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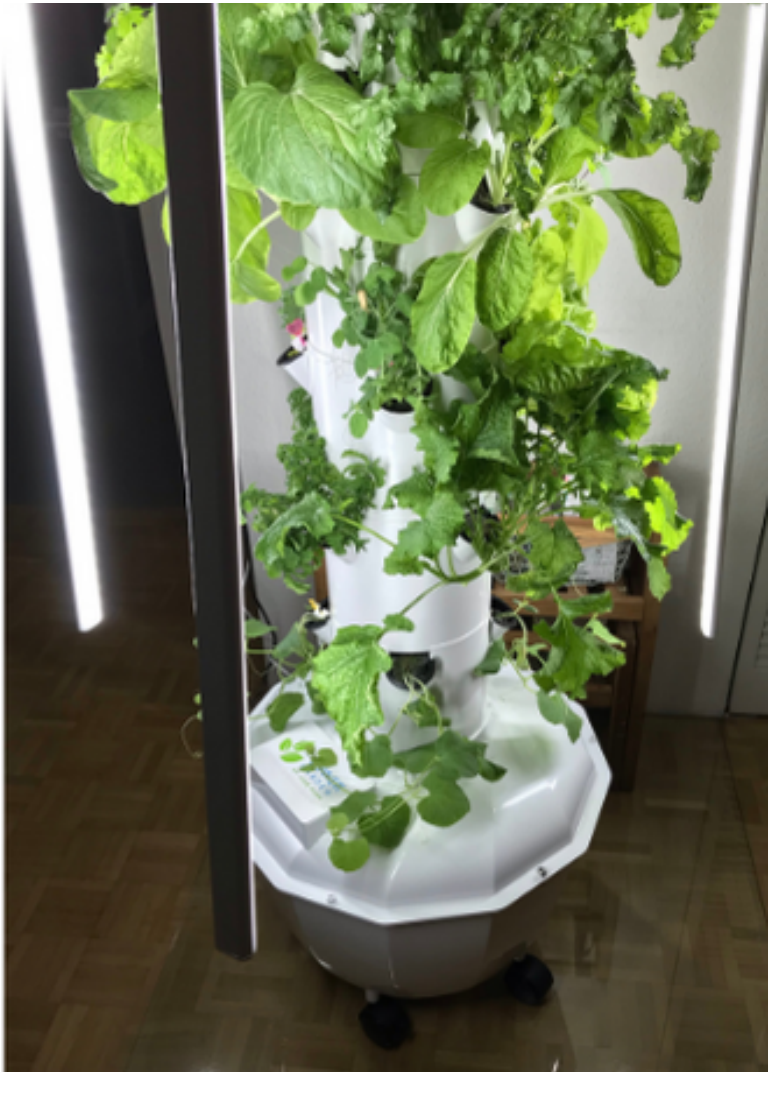

Wissenschaftliche Herbsttagung der Österreichischen Gesellschaft für Knochen und Mineralstoffwechsel (ÖGKM) 25. und 26. November 2011, Wien

Abstracts von Vorträgen und Postern*

Regeneration von oralem Gewebe: Die Regulation von Vascular Endothelial Growth Factor, Sklerostin und Dickkopf-1 in parodontalen Fibroblasten

H. Agis[1], L. Hueber[1], M. Gruber[1], M. Silver[2], G. Watzek[1], R. Gruber[1]
[1]Abteilung für Orale Chirurgie, Medizinische Universität Wien, und Austrian Cluster for Tissue Regeneration, Österreich; [2]Harvard School of Dental Medicine, Boston, USA

Die Gefäßneubildung ist essenziell für die Regeneration von Knochen und Weichgewebe. Es ist bekannt, dass die Reduktion des Sauerstoffpartialdrucks, also Hypoxie, die Entstehung neuer Gefäße auslöst. Ein rezenter Ansatz zur Förderung der Regeneration von Knochen und Weichgewebe ist daher die pharmakologische Simulation von Hypoxie durch so genannte „small molecules". Die Wirkung dieser „small molecules" auf parodontales Gewebe ist aber noch ungeklärt. Wir untersuchten daher die Wirkung von „small molecules" in Bezug auf die Produktion von pro-angiogenem „vascular endothelial growth factor" (VEGF) durch parodontale Fibroblasten. Es zeigt sich, dass „small molecules" *in vitro* die VEGF-Produktion steigern können, ohne die inflammatorische Antwort substanziell zu modulieren. Unsere Untersuchungen über die Wirkung von Knochenersatzmaterial, welches mit „small molecules" funktionalisiert wurde, zeigen, dass diese Prozessierung die Produktion von VEGF steigern kann. Ob diese pro-angiogene Wirkung der „small molecules" zu einer Steigerung der Gewebsregeneration führt, ist Gegenstand präklinischer Untersuchungen.

Sklerostin und Dickkopf-1 sind zentrale Regulatoren der Neubildung von Knochen, indem die beiden Faktoren den Wnt-Signalweg blockieren. Sklerostin und Dickkopf-1 sind möglicherweise auch an jenen Prozessen beteiligt, die den Erhalt der Zähne ermöglichen, aber auch pathophysiologische Wirkungen sind nicht auszuschließen. Die Frage nach der Regulation der Expression kann als erster Schritt zum Verständnis der Wirkung von Sklerostin und Dickkopf-1 angesehen werden. Basierend auf dieser Frage wurden parodontale Fibroblasten mit Wachstumsfaktoren und inflammatorischen Faktoren, die im Parodont vorkommen, inkubiert. Untersucht wurde die Produktion von Sklerostin und Dickkopf-1 auf mRNA- und Proteinebene. Wir konnten zeigen, dass „transforming growth factor beta" die Produktion von Sklerostin in parodontalen Fibroblasten sowohl auf mRNA- als auch auf Proteinebene steigert. Weiters fanden wir, dass „tumor necrosis factor alpha" die Produktion von Dickkopf-1 in parodontalen Fibroblasten sowohl auf mRNA- als auch auf Proteinebene steigert. Zusammengefasst zeigen diese Daten, dass sowohl Sklerostin als auch Dickkopf-1 in parodontalen Zellen durch unterschiedliche Faktoren reguliert werden. Sklerostin und Dickkopf-1 könnten demnach unterschiedliche funktionelle Bedeutungen im Zahnhalteapparat haben. Diese Annahme ist insofern interessant, da zukünftige Therapien mit Anti-Sklerostin- und Anti-Dickkopf-1-Antikörpern eine Auswirkung auf das Parodont haben könnten.

Sclerostin and Its Association with Age, Gender, Body Composition, Physical Activity, and Bone Mineral Content in Healthy Adults

K. Amrein[1], S. Amrein[2], C. Schnedl[1], C. Drexler[3], H. P. Dimai[1], H. Dobnig[1], A. Tomaschitz[1], T. R. Pieber[1], A. Fahrleitner-Pammer[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, [2]Department of Anaesthesiology and Intensive Care Medicine, and [3]University Clinic of Blood Group Serology and Transfusion Medicine, Medical University of Graz, Austria

Background Sclerostin is produced by osteocytes and inhibits bone formation through the Wnt/β-catenin signalling pathway. Only limited data is available on circulating sclerostin levels in healthy subjects. Therefore we aimed to evaluate the correlation between sclerostin and physical activity (PA), anthropometric and biochemical variables in healthy adult men and premenopausal women.

Methods Serum sclerostin levels were measured in 161 healthy adults aged 19–64 years (mean 44 ± 10). Body composition and bone mineral density (BMD) were assessed with dual x-ray absorptiometry. Physical activity was recorded with a standardized questionnaire.

Results A positive correlation between age and sclerostin in both men ($r = 0.37$; $p < 0.001$) and premenopausal women ($r = 0.66$; $p < 0.001$) was found. Men had significantly higher sclerostin levels than women (49.8 ± 17.6 vs 37.2 ± 15.2 pmol/l; $p < 0.001$). However, after adjustment for age, bone mineral content (BMC), PA, BMI, and renal function sclerostin levels did not differ ($p = 0.543$). Partial correlation analysis adjusted for age, gender, and kidney function revealed a significantly positive correlation between sclerostin levels and BMC, BMD, BMI, and android/gynoid fat and a significantly negative correlation with serum osteocalcin and calcium. The most physically active quartile had significantly lower sclerostin levels compared to the least active quartile in an univariate analysis.

Conclusion In healthy adults, sclerostin serum levels correlate positively with age, Body Mass Index, and bone mineral content and negatively with osteocalcin and calcium. Further studies in larger populations are needed to confirm our findings and to better understand their clinical implications.

Einfluss unterschiedlicher Thrombozytenpräparationen auf die osteogene Differenzierung

M. Eichholzer[1], M. Silver[2], G. Watzek[1], H. Agis[1], R. Gruber[1]
[1]Abteilung für Orale Chirurgie, Medizinische Universität Wien, und Austrian Cluster for Tissue Regeneration, Österreich; [2]Harvard School of Dental Medicine, Boston, USA

Die klinische Anwendung von „platelet-rich plasma" (PRP) hat das allgemeine Interesse geweckt, die Funktion der Thrombozyten im Rahmen der Knochenregeneration besser zu verstehen. Die Knochenregeneration ist ein hochkonservierter, mehrstufiger Prozess, der auf dem Zusammenwirken von spezialisierten Zellen beruht. Hier kommt den knochenaufbauenden Osteoblasten eine zentrale Bedeutung zu. Um die Wirkung von Thrombozyten auf die Bildung der Osteoblasten besser zu verstehen, wurden In-vitro-Studien initiiert, in wel-

*Reihung alphabetisch nach Erstautor

chen der Effekt von Thrombozytenpräparationen auf den Prozess der Osteoblastogenese untersucht wurde. Die Ergebnisse der vorliegenden Studien sind scheinbar widersprüchlich. Untersuchungen über den Einfluss von aktiviertem PRP zeigen, dass PRP die Entstehung von Osteoblasten fördert. Untersuchungen über den Einfluss von Wachstumsfaktoren aktivierter Thrombozyten („platelet-released supernatant", PRS) können das hingegen nicht bestätigen. Eine Studie, die einen direkten Vergleich ermöglicht, liegt nicht vor. Ziel dieser Studie war es, die Wirkung von PRP und PRS auf die Osteoblastogenese direkt zu vergleichen. Hierzu wurden osteogene Zellen der Zelllinie MG63 und primäre osteogene Zellen mit folgenden 3 Präparationen stimuliert: (i) Überstände von PRP, welche sowohl Wachstumsfaktoren von Thrombozyten als auch Serumbestandteile enthalten; (ii) PRS, welches Wachstumsfaktoren von Thrombozyten enthält; (iii) Überstände von „fresh frozen plasma" (FFP), welches Serumbestandteile enthält. Die Wirkung auf die osteogene Differenzierung der Zellen wurde mittels histochemischer Färbung der alkalischen Phosphatase bestimmt. Weiters wurde die Wirkung auf die Proliferation der Zellen mittels [^{3}H]Thymidin-Einbau gemessen. Unsere Ergebnisse zeigen, dass Überstände von PRP sowie FFP, also die Präparationen, die Serumbestandteile enthalten, die osteogene Differenzierung von MG63 steigern können. PRS hingegen, welches keine Serumbestandteile enthält, führt nicht zu einer derartigen Steigerung. Ein ähnliches Bild zeigt sich auch bei primären osteogenen Zellen. Zusammengefasst weisen unsere Ergebnisse darauf hin, dass die Gegenwart von Serum in den Thrombozytenpräparationen den Effekt auf die osteogene Differenzierung beeinflussen kann. Diese Tatsache sollte bei der Wahl von In-vitro-Modellen zur Untersuchung der Wirkung von PRP und Thrombozyten auf osteogene Zellen berücksichtigt werden.

Epigenetic Regulation of the Calcium-Sensing Receptor

I. Fetahu, J. Höbaus, D. Hummel, T. Manhardt, E. Kallay
Division of Comparative Immunology and Oncology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

Epidemiological studies suggest a cancer-preventive effect of calcium in colorectal cancer (CRC). The calcium-sensing receptor (CaSR) probably mediates the anti-proliferative features of calcium in colon. The CaSR is a G protein-coupled receptor involved in several signalling pathways. There is an inverse relationship between CaSR expression and tumour progression in human CRC. We reasoned that epigenetic mechanisms, such as DNA hypermethylation and histone deacetylation, might be responsible for reduction or loss of CaSR expression in colorectal tumours. We analyzed CaSR mRNA and protein expression in CRC patients and cell lines by real time RT-PCR and immunofluorescence. In CRC patients (n = 61) we saw a down-regulation of CaSR expression in tumour compared with the respective adjacent mucosa. Bisulfite sequencing of 2 promoter regions of the CaSR in CRC cell lines showed dense methylation of the second promoter. However, in patients the methylation of CpG islands was very low and there was no difference in methylation pattern between tumour and respective adjacent mucosa. We treated an adenoma and 3 CRC cell lines with 5-aza-2-deoxycytidine (5-aza-dC), a DNA methyltransferase inhibitor, and/or Trichostatin A (TSA), a histone deacetylase inhibitor. Treatment with 5-aza-dC and TSA caused only modest increase of the CaSR expression, despite the presence of densely methylated CpG islands in the promoter of the CaSR.

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Gene Expression and Bone Architecture in Men with Osteoporotic Hip Fractures

U. Föger-Samwald[1], J. M. Patsch[1,2], J. Deutschmann[1,2], L. Hornek[3], S. Salem[4], P. Becker[5], P. Pail[1], D. Schamall[1], M. Mousavi[3], S. Becker[5], F. M. Kainberger[2], P. Pietschmann[1]
[1]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, and [2]Department of Radiodiagnostics, Medical University of Vienna; [3]Department of Trauma Surgery, Danube Hospital, Vienna; [4]Department of Orthopaedics, St. Vincent Hospital, Vienna; [5]Department of Spine Surgery, Orthopaedic Hospital Speising, Vienna, Austria

Background Osteoporosis is extremely frequent in post-menopausal women; nevertheless, osteoporosis in men is a severe, frequent, and often underestimated disease. In a previous study we found evidence of osteoblast dysfunction in middle-aged men with idiopathic osteoporosis. The aim of this study was to investigate gene expression and bone architecture in bone samples derived from elderly osteoporotic men with hip fractures in comparison to bone samples from age-matched non-osteoporotic controls.

Methods Femoral heads and adjacent neck tissue were collected from 10 men with low-trauma hip fractures (mean age 81.9 ± 7.1) and consecutive surgical hip replacement. 14 bone samples of age-matched patients undergoing hip replacement due to osteoarthritis served as controls. One half of the bone samples was subjected to RNA extraction, reverse transcription, and real time polymerase chain reactions. The second half of the bone samples of each patient was analyzed by *ex vivo* dual x-ray absorptiometry and by high resolution peripheral micro-computed tomography.

Results We could show a significantly decreased runx2, osterix, sclerostin, and osteocalcin expression in bone samples from hip fracture patients compared to controls. Areal bone mineral density was significantly lower in fracture samples. Comparing local bone microstructure, the femoral head displayed significantly lower BV/TV and trabecular thickness in the fracture when compared to the osteoarthritic bone samples.

Conclusion Decreased local gene expression of runx2, osterix, and osteocalcin in men with hip fractures strongly supports the concept of osteoblast dysfunction in male osteoporosis.

Acknowledgment This work was supported by a grant from the Österreichische Nationalbank (OeNB Project No. 12544).

Abnormally High Bone Matrix Mineralization in Children with Dominant and Recessive Osteogenesis Imperfecta

N. Fratzl-Zelman, P. Roschger, K. Klaushofer
Ludwig Boltzman Institute of Osteology at Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria

Introduction Osteogenesis imperfecta (OI) is genetically and clinically a heterogeneous disorder characterized by reduced bone mass and increased bone fragility. Most cases of OI are caused by autosomal dominant defects in the genes encoding type I collagen (OI type I–IV). Moreover, recessive forms of OI have been described (OI type VI–XI) to be caused by deficiency of proteins that affect post-translational modifications of collagen. In particular, OI types VII, VIII, and IX are associated with mutations in any of the 3 components of the collagen prolyl 3-hydroxylation complex, which appears to be critical for proper collagen processing [1]. Very recently, a distinctive OI phenotype with bone fragility but high bone mass has been described. This new OI phenotype is caused by a defect in the COL1 C-proteinase cleavage site whereas the pericellular procollagen processing is impaired leading to incorporation of altered collagen molecules into the matrix [2].

The phenotypic consequences of a particular mutation at the bone material level are not fully understood.

Purpose and Methods To get insights into the bone material quality in OI we evaluated bone mineralization density distribution (BMDD, measured by quantitative backscattered electron imaging) in bone biopsies from affected children (age 2–17.5 years) with different clinical severity and/or different mutation types: OI type I [2] (quantitative mutations: n = 13, qualitative mutations: n = 6), OI type III (n = 3), OI type IV [4] (n = 8), hypomorphic OI type VII [5] (n = 4), and high bone mass OI (n = 2) with specimens from a control population (n = 54, age 1.5–23 years) [6].

Results The analyses revealed that the mean calcium content (Ca_{Mean}) in each group was significantly higher than in controls: OI type I, quantitative mutation: + 7.4 %, $p < 0.001$; OI type I, qualitative mutation: + 6.4 %, $p < 0.001$; OI type III: + 7.3 %, $p < 0.01$; OI type IV: + 7.9 %, $p < 0.001$; OI type VII: + 3.3 %, $p < 0.05$. In addition, there was no statistical difference between both OI type I forms and between OI types III, IV, and VII. Only the most frequent calcium content (Ca_{Peak}) was lower in the hypomorphic OI type VII than in OI type I: – 4.1 %, $p < 0.05$. Very interestingly both high bone mass OI cases showed also a significant increase in BMDD, whereas one patient had a Ca_{Mean} (+ 7.1 %) and Ca_{High} (+ 737 %) exceeding classical OI bone.

Conclusion The results show that the bone matrix in children with OI has an abnormally high mineral content and that this effect is independent of type-I collagen alterations and clinical severity suggesting possibly a fundamental cellular dysfunction in OI.

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Effects of One Year Daily Teriparatide Treatment on Trabecular Bone Material Properties in Postmenopausal Osteoporotic Women Previously Treated with Alendronate or Risedronate

S. Gamsjäger[1], B. Hofstetter[1], R. Phipps[2,3], K. Klaushofer[1], E. P. Paschalis[1]
[1]Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria; [2]Procter & Gamble Pharmaceuticals, Mason, Ohio, USA; [3]Husson University School of Pharmacy, Bangor, Maine, USA

Given the recent concerns regarding long-term bisphosphonate use in the management of osteoporosis, teriparatide (TPTD) therapy following bisphonate (BP) administration becomes an attractive clinical option.

The purpose of the present study was to investigate TPTD effects on bone material properties (specifically mineral-to-matrix ratio, corresponding to ash weight measurements; mineral crystallite maturity/crystallinity, indicative of the mineral crystallite chemistry and stoichiometry, and having a direct bearing on crystallite shape and size; relative proteoglycan content, regulating mineralization commencement; and the ratio of 2 of the major enzymatic collagen cross-links, namely pyridinoline to divalent collagen cross-links) as a function of prior BP (alendronate [ALN] or risedronate [RIS]) therapy, independent of bone turnover. To do this we used Fourier Transform Infrared Imaging (FTIRI) and Raman spectroscopy to examine 32 paired iliac crest biopsies (before and after one year teriparatide) from 16 osteoporotic women previously treated with either ALN (either 10 mg/day or 70 mg/week) or RIS (5 mg/day or 30–35 mg/week) for at least 24 months (mean duration of therapy 37.2 months for ALN and 38.0 months for RIS). They stopped their BP therapy and were then treated with TPTD (20 µg/day subcutaneously) for 12 months. These iliac crest bone biopsies were collected during the OPTAMISE study. In addition, we compared the 2 BPs prior to TPTD treatment. Analysis was focused on formation sites, and specifically between fluorescent double labels. This selection of anatomical areas ensures that bone of similar tissue age was analyzed, and thus the results are independent of bone turnover.

TPTD produced significant changes in mineral/matrix ratio, mineral maturity/crystallinity and collagen cross-link ratio. TPTD-induced changes in relative proteglycan content were observed only in the prior RIS-treated group. Collagen cross-link ratio was also significantly different between the 2 BP groups at baseline.

In conclusion, the results of the present study have shown a response to TPTD treatment in newly forming bone material in patients who had been treated with BPs. There were no statistically significant differences between the prior ALN and prior RIS groups in any of the outcomes after 12 mo TPTD treatment. On the other hand, there was a significant difference at baseline in the collagen cross-link ratio between ALN- and RIS-treated patients.

A Machine Learning-Based Approach to Automatically Discriminate *In Silico* Murine Osteoclasts and Their Precursor Cells by Nuclei-Based Features

A. Heindl, M. Schepelmann, A. Seewald, K. Burger, G. Bises, R. Rogojanu, R. Ecker, P. Pietschmann, T. Thalhammer, I. Ellinger
Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

Reliable *in silico* quantification of osteoclasts (OC) may yield new insights into their formation and function under the influence of physiological or pharmaceutical substances. This knowledge may further lead to new treatments of bone-related diseases such as rheumatoid arthritis and osteoporosis. Current cell-analysis software provides essential support for the life scientist, though automated discrimination of OC and their precursor cells is still a challenging task for image-processing algorithms. Our recently developed automated cell marker-based OC identification system which is based on a specific target protein expression can now be further improved by a machine learning-based classifier that discriminates OC and their precursor cells in culture only by the specific appearance of their nuclei labeled with DAPI. During this process, ellipses are inscribed in the perimeter of each nucleus. Ellipse features such as eccentricity, area, mean intensity, standard deviation of intensities, a- and b-axis of the ellipse, are then computed and combined to distinguish between OC and their precursors. Thousands of OC and non-OC nuclei mark-ups, provided by 2 human experts, were used for training the machine learning-based classifier. An independent set of mark-ups was used to validate the performance of the algorithm in comparison to the experts. This was done by showing only single nuclei without cellular context to each. Comparing these results with the ground truth yielded a significantly better (2.5-fold) performance of the classifier than 2 human experts. The combination of our previously designed automated marker-based OC detection system with the presented method will allow more reliable evaluation of large-scale experiments investigating the influence of distinct compounds on osteoclast formation and growth.

Das Doktoratsprogramm „Bone and Joint Regeneration“: Informationen aus erster Hand

B. Hirschegger, H. Agis, R. Gruber
Abteilung für Orale Chirurgie, Medizinische Universität Wien, und Austrian Cluster for Tissue Regeneration, Österreich

Das Doktoratsprogramm „Bone and Joint Regeneration“ wird seit 2006 an der Medizinischen Universität Wien in Zusammenarbeit mit dem Ludwig-Boltzmann-Institut für Experimentelle und Klinische Traumatologie und dem Ludwig-Boltzmann-Institut für Osteologie durchgeführt. Es richtet sich an Absolventen der Medizin, Zahnmedizin sowie anderer naturwissenschaftlicher und technischer Disziplinen. Ziel des Doktoratsprogramms ist die Befähigung zu selbstständiger wissenschaftlicher Arbeit im weiten Bereich der muskuloskelettalen Erkrankungen.

In 6 Semestern werden Lehrveranstaltungen im Ausmaß von 38 Semesterstunden angeboten, die das praktische und theoretische Ar-

beiten an der Dissertation begleiten. Insgesamt werden damit 180 ECTS-Punkte erreicht. Besonderer Wert wird auf Interdisziplinarität gelegt, insbesondere in Bezug auf die Themenwahl und die theoretische und praktische Bildung der Studierenden. Die Interdisziplinarität zeigt sich auch in der Wahl des Dissertationskomitees, bestehend aus dem Betreuer und 2 weiteren Mitgliedern, die gemeinsam mit den Studierenden den Dissertationsplan erstellen.

Betreuer können „Principal Investigators" und „Junior Supervisors" sein. „Principal Investigators" verfügen über Erfahrung in der Betreuung von Studierenden, sind Initiatoren aktiver wissenschaftlicher Forschungsprogramme sowie erfolgreich in der Akquise von Forschungsförderung. „Junior Supervisors" sind zukünftige „Principal Investigators", die bereits erfolgreich wissenschaftlich arbeiten. „Lecturers" unterstützen das Programm durch Lehrveranstaltungen in ihrem Forschungsbereich.

Derzeit sind 45 Studierende im Doktoratsprogramm aktiv. 6 Studierende haben das Programm abgeschlossen. Betreut werden die Studierenden von 18 „Principal Investigators", 6 „Junior Supervisors" und 5 „Lecturers". Neben den beiden Ludwig-Boltzmann-Instituten sind 8 Abteilungen der Medizinischen Universität Wien an der Ausrichtung des Programms beteiligt. Die Absolventen erhalten den Titel „Doctor scientiae medicinae" (Dr. scient. med.). Derzeit laufen Bestrebungen, die Voraussetzungen für die Verleihung des Titels „Doctor of Philosophy" (PhD) zu schaffen.

Weitere Informationen: www.meduniwien.ac.at/n790-bjr

Overexpression and Methylation of the Vitamin D Degrading Enzyme CYP24A1 in Colorectal Cancer

J. Höbaus, I. Fetahu, D. Hummel, T. Manhardt, E. Kallay
Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Austria

There is an inverse association between colorectal cancer and prediagnostic vitamin D levels (25-OHD_3), underlining the importance of vitamin D in the prevention of colon cancer. 1,25$(OH)2D_3$ ($1,25D_3$) is the most active form of vitamin D and exhibits anti-proliferative, pro-differentiating, and anti-angiogenic functions. Both $1,25D_3$ and its precursor $25(OH)D_3$ can be catabolized by the 25-hydroxyvitamin D_3-24-hydroxylase (CYP24A1). CYP24A1 is expressed at very low basal levels in the colon but is strongly induced by $1,25D_3$. $1,25D_3$ forms a complex with the vitamin D receptor, together they bind to vitamin D responsive elements (VDRE) located within the CYP24A1 promoter region and lead to the induction of CYP24A1 expression. A high expression of the Vitamin D catabolizing enzyme CYP24A1 implies faster degradation of $1,25D_3$, restricting its anti-tumourigenic functions. A genomic copy number gain and overexpression of CYP24A1 on mRNA level have been observed in different malignancies, such as colon and breast.

We investigated the role of epigenetic modulations in the overexpression of CYP24A1 in tumours. Bisulfite sequencing of the CYP24A1 promoter of different colon cancer cell lines revealed different degrees of DNA methylation. Treatment with the methyltransferase inhibitor 5-aza 2-deoxycytidin resulted in a strong increase of CYP24A1 expression in 3 out of 4 cell lines. Bisulfite sequencing of human colorectal tumours and their adjacent mucosa showed no methylation of the CYP24A1 promoter. In conclusion, CYP24A1 itself is not silenced by promoter methylation, however, de-methylation of upstream activators of CYP24A1 seems to lead to an induction of CYP24A1 expression.

Impact of High Dietary Vitamin D on the Effect of AOM and DSS Treatment in Mouse Colon and Kidney Tissue

D. Hummel, J. Höbaus, U. Thiem, I. Fetahu, T. Manhardt, E. Kallay
Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

Colorectal cancer (CRC) is one of the leading causes of cancer morbidity and mortality in the western world. The most active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 ($1,25\text{-}D_3$), has anti-tumourigenic effects and is considered protective against CRC. High tissue levels of $1,25\text{-}D_3$ in organs prone to sporadic cancer are considered to counteract the development of incipient neoplasms.

The aim of this study is to investigate the effect of high vitamin D diet on tumour development *in vivo*.

We fed C57BL/6J mice with vitamin D concentrations ranging from 100 to 5000 IU/kg to determine the sufficient concentration to prevent the development of chemically induced abberant crypt foci (ACF), a precursor of CRC. To induce ACF we injected mice once with 10 mg/kg azoxymethane (AOM) intraperitoneally, followed by 3 cycles of dextran sodium sulfate (DSS) salt in the drinking water.

The colon of mice fed with low vitamin D diet showed significantly more and further progressed dysplasia compared with mice fed with high vitamin D diet. Dysplasia is characterized by a reduced cytoplasm-to-nucleus ratio, a shift of the nuclei from the basal side of the cells to the center and a disorganized shape of the crypts. No statistically significant changes could be observed in the expression of VDR and CYP27B1 in the kidney. CYP24A1 expression decreased significantly between the kidney of mice receiving the lowest vitamin D diet (100 IU/kg) compared with groups fed with 2500 IU/kg and 5000 IU/kg cholecaliferol.

High vitamin D concentrations in the diet are able to reduce the development of chemically induced ACF.

Cardiovascular, Bone, and Kidney Parameters in Osteoporotic Patients

D. Karau, K. Ez-Zenje, B. Eichinger, G. Hawa
Biomarker Design ForschungsGmbH, Vienna, Austria

Introduction In osteoporotic patients, reduced bone mineral density is associated with cardiovascular disease (CVD). These 2 conditions may be sustained by disease mechanisms [1, 2], of which increased osteoprotegerin (OPG) levels in serum are an indicator [3]. Disordered phosphate metabolism could be part of this disease mechanism, as OPG levels and vascular adverse effects may also be affected in chronic kidney disease/mineral bone disorder characterized by increased FGF23 levels [4]. Using blood samples of osteoporotic patients, we determined the bone (calcification) markers sclerostin and DKK1, the CVD parameter BNP fragment, and the levels of FGF23 (an osteocyte hormone regulating phosphate resorption in the kidney).

Methods Blood samples of 57 osteoporotic patients were analyzed by ELISAs for BNP-fragment, OPG, sclerostin, DKK1, and FGF23.

Results Of the 57 patients analyzed, 99 % had above-normal sclerostin levels (> 30 pM) and 87 % had below-normal DKK1 levels (< 12 pM). FGF23 levels were above-normal (> 45 pM) in 35 % of these patients and OPG levels were above-normal in 51 %. Only 50 % of the high-FGF23 patients were in the high OPG group, and vice-versa. Among the 37 patients with above-normal levels (> 2000 pM) of BNP fragment, 30 patients had elevated levels of *either* OPG or FGF23, but only 9 had elevated levels of *both* FGF23 and OPG.

Conclusion In osteoporotic high-sclerostin/low-DKK1 patients with elevated levels of BNP, increases in blood levels of FGF23 or OPG appear to be independent of one another. We will present additional analyses of these parameters in kidney-dialysis patients.

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Bone Metabolism in Patients with Primary Hyperparathyroidism Before and After Surgery

K. Kerschan-Schindl[1], P. Riss[2], C. Krestan[3], M. Rauner[4, 5], C. Bieglmayer[6], A. Gleiss[7], V. Fialka-Moser[1], B. Niederle[2], P. Pietschmann[8]
[1]Department of Physical Medicine and Rehabilitation, [2]Department of Surgery and [3]Department of Radiology, Medical University of Vienna; [4]Ludwig Boltzmann Institute of Ageing Research, Vienna, Austria; [5]Division of Endocrinology, Diabetes, and Metabolic Bone Diseases, Department of Medicine III, Technical University, Dresden, Germany; [6]Department of Biochemistry, [7]Center for Medical Statistics, Informatics, and Intelligent Systems and [8]Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria

Background Primary hyperparathyroidism (PHPT) is accompanied by a reduced bone mineral density (BMD) and an increased risk of fracture. Surgery is the only option for cure.

Objective To investigate the effect of parathyroidectomy (PTX) on bone metabolism in patients with PHPT.

Design Prospective observational study.

Patients 52 patients with PHPT who underwent surgery.

Measurements Biochemical analyses were performed at baseline and 1, 4, and 7 days, 6 weeks, 3, 6, and 12 months after the operation. BMD was measured before surgery and after one year.

Results There seem to exist some gender-specific differences in patients with PHPT. Bone resorption decreased immediately after PTX, bone formation more slowly. The RANKL (receptor activator nuclear factor kappa B ligand)/OPG (osteoprotegerin) ratio as well as cathepsin K did not show significant changes within the first year. BMD of the lumbar spine but not BMD of the femoral neck increased significantly. Moderate correlations existed between the changes of calcium, ionised calcium, as well as bone-specific alkaline phosphatase and changes of the lumbar BMD. Patients who needed postoperative supplementation with calcium and vitamin D had significantly higher parathyroid hormone (PTH) levels.

Conclusion In patients with PHPT, males appear to be more affected than females. Within the first year after PTX bone metabolism normalized, BMD of the lumbar spine increased. Patients who needed a supplementation with calcium and vitamin D after PTX preoperatively had higher serum levels of PTH.

Cathepsin S-Deficient Mice Exhibit a Bone and Fat Phenotype

M. F. Kurz[1], M. Rauner[2], C. Brünner-Kubath[1], A. Kapfenberger[1], U. Föger-Samwald[1], D. Schamall[1], P. Varga[3], S. Kudlacek[4], A. Wutzl[1], H. Höger[1], W. Sipos[5], P. K. Zysset[3], G. P. Shi[6], P. Pietschmann[1]
[1]Medical University of Vienna, Austria; [2]Technical University Dresden, Dresden, Germany; [3]Vienna University of Technology; [4]Krankenhaus der Barmherzigen Brüder, Vienna; [5]University of Veterinary Medicine Vienna, Austria; [6]Harvard Medical School, Boston, USA

Background Cathepsin S is a cysteine protease that regulates the differentiation of fat cells. Recently, the interactions between fat and bone have received much attention. Therefore the aim of our study was to assess the bone and fat phenotypes of cathepsin S-deficient mice.

Methods The fifth lumbar vertebrae of cathepsin S knockout and matched wild-type mice were analyzed by micro-computed tomography (μCT40, Scanco Medical, Switzerland). Moreover, the weight of the subscapular and gonadal fat pads was determined.

Results In young knockout mice trabecular thickness was decreased by 9.5 % compared to wild-type mice. In adult mice (6 months old) trabecular thickness was not significantly different in the knockout mice when compared to the wild-type mice. When young and adult mice were analyzed together, a significant decrease of 2.8 % of trabecular tissue bone mineral density was seen in the knockout mice. Significant differences in trabecular tissue bone mineral density were also observed irrespective of the sex of the mice. Although the body weight of the knockout and the wild-type mice was similar, cathepsin S knockout mice had significantly lower weights of the subscapular fat pads (– 22 %). Male cathepsin S-deficient mice had 23 % lighter gonadal fat pads.

Conclusion Cathepsin S knockout mice have a fat and discreet but reproducible bone phenotype. Recently, inhibitors of cathepsin S have been proposed for the treatment of obesity and its complications. Nevertheless, the results of our study suggest the possibility that cathepsin S inhibitors could be associated with adverse skeletal effects.

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Reconsidering Osteoporosis Therapy in Gaucher's Disease? First Report of Osteoanabolic Therapy in a Patient with Gaucher's Disease After Failure with Bisphosphonates

P. Mikosch[1,2], O. Bodamer[3], S. Becker[4], K. Klaushofer[1,2], D. A. Hughes[5]
[1]Department of Internal Medicine 1, General Hospital Klagenfurt; [2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA, Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna; [3]University Children's Hospital, Paracelsus University, Salzburg; [4]Department of Orthopedics III, Orthopedic Hospital Speising, Vienna, Austria; [5]Department of Haematology, LSD Unit, Royal Free Campus, University College Medical School, London, UK

A 56-year-old female patient with Gaucher's disease (GD) type 1, who had undergone splenectomy at the age of 35 due to pancytopenia and bleeding complications, presented with extensive bone involvement including osteoporosis. After vertebral fracture enzyme replacement, therapy was started and chitotriosidase levels decreased significantly within one year. However, bone mineral density further deteriorated although the patient was also on therapy with bisphosphonates beside calcium and vitamin D supplementation. Osteological therapy was thus changed to parathyroid hormone 1-34 (teriparatide 20 μg subcutaneously daily), an osteoanabolic substance, for a period of 2 years. Significant improvement of bone mineral density (BMD change + 15 % lumbar spine, + 9 % femoral neck) could be observed while the patient was on therapy with teriparatide and no further fractures occurred.

The pathophysiological background of bone pathologies including osteoporosis in GD is still not fully understood. Until recently the pathogenesis of osteoporosis development in GD was based on the thesis of increased bone resorption. The case report showed for the first time that the osteoanabolic agent teriparatide could effectively stimulate osteoblastic function in a patient with GD. This clinical outcome matches to recent research results indicating that bone loss in GD may be mainly due to impaired osteoblastic function. As currently only data for bisphosphonate therapy are available for therapy of osteoporosis in GD, osteoanabolic agents, such as parathyroid hormone, may enrich the current osteological therapeutic options in GD. Further studies are warranted to investigate osteoblast dysfunction and the effect of osteoanabolic therapies in GD.

„Small molecules" steigern die Produktion von Vascular Endothelial Growth Factor durch humane Zahnpulpazellen

H. Müller, G. Watzek, R. Gruber, H. Agis
Abteilung für Orale Chirurgie, Medizinische Universität Wien, und Austrian Cluster for Tissue Regeneration, Österreich

Traumata, Karies und zahnärztliche Eingriffe können zur Exposition von Gewebe der Zahnpulpa führen. Die derzeitige Strategie zur Vitalerhaltung der Zähne bei eröffneter Pulpa ist die direkte Überkappung. Therapiestrategien, die das regenerative Potenzial der Pulpa fördern, sind wünschenswert. Es ist bekannt, dass durch pharmakologisch simulierte Hypoxie die Regeneration von Knochen sowie die Wundheilung gefördert werden können. So genannte „small mole-

cules“ werden hierbei zur Steigerung der Angiogenese angewendet, welcher eine zentrale Rolle in der Gewebsregeneration zukommt. Ob „small molecules“ auch die Regeneration der Pulpa fördern, ist unbekannt. Folglich stellten wir uns die Frage, ob „small molecules“ das pro-angiogene Potenzial der Zellen in der Pulpa steigern können.

Um diese Frage zu klären, isolierten wir Zellen der Pulpa und stimulierten sie mit den „small molecules“ Dimethyloxaloylglycin, Desferrioxamin, L-Mimosin und Kobaltchlorid. Die Wirkung von „small molecules“ auf die Vitalität, Proliferation und Proteinsynthese wurde über die Bildung von Formazankristallen sowie den Einbau von [^{3}H]Tymidin und [^{3}H]Leucin bestimmt. Die Wirkung auf das pro-angiogene Potenzial der Pulpazellen wurde über die Messung von „vascular endothelial growth factor“ bestimmt.

Unsere Daten zeigen, dass „small molecules“ in millimolaren Konzentrationen toxisch wirken können. In nicht-toxischen Konzentrationen steigern „small molecules“ die Produktion von „vascular endothelial growth factor“. Weitere Experimente über die Freisetzung von „small molecules“ aus funktionalisiertem Überkappungsmaterial sind geplant.

Zusammengefasst zeigen unsere Ergebnisse, dass „small molecules“ das pro-angiogene Potenzial von Zellen aus der Zahnpulpa steigern können. Ob „small molecules“ über diesen Mechanismus auch die Pulparegeneration steigern und inwiefern sich Überkappungsmaterial mit „small molecules“ funktionalisieren lässt, werden weiterführende Studien zeigen.

The Discriminatory Capacity of Clinical Risk Factors and BMD Measurements at Different Skeletal Sites for Detecting Patients with Vertebral Fractures

C. Muschitz[1], F. Kühne[1], R. Kocijan[1], C. Bittighofer[1], A. Trubrich[1], R. Waneck[2], H. Resch[1]

[1]Medical Department II – The VINFORCE Study Group and [2]Department of Radiology, St. Vincent Hospital, Vienna, Austria

Purpose Since the clinical outcome of osteoporosis is bone fracture, attention is focused on the identification of patients at high risk for fractures rather than the identification of people with osteoporosis defined by BMD. We investigated measurements of BMD of the calcaneus by DXL as a possible alternative device for the assessment of BMD together with the implementation of clinical risk factors (CRF) compared to DXA at standard skeletal sites.

Methods Baseline characteristics of 494 outpatient treatment-naïve postmenopausal women (67.9 ± 15.0 ys) and 97 men (61.5 ± 12.7 ys) were evaluated for a pre-planned 5 year follow-up period. All subjects had prior spine x-rays and evaluation of CRF according to the FRAX guidelines. Each subject had DXA of spine/hip and DXL of the calcaneus on the same day. We calculated receiver-operating characteristics (ROC) expressed as area under the curve (AUC) to describe the sensitivity and specificity of DXA of spine/hip and for DXL at calcaneus to discriminate between patients at risk for vertebral fracture. Furthermore, we generated a univariate multiple logistic regression model for CRF alone and for CRF including BMD by DXA or DXL.

Results Out of 591 subjects, 160 patients had prevalent vertebral fractures (72.6 ± 12.1 vs 61.4 ± 9.3 ys; $p < 0.001$). In the ROC analysis the AUC of BMD alone was 0.669 for DXA femoral neck, 0.665 for DXL calcaneus, 0.661 for DXA total hip, and 0.598 for DXA lumbar spine ($p = 0.034$). The AUC for CRF without BMD was 0.805. In this model, significant CRF ($p < 0.05$) were previous fragility fractures (OR 24.5; 95 % CI 13.4; 44.6), age (OR 1.04; 95 % CI 1.02; 1.06), and BMI (OR 1.05; 95 % CI 1.00; 1.90). After combination of DXA femoral neck and CRF the AUC improved to 0.869. Significant ORs ($p < 0.05$) were previous fractures (20.9; 95 % CI 10.5; 41.6), alcohol use (3.9; 95 % CI 1.05; 14.46), and BMD femoral neck (0.68; 95 % CI 0.48; 0.94). The AUC for DXL calcaneus and CRF was 0.861 including significant factors ($p < 0.05$) such as age (OR 1.03; 95 % CI 1.00; 1.05), previous fracture (OR 23.9; 95 % CI 12.5; 45.7), alcohol use (OR 2.94; 95 % CI 0.86; 10.02), and BMD calcaneus (OR 0.77; 95 % CI 0.57; 1.04; $p = 0.09$).

Conclusions Our data clearly demonstrate that the predictive value of specific CRFs and BMD measured by DXA or DXL is similar and improves the discriminatory capacity for detecting patients with vertebral fractures compared to BMD measurements or assessment of CRF alone.

Teriparatide and Antiresorptive Combination Treatment Subsequent to 9 Months of Teriparatide Monotherapy

C. Muschitz[1], R. Kocijan[1], A. Fahrleitner-Pammer[2], C. Bittighofer[1], A. Trubrich[1], F. Kühne[1], R. Waneck[3], H. Resch[1]

[1]Medical Department II – The VINFORCE Study Group, St. Vincent Hospital, Vienna (Academic Teaching Hospital of Medical University of Vienna); [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University of Graz; [3]Department of Radiology, St. Vincent Hospital, Vienna, Austria

Purpose Increased bone formation in the absence of accelerated resorption is resulting in a marked anabolic response to teriparatide (TPTD) during the early phase after treatment initiation. Months later, due to a coupling mechanism, the sustained increase of bone formation and ongoing anabolic effects are accompanied by significantly increased bone resorption as well. Antiresorptives influence the balance of bone formation and resorption. Therefore our aim was to investigate the effects of the addition of antiresorptives to the second half of the TPTD cycle when resorption is already markedly elevated.

Methods We prospectively randomized 117 postmenopausal women (mean age 71.7 ± 8.5 years, T-score L1–L4 2.45 ± 1.38; 91.5 % with prevalent fractures) after 9 months of TPTD treatment into 3 different open-label groups for another 9 months: either alendronate (ALN, 70 mg/week, 41 patients), raloxifene (RAL, 60 mg/day, 34 patients) or no medication (TPTD mono, 42 patients) on top of ongoing TPTD treatment. All subjects received daily supplementation of 1000 mg calcium and 800 IU vitamin D. Serum level of intact amino terminal propeptide of type I procollagen (PINP) and type 1 collagen cross-linked C-telopeptide (CTX) as well as DXA measurement at the spine, total hip, and femoral neck BMD were evaluated at TPTD treatment initiation, at baseline of randomization to antiresorptive therapy as well as at 3, 6, and 9 months during combination treatment.

Results At the end of the 18-month TPTD treatment, the increase in lumbar spine BMD was significantly higher in the ALN (+ 0.08 gcm^2, $p = 0.046$) and RAL (+ 0.09 gcm^2, $p = 0.026$) group when compared with the TPTD mono group (+ 0.05 gcm^2). In the total hip region, addition of RAL (+ 0.03 g/cm^2) did not alter the BMD effects of TPTD monotherapy (+ 0.03 g/cm^2), but addition of ALN induced a more pronounced increase in total hip BMD (+ 0.05 g/cm^2) than RAL ($p = 0.026$) or TPTD alone ($p = 0.048$). The elevation of PINP and CTX was significantly lower in the ALN and RAL groups at the end of the 18 months treatment when compared to TPTD treatment alone.

Conclusions Our data suggest that addition of ALN to the second 9 months of TPTD treatment cycle results in augmented BMD increase. This BMD increase may reflect either favourable balance of bone formation and resorption towards more formation or increased secondary mineralization of newly formed bone matrix.

Treatment with PTH 1-84 in Male Patients with Severe Osteoporosis. Results from a Prospective 24 Month Open-Label Trial

C. Muschitz[1], J. Haschka[1], R. Kocijan[1], Ch. Bittighofer[1], A. Trubrich[1], A. Fahrleitner-Pammer[2], G. Nirnberger[3], H. Resch[1]

[1]Medical Department II – The VINFORCE Study Group, St. Vincent Hospital, Vienna (Academic Teaching Hospital of Medical University of Vienna); [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University of Graz; [3]Bioconsult Ltd, Perchtoldsdorf, Austria

Purpose Osteoporosis in men is an important and often underestimated problem. The administration of antiresorptives which influence bone metabolism by reducing bone resorption and increasing bone mineral density (BMD), is generally accepted. Usually anabolic

treatment by parathyroid hormone (PTH) is reimbursed when new osteoporotic fractures occur during antiresorptive treatment, in glucocorticoid-induced osteoporosis (GIOP), and in patients with low BMD and multiple clinical risk factors (CRF).

Methods We prospectively assigned 26 men (mean age 63.3 ± 14.3 ys) to daily subcutaneous injections of 100 µg of PTH 1-84 for 24 months. All pts received daily supplementation of 1000 mg calcium and 800 IU vitamin D. Mean T-score was –3.24 ± 0.92 at L1–L4 and –2.31 ± 0.94 at hip. 81 % had prevalent fractures including vertebral fractures (20 pts, mean 3 ± 1.8), non-vertebral fractures (11 pts) and hip fractures (5 pts). 6 pts had long-term steroid medication (9.3 ± 5.3 ys). 20 pts had prior antiresorptive treatment (6.3 ± 2.6 ys) and 6 were treatment naïve (low BMD, ≥ 2 CRFs).

Primary objectives were the gain of BMD at lumbar spine and hip and the changes of bone turnover markers (P1NP, S-CTX). Secondary objectives were the reduction of new osteoporotic fractures and the evaluation of safety and tolerability.

Patients had BMD measurements (DXA), x-ray of spine, assessment of P1NP, and S-CTX at baseline and at months 6, 12, 18, and 24. A visual analogue scale (VAS) was assessed for general health.

Results The increase of lumbar BMD at month 12 was 6.51 % and at month 24 finally 13.98 %, at femoral neck 1.17 % and 8.5 %, and at total hip 1.5 % and 7.72 %, compared to baseline ($p < 0.0001$ for all). P1NP rapidly increased up to 421 % at month 12 and remained till to the end of the observation peroid; S-CTX increased between 181 % and 322 % (maximum 347 % at month 12; $p < 0.001$ for all).

One patient (GIOP) had a new morphometric vertebral fracture and one patient sustained a hip fracture after a major trauma (accident).

PTH 1-84 was generally well tolerated. VAS score declined from 7.5 to 3.2 ($p < 0.05$).

Conclusions Our data suggest that PTH 1-84 rapidly increases BMD at different skeletal sites regardless of prior long-term treatment with bisphosphonates or steroid medication. The bisphosphonate-induced suppression of the bone turnover does not seem to reduce the anabolic effect of PTH in male patients with severe osteoporosis.

Influence of Growth Hormone Treatment on Bone Matrix Mineralization in Children with Chronic Kidney Disease

K. Nawrot-Wawrzyniak[1], N. Fratzl-Zelman[1], B. M. Misof[1], P. Roschger[1], S. Lueger[1], M. Pańczyk-Tomaszewska[2], H. Ziółkowska[2], K. Klaushofer[1]
[1]Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria; [2]Department of Pediatrics and Nephrology, Medical University of Warsaw, Poland

Patients with chronic kidney disease (CKD) develop renal osteodystrophy (ROD) that is classified by the turnover/mineralization/volume-system. Treatment with recombinant human growth hormone (rhGH) was shown to be a safe and efficacious method to improve height deficits in children with CKD. The influence of rhGH treatment on bone matrix mineralization, an important determinant of bone material quality, is unknown.

We studied bone histomorphometry and matrix mineralization in 20 children (4–16 years) with CKD before and after treatment with rhGH in paired transiliac bone biopsies (with informed consent from the parents). Bone mineralization density distribution (BMDD) was determined by quantitative backscattered electron imaging. Following parameters were used to characterize the BMDD curve: weighted mean mineral concentration (Ca_{Mean}), most frequently measured mineral concentration (Ca_{Peak}), heterogeneity of mineralization (Ca_{Width}) and portion of fully (Ca_{High}) and low (Ca_{Low}) mineralized bone areas. All BMDD data were compared to previously published reference data of healthy children.

Before rhGH treatment, the bone samples were classified to adynamic bone disease (ABD, n = 5), osteomalacia (OM, n = 2), normal bone (NB, n = 4), mixed uraemic osteodystrophy (MUO, n = 9), and osteitis fibrosa (OF, n = 0). In general, indices of bone formation and bone resorption were decreased or normal, while bone matrix mineralization was normal or increased.

After rhGH treatment, the ROD classification was: ABD (n = 1), OM (n = 1), NB (n = 6), MUO (n = 11), OF (n = 1), and bone turnover indices were normal or increased. The BMDD results showed that Ca_{Mean} (– 4.1 %; $p < 0.03$), Ca_{Peak} (– 4.4 %; $p < 0.01$), and Ca_{High} (– 70.6 %; $p < 0.001$) were decreased, whereas Ca_{Width} (+ 16.6 %, $p < 0.01$) was increased compared to before treatment. There were significant correlations between bone turnover variables (BFR/BS, MS/BS) and BMDD parameters. In particular, the correlations within the former ABD subgroup were remarkably strong: Spearman coefficients ranged from –0.71 to –0.88 for Ca_{Mean}, Ca_{Peak} and Ca_{High}, and from 0.74 to 0.88 for Ca_{Width} and Ca_{Low} (p-value from < 0.001 to < 0.05) after treatment.

The results revealed an anabolic effect of rhGH on bone. In particular in the ABD group with abnormally high bone matrix mineralization, there was a shift towards normal BMDD. In conclusion, rhGH treatment in paediatric CKD patients seems to improve bone material quality.

Trace Element Distribution in Trabecular and Cortical Bone of Fractured Femoral Necks of Postmenopausal Osteoporotic Women: A Synchrotron Micro X-Ray Fluorescence Imaging Study

B. Pemmer[1], A. Roschger[1, 2], J. G. Hofstätter[2,3], P. Wobrauschek[1], R. Simon[4], P. Roschger[2], K. Klaushofer[2], C. Streli[1]
[1]Atominstitut, Vienna University of Technology, Austria; [2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna; [3]Department of Orthopaedic Surgery, Medical University of Vienna, Austria; [4]Institute for Synchrotron Radiation, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany

Osteoporosis (OP) is characterized by a low bone mass and a microarchitectural deterioration of bone tissue leading to increased bone fragility and fracture incidence. The general prevalence of OP in women rises from 5 % at age of 50 years to 50 % at age of 85 years. Many risk factors have been found which are associated with osteoporotic fracture, including low peak bone mass, hormonal factors, the use of certain drugs (eg glucocorticoids), cigarette smoking, low physical activity, low intake of calcium and vitamin D, race, small body size, and a personal or a family history of fracture. However, it is likely that many environmental factors are still unknown. In a recent study an association with blood Pb levels and fractures and lower total hip BMD (bone mineral density) was shown. Since Pb is predominantly accumulated in the skeleton, where approximately 95 % of the total body burden of Pb is present, diseases with increased bone turnover, such as osteoporosis (OP), are associated with increased mobilization of Pb from the skeleton. Little is known about where Pb and other trace elements, eg, zinc (Zn), are incorporated within the bone tissue. Previous studies on the local distribution of Pb in bone could only be differentiated between cortical und trabecular bone tissue.

A set of 10 femoral necks from women suffering an osteoporotic femoral neck fracture and from age-matched non-fractured controls was analyzed. The undecalcified samples were examined by quantitative Backscattered Electron Imaging (qBEI) using a pixel resolution of 1 µm. Grey-level images were generated to differentiate between bone packets and osteons of different mineral content separated by cement lines. Areas of interest were analyzed with synchrotron radiation induced confocal micro x-ray fluorescence analysis (SR µ-XRF) to determine the distribution of Ca, Sr, Zn, and Pb in trabecular and cortical bone. Measurements were performed at the FLUO beamline at ANKA using a beam size of 17 × 12 µm and a depth resolution of 19 µm at Au-Lα, with primary excitation energy of 16.7 keV. Pb and Sr were found to correlate positively with the Ca content among the bone packets, while Zn did not show any correlation. Furthermore an accumulation of Pb and Zn was found specifically in the cement lines as identified in the XRF-maps by overlaying them with the

corresponding qBEI. No significant differences in the elemental distributions between fractured and controls could be observed.

Differential Accumulation of Lead in Double-Tidemarks in Articular Cartilage of Osteoarthritic Human Joints

A. Roschger[1,2], B. Pemmer[1], J. G. Hofstaetter[2,3], N. Zoeger[1], P. Wobrauschek[1], R. Simon[4], G. Falkenberg[5], P. Roschger[2], K. Klaushofer[2], C. Streli[1]

[1]Atominstitut, Vienna University of Technology; [2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna; [3]Department of Orthopaedic Surgery, Medical University of Vienna, Austria; [4]Institute for Synchrotron Radiation, Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen; [5]Hamburger Synchrotronstrahlungslabor HASYLAB DESY, Hamburg, Germany

Long-term exposure to increased lead (Pb) concentrations is associated with chronic diseases of the central nervous, the skeletal-hematopoietic, renal, and endocrine systems. Approximately 95 % of the total burden of Pb is stored in the skeleton. Normal environmental exposure to Pb derives mainly from air, drinking water, and food. In a recent study we found that Pb was accumulated up to 18-fold in the tidemark of articular cartilage compared to bone tissue. However, the underlying mechanism remains unclear. In joints the articular cartilage is tightly bound to bone by an about 100 µm wide region of mineralized cartilage. The transition zone between mineralized and non-mineralized cartilage is called tidemark (TM), because of its characteristic histological appearance. Especially in osteoarthritis associated with damages in the articular cartilage, an initiation of an additional mineralization phase can lead to a second tidemark. Our hypothesis is that Pb is accumulated after the mineralization front has stopped to advance and the Pb concentration is increasing with time. Thus, if there are 2 TMs, the inner (older) TM should have the higher and the outer (younger) TM should have the lower Pb level.

Undecalcified tissue from osteoarthritic human joints (patella and hip heads) was examined by quantitative backscattered electron imaging using a pixel resolution of 1 µm to localize the TMs within the cartilage and the subchondral bone region. Areas of interest were analyzed with synchrotron radiation-induced confocal micro x-ray fluorescence analysis (SR µ-XRF) to determine the distribution of Ca, Zn, and Pb in trabecular and cortical bone. Measurements were performed at the FLUO beamline at ANKA and HASYLAB beam L using a beam size of 15 × 10 µm and a depth resolution of 20 µm at Au-Lα, with primary excitation energy of 17 keV.

The evaluation of Pb intensities in double TM revealed in average a 2.6-fold higher level in the inner TM compared to the outer TM, whereby the Pb in the outer TM showed a high variation with respect to the inner TM (1.4–3.9-fold). The element Zn, which is also accumulated in the TMs, did not show differences between inner and outer TM. The data confirm our hypothesis made above. The biochemical composition of the TM seems to be able to selectively accumulate continuously Pb from the interstitial fluid of articular cartilage. In contrast, Zn seems to be a fixed constituent of the TM. The results might be of clinical importance, when during degenerative processes in osteoarthritis the tidemark region is destroyed and accumulated Pb is released again into the body fluid.

Strontium-Ranelate Treatment Leads to Uptake of Strontium into the Mineral of Newly Formed Bone

P. Roschger[1], B. Pemmer[2], P. Fratzl[3], K. Klaushofer[1]

[1]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Center Meidling, 1st Medical Department, Hanusch Hospital, Vienna; [2]Atominstitut, Vienna University of Technology, Austria; [3]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Based on results of recent clinical trials showing reduced vertebral and non-vertebral fracture risk, strontium ranelate (SrR) has been approved in several countries for the treatment of postmenopausal osteoporosis. This treatment is accompanied by uptake of strontium (Sr) into bone as the latter is a bone-seeking element. Spatial resolved techniques like backscattered electron imaging, scanning synchrotron radiation micro x-ray fluorescence, and small- and wide-angle x-ray scattering were applied to bone biopsy samples from the SOTI and TROPOS studies and to vertebral bone of an OVX rat model. The following results were obtained: (i) Sr was preferentially taken up into new bone formed during SrR treatment. (ii) The fraction of Sr to Ca was proportional to the Sr serum levels. (iii) Dietary deficiency of Ca caused a strong increase in Sr serum level and consequently an increased Sr uptake to bone. (iv) Sr was incorporated into the crystal lattice of the bone mineral, by replacing about 5 atom % Ca by Sr at a therapeutic SrR dose of 2 g/d.

In consequence, global Sr content of bone is expected to increase continuously with duration of treatment until all old bone is replaced by new bone due to remodelling. Thus, results of DXA measurements are difficult to interpret, because of the higher x-ray attenuation of Sr (Z = 38) compared to Ca (Z = 20). Potential changes in BMD values have to be considered as the sum of changes in bone volume, degree of matrix mineralization, and additionally of Sr content. After stopping of SrR treatment, Sr release from bone will occur only via bone resorption by osteoclasts.

Osteoclasts Create Resorption Trails by Resorbing the Mineralized Matrix Layer by Layer and Show a Different Resorption Potential on Bone and Dentin

M. Rumpler[1], T. Würger[1], P. Roschger[1], E. Zwettler[1], P. Fratzl[2], K. Klaushofer[1]

[1]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Center Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria; [2]Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Bone resorption by osteoclasts results in the formation of pits and trails, which includes dissolution of the mineral and organic phase and moving of the cell simultaneously in case of trails. For this resorption the cytoskeleton undergoes a unique organization by forming an actin ring at the sealing zone, bordering the extracellular compartment, which represents the resorption organelle. We analysed the resorption process, leading to trail formation in detail, and compared the resorption potential of osteoclasts on 2 different mineralized substrates, namely bone and dentin. 3D analyses with confocal laser scanning microscopy showed that resorption trail formation happens by a mechanism where the actin ring attaches at one side outside the existing pit, a little bit ahead of the trail margin. The other part attaches inside the existing pit, thus sparing that area which is going to be resorbed next. As a consequence, resorption trails are formed by a periodical process including starting resorption from the top and going down into depth. This anatomical configuration of the resorption organelle does not allow a simple "drilling movement" in a worm-like style, but only a resorption of a thin layer of the material from the surface into depth. By repeating this cycle, the material is degraded layer by layer, and at the end it appears as a longitudinal trace under the reflection light microscope.

Furthermore, we compared osteoclastic resorption on bone and dentin using histomorphometric parameters and found that resorption (resorbed area, %) was 11-times higher on dentin than on bone. The number of pits/cm² was 7-times higher on dentin compared to bone; however, average pit size was similar on both materials. This indicates that, once resorption has started, it runs independent from the material. Additionally, analyses of gene expression patterns revealed that mature osteoclast-specific genes (eg, MMP9, TRAP, RANK, ATPase, CLCN7, Integrin alpha), cytoskeleton-associated genes (tubulins, myotubularin, alpha actinin 2), and fusion-related genes are upregulated on dentin compared to bone. Thus, dentin exhibits a positive material-dependent effect on initiation of osteoclastic differentiation and resorption.

Verbesserung der Sekundärprävention bei Patienten nach osteoporotischer Fraktur: 2-Jahres-Daten eines interdisziplinären Netzwerkes

E. Sampl, H. Dobnig, B. Obermayer-Pietsch, H. P. Dimai, T. Pieber, A. Fahrleitner-Pammer
Klinische Abteilung für Endokrinologie und Stoffwechsel, Universitätsklinik für Innere Medizin, Medizinische Universität Graz, Österreich

Hintergrund Weltweit sind große Defizite in der Diagnostik und Therapie der Osteoporose vorhanden. Auch nach einer stattgehabten Fragilitätsfraktur werden die meisten Patienten nicht weiter osteologisch abgeklärt. Diese Auswertung soll die Auswirkungen eines „Fracture-Liaison-Dienstes" (FLD) über einen einjährigen Beobachtungszeitraum zu der Situation vor dem Projektbeginn analysieren.

Methoden Alle Patienten ab dem 50. Lebensjahr, die zwischen November 2008 und Oktober 2010 aufgrund einer Fraktur nach Bagatelltrauma an einer der 4 Unfallchirurgien der KAGES (Steiermärkische Krankenanstaltengesellschaft) in stationärer Betreuung waren, wurden anhand der „International Classification of Diseases"-(ICD-) Codierung über das Krankenhausinformationssystem identifiziert. Die Frakturpatienten aus dem Zeitraum November 2008 bis Oktober 2010 wurden im Zuge des „Fracture-Liaison-Dienstes", welcher 1× wöchentliche Besuche jeder teilnehmenden Unfallchirurgie vorsah, erfasst.

Ergebnisse In den beiden Jahren von November 2008 bis Oktober 2010 wurden 2186 Patienten, davon 1734 Frauen (79 %) und 452 Männer (21 %), mit einem durchschnittlichen Alter von 79 ± 10 Jahren stationär aufgrund einer osteoporosetypischen Fraktur nach Bagatelltrauma behandelt. Im Kollektiv vor Einführung des FLD wurde bei 42 Patienten (3,8 %) eine osteoprotektive Therapie empfohlen, wobei 27 Patienten ein orales Bisphosphonat, 2 Patienten ein intravenöses Bisphosphonat und 13 Patienten ausschließlich Vitamin D und/oder Kalzium verordnet bekamen. Im Rahmen des FLD konnten innerhalb eines Jahres 835 Patienten (76,9 %) erfasst werden, wovon 744 Patienten (89,1 %) in das Projekt aufgenommen wurden, 69 Patienten (8,3 %) eine Abklärung ablehnten und 22 Patienten (2,6 %) noch während des stationären Aufenthaltes verstarben. Insgesamt konnte durch Einführung des FLD der Prozentsatz an Patienten, welche einen Therapievorschlag bzw. eine weitere Abklärung erhielten, signifikant von 3,8 auf 68,5 % gesteigert werden ($p < 0{,}0001$).

Schlussfolgerung Die enorme Unterversorgung im Jahr vor Einführung des FLD weist auf den dringenden Handlungsbedarf hin. Anhand der deutlichen Steigerung des Prozentsatzes an weiter abgeklärten Patienten im Rahmen des FLD konnte die erfolgreiche Umsetzung solcher Maßnahmen demonstriert werden.

Monteggia Fracture and Multiple Traumata in Mediaeval Skeletal Remains Analysed by µCT and Histology

D. Schamall[1,2], P. Pietschmann[1], D. Moser[3], M. Dockner[4], M. Teschler-Nicola[2,4]
[1]Department of Pathophysiology and Allergy Research, Centre for Pathophysiology, Infectiology and Immunology, Medical University of Vienna; [2]Department of Anthropology, Natural History Museum Vienna, Austria; [3]Department of Cranio-, Maxillofacial and Oral Surgery, Medical University of Vienna; [4]Department of Anthropology, University of Vienna, Austria

During an archaeological excavation in Altenberg/Linz (Upper Austria) the well-preserved skeletal remains of a mature male dated to the 13th century were recovered. Several elements of the skeleton yielded alterations caused by trauma: Beside a malunion of the left ulna which was accompanied by shortening of the diaphysis, a luxation and deformation of the left radial head was observed (Monteggia-type lesion, Bado-type I). Moreover, at the anterior aspect of the corresponding humerus a chalice-shaped, newly built bone structure that framed the displaced capitulum radii was visible. This structure formed a sort of "alternative joint" that functionally even allowed some movements, although considerably restricted in regard to flexion/extension and even more in pronation/supination.

To verify the assumption of a "single event", we not only investigated the concerned skeletal portions by gross-anatomical examination, but also by non-invasive conventional radiological and µCT techniques. Additionally, we also carried out an invasive histological examination: Small samples of the proliferative new "joint-bone" and callus formations were taken and embedded in methylmethacrylate; undecalcified thin-ground sections were prepared, subjected to Goldner-staining, and studied for their micro-structural composition and documented by light-microscopical inspection.

Up to now, many clinical case studies dealt with Monteggia fractures and their consequences, eg, the restriction of movability due to bone proliferation; nevertheless, a similar massive new bone formation as we observed in the historical specimen has never been reported. The latter implies long-term survival and – because of the severe handicap – social care by members of the community. Taking the (similar) degree of remodelling at the injured skeletal elements into account, we assume and discuss the lesions as being caused by a complex, single event/accident.

Vitamin D in Urine as an Alternative to Serum Determination

V. Schwetz[1], E. Lerchbaum[1], H. Siggelkow[3], O. Trummer[1], N. Hacker[1], P. Kieslinger[2], H.-J. Gruber[2], B. Obermayer-Pietsch[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine and [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria; [3]Endokrinologikum, Medical University of Göttingen, Germany

Background Low 25-hydroxyvitamin D_3 (25[OH]D) status is common in nursing home residents due to reduced vitamin D intake, diminished production of vitamin D in the aging skin, and little sun exposure. Especially in these patients, vitamin D deficiency enhances bone loss, falls, and fractures. Vitamin D sufficiency in neonates and infants has been acknowledged to have a long-lasting positive clinical outcome and therefore supplementation especially in the first year of life is recommended worldwide. Whether supplementation should be continued beyond the first 12 months is subject to debate. In nursing home residents as well as in young children, drawing of blood samples for vitamin D status determination is sometimes problematic.

Aim The aim was to determine whether urine measurement of 25(OH)D could serve as an alternative method to serum 25(OH)D measurement.

Methods 25(OH)D was measured both in serum and spot as well as in 24 h-urine samples using an adapted enzyme immunoassay (IDS, Boldon, UK) in 243 patients. 25(OH)D was additionally measured repeatedly in further 305 patients with 2–3 24-h-urine samples each in order to determine reproducibility of 4 measurements.

Results Urine 25(OH)D normalised to urine creatinine correlated with serum 25(OH)D ($r = 0.494$; $p < 0.001$), with urine calcium ($r = –0.498$; $p < 0.001$), with urine protein ($r = –0.546$; $p < 0.001$), and with the urine protein-creatinine-ratio ($r = 0.412$; $p < 0.001$). The three 24-h-urine sample measurements highly correlated with each other ($r = 0.697$; $r = 0.728$; $r = 0.765$; $p < 0.0001$).

Conclusion Urine vitamin D measurement is a cheap, feasible, and stable alternative to determine overall vitamin D status especially in nursing home residents and children and in any other patients at risk of vitamin D deficiency.

Active DNA Demethylation Shifts the Phenotype of Osteoblastic MC3T3-E1 Cells

R. Thaler[1], S. Spitzer[1], H. Karlic[2], K. Klaushofer[1], F. Varga[1]
[1]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Center Meidling, 1st Medical Department, Hanusch Hospital, Vienna; [2]Ludwig Boltzmann Institute for Leukemia Research and Hematology, Hanusch Hospital, Vienna, and Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Austria

Methylation of cytosine-guanine dinucleotides (CpGs) of the DNA is an important epigenetic mechanism regulating gene expression,

tissue development, pathogenesis, and stabilization of chromatin [1]. Very recently, groundbreaking insights were published regarding the long-standing enigma of active CpG demethylation [2]. Thereby, it was demonstrated that this process occurs via CpG hydroxymethylation by members of the TET gene family. This process is dynamically regulated and is essential for early differentiation in embryonic stem cells [3, 4]. Dimethyl sulfoxid (DMSO) has been described to cause phenotypic changes in embryonic stem cells altering the genome-wide DNA methylation profiles and to affect differentiation of several cell lines.

As a model system, we used the heterogeneous pre-osteoblastic MC3T3-E1 cell-line because of its potential to differentiate from pre- into mature osteoblasts. Treatment of MC3T3-E1 cells with 0.5–2 % DMSO in culture medium for 24 hours resulted in attenuation of cell multiplication and increased caspase 3/7 and 8 activity. As shown by qRT-PCR, DMSO increased expression of Fas, Dlx5, Runx2, and Bglap2 indicating both apoptosis and differentiation, respectively. Already after 12 hours but much stronger after 24 hours, we observed decreased cytosine methylation and increased hydroxymethylation of the DNA of Fas and Dlx5 promoter, which was abrogated by Tet1-siRNA. In parallel expression of genes important for DNA methylation (Dnmt1, Dnmt3b, Hells) decreased while those of gene families involved in hydroxymethylation (TET) and nucleotide excision repair (GADD45) increased.

Compared to controls, after 5 days of treatment promoter-methylation of Fas, depending on the analyzed region, was slightly increased or decreased, while the Dlx5 promoter was left unchanged. Moreover, activities of the caspases 3/7 were unchanged while those of caspase 8 were only slightly increased indicating downregulation of apoptosis, leaving cells with decreased Runx2 and Bglap2 and increased expression of genes important for matrix synthesis (Col1a1, Lox).

We demonstrate that DMSO by active DNA demethylation firstly stimulates differentiation of MC3T3-E1 cells, which then apoptose leaving a cell population of less differentiated osteoblasts featuring an extra-cellular matrix producing phenotype. Further, we show for the first time a chemical compound capable to induce methylcytosine hydroxylation.

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In Vitro Effect of 1,25-Dihydroxyvitamin D_3 on the Catabolism of Tryptophan

U. Thiem[1,2], J. Höbaus[1], D. Hummel[1], T. Nittke[1], I. Fetahu[1], T. Manhardt[1], K. Borchhardt[2], E. Kallay[1]
[1]Division of Comparative Immunology and Oncology, Department of Pathophysiology and Allergy Research, and [2]Division of Nephrology and Dialysis, Medical University of Vienna, Austria

The active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3, has immunomodulatory properties. However, the underlying mechanisms are not yet fully understood. One putative mechanism involves the effect of 1,25-dihydroxyvitamin D_3 on the catabolism of tryptophan via the enzymes indoleamine 2,3-dioxygenase (IDO) 1 and 2. The induction of IDO leads to the depletion of tryptophan and to the accumulation of its catabolites, the kynurenines. This microenvironment of low tryptophan and high kynurenine levels favors the development of regulatory T cells on the one hand and is considered to exert antimicrobial activity on the other hand.

Our aim was to investigate the effect of 1,25-dihydroxyvitamin D_3 on the tryptophan catabolizing enzymes *in vitro*. For our studies we used the human monocytic leukemia cell line THP-1 which can be differentiated into macrophage-like cells upon stimulation with phorbol 12-myristate 13-acetate (PMA). We treated both undifferentiated and PMA-differentiated THP-1 cells with 10 nM of 1,25-dihydroxyvitamin D_3 between 1 and 24 hours and analyzed gene expression of IDO1 and IDO2 by qRT-PCR.

In PMA-differentiated THP-1 cells we observed an upregulation of IDO2. However, the effect on IDO1 was not significant. In undifferentiated THP-1 cells, we could not observe a clear effect of 1,25-dihydroxyvitamin D_3.

In conclusion, 1,25-dihydroxyvitamin D_3 seems to modulate PMA-differentiated THP-1 cells by upregulation of IDO2.

Vitamin D Insufficiency Genotypes, Bone Mineral Density, and Past and Prospective Bone Fractures

O. Trummer[1], V. Schwetz[1], D. Walter[1], E. Wehr[1], M. Gugatschka[2], A. Fahrleitner-Pammer[1], H. Dobnig[3], W. Renner[4], T. R. Pieber[1], B. Obermayer-Pietsch[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, and [2]ENT University Clinic, Department of Phoniatrics, Medical University of Graz; [3]Diagnostikinstitut Univ.-Prof. Dr. H. Dobnig GmbH, Graz; [4]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Austria

Background Low 25-hydroxyvitamin D (vitamin D) status is known to play an important role in many diseases with focus on bone health. A genetic background for bone characteristics such as bone density and structure is well established. Whether vitamin D genetics might play an important role in skeletal determinants is discussed controversially.

Aims Based on recently reported genetic determinants of vitamin D insufficiency, we investigated genetic variants of group-specific component (GC), 7-dehydrocholesterol reductase (DHCR7), and cytochrome P450IIR-1 (CYP2R1) for their potential role in predicting vitamin D levels and bone mineral density (BMD) as well as bone fractures.

Methods Replication was performed in 2 cohorts, in a cross-sectional study of 541 subjects (mean age 56 ± 13 years) and in a prospective study of 1119 nursing home subjects (mean age 84 ± 6 years), both with clinical and biochemical patient characterisation.

Results Mean 25(OH) vitamin D levels were 33 ± 13 ng/ml in the cross-sectional and 9 ± 6 ng/ml in the prospective nursing home subjects. GC genotypes were significantly associated with lower mean 25(OH) vitamin D levels in both cohorts ($p = 0.001$ and $p = 0.048$, respectively).

There was no significant association of BMD with any of the genotypes and no relationship to past fractures. However, prospective fractures were different between DHCR7 ($p = 0.018$) and GC genotype groups ($p = 0.023$) but not between CYP2R1 groups. Moreover DHCR7 variants were significantly associated with months to hip fracture ($p = 0.04$, risk ratio of recurrence [RR] 0.73 [95 % CI 0.53–0.99]) and with time to fracture by Kaplan-Meier analysis.

Conclusion In our 2 independent cohorts, GC genotypes were associated with 25(OH) vitamin D status, but not with BMD. However, prospective fracture risk might be related to these gene variants.

Paired Bone Biopsies – How Valid Are They? A Prospective Controlled Study

D. Wagner[1], H. P. Dimai[2], K. Amrein[2], H. Dobnig[2], T. Pieber[2], P. Stiegler[1], M. Sereinigg[1], C. Beham Schmid[3], A. Fahrleitner-Pammer[2]
[1]Department of Transplantation Surgery, [2]Division of Endocrinology and Metabolism, Department of Internal Medicine and [3]Department of Pathology, Medical University of Graz, Austria

Iliac crest biopsies are requested for all osteoporotic treatment agents in clinical use to ensure that bone histology remains normal under treatment. Paired bone biopsies are also used to evaluate the effect of an osteoactive compound over an administration period. Data about a potentially existing side-to-side variance between paired biopsies are scarce.

Aim of the current study was to analyze the variance of paired iliac crest biopsies that had been obtained under ideal conditions. Samples were taken in a population of brain-dead organ donors as part of explant surgery. The 2 biopsies were performed by the same surgeon directly after organ explantation. Consecutive organ donors between 10/2009 and 12/2010 were included into the study. The included donors were very carefully defined and healthy; donors with a history of fractures or who had received any osteoporotic treatment or medication with impact on bone metabolism were excluded from the presented study. Histomorphometric analyses were performed in duplicates by the same technician who was blinded to patient's identity.

More than half of the patients were male (n = 27), median age was 52.5 ± 13 with a BMI of 23 ± 2.1. All parameters investigated were comparable between men and women. Intraindividual variation coefficients ranged between 4.2 % (BV/TV) and 9.8 % (TbPf). The results of the histomorphometric analysis are listed in table 1. No differences between the sides of the paired biopsies were observed variance ranged from 0.9 % (QS/BS) to 22 % (ES/BS).

Histomorphometric analysis of bone biopsies – at least performed under standardized conditions – is a highly sensitive approach to assess bone metabolism. As bone biopsies showed comparable results between both sides, they might represent a possibility to investigate effects of specific medications even in small populations.

Table 1: Wagner D et al., Results of the histomorphometric analysis

	Left	Right	Delta (%)	p-value
BV/TV (%)	24.4 ± 4	24.5 ± 5	3 ± 5	0.79
BS/BV (%)	18 ± 3	17 ± 2	5 ± 5	0.14
OS/BS (%)	6.4 ± 2.3	6.6 ± 2.1	6 ± 15	0.32
ES/BS (%)	2.4 ± 1	2.3 ± 1	3.4 ± 2.7	0.79
QS/BS (%)	91 ± 3	91 ± 2	0.9 ± 1	0.74
Tb.N. (mm^{-1})	1.8 ± 0.4	1.7 ± 0.3	7 ± 4	0.37
Tb.Th (µm)	142 ± 26	140 ± 25	3.6 ± 7	0.22
Tb.Sp (µm)	420 ± 87	426 ± 100	4.5 ± 7	0.31

BV/TV = bone volume/tissue volume, BS/BV = bone surface/bone volume, OS/BS = osteoid surface/bone surface, ES/BS = eroded surface/bone surface, QS/BS = quiescent surface/bone surface, Tb.N = trabecular number, Tb.Th = trabecular thickness, Tb.Sp = trabecular separation rate

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