasmic reticulum stress, pro-inflammatory cytokines secretion and apoptosis in human mesenchymal stem cells (hMSC). On the contrary, mono-unsaturated FFAs (MUFAs) like oleate are nontoxic and abolish the deleterious effects of SFAs. Therefore, we postulated that increasing the activity of stearoyl-Co-A desaturase-1 (SCD1), the enzyme transforming SFAs into MUFAs, could protect hMSC from lipotoxicity.
Objective: To analyze whether upregulation of SCD1 expression may counteract lipotoxicity induced by SFAs in hMSC.
Methods: Gene and protein expression were determined by RT-qPCR and western blot, respectively. Viability was observed by Hoechst/PI staining. Caspases-3/7 activity was determined using the Caspase-Glo® 3/7 assay.
Results: We showed that hMSC express Liver X Receptor (LXR), a transcription factor regulating SCD1 expression. Treatment of hMSC with the LXR agonist To901317 increased SCD1 expression at both the mRNA and protein level. We further demonstrated that the treatment with To901317 protected hMSC from palmitate-induced cell death and caspase 3/7 activation. In the presence of a SCD1 inhibitor, the LXR agonist failed to prevent the deleterious effects of palmitate. Finally, we established that To901317 counteracted the upregulation of markers of endoplasmic reticulum stress (BiP and CHOP) and inflammation (IL6 and IL8).
Conclusion: Induction of SCD1 expression protects hMSC from lipotoxicity probably via modulation of the ratio of saturated/monounsaturated FFAs.

Bram C.J. van der Eerden

Osteogenic differentiation of human mesenchymal stromal cell relies on autocrine/paracrine leptin activity/action

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Leptin is an important molecule linking energy to bone metabolism. Although the central and peripheral effects have been extensively studied, the role of locally produced leptin by bone itself has not yet been fully explored. Therefore, we assessed the role of endogenous leptin on osteogenic differentiation of human mesenchymal stromal cells (MSC).
Leptin and leptin receptor (LEPR) mRNA were expressed in both MSC-derived osteoblasts and adipocytes but the level of leptin in osteoblasts was 10-fold higher compared to adipocytes. This was confirmed at the protein level, using ELISA. Immunocytochemically, leptin was located throughout the cytoplasm. Next, we assessed whether leptin produced by osteoblasts had an autocrine/paracrine effect on osteoblast differentiation by either blocking the binding to LEPR, using a leptin neutralising antibody (nAb), or by using short hairpin RNA (shRNA) against the LEPR. Compared to untreated osteoblasts, mineralization was strongly reduced following nAb treatment (-80% at day 14, -40% at day 17 of culture). This was corroborated by inhibition (60-100%) of mineralization by 3 different LEPR shRNAs. Osteoblast marker genes were unaffected in the first 10 days of culture compared to controls by nAb treatment. Interestingly, while the expression of these genes started to decrease in the control condition after 10 days at the onset of mineralization, neutralizing leptin led to a persistent high expression: collagen I (+300% and +400% at days 14 and 17), alkaline phosphatase (+150% at days 14 and 17) and RUNX2 (+300% at day 17) compared to control.
In conclusion, osteoblast maturation and mineralisation require endogenously produced leptin, thereby adding complexity to the role of leptin in bone metabolism. We hypothesize that leptin signaling plays a role in transition to the mineralisation phase and that lack of leptin signaling prevents the necessary downregulation of osteoblast differentiation genes and thereby inhibits the differentiation and delays mineralisation.

Olfa Ghali

Bone marrow adiposity and energy deficit in mouse: no simple relationships

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Background: An increase in bone marrow adiposity (BMA) is usually described in anorexia nervosa (AN) patients and in calorie restriction models. This BMA could be involved in the development of the osteoporosis often described in AN. However, mice submitted to a long-term protocol associating separation and time-restricted feeding to induce a body weight decrease around 25% (Separation-based anorexia - Ethical approval CEEA #022012), did not display any obvious alteration in BMA despite a severe body weight loss and a low bone mass.
Objectives: Therefore we explored the relationships between the severity of body weight loss, bone mass and BMA in this model.
Methods: The SBA model was adapted to target various levels of body weight loss. At the end of ten week protocol, tibia bone parameters and changes in BMA (OSO4 staining) were assessed by using microCT. Differentiation capabilities of bone marrow stromal cells (BMSCs) from control and SBA mice were determined in a co-differentiation medium allowing the commitment in both differentiation pathways. Plasma adipokines were assessed by ELISA methods.
Results and discussion: Our results showed that bone alterations take place without increase in BMA and even when BMA is decreased. Moreover, the mildest body weight losses (0% and -12%) induced BMA lower than in control mice, while the more severe (-24%) was associated with a normalization of BMA. Interestingly, BMSCs from SBA mice displayed a dramatically increase in adipogenic differentiation at the expense of osteogenic differentiation, whatever the severity of the SBA protocol. To understand this phenomenon, plasma levels of several key factors are being assessed.
Conclusion: This study suggests that relationships between body weight loss, bone mass, bone microarchitecture and bone marrow adiposity are very complex. Furhter more specific studies will be needed to better understand these relationships and to design in the future efficient anti-osteoporotic strategies targeting BMA.

Louise Grahnemo

Low adipogenesis results in high bone mass in mice

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Background: Bone and fat are linked in many ways. For example, expansion of bone marrow adipose tissue (MAT) is often associated with low bone mass, possibly because adipocytes and osteoblasts competitively differentiate from the same stem cell.
Objective: To test the hypothesis that reduction of adipocyte numbers, via low adipogenesis, results in high bone mass.
Methods: Adipocyte numbers were reduced by using the RID transgenic (tg) mouse that has low visceral adipogenesis, and possibly also low MAT adipogenesis. High fat diet (HFD) was fed for 11 weeks to female and male RID tg mice and wildtypes. Bone mineral density (BMD) was determined by dual energy X-ray absorptiometry during the study. At termination, femur and humerus were collected for analysis by peripheral quantitative computed tomography and three-point bending, respectively.
Results: Male RID tg mice had higher total BMD at 4 (+5.0%, p=0.002), 8 (+5.4%, p<0.001), and 11 (+3.6%, p=0.006) weeks of HFD, as well as higher lumbar BMD at 4 (+10.6%, p=0.002), 8 (+16.8%, p<0.001), and 11 (+7.5%, p=0.02) weeks of HFD, compared with wildtypes. Male RID tg mice also had higher femoral trabecular BMD (+9.1%, p=0.02) and cortical content (+9.7%, p=0.02) than wildtypes. For female mice, the bone phenotype was not apparent until 11 weeks of HFD, when female RID tg mice had higher lumbar (+16.5%, p<0.001), but not total, BMD than wildtypes. Female RID tg mice also had higher femoral trabecular BMD (+10.8%, p=0.01) and cortical content (+8.2%, p=0.005) than wildtypes. Three-point bending of humeri indicated that male and female RID tg mice had higher bone strength (+44% and+47% respectively, p<0.01) than wildtypes.
Conclusion: RID tg mice with low adipogenesis had higher BMD and bone strength than wildtypes. However, it remains to be investigated if this is caused by their low visceral adipogenesis or a possible low MAT adipogenesis.

Alex Jacobs

Vanadate reduces adipocytic differentiation of mesenchymal stem cells derived from different regions within the rat femur.

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Background: Chronic systemic glucocorticoid (GC) treatment can lead to osteoporosis and increase marrow adiposity. GC-induced osteoporosis (GIO) may be due to increased osteoblast apoptosis and/or skewing of the differentiation of mesenchymal stem cells (MSCs) away from osteogenesis towards adipogenesis, resulting in fewer osteoblasts. Vanadate, a protein tyrosine phosphatase inhibitor, inhibits adipogenesis in 3T3-L1 pre-adipocytes and also prevents GIO in vivo in rats, suggesting that vanadate may avert marrow adiposity associated with GIO. To investigate this hypothesis, we examined the effects of GCs and vanadate on MSCs isolated from the bone marrow and the proximal region of the femur, as this latter region is typically fractured during osteoporosis.
Methods and Results: Rat femora were harvested and MSCs isolated from the proximal femur region (pfMSCs) and the diaphyseal marrow (bmMSCs). Both cell-types were found to express the MSC cell surface markers CD90 and CD106, but not the haematopoietic marker CD45. Upon treatment with an adipogenic cocktail (dexamethasone, IBMX, indomethacin, insulin, ascorbic acid), pfMSCs rapidly differentiated into adipocytes within 7 days, compared to 21 days required by bmMSCs. Conversely, when treated with an osteogenic cocktail (ascorbic acid, beta-glycerophosphate, dexamethasone), bmMSCs rapidly differentiated into osteoblasts, while pfMSCs exhibited a weak osteogenic response. Neither cell-types accumulated lipid in response to GCs (1 µM dexamethasone), but the pfMSCs displayed a rapid loss of cell viability which was not observed in bmMSCs. The dexamethasone-induced loss of cell viability was associated with an increase in apoptosis, but could not be prevented with vanadate co-administration. Vanadate treatment reduced lipid accumulation and expression of adipogenic markers (PPARγ2, aP2, adipsin) during adipogenesis of both bmMSCs and pfMSCs.
Conclusion: It seems unlikely that vanadate prevents GIO by inhibiting apoptosis of osteoprogenitor cells, but data presented here supports the notion that vanadate may reduce marrow adiposity associated with GIO.

Karin Jöhrer

Myeloma-adipocyte crosstalk promotes tumor growth and modulates cancer metabolism

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Background: Multiple myeloma is a plasma cell malignancy that is almost restricted to the bone marrow. There, crosstalk with adipocytes might influence tumor establishment and progression as well as development of drug resistance.
Objective: Utilizing cell lines and primary cells we investigated proliferation, migration and drug resistance in myeloma-adipocyte cocultures. In addition, we tested the effect of the inhibitor of fatty acid synthesis, Orlistat, in this setting.
Methods: Myeloma cell lines NCI-H929, OPM-2, U266 were used as tumor models and cocultured with SGBS cell line, primary adipose-tissue derived stem cells (ASC) and human multipotent adipose-derived stem cells (hMADs) which were also further differentiated to adipocytes and used in coculture, respectively. Primary adipocytes isolated from peripheral fat tissue were also utilized for cocultures. Proliferation was measured by 3H-thymidine incorporation and migration by transwell assays. Drug-induced cell death rates in cocultures were calculated according to AnnexinV/7-AAD staining analyzed by flow cytometer. Cell culture supernatants were analyzed by protein arrays. Expression of enzymes representing main metabolic pathways was investigated by qPCR.
Results and Conclusion: Myeloma cells showed increased proliferation rates under coculture conditions with all tested models of pre- and mature adipocytes. Whereas ASCs rescued myeloma cells from drug-induced cell death, primary adipocytes even induced cell death, depending on the cell numbers utilized. Fatty acids derived from patients increased proliferation of myeloma cells. The impact of Orlistat on myeloma cell proliferation was essentially neutralized in the coculture setting. Migration of myeloma cells was enhanced by the conditioned media of ASCs and myeloma cells. Protein arrays of the different cell types and cocultures revealed a specific profile that might contribute to the observed effects.
From our data we conclude that myeloma-adipocyte crosstalk promotes proliferation, migration and partially drug resistance of the malignant cells. Inhibitors like Orlistat might not be efficient in the myeloma microenvironment.

Stéphanie Lucas

Contribution of bone marrow adipocytes to the altered bone remodeling of the ovariectomy model

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Background: In the fat-bone interaction, Bone Marrow Adipocytes (BMA) are sensed to be critical players. Indeed, the BMD loss in osteoporosis is paralleled by a severe increase in BM adiposity; adipokines can in vitro alter the differentiation, function and survival of osteoblasts or osteoclasts. However primary mature BMA remain poorly studied: their relative capacity compared to extramedullary adipocytes to release adipokines and to interfere with bone remodeling has not been clearly established in vivo.
Objective: Our aim is to characterize the phenotype of BMA in the ovariectomy model.
Methods & results: Sham-operated/ovariectomized mice were analyzed after 4 or 14 weeks following the surgery. As the trabecular bone volume declines at the two time points following ovariectomy, the adiposity percentage increases in the tibia metaphysis. Real-time PCR analysis was performed to compare BMA isolated from femurs and tibias to isolated perigonadal adipocytes in the ovariectomized mice. At both time points, BMA have decreased expression levels of transcriptional factors involved in adipogenesis and classical adipokines compared to visceral adipocytes. However, some MMPs, RANKL and three Wnt-signaling inhibitors (sFRP4, sFRP1 and DKK1) are found highly expressed in the BMA. Moreover, the mRNA levels of these factors increase in the BMA between 4 and 14 weeks of ovariectomy. Importantly, the sFRP4 protein expression is confirmed in the BMA by immunohistochemistry. Whereas the protein is undetected in plasma using western-blot, sFRP4 level increases in the tibia BM fluids after 14 weeks of ovariectomy.
Conclusions: Compared to peripheral adipocytes, BMA exhibit a specific phenotype characterized by the expression of several factors known to interfere with osteoblastogenesis and osteoclastogenesis. Our data also indicate that the BMA pattern evolves as the bone loss worsens and can modify the BM microenvironment. Altogether, our study supports that BMA can contribute to the bone loss in a model of postmenopausal osteoporosis.

Domenico Mattiucci

Identification of different adipocytes populations within human marrow microenvironment

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The bone marrow (BM) microenvironment contains a variety of cell types at different maturation stages. Among haematopoietic elements, adipocytes (BM-A) represents the most abundant stromal component. They progressively increase with ageing, and eventually occupy up to 50 % of BM cavity.
We studied adipocytes from femoral head of hip surgery patients. BM-A morphology was different depending on their location, we observed several smaller adipocytes interspersed within the hematopoietic cells and larger cells in low hematopoietic areas. Electron microscopy observation showed that BM-A presented the same morphology of white adipocytes, some cells were surrounded by slender cytoplasmic extensions owing to mesenchymal like cells.
We also isolated BM-A after collagenase digestion and filtration and we cultured them through the ceiling culture method. We observed that, while cells coming from rich hematopoietic femoral head compartment maintained in culture their morphological features, BM-A derived from some older patients, that presented higher amount of yellow marrow, de-differentiated in culture into fibroblast-like cells, as usually observed with adipose tissue derived adipocytes (AT-A). Notably when BM-A were isolated from human iliac crest no de-differentiation processes were observed.
We study the gene expression profile of BM-A coming from red marrow of femoral head compared them with AT-A. BM-A displayed a different gene expression profile respect to AT-A and the analysis of pathways involved in haematopoiesis regulation showed that BM-A are closely related to BM-MSC. Cytokines secretion showed that critical molecules as CXCL12, IL3, IL8, G-CSF and LIF, were expressed at similar level in BM-A and BM-MSC.
Future studies of these BM-A populations will be important to link BM-A to hematopoiesis, inflammatory disease and ageing.

Gina Woods

Are Sex Hormones Negatively Associated with Vertebral Bone Marrow Fat?

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Background: Higher bone marrow fat (BMF) is associated with osteoporosis, diabetes, and reduced hematopoiesis. However, the mechanisms controlling BMF are not well understood. Exogenous estradiol reduces BMF accumulation in older women, but effects of endogenous sex hormone levels on BMF are unknown.
Objective: To examine the cross-sectional association between endogenous sex hormones and BMF using data from the Iceland AGES-Reykjavik cohort of older adults.
Methods and results: Vertebral BMF was measured with 1.5T scanner using single voxel proton magnetic resonance spectroscopy (1H-MRS). Participants using bone-active medications (oral glucocorticoids, osteoporosis medications, hormone replacement therapy, anti-androgens, and aromatase inhibitors) were excluded. Sex hormone blinding globulin (SHBG), estradiol, and testosterone were measured on archived serum from 249 participants who attended a clinic visit in 2011 and from an additional 238 during a visit in 2015. Free estradiol and testosterone levels were estimated using Mazer's method. One participant was excluded due to exceptionally high hormone levels. Linear regression models were adjusted for age, BMI and visit date. Analyses included 253 men, mean age 79 (SD 3.7) years, and 233 women, mean age 78 (SD 3.7) years. Mean BMF was 54% (SD 8.7) (men) and 55% (SD 8.1) (women). In unadjusted models, BMF was negatively associated with free estradiol in men [-1.15 difference in BMF (%) per 1 SD change in free estradiol (95% CI -2.20, -0.09)] but not in women [-0.56% (95% CI: -1.60, 0.47)]. After adjustment, BMF remained negatively associated in men [-1.17% (95% CI: -2.26, -0.09)], and was further attenuated in women [-0.14% (95% CI: -1.28, 1.00)]. Free testosterone and SHBG were not associated with BMF in either sex.
Conclusion: Higher endogenous estradiol levels were associated with lower bone marrow fat in older men but not in women. These results suggest a gender-specific role for estradiol in regulation of marrow fat.

Karla Suchaki

Mice lacking 11β-hydroxysteroid dehydrogenase 1 (*11βHsd1*) have no alteration in bone marrow adipose tissue despite intracrine activation of glucocorticoids

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Background: Bone marrow adipose tissue (MAT) accounts for over 10% of total fat mass in healthy adults and further increases in diverse clinical contexts. These include conditions of glucocorticoid (GC) excess, such as Cushing's disease, chronic GC therapy, and in anorexia nervosa. Indeed, our recent work suggests that GC excess contributes to MAT expansion during caloric restriction (CR). The enzyme 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) mediates intracellular GC reactivation, thereby potentiating GC activity. One study found that MAT is absent in tibiae of mice lacking 11βHSD1 (*Hsd11β1*$^{-/-}$); however, whether 11βHSD1 deficiency impacts MAT formation elsewhere, or influences CR-associated MAT expansion, remains unknown.
Objective: To determine the effect of 11βHSD1 ablation on MAT formation at multiple skeletal sites, thereby establishing the impact of 11βHSD1 on global MAT development.
Methods and results: We characterised MAT in humeri, femurs, tibiae and vertebrae of male and female wild-type (WT) and *Hsd11β1*$^{-/-}$ mice. These genotypes had similar body and fat masses, while X-ray analysis revealed no genotypic differences in bone length or bone mineral density of femurs or tibiae. Based on immunoblotting and qPCR, expression of adipocyte markers in whole bones was unchanged between the WT and *Hsd11b1*$^{-/-}$ mice. Preliminary µCT analysis of osmium tetroxide-stained bones further suggested no difference in MAT volume between these genotypes. Finally histomorphometry of vertebral bone marrow adipocyte size and distribution was not significantly different between WT and *Hsd11β1*$^{-/-}$ mice.
Conclusion: Mice lacking 11βHSD1 exhibit no significant differences in MAT content, which stands in contrast to previous data suggesting that 11βHSD1 is required for MAT formation. However this previous study used a different strain of *Hsd11b1*$^{-/-}$ mice and focused only on tibiae, underscoring the importance of MAT analysis at multiple skeletal sites. Further work is required to characterise the MAT phenotype in CR providing key insights into glucocorticoid function in CR.

Richard Sulston

Increased circulating adiponectin in response to thiazolidinediones: investigating the role of bone marrow adipose tissue

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Background: Bone marrow adipose tissue (MAT) contributes to increased circulating adiponectin, an insulin-sensitising hormone, during caloric restriction (CR), but whether this occurs in other contexts remains unknown. MAT expansion and hyperadiponectinemia also occur during treatment with thiazolidinediones (TZDs), a class of anti-diabetic agents with beneficial metabolic effects. Notably, TZDs can increase circulating adiponectin without altering adiponectin expression in white adipose tissue (WAT).
Objective: To assess the contribution of MAT expansion to TZD-associated hyperadiponectinemia.
Methods: We addressed this by investigating effects of rosiglitazone, a prototypical TZD, in wild-type (WT) or Ocn-Wnt10b mice; the latter resist MAT expansion during CR, leading us to postulate that they would also resist this effect of rosiglitazone.
Results: In WT mice, rosiglitazone induced hyperadiponectinaemia and led to marked MAT expansion, as assessed by osmium tetroxide staining and adipocyte marker expression. In comparison, MAT volume in Ocn-Wnt10b mice was decreased in distal tibiae but only partially in proximal tibiae; however, interpretation was complicated by leakage of osmium tetroxide from ruptures in some tibiae. Despite decreased MAT volume in Ocn-Wnt10b mice, circulating adiponectin concentrations were generally similar to those in WT mice; however, in female mice receiving rosiglitazone for 4 weeks, hyperadiponectinaemia was blunted in Ocn-Wnt10b compared to WT mice. Notably, this was also the only group in which tibial adiponectin expression was lower than in WT mice, suggesting a close association between MAT adiponectin production and circulating adiponectin. However, rosiglitazone increased adiponectin protein expression in WAT, suggesting that WAT also contributes to hyperadiponectinemia in this context.
Conclusions: Together, our observations support the conclusion that TZD-induced hyperadiponectinemia is closely associated with increased adiponectin production in MAT, but is not prevented by the partial loss of MAT that occurs in Ocn-Wnt10b mice. Thus, more robust loss-of-MAT models are required for future studies to better establish MAT's elusive functions.

Michaela Tencerova

Study on impact of obesity on cellular and molecular characteristics of bone marrow stromal stem cells in healthy men

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Background: Obesity is a risk factor for development of insulin resistance, Type 2 Diabetes and other metabolic diseases. Also obesity is associated with increased bone fragility, bone fractures and osteoporosis. This suggests that bone marrow stromal (skeletal) stem cells (BMSC) may be affected by metabolic status of obese patients. However, little is known on changes of the cellular and biological characteristics of BMSC associated with obesity.
Objective: To investigate in a case-control study, whether obesity is associated with intrinsic changes in cellular and molecular characteristics of BMSC.
Material and Methods: Participants including lean (n=15), overweight (n= 6) and obese metabolically healthy (n= 8) men (age 32□ 2 years; body mass index (BMI) 21-40 kg/m2) were recruited from the local community. Bone mass, body composition and metabolic characteristics were analyzed using DXA scan, oral glucose tolerance test (OGTT) and standard biochemical analysis. Iliac crest bone marrow aspirates were obtained and in vitro cell cultures of BMSC were established. Cellular phenotype, cell growth and differentiation into adipocytes and osteoblasts were determined including measuring alkaline phosphatase activity, Alizarin red staining for mineralized matrix formation and Oil-Red O staining for mature adipocytes. Immunophenotypization of BMSC was performed by flow cytometry.
Results: The groups of overweight and obese men differ from the group of lean participants in investigated anthropometric characteristics. Basic cellular characteristics of BMSC in overweight and obese men did not show any difference compared to lean (Table 1). Currently data regarding cell proliferation and responsiveness to osteoblast and adipocyte differentiation-induction signals are being processed.
Conclusion: No differences were observed in the amount of bone marrow obtained or in baseline characteristics of BMSC obtained from obese and lean healthy individuals. Studies of differentiation responsiveness of BMSC of these groups are likely to yield information regarding the presence of intrinsic cellular and molecular changes associated with obesity.

Josefine Tratwal

A semi-automated image analysis tool for quantitative assessment of bone marrow in histological sections

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Background: If bone marrow (BM) was long considered a source of homogeneous tissue, we now know that depending on skeletal location, age, and physiological conditions, it exists heterogeneously as hematopoietic (red) or adipocytic (yellow) marrow. BM adipocytes are proposed to inhibit hematopoiesis, and BM failure is accompanied by massive adipocytic infiltration (red-to-yellow transition). Following hematopoietic stem cell (HSC) transplantation, a yellow-to-red transition occurs, with hematopoietic recovery assessed by pathologists' scoring of BM cellularity. However, accessibility to this expertise can be problematic outside clinical laboratories.
Objective: We have developed and optimized a semi-automated image analysis plugin for ImageJ, MarrowQuant, to systematically quantify BM in histological sections.
Methods and Results: Based on variations in color and texture of H&E staining on histological sections, MarrowQuant distinguishes and quantifies area of adipocyte ghosts (with circularity and size parameters), hematopoietic cells, red blood cells, and bone. We find that MarrowQuant analysis correlates directly with scoring by three independent clinical pathologists and is comparable to volumetric quantification by microCT. In BM sections of homeostatic C57BL6 mice, we observe the red-to-yellow marrow transition prominently in the vertebrae of the caudal tail and proximal-to-distal tibia. With age, regions of BM adiposity increase and appear in the femur. Following HSC transplantation, adipogenesis inversely correlates with kinetics of hematopoietic recovery. Indeed, BM adipocytes reach maximum expansion at 17 days, and BM hematopoiesis recovers after 25 days when adipogenesis recedes (yellow-to-red transition), consistent with the exit of severe neutro- and thrombocytopenia, and recovery of pre-transplant cell blood counts.
Conclusion: Quantification of BM cellularity by MarrowQuant correlates directly with expert scoring by independent clinical pathologists from different countries. Together with volumetric comparison by microCT, this supports MarrowQuant as a valid quantification tool for histological sections, opening avenues for its application in an experimental or clinical context.

Bone chip is the main source of mesenchymal stem/progenitor cells in adult mouse bone marrow

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Background: Hematopoietic stem cells (HSCs) produce all blood cells. They reside in a specialized niche of the bone marrow (BM) where they interact with mesenchymal stem/progenitor cells (MSPCs). When HSC function is impaired, BM failure installs, which is characterized by important infiltration of adipocytes. It was shown that mature adipocytes inhibit, whereas progenitors (including MSPCs and *Pref1* expressing preadipocytes) support hematopoiesis. Moreover, MSPCs exert a strong regulatory effect on immune cells that may impact engraftment and BM repopulation by protecting tissues from insult of alloreactive cells. Together, this suggests that the MSPC-to-adipocyte differentiation axis regulates HSC functions.
Objective: The purpose of this study is to identify and characterize BM MSPC/preadipocyte populations with hematopoietic-supportive and immunomodulation potentials.
Methods and results: We have isolated three cell fractions from mouse femurs and tibias: flushed BM, cells released from collagenase-digested bones, and cultured cells from digested bone chips (BC). We first assessed the presence of MSPCs in each fraction by quantifying the number of colony forming unit-fibroblasts (CFU-Fs). We found that BC is the main source of CFU-Fs. Phenotypically, cells in the BC fraction are heterogeneous for the expression of MSPC markers (SCA-1, CD105 and PDGFRα). Using a *Pref1-CreER* mouse model, we observed that BC also contains *Pref1*$^+$ cells, suggesting the presence of preadipocytes. These results are in line with high adipocytic differentiation potential by these cells, as assessed by quantification of Oil Red O staining.
Conclusion: Our results suggest that the BC fraction contains MSPCs and preadipocytes. Indeed, BC is the main source of CFU-Fs, contains cells expressing preadipocyte marker *Pref1,* and are proficient in adipocytic differentiation. However, this cell population is heterogeneous for the expression of MSPC markers, suggesting that the BC fraction could be further subdivided in phenotypic populations. We are presently characterizing subpopulations for their differentiation capacity and immunomodulation potential.

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