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Wissenschaftliche Herbsttagung der Österreichischen Gesellschaft für Knochen und Mineralstoffwechsel (ÖGKM)

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Loss of the Calcium-Sensing Receptor Provides a Growth Advantage to Colon Cancer Cells

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Background The classical role of the calcium-sensing receptor (CaSR), an extracellular G protein-coupled receptor, is regulation of calcium homeostasis. In addition, it is involved in several cellular processes such as proliferation, differentiation, and apoptosis. The CaSR has been suggested to mediate the effects of calcium in delaying the onset of colorectal cancer (CRC). We have previously demonstrated that CaSR expression is downregulated in CRC, leading us to hypothesize that loss of CaSR might provide a growth advantage to transformed cells, conferring them resistance to calcium-mediated growth inhibition. Therefore the aim of the study was to understand the consequences of losing CaSR expression in CRC.

Methods and Results We analysed mRNA (qRT-PCR) and protein (immunofluorescence) expression of CaSR in two CRC cell lines: Caco-2/15 (well-differentiated adenocarcinoma) and HT-29 (moderately differentiated adenocarcinoma). HT-29 cells showed lower CaSR levels when compared with Caco-2/15 cells (30-fold lower mRNA expression), similar to our CRC patient cohort (n = 60), where we found significantly less CaSR expression in tumours compared with respective adjacent mucosa ($p < 0.001$).

To investigate the possible impact of CaSR on growth, we used a positive allosteric CaSR modulator, NPS R-568, that specifically modifies the CaSR structure, making it more susceptible to the effects of calcium. Using bromodeoxyuridine cell proliferation assay we established that Caco-2/15 cells, which express CaSR endogenously, had stricter proliferation control by calcium (2.25 times slower growth-rate, $p < 0.05$), whereas HT-29 cells were insensitive to this treatment. In Caco-2/15 cells this effect could be reversed with the negative allosteric CaSR modulator, NPS-2143.

To understand the consequences of losing CaSR expression in CRC, we transfected the HT-29 and Caco-2/15 cells to obtain cells overexpressing either the wild-type CaSR (OE-CaSR) or a CaSR harbouring an inactivating mutation (IM-CaSR). The cells transfected with the IM-CaSR construct grew significantly faster than the cells overexpressing the CaSR (Caco-2/15: 2.8 times faster; HT-29: 1.7 times faster; $p < 0.0001$). Calcium treatment (2 mM calcium vs 0 mM calcium) led to a strong proliferation inhibition in cells overexpressing the CaSR, which was lost in the IM-CaSR cells ($p < 0.001$).

Conclusion These results indicate that loss of CaSR expression gives CRC cells a growth advantage and sustains a malignant phenotype. These observations further strengthen our understanding of the molecular mechanism by which colon cancer cells lose their anti-proliferative features where expression of CaSR is lost.

Acknowledgments This project was funded by the Marie Curie Initial Training Network grant number FP7-264663. The authors would like to thank Prof. Romauld Mentaverri for the CaSR constructs and Prof. Sabina Baumgartner-Parzer for sequencing the cells for conformation of transfection.

Influence of the Organic Matrix of Mineralized Tissues on Their Dynamic Mechanical Properties Assessed by Scanning Acoustic Microscopy

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Mineralized tissues like bone, articular calcified cartilage, or mineralized turkey leg tendon (MTLT) are built by a composite of hydroxyapatite nano-particles and organic matrix. In bone and MTLT the matrix is formed by collagen type-I, but in contrast to bone in MTLT the collagen is uniaxially orientated, while in cartilage the matrix consists of collagen type-II and proteoglycans.

Composition/orientation differences were investigated by a new scanning acoustic microscopy method (SAM-TOF). Time-of-flight differences of ultrasound pulses obtained from human femoral head and distal MTLT samples with known thickness (30 microns) were determined with 0.125 ns time resolution to obtain sound velocity maps with 2 µm pixel resolution using a 330-MHz lens (kibero Gmbh). The velocity maps were combined with calcium content maps obtained by quantitative backscattered electron imaging to extract dynamic elastic moduli (E) maps.

Bone was found to require a lower mass density (–4.3 %) than cartilage to achieve similar velocity (range 3700–4300 m/s) or elastic modulus (range 22–30 GPa), which is qualitatively in line with nanoindentation results. In the circumferential compartment of MTLT, an axial/transversal velocity ratio of 1.13 and E ratio of 1.28 and in the interstitial compartment 1.16 and 1.32 ratios, respectively, were found. This anisotropy is clearly due to the preferred orientation of collagen. However, the higher E in cartilage-bone and lower ratio in MTLT compared with what is typically measured with (quasi-) static mechanical test such as uniaxial tension or nanoindentation could indicate an influence of relaxation processes.

These first results suggest that TOF scanning acoustic microscopy may be able not only to provide mechanical maps of mineralized tissues but to extend our understanding of the mechanical properties of bone and cartilage to the region of high loading rates, which may be highly relevant for the fracture resistance under an impact, eg, during a fall.

Bone Turnover Markers in Normal Pregnancy

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Objective The aim of this study was to investigate the concentrations of bone turnover markers in normal pregnancy. Therefore osteoprotegerin (OPG), free soluble RANKL, sclerostin, dickkopf-1

* Reihung alphabetisch nach Erstautor

(DKK-1), and cathepsin K serum concentrations of healthy pregnant women were measured and compared to serum levels of healthy non-pregnant women.

Pregnancy and lactation are heavily associated with alterations in the calcium metabolism. To which extent the maternal bone mineral contributes to the developing skeleton of the fetus is as discussed as the degree to which pregnancy affects changes in long-term bone mineral density (BMD) and the resulting risk of osteoporosis later in life. Previous studies demonstrated that serum parathyroid hormone (PTH) levels are suppressed at the beginning of pregnancy but return to normal levels by the end of pregnancy [1]. Other groups suggested that OPG and beta crosslaps (CTx) may be involved in the regulation of bone turnover during pregnancy [2, 3] as elevated levels were found in pregnant women. Also significant higher concentration of sRANKL and OPG were found in the second trimester of normal pregnancy when compared to the first and third trimesters [4].

Study Design and Results The sera of 30 healthy pregnant women (kindly provided by B. Svejda, Klagenfurt, Austria) and of 9 healthy non-pregnant women about the same age were assayed by ELISA (Biomedica, Austria). While serum concentrations of cathepsin K and sclerostin remained constant, the median OPG and free sRANKL concentrations were significantly higher in pregnant women (OPG: median 5.15 pmol/L vs 3.5 pmol/L, $p = 0.0232$; free sRANKL: median 0.68 pmol/L vs 0.12 pmol/L, $p = 0.0455$) and serum concentrations of DKK-1 significantly decreased (median 16.6 pmol/L vs 34 pmol/L, $p = 0.0003$).

Conclusion Serum levels of OPG, free sRANKL, and DKK-1 were found to be changed significantly between pregnant and non-pregnant women. To obtain more meaningful data these markers should be monitored in a time-dependent manner during pregnancy and lactation.

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Renal Elimination of Sclerostin Increases as Kidney Function Declines

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Background Sclerostin is a soluble inhibitor of canonical Wnt-signalling, which is crucial for bone biology. Currently, anti-sclerostin antibodies are in clinical development for the treatment of osteoporosis. Osteoporosis and chronic kidney disease (CKD) often occur simultaneously. Sclerostin serum levels are increased in patients with CKD. It is unclear whether the increase of sclerostin serum levels in CKD patients is due to increased sclerostin production and/or decreased renal elimination.

Patients and Methods In this study, 120 patients with impaired kidney function were recruited for measurements of sclerostin in serum and urine (ELISA), renal function (eGFR), electrolytes, α1-microglobulin, parathyroid hormone, vitamin D, and markers of bone turnover. Eight human kidney biopsies were stained for sclerostin using immunohistochemistry.

Results Urinary sclerostin excretion increased with declining eGFR ($R = –0.75$, $p < 0.001$), from 10.4 (± 12.7) pmol/l in patients with eGFR > 90 ml/min/1.73 m² (CKD stage 1) to 117.9 (± 65.4) pmol/l in patients with eGFR < 15 ml/min/1.73 m² (CKD stage 5, $p < 0.001$). Fractional excretion of sclerostin ($FE_{Sclerostin}$) increased with declining eGFR ($R = –0.83$, $p < 0.001$) from 0.45 (± 0.6) % in CKD 1 to 26.3 (± 17.6) % in CKD 5 ($p < 0.001$). $FE_{Sclerostin}$ correlated with fractional excretion of α1-microglobulin ($FE_{\alpha1\text{-microglobulin}}$, $R = 0.82$, $p < 0.001$). Sclerostin was detected in proximal tubular cells, showing a diffuse cytoplasmic staining pattern.

Conclusion Increased sclerostin serum levels in CKD patients are not due to decreased renal elimination. On the contrary, renal elimination increases with declining kidney function. Whether this has consequences on anti-sclerostin antibody dosing, efficacy, or safety in patients with CKD remains to be determined.

Serum Levels of Sclerostin and Dickkopf-1 in Geriatric Patients with Osteoporotic Hip Fractures

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Background Sclerostin (SOST) and dickkopf-1 (Dkk-1) are inhibitors of the canonical Wnt-signalling pathway. Wnt-signalling has been shown to play a significant role in osteoblastogenesis; inhibition of Wnt-signalling by SOST and Dkk-1 will therefore impair bone formation. Since osteoblast dysfunction is an important aspect of age-related osteoporosis, the aim of this study was to evaluate SOST and Dkk-1 serum levels in an osteoporotic study cohort and compare them with geriatric and young control subjects.

Methods We studied serum levels of the aforementioned Wnt inhibitors in 164 patients with osteoporotic hip fractures (mean age: 81 ± 8 years), in 119 geriatric control subjects, and in 68 young control subjects (mean age: 23 ± 5 years). Serum concentrations of sclerostin and dickkopf-1 were measured by ELISA (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria).

Results Dkk-1 levels were significantly higher in hip fracture patients than in geriatric (96.0 ± 3.6 pmol/l versus 76.6 pmol/l, $p < 0.001$) or young controls (96.0 ± 3.6 pmol/l versus 19.6 ± 1.0 pmol/l, $p < 0.001$). Serum Dkk-1 levels increased significantly with age ($\tau = 0.417$, $p < 0.001$). There was no significant difference of SOST serum levels in hip fracture patients compared to geriatric or young controls. However, in women with hip fractures serum sclerostin was significantly lower than in young control women (15.9 ± 1.3 pmol/l versus 16.3 ± 0.9 pmol/l, $p < 0.05$). In men a significant increase of serum SOST levels with age was seen (20.7 ± 1.4 pmol/l versus 28.8 ± 2.7 pmol/l, $p < 0.05$).

Conclusion The determination of sclerostin and dickkopf-1 is useful for the study of age-related bone fragility. Nevertheless, sclerostin and dickkopf-1 levels in the peripheral circulation appear to reflect different biologic events.

Low Bone Mineral Density in Friedreich Ataxia

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Background Friedreich ataxia (FRDA) is the most common inherited neurodegenerative ataxia. Apart from predominant neurological features an involvement of the skeletal system in terms of scoliosis and foot deformities is frequent. Disease-related falls, mobility restrictions, and wheelchair-dependency in later disease stages might additionally compromise bone structure in FRDA. The aim of this pilot study was to systematically evaluate the bone status in a representative FRDA cohort.

Methods 28 FRDA patients were enrolled in this cross-sectional study. The mean age of the whole study population was 37.6 years, ranging from 22 up to 62 years. Neurological assessment, a questionnaire comprising the history of fractures and osteoporosis, as well as osteodensitometric measurements complemented with gen-

eral and bone-specific laboratory parameters were performed. The WHO Fracture Risk Assessment tool (FRAX®) was applied, calculating the 10-year risk of suffering an osteoporotic fracture.

Results Six patients (21.4 %) presented with a bone mineral density below the expected range for age in at least one of the examined sites (femoral neck, lumbar spine, and forearm) irrespective of their gender. Corresponding *Z*-scores were significantly lower compared to normative values for the femoral neck and lumbar spine. Vitamin D status was insufficient in 11 and deficient in 8 FRDA patients. There was a strong negative correlation between ataxia severity, GAA repeat expansion, and bone density in the femoral neck of FRDA patients.

Conclusions This is the first report of an increased rate of low bone mineral density in FRDA. Given the increased risk of falls, this data rectifies routine bone mineral density measurements in FRDA, which may help to initiate therapeutic interventions to prevent this condition.

Role of DNA Me thylation and Histone Modifications on Calcium-Sensing Receptor Expression in Colorectal Cancer

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Numerous epidemiological studies have shown that low intake of calcium is related with higher incidence of colorectal cancer. The anti-proliferative effects of calcium in the colon are partially mediated by the calcium-sensing receptor (CaSR). In colorectal cancer the expression of the CaSR is lost as the tumour progresses. We hypothesized that DNA hypermethylation and imbalance of transcriptionally permissive/repressive histone alterations lead to loss of CaSR in colorectal tumours.

CaSR mRNA and protein expression was analyzed by qRT-PCR and immunofluorescence. We determined the methylation pattern of CaSR promoter by pyro- and bisulfite sequencing, methylation of lysine 4 (K4) and acetylation of lysine 9 (K9) on histone 3 (H3) were assessed by chromatin immunoprecipitation.

In colorectal tumours we observed significantly lower CaSR mRNA expression ($n = 65$, $p < 0.001$) compared with adjacent mucosa from the same patient. Immunofluorescence staining confirmed downregulation of CaSR protein in tumours. The CaSR promoter was higher methylated in tumours compared with the adjacent mucosa ($n = 45$, $p < 0.001$) and correlates inversely with mRNA expression ($n = 45$, $\rho = -0.588$, $p < 0.001$). Treatments with 5-aza-2-deoxycytidine, a DNA methyltransferase inhibitor, and with two different histone deacetylase inhibitors, Trichostatin A or suberoylanilide hydroxamic acid, restored the expression of CaSR in several colon cancer cell lines, in a compound- and cell line-dependent manner. Inhibition of lysine-specific demethylase 1, to prevent demethylation of mono- and dimethylated H3K4, caused only modest increase of the CaSR expression.

Our results suggest that in colorectal tumours loss of the CaSR expression is caused partially by hypermethylation of its promoter and by deacetylation of lysine 9 on histone 3.

Bone Microarchitecture in Men with Osteoporotic Hip Fractures

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Background Approximately one third of postmenopausal women have osteoporosis; nevertheless, also men suffer from this disorder and about one third of osteoporotic hip fractures worldwide occur in the male population. However, there is limited information about microstructural changes associated with osteoporotic hip fractures in men. The aim of this study was to investigate changes in trabecular bone structure in men with osteoporotic hip fractures.

Methods Femoral heads and adjacent bone tissue from 11 men with low-trauma hip fractures (mean age 82 ± 7 years) and consecutive surgical hip replacement were collected. Bone samples from age-matched men undergoing hip replacement due to osteoarthritis served as controls. Bone samples were taken from 4 different regions (central and subcortical region of the femoral head, central and subcortical region of the femoral neck). All samples were analyzed separately by static histomorphometry. The following parameters were evaluated: trabecular bone volume (BV/TV), marrow cavity volume (MaV/TV), bone surface density (BS/BV), trabecular number (TbN), trabecular thickness (TbTh), and trabecular separation (TbSp).

Results Major microarchitectural changes were seen in the subcortical region of the femoral neck. In the fracture group BV/TV and TbN were significantly decreased (BV/TV: 26.5 ± 2.2 % versus 62.1 ± 13.6 %, $p = 0.001$; TbN: 3.2 ± 0.2 mm^1 versus 4.7 ± 0.6 mm^{-1}, $p = 0.007$), whereas TbSp and MaV/TV were significantly increased (TbSp: 249.8 ± 24.1 µm versus 114.2 ± 19.4 µm, $p = 0.001$; MaV/TV: 76.1 ± 2.7 % versus 54.4 ± 5.4 %, $p = 0.006$). In the 3 other regions analyzed no significant differences were detected for these parameters. For TbTh and BS/BV no significant differences were observed in all 4 regions.

Conclusion Changes in the trabecular structure associated with osteoporotic hip fractures in men are localized in the subcortical region of the femoral neck and are (as has been reported in women) characterized by decreased BV/TV and by loss of trabeculae. This information might help to identify persons at higher risk for osteoporotic hip fractures.

Effects of Growth Hormone Therapy on Bone Turnover and Bone Matrix Mineralization in Children with Chronic Kidney Disease

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Background and Methods A specific skeletal complication in children with CKD is growth impairment. The effects of recombinant human growth hormone (rhGH) therapy on bone turnover and bone material quality are not well understood. Bone matrix mineralization density distribution (BMDD) as assessed by quantitative backscattered electron microscopy (qBEI) is an important determinant of bone material quality and reflects bone turnover (average tissue age) and mineralization kinetics of the individual bone packets of the sample.

We have evaluated a cohort of 20 paediatric patients (CKD stage 4: n = 2; end-stage renal disease treated by dialysis: n = 18). Bone histomorphometry was performed and all biopsies were classified according to the new TMV system.

Results Prior treatment, strong associations between bone matrix mineralization and bone turnover were found: the mean bone matrix mineralization was abnormally high while bone formation rates were low compared to reference data.

After rhGH treatment, the mean height acquisition was about 8.5 cm, serum ALP levels and bone turnover indices were significantly increased compared to baseline, and bone matrix mineralization in cancellous and cortical compartments was significantly decreased towards normal range.

A striking exception was the case of an adolescent with difficulties to adhere to therapy, who developed severe hyperparathyroidism and osteitis fibrosa concomitantly with abnormally low bone matrix mineralization.

Conclusion Our data show that bone turnover rate is a strong predictor of the bone matrix mineralization in young patients with CKD and growth deficiency. At baseline, our cohort had low bone turnover and increased bone matrix mineralization, indicating further normal mineralization kinetics in these patients with CKD. The data suggest that rhGH treatment in children with CKD does not only increase height but also bone turnover, which appears beneficial for bone matrix mineralization.

Analysis of BMD, Hip Geometry, and Trabecular Bone Score in Relation to Body Composition and Biochemical Markers in Adult Females with Severe Anorexia Nervosa

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Purpose Anorexia nervosa (AN) is a psychiatric eating disorder associated with reduced body mass, estrogen deficiency, malnutrition, and amenorrhea. The aim of the study was to investigate differences in hip geometry (HSA) and spinal bone microarchitecture as measured indirectly by trabecular bone score (TBS) in relation to body composition in patients with AN. TBS, a new texture measurement that can be applied to X-ray images and DXA, quantifies local variations in gray level and characterizes bone microstructure. HSA, derived from DXA, provides information about hip geometry and biomechanical strength.

Methods DXA scans of femoral neck, lumbar spine and body composition (lean body mass [kg], body fat percentage [%], BMC [kg]), and HSA (CSMI: cross-sectional moment of inertia, CSA: cross-sectional area) were assessed by iDXA (GE Lunar) in 34 patients with AN (23.5 ± 4.5 y, BMI 14.7 ± 1.3) and 26 controls (24.4 ± 2.5 y, BMI 23.5 ± 3.7). The raw data of spinal DXA (L1–L4) were extracted and analysed with TBS iNsight software (v1.9, Medimaps SA, France). Serum parameter for bone formation (P1NP) and bone resorption (CTX), sclerostin levels (Scl), 25(OH)Vit. D, estradiol, and cortisol were assessed.

Results In patients with AN BMD was significantly decreased at all sites (femoral neck 0.850 ± 0.14 vs 1.068 ± 0.11; L1–L4 0.973 ± 0.15 vs 1.292 ± 0.15, $p < 0.001$). Consequently mean TBS spine was significantly lower in AN (1.35 ± 0.12 vs 1.56 ± 0.08, $p < 0.001$). CSMI (7.6 ± 1.8 vs 10.8 ± 2 .7) and CSA (121.2 ± 21.7 vs 161.6 ± 19.7) were significantly lower in AN ($p < 0.001$ for both). Body composition showed significantly lower BMC (2.0 ± 0.3 vs 2.5 ± 0.5), lean body mass (32.7 ± 4.0 vs 42.6 ± 5.1), and body fat percentage (14.1 ± 8.0 vs 36.1 ± 5.2, $p < 0.001$ for all) in AN. In AN, lean body mass, BMC, and hip BMD correlated positively with CSMI and CSA. Patients with AN had significantly higher Scl (41.7 ± 17.7 vs 28.8 ± 11.9, $p < 0.05$) and CTX (0.767 ± 0.4 vs 0.446 ± 0.2, $p < 0.001$) levels, while there was no difference in P1NP and cortisol. Vit. D (26.7 ± 11.0 vs 32.9 ± 9.5) and estradiol (25.9 ± 25.6 vs 75.4 ± 81.1, $p < 0.05$ for both) were significantly lower in patients with AN.

Conclusion Apart from the expected significant decrease in body composition and BMD, our data suggest strong deteriorations of microstructure and bone geometry in young adults with AN. Moreover, high Scl levels, elevated bone resorption markers, but unchanged formation markers give evidence of an uncoupling in bone turnover.

Altered Material Properties in Sclerostin-Deficient Bone

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High bone mass induced by Sclerostin (Sost) deficiency is not associated with a prevalence of fragility fractures as in other sclerosing bone disorders. Beneficial alterations in bone material properties may be responsible for that. Thus, bone of Sost-KO mice (n = 9, 16 weeks old) and patients (young [4–16 yrs old], n = 4, and adults [24 and 43 yrs old], n = 2) with sclerosteosis was investigated by means of quantitative backscattered electron imaging and Raman microspectroscopy and compared to healthy bone. In Sost-KO mice the bone formed by osteoblasts of the endosteal compartment exhibited altered material properties, unlike the periosteal surfaces. Comparing bone tissue of same tissue ages (5–15 and 55–65 days) as defined by fluorescence labelling the average bone matrix mineralization was reduced (–1.9 %, $p < 0.0001$ and –1.5 %, $p < 0.05$, respectively), and the relative proteoglycan content was significantly increased. In sclerosteosis patients, where only chips of compact bone without any fluorescence labelling were available, the bone matrix mineralization density distribution was shifted towards lower matrix mineralization, coupled with an increase in mineralization heterogeneity in the young population. The relative proteoglycan content was also increased in the bone samples of children.

The present study revealed altered bone material properties at the endocortical envelope in Sost-Ko mice and also indications for alterations in sclerosteosis patients. We suggest that these alterations in bone material properties may contribute to the absence of fragility fractures in patients with sclerosteosis.

Consequences of the Overexpression of the Vitamin D-Degrading Enzyme CYP24A1 *In Vivo* and *In Vitro*

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Low serum vitamin D level increases risk for colorectal cancer in humans. The vitamin D-degrading enzyme CYP24A1 is severely overexpressed in colorectal tumours further depleting vitamin D metabolites. In the colon, vitamin D metabolites are thought to regulate proliferation, angiogenesis, apoptosis, and differentiation. Therefore, depletion of these metabolites reduces the anti-tumorigenic activities of vitamin D. Recently, we found strong correlations between the expression of the vitamin D-degrading enzyme CYP24A1 and proliferation markers. This led us to speculate that tumours with high CPY24A1 expression may have an increased proliferative potential. To investigate this, we stably transfected HT-29 cells (low basal CYP24A1 transcription) with a CYP24A1 overexpression vector. The cell line HT-29CYP24A1-GFP shows more than 20,000-fold higher mRNA expression of CYP24A1 and significantly increased protein expression. Both mRNA and protein expression can be further induced by vitamin D (1,25-D_3) treatments. To investigate the effect of CYP24A1 overexpression on tumour growth *in vivo*, we injected xenografts into male SCID mice. The animals received high or low vitamin D supplementation with either high or low soy protein. Soy contains high amounts of genistein, a CYP24A1 inhibitor. Overall, HT-29CYP24A1-GFP xenografts grew faster and penetrated skin earlier than control HT-29GFP xenografts. Soy-rich diet reduced tumour growth in control cells but not in HT-29CYP24A1-GFP xenografts. Our preliminary results suggest that a soy-rich diet slows xeno-

graft growth in SCID mice, while CYP24A1 overexpression counteracts this effect.

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Vitamin D und Osteocalcin als Modulatoren der männlichen Steroidogenese

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Einleitung Vitamin D (VD) ist ein vielseitiges Hormon und spielt neben seiner Funktion in der Kalzium-Homöostase u. a. in der Insulinsekretion und im Androgenstoffwechsel eine wichtige Rolle. Osteocalcin (OC), ein Peptidhormon, das in den Osteoblasten im Knochen gebildet wird und vorwiegend als Marker des Knochenaufbaus dient, scheint nach neuen Erkenntnissen in Zusammenhang mit Insulinausschüttung und männlicher Fertilität zu stehen.

Ziel Ziel dieser Studie ist es, Auswirkungen der Hormone Vitamin D bzw. Osteocalcin auf die Genexpression humaner Hodenzellen zu zeigen.

Methoden Für die Untersuchungen wird eine testikuläre Krebszellinie (Ntera 2/d1) herangezogen. Die Expression von Genen, die in den Androgenstoffwechsel involviert sind (Androgenrezeptor [AR], Aromatase [CYP19A1], 17α-Hydroxylase/17,20 Lyase [CYP17A1], 3-β-Hydroxysteroid-Dehydrogenase [HSD3B2], 17β-Hydroxysteroid Dehydrogenase [HSD17B1]), wird basal und nach Supplementierung der o. g. Hormone in physiologischen Konzentrationen mittels quantitativer RT-PCR (TaqMan-Assays) analysiert.

Ergebnisse Ntera-2/d1-Zellen zeigten große Unterschiede in der Expression von Genen des Androgenstoffwechsels abhängig von der VD- bzw. OC-Supplementation. Der physiologische Zusatz von luteinisierendem Hormon (LH) allein, das *per se* die männliche Testosteronsynthese in Hoden stimuliert, zeigte hingegen keine signifikanten Auswirkungen auf die Expression der untersuchten Gene. Ein signifikanter Anstieg der Steroidogenese in Ntera-2/d1-Zellen zeigte sich erst in der kombinierten Supplementation aus LH, VD und OC.

Schlussfolgerung Wir können erstmals eine reproduzierbare Beteiligung von VD und OC an der Androgensynthese in humanen Testeszellen nachweisen. Vermutlich ist die Aktivierung des Vitamin-D-Rezeptors (VDR) ein wichtiger Initiator der Androgen-Transkription. Diese Ergebnisse unterstützen neueste Forschungsansätze, wonach der Knochen als endokrines Organ und speziell sezerniertes OC und VD wichtige Faktoren der männlichen Fertilität sind. Details der Signalwege von VD und OC in der Androgensynthese bleiben zu klären.

Role of the Local Vitamin D Metabolism in Pancreatic Tumours

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Background The vitamin D system is deregulated during tumour development and progression in several cancer types. Data on the characteristics of the vitamin D system in the normal and diseased pancreas are missing.

Aim The aim of this study was to investigate the expression of the vitamin D receptor (VDR), 1,25-dihydroxyvitamin D_3 24-hydroxylase (CYP24A1), 25-hydroxyvitamin D_3-1-alpha hydroxylase (CYP27B1), and the calcium-sensing receptor (CaSR) in normal pancreatic tissue and in pancreatic tumours.

Methods The expression of vitamin D system genes and the CaSR at mRNA and protein level was investigated with quantitative real-time RT-PCR and immunohistochemistry.

Results mRNA expression of CYP24A1 and VDR was increased significantly in tumours compared with the adjacent normal tissue ($p < 0.05$ for both). We could observe a trend towards a decrease of CYP27B1 and CaSR expression in tumours compared with adjacent normal tissue. On protein level, we could see an increase in expression of the vitamin D-degrading enzyme CYP24A1 in ductal adenocarcinoma compared with normal ducts ($p < 0.05$). In addition, a high expression rate of the VDR protein was observed in endocrine cells in both normal pancreas and pancreatic tumours.

Conclusions The main finding of our study is an upregulation of CYP24A1 expression in pancreatic ductal adenocarcinoma compared with normal pancreatic tissue from the same patient. We could show different expression patterns of CYP24A1, VDR, and CaSR in functionally different regions of the pancreas.

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Bone Microstructure, Density, and Geometry Are Affected in Patients with Rheumatoid Arthritis

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Objectives Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by local and systemic bone loss. Proinflammatory cytokines as well as antibodies directed against citrullinated proteins (ACPA) can stimulate osteoclast differentiation in RA. Therefore, RA is an independent risk factor for secondary osteoporosis and osteoporosis-related fractures. However, the impact of chronic inflammation as well as conventional and biological disease-modifying anti-rheumatic drugs (DMARDs) on bone structure, density, and geometry remains unclear.

Methods We performed HR-pQCT (XtremeCT) measurements at the distal radius (DR) and the ultra-distal radius (UDR), close to the joint gap, in 90 patients with RA (60 female, 30 male). Data were compared to age- and gender-matched controls (CTRL, n = 70; 40 female, 30 male). Volumetric bone mineral density (vBMD), bone geometry, and bone microstructure including trabecular bone volume fraction (BV/TV), trabecular number (Tb.N, 1/mm), cortical thickness (Ct.Th, mm), and cortical porosity (Ct.Po, %) were assessed at the DR. Since cortex is too thin at the UDR, only trabecular parameters were analyzed.

Results Patients in the RA group were comparable to CTRL regarding age (53.6 ± 12.8 vs. 52.3 ± 12.6 yrs), height, weight, and BMI. At the DR, trabecular ($p = 0.005$ and $p < 0.001$) and cortical BMD ($p < 0.001$ and $p < 0.001$) were significantly decreased in male and female patients with RA, respectively. Similar results for trabecular BMD were found for both sexes at the UDR. We found a decreased BV/TV at the DR and UDR, mainly caused by a decrease in trabecular number in female RA ($p < 0.001$ for both sites) and by trabecular thinning ($p = 0.034$ and $p = 0.005$) in male RA, respectively. Moreover, cortical thinning ($p = 0.018$ and $p = 0.002$) but not cortical porosity ($p = 0.070$ and $p = 0.275$) was common in male and female RA patients at the DR. Cortical perimeter was increased in male and female RA patients at both sites. These results were supported by a multi-regression analysis, showing a strong association between trabecular and cortical bone loss and "the diagnosis of rheumatoid arthritis" as well as disease duration. Glucocorticoids (> 7.5 mg prednisolone daily) negatively influenced bone microstructure, whereas

low-dose glucocorticoids (< 5 mg prednisolone daily) did not influence bone quality or quantity. A strong influence of conventional and biological DMARDs on bone could not be demonstrated.

Conclusion Both trabecular and cortical bone are severely affected in RA. The increase in cortical perimeter in RA reflects a compensatory mechanism to counteract cortical thinning and to restore bone strength. Our data suggest that bone quantity and quality are significantly decreased in the appendicular skeleton of RA patients at both periarticular and non-periarticular sites.

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Datenmanagement und Plattformbildung für Osteoporose

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Einleitung Digitale Verwaltung und Management klinischer Daten wird zunehmend zur absoluten Anforderung an die moderne Medizin und medizinische Forschung. Sammlungen klinischer Fälle von Osteoporose stellen dabei aufgrund des heterogenen Krankheitsbildes eine besondere Herausforderung dar.

Materialien und Methoden Vorstellung einer Web-basierten, Osteoporose-spezifischen Datenbanklösung mit wissenschaftlichem Plattform-Charakter.

Resultat „Osteoporosis on SciCoMed“ stellt sich dieser Herausforderung und wurde von Experten für die speziellen Bedürfnisse eines Osteoporosezentrums entwickelt und umgesetzt. Bereits in voller Verwendung stehend, bietet es standortunabhängiges pseudoanonymisiertes Datenbankmanagement mit standardisierten Eingabemasken für Symptomatik, Diagnostik, Therapie usw. Die Verwaltung der Datensätze erfolgt passwortgeschützt durch den Datamanager und seine Sub-User und umfasst auch eine Exportfunktion in Excel-Format zur statistischen Auswertung. Ein Profilmodus bietet Gelegenheit zur Netzwerkbildung.

Conclusio Osteoporose-spezifisches internetbasiertes Datenmanagement mit einheitlicher Dokumentation schafft eine ideale Voraussetzung für Plattformbildung und effiziente wissenschaftliche Arbeit und Kooperation.

Micro-CT Analyses of Historical Osteomyelitic Bone Samples from the “Narrenturm” (NHM)

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Introduction Osteomyelitis is defined as an inflammation of the bone marrow mainly caused by bacteria such as staphylococcus aureus. It mainly affects long bones, eg, femora, tibiae, and humeri. Recent micro-computed tomography (µCT) techniques offer the opportunity to investigate the microarchitecture of bone in great detail. Since there is no information on long bone microarchitecture in osteomyelitis, the aim of our study was to study historical bone samples with osteomyelitis by µCT.

Materials and Methods We investigated 24 femora of 23 individuals with osteomyelitis derived from the Collection of Anatomical Pathology in the “Madhouse Tower”, NHM (mean age: 44 ± 19 years); 9 femora from the Department of Applied Anatomy of the Medical University of Vienna were studied as controls. Bone microstructure was assessed by µCT VISCOM X 8060 II with a minimal resolution of 18 µm.

Results In the osteomyelitic femora most prominent alterations were seen in the cortical compartment, in 73 % of the individuals with osteomyelitis cortical porosity occurred. In 64 % trabecularisation of cortical bone was observed. 59 % of the individuals showed cortical thinning. Remarkably, we also noticed microstructural changes in areas that did not show any pathology macroscopically.

Conclusion Osteomyelitis is associated with severe alterations of cortical bone structure typically observed in old age such as cortical porosity and cortical thinning.

Sturzrisiko und Reduktion

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Einleitung Zunehmendes Alter ist mit steigendem Sturzrisiko vergesellschaftet. In der Altersgruppe > 65 stürzt jeder zumindest einmal im Jahr, in der Altersgruppe > 80 sind es bereits 40 %.

Sturz ist nicht gleich Sturz.

Material und Methode Wir führen in unserer Reha-Klinik seit 2005 Aufzeichnungen über Stürze. So lagen die Sturzzahlen in den Jahren 2005–2009 in einer Bandbreite zwischen 257 und 303.

Die Sturzereignisse haben unterschiedliche Folgen, die in den Sturzereignisprotokollen dokumentiert sind.

Die unserer Auswertung zugrunde gelegte Einteilung basiert auf nachstehendem Schema:
- Grad 0: Keine sichtbaren Verletzungen
- Grad 1: Kleine Verletzung (Hämatom, Prellung etc,), die keiner ärztlichen Hilfe bedürfen
- Grad 2: Mäßige Verletzungen (Rissquetsch-, Platzwunde etc.), die ärztlicher Hilfe bedürfen
- Grad 3: Frakturen und Luxationen

In all den Jahren unserer Aufzeichnungen waren gut $^{2}/_{3}$ der Sturzfolgen dem Grad 0 zuzuordnen. Doch jeder Sturz ist ein Sturz zu viel.

In den Folgejahren kam es zu einem deutlichen Rückgang dieser Sturzereignisse: 2010 auf 286, 2011 auf 240 und 2012 auf 169. Der Grund dafür lag in einem völlig neu eingeführten und in den Aufnahmeprozess der Pflege eingepflegten Qualitätsstandard. Die Patienten werden zum Zeitpunkt der stationären Aufnahme nach der modifizierten Sturzrisikoskala nach S. Huhn gescreent. In diesem Tool werden Alter, mentaler Zustand, Ausscheidung, Zahl der vorangegangenen Stürze, Einnahme von Medikamenten, körperliche Aktivität sowie Gang und Gleichgewicht als auch das Sehvermögen mit unterschiedlicher Punkteanzahl bewertet. Aus diesem Punktwert ergibt sich entweder ein geringes, hohes oder sehr hohes Sturzrisiko. Entsprechend dieser Risikostratifizierung kommen unterschiedliche Maßnahmen zum Einsatz. Besteht ein geringes Risiko, wird mit dem Patienten ein Sturzpräventionsgespräch am Anreisetag geführt und der Patient wird zum „Sturzvortrag“ eingeplant (Therapieplan). Darüber hinaus erhält der Patient eine Infobroschüre, wie er am besten Stürze vermeiden kann, und eine DIN-A4-seitige Auflistung über die häufigsten Stolperfallen. Zeigt sich ein hohes Risiko, so kommen zu diesem Basispaket noch ein Aufklärungsgespräch, engmaschige nächtliche Kontrollgänge durch die Pflege, die Betttiefstellung und die Empfehlung des Tragens einer Hüftprotektorenhose hinzu. Darüber hinaus wird das im interdisziplinären Team besprochen und zusätzlich erfolgt eine Markierung mit einem blauen Punkt auf dem Therapieplan sowie auch auf der Patientenakte und dem Kadex. Wird ein sehr hohes Risiko festgestellt, so kommen weitere Maßnahmen wie Sitzhose und/oder Steckgitter hinzu.

Ergebnisse Durch die Einführung dieser Maßnahmen konnte die Zahl der Stürze in den Jahren 2010–2012 deutlich reduziert werden: 2010 auf 286, 2011 auf 240 und 2012 auf 169.

Den Patienten soll einerseits das Sturzrisiko vor allem im Alltag nähergebracht werden und andererseits sollen sie lernen, ihre körperliche Aktivitäten dem Alter anzupassen.

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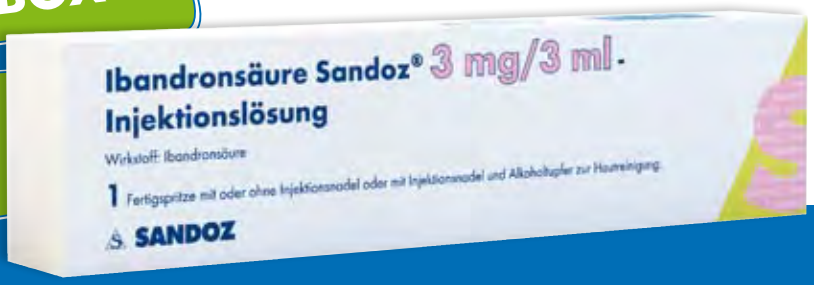

- **Back-Stop** System schützt beim Aspirieren
- **Nadelschutz** bewahrt vor Verletzungen
- **Versiegelte Verpackung**

SANDOZ
Eine gesunde Entscheidung

1 Eisman JA et al., J Rheumatol. 2008 Mar;35(3):488-497

Fachkurzinformation siehe Seite 14

OPO/SDZ/2013/4/3

Overlapping and Follow-Up of Alendronate to Teriparatide Results in Continuing Volumetric Bone Mass Increase Measured by Quantitative Computed Tomography

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Purpose After 9 months of teriparatide (TPTD) treatment the combination of alendronate (ALN) and TPTD for the consecutive 9 months results in enhanced bone mineral density (BMD) gain when compared with 18 months of TPTD monotherapy. Use of raloxifene (RAL) instead of ALN was less advantageous. During a subsequent 12-month maintenance therapy, hip BMD only increased in the ALN group. Our aim was to investigate changes of cortical and trabecular bone by quantitative computed tomography (QCT).

Methods 125 postmenopausal women (mean age 71.7 ± 8.5 ys, 91.5 % fracture prevalence, 95.7 % prior antiresorptive treatment > 2 ys) were randomized after 9 months of TPTD treatment into 3 open-label groups for another 9 months: ALN (70 mg/week, 41 pts) or RAL (60 mg/day, 37 pts) added to TPTD treatment or TPTD monotherapy/Ca+Vit D group (47 pts). After TPTD termination, patients were maintained on their respective treatment regime for another 12 months. All subjects received 1 g calcium/800 IU vitamin D daily. Trabecular bone in 2nd lumbar vertebra (L2), cortical and trabecular bone in total hip and femoral neck were measured at randomization, end of TPTD, and end of maintenance by QCT (Siemens Somatom Definition AS+).

Results L2 bone volume changed by (mean % ± SD) 12.1 ± 4.5, 14.6 ± 8.9, and 7.2 ± 7 during combination and 21.9 ± 8.3, 23.6 ± 10, and –3.9 ± 5.7 % ($p < 0.001$ for all) at the end of maintenance treatment for the ALN, RAL, and Ca+Vit D groups, respectively. Ca+Vit D group was inferior to the other groups at both time points ($p < 0.01$). The corresponding changes of trabecular bone in the total hip were 10.7 ± 4.4, 5.1 ± 2.3, and 4.8 ± 2.6 as well as 16.7 ± 4.4, 8 ± 3.2 ($p < 0.001$ for all), and –0.4 ± 4 % (n.s.), respectively. Changes in both time points were superior in the ALN group. The increase of the RAL was greater than of the Ca+Vit D group at the end of the maintenance ($p = 0.02$). In contrast, cortical bone in the total hip changed by 6.6 ± 2.2 ($p < 0.001$), –1.7 ± 5.8 ($p = 0.01$), and 2.8 ± 3.1 ($p = 0.002$) and 13.8 ± 2.9, –3.9 ± 6 ($p < 0.001$ both), and 0.7 ± 3.3 % (n.s.), respectively. In both intervals, cortical bone increased in the ALN group significantly more than in the other groups ($p < 0.001$). The decrease observed in the RAL group was significantly different from changes in the Ca+Vit D group.

Conclusion Continuation of ALN after its addition to the second 9 months of an 18-month TPTD treatment cycle resulted in a robust increase especially in cortical bone of the hip in severe postmenopausal osteoporosis.

Intravenous Ibandronate Increases Femoral and Vertebral Strength Measured by Finite Element Analysis in Male Patients with Idiopathic Osteoporosis and Fragility Fractures

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Purpose Male idiopathic osteoporosis (MIO) is a metabolic bone disease characterized by low BMD, micro structural alterations, and reduced mineralization of cortical structures resulting in increased fracture risk in otherwise healthy men. The evidence of antiresorptive treatment for MIO is lower than those for postmenopausal osteoporosis. Volumetric BMD measurements and strength predictions using voxel-based finite element (FE) simulations of the vertebral body and the proximal femur predict alterations in strength and stiffness. The hypothesis of this study was to test if ibandronate (IBN) improves biomechanical properties of femoral and vertebral bone in MIO.

Methods 13 treatment-naïve MIO patients from a prospectivejy paired biopsy study – mean age 53.0 (44.5; 57.0) years – with prevalent fragility fractures and low BMD received 3 mg intravenous IBN for 24 months quarterly. QCT (baseline/month 24) of Th12 and hip were assessed to compare BMC, vBMD, BV/TV, areal bone volume fraction (aBMD), stiffness, and ultimate force by using FE simulations for fall (femur) and compressive (vertebra) loading.

Results At the proximal femur the average strength increase (%) was 4.9 ± 8.9 (range –8.4 to 16.5, $p < 0.01$; 3 patients below 0). Pronounced increases were observed for trochanteric aBMD (2.9 ± 2.6) and BV/TV of FE models (3.7 ± 1.7, $p < 0.05$), with less effect in the neck region (0.7 ± 1.5). Most hip fracture types observed by FE-simulated side-fall scenarios were discovered in the trochanteric region. Regressions between strength increase and vBMD increases showed no correlation for BMC, BMD, BV/TV, or aBMD ($R^2 < 0.04$). In the vertebral body the mean strength increase was 7.3 ± 9.2 (range –2.7 to 31.9, 2 patients below 0) with most pronounced increase for BMC (9.4 ± 10.5 %); BV/TV distributions increased both at cortical shell and trabecular center ($p < 0.01$). A strong correlation was found for increase in strength and BV/TV, ($R^2 = 0.80$) and between BV/TV, BMD, and BMC ($R^2 > 0.65$). Predicted fracture locations based on damage patterns by FE simulation were similar between baseline and follow-up.

Conclusions This study clearly shows decisive positive effects of intravenous IBN treatment on biomechanical properties at the hip and spine in patients with MIO comparable postmenopausal osteoporosis. FE simulations are more sensitive and provide improved detailed insight than BMD measures alone, especially for side-fall simulations, since the fracture position and the local BMD increase can be considered.

Metabolic Changes After Bariatric Surgery – Baseline and Short-Term Follow-Up Results from the BABS Study

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Introduction An increasing number of obese patients are subject to laparoscopic gastric bypass or sleeve surgery, which leads to rapid weight loss. Less data is available on baseline and follow-up changes of body composition or metabolic bone alterations due to the modification in eating behaviour and intestinal resorption caused by surgery.

The aim of this prospective study is the investigation of baseline values of female and male patients prior to surgery for a pre-planned period of 2 years.

Material and Methods Prior to surgery all patients had a complete clinical work-up including medical history and verification of the eligibility criteria by the Austrian health authorities. Bone mineral density (BMD) and total body composition (% fat mass, lean mass) were measured by dual-energy X-ray absorptiometry (GE Lunar iDXA). Fasting serum levels of intact amino propeptide of type 1 procollagen (P1NP) and type 1 collagen cross-linked C-telopeptide (CTX), as well as intact parathyroid hormone (iPTH) and 25-OH vitamin D_3 were measured quarterly. Baseline and 6-month follow-up data are presented.

Results Between February 2012 and March 2013 a total of 144 (102 female and 42 male) patients were assigned to bariatric surgery. At baseline mean age was 43.3 ± 13.4 years; mean body weight was

127.6 ± 25.1 kg (BMI 44.8 ± 8.7) with a mean percentage of body fat of 50.4 ± 5.7 %, a mean lean mass of 58.6 ± 10.7 kg, and a total body BMD of 1.27 ± 0.13 g/cm^2. BMD at femoral neck (1.13 ± 0.18 g/cm^2) and lumbar spine (1.23 ± 0.15 g/cm^2) were within normal range. iPTH levels were close to the upper limit of normal (71.2 ± 35.4 ng/ml) whereas 25-OH vitamin D levels were in insufficient range (18.4 ± 8.9 ng/ml). P1NP and CTX values did not show any signs of accelerated bone metabolism.

Six months after surgery the mean percentage of body fat significantly decreased to 35.5 ± 11.4 % ($p < 0.01$, Δ 29.4 %), lean mass was reduced to 48.2 ± 5.4 kg ($p < 0.05$, Δ17.7 %), but total body BMD did not change (1.22 ± 0.13 g/cm^2, p = n.s.). At the femoral neck there was a significant decline in BMD (1.00 ± 0.14 g/cm^2, $p < 0.05$, Δ 11.5 %).

Conclusions We conclude that bariatric surgery leads to a rapid and early reduction of body fat and lean mass after 6 months of observation. Total body BMD does not seem to be susceptible to these early changes and vitamin D deficiency is ubiquitous. The decline in hip BMD as a weight-bearing bone site can partly be explained by the body mass changes, but must be subject of further and prolonged investigation.

Do Dietary Protein Amount, Source of Protein, High-Intensity Exercise, and Anabolic Androgenic Steroid Administration Affect Bone Microarchitecture in Rats?

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Background The amount and source of dietary proteins, high-intensity exercise (HIE), and anabolic androgenic steroid (AAS) administration may affect bone status.

Objective To analyze the effects of the dietary protein amount and source of protein, HIE, and AAS on bone trabecular microarchitecture in rats.

Material and Method Adult male Wistar rats were randomly distributed into 8 experimental groups corresponding to groups fed normal-protein (NP) or high-protein (HP), soy protein or whey protein, trained or sedentary, with or without AAS administration. Diets were based on commercial hydrolyzates of whey or soy protein. A 45-% protein content was selected for the HP diet groups, whereas a 10-% protein content was chosen for the NP diet groups. The exercised groups carried out a training protocol previously described by Aparicio et al. [1]. AAS administration consisted of weekly intramuscular injections of 10 mg/kg body weight of nandrolone decanoate. At the end of the experimental period (12 weeks) femurs were extracted. Bone microarchitecture parameters of the distal femora from a total number of 90 rats were analyzed by micro-computed tomography (μCT) using a μCT 35 device (Scanco Medical AG, Switzerland).

Results Trabecular thickness was higher in the HP groups when compared to the NP groups (0.088 ± 0.0006 vs 0.085 ± 0.0008 mm, $p \leq 0.001$). Connectivity density (ConnD), trabecular number (TbN), apparent bone mineral density (AppBMD), and tissue bone mineral density (tissBMD) were significantly higher in the HIE groups when compared to the control groups (552.4 ± 48.6 vs 426.2 ± 24.9 1/mm^3 for ConnD, $p \leq 0.05$; 11.4 ± 0.4 vs 10.2 ± 0.3 1/mm for TbN, $p \leq 0.05$; 387.4 ± 7.2 vs 369.2 ± 5.2 mg HA/ccm for AppBMD, $p \leq 0.05$, and 874.7 ± 3.8 vs 860.9 ± 3.6 mg HA/ccm for tissBMD, $p \leq 0.05$). No differences between groups were observed regarding the source of protein and AAS administration.

Conclusion In contrast to protein source and anabolic steroids, high-intensity exercise has a beneficial effect on femoral trabecular microarchitecture.

References:

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Signalling Pathways Regulated by the Calcium-Sensing Receptor in Colon Cancer Cells

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Background The G protein-coupled receptor, calcium-sensing receptor (CaSR), is one of the main molecules that sense changes of extracellular calcium levels. Not only calcium, but a plethora of other ligands such as amino acids, polyamines, antibiotics, and divalent cations can activate the CaSR regulating numerous downstream signalling pathways. As a result, various cellular functions such as cell proliferation and survival are affected. The signalling pathways regulated by the CaSR seem to be tissue- and ligand-dependent.

Aim This study examines the signalling pathways which are regulated by the activated CaSR in colon cancer cells. We have investigated the impact of CaSR activation on phosholipase C (PLC) and on the mitogen-activated protein kinase (MAPK) signalling cascade.

Materials and Methods Extracellular calcium and spermine were used as primary CaSR ligands for the study. Live cell intracellular calcium imaging with Fluo-4 AM fluorescent dye was performed to determine the activation of PLC. Phosphorylation of downstream kinases was studied by western blotting to investigate the CaSR influence on the MAPK pathway.

Results Intracellular fluorescence intensity was increased 2.5-fold in cells challenged with extracellular calcium and 4.5-fold when treated with spermine, reflecting the increase in intracellular calcium level upon treatment. Western blot analysis revealed that ERK and p38 phosphorylation was increased 3.3- and 2.4-fold upon calcium treatment whereas JNK phosphorylation was not affected. Interestingly, spermine had no significant effect on ERK activation but increased JNK (2.9-fold) and p38 (4.3-fold) phosphorylation significantly. The involvement of CaSR in these processes was shown in cells overexpressing the CaSR exogenously.

Conclusion Orthosteric CaSR ligands, calcium and spermine, increase intracellular calcium in colon cancer cells suggesting the activation of the Gαq/PLC pathway. The differences in the kinase phosphorylation pattern between calcium and spermine treatment shows that signalling through the CaSR is ligand-dependent, supporting the recognition that CaSR exhibits "ligand-biased signalling" a characteristic shared by several GPCRs.

Vitamin-D-Status nach Nierentransplantation: Zentrumserfahrungen mit 920 Patienten

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Background Zwischen 70 und 90 % der Patienten mit chronischer Niereninsuffizienz weisen eine Hypovitaminose D auf, d. h. 25-Hydroxyvitamin D ≤ 75 nmol/l. Bis dato gibt es nur wenige Studien, die den Vitamin-D-Status im Verlauf nach erfolgreicher Nierentransplantation untersuchen.

Methoden In einer Querschnittsanalyse wurde der aktuelle Vitamin-D-Status aller an der Klinischen Abteilung für Nephrologie und Dialyse, Medizinische Universität Wien, geführten nierentransplantierten Patienten evaluiert, bei denen im Jahr 2010 eine einzige Messung des 25-Hydroxyvitamin-D-Spiegels durchgeführt wurde (n = 920). In einer nach Jahreszeiten gematchten Subgruppe von 303 Patienten wurde der Verlauf von 25-Hydroxyvitamin D innerhalb

von 2–6 Jahren retrospektiv analysiert. Eine Therapie mit Vitamin D wurde in diesem Zeitraum nicht routinemäßig durchgeführt.

Ergebnisse Die Gesamtpopulation bestand aus 920 nierentransplantierten Patienten (575 Männer und 345 Frauen) mit einem medianen (Spanne) Alter von 52 (20–88) Jahren. Im Median lag die letzte Nierentransplantation 6,6 (0–37) Jahre zurück. Der mediane 25-Hydroxyvitamin-D-Spiegel im Jahr 2010 lag bei 34 (5–166) nmol/l, mit signifikant höheren Werten im Sommer verglichen mit allen anderen Jahreszeiten ($p < 0{,}001$). 74 % der Patienten wiesen eine Defizienz (25-Hydroxyvitamin D ≤ 50 nmol/l) und 19 % eine Insuffizienz (25-Hydroxyvitamin D 51–75 nmol/l) auf. Eine Subgruppenanalyse in einem nach Jahreszeiten gematchten Kollektiv zeigte, dass der 25-Hydroxyvitamin-D-Spiegel im Verlauf von 2–6 Jahren signifikant abnahm (48 [4–140] nmol/l vs. 35 [5–106] nmol/l, $p < 0{,}0001$). Im Verlauf ist der Anteil der Patienten mit Hypovitaminose D von 80 % auf 95 % angestiegen.

Schlussfolgerungen Aus den erhobenen Daten schließen wir, dass ein Vitamin-D-Mangel nach Nierentransplantation häufig auftritt und es im Verlauf nach Transplantation zu keiner spontanen Verbesserung kommt.

Computer-Assisted Morphological Sex Estimation on Crania

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Objective The aim was to develop a computer-assisted age-dependent sex estimation scheme of cranial bones for improving visual assessments.

Material and Methods A computer-assisted surface analysis scheme for assessing the bony relief was developed on 5 skulls (3 males, 2 females) basing on Koenderink's Shape Index. The feasibility of this technique was tested on 24 skulls of European individuals (12 males, mean age 45.58 ± 16.26 years, and 12 females, mean age 46.17 ± 18.31 years) from local historical collections. Scans were performed with a 64-row multidetector CT (pitch = 0,64) and modelled with AMIRA 5.4. Concavities and convexities were measured with the Shape Index on preselected regions of the frontal, zygomatic, petrous, occipital bones, and the mandible. Results were correlated to visual assessments with the well-established Knussmann's scheme performed by two raters. Age-dependent changes were analysed by using convexity-concavity plots.

Results Employing the Shape Index on refined post-processed CT data sets with additional regression analysis provides a new computer-assisted morphological sex estimation scheme. The specificity was 94.43 % and the sensitivity was 88.1 %, while the application of the Knussmann scheme had a specificity of 36.9 % and a sensitivity of 73.8 %.

Conclusion CAMSAS is a feasible computer-assisted analysis tool for improving the subjective age-dependent sex estimation of cranial bones.

Galectin-3 Is a Component of Circulating Microvesicles and Boosts Osteogenic Differentiation of Mesenchymal Stem Cells

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Introduction Aging is a complex process that results in the decline of physiologic functions due to accumulation of damage in cells and tissues as well as in reduced repair capacities. The regenerative power of stem and progenitor cells has been found to decline with age and to be influenced by the systemic environment. In particular osteogenic differentiation capacity of mesenchymal stem cells (MSCs) has been shown to decrease with age, thereby contributing to slowed down bone formation that might contribute to osteopenia or osteoporosis. Here, we set out to identify circulating factors of the aged systemic environment that influence the functionality of adult stem cells.

Results While searching for systemic factors which are deregulated in old age and influence osteogenic differentiation capacity of mesenchymal stem cells (MSCs), Galectin-3 was found. Overexpression of Galectin-3 was shown to boost osteogenic differentiation capacity of MSCs while reducing its expression by small interfering RNA inhibited osteogenesis. We could demonstrate that Galectin-3 is secreted within microvesicles (MVs) by endothelial cells *in vitro*. Furthermore a possible genetic transfer of endothelial microvesicular Galectin-3 to MSCs was confirmed, since coincubation of GFP-containing endothelial MVs and MSCs resulted in an uptake of MVs via endocytosis. The fact that endothelial cells line the blood vessels supports the notion that secretion of Galectin-3 within vesicles may also occur *in vivo*. This was proven by analysing human plasma samples of donors older than 55 and younger than 25 years. *Ex-vivo* derived circulating MVs isolated from human plasma of young individuals exhibited elevated Galectin-3 levels and boosted osteogenic differentiation capacity of MSCs compared to MVs of elderly donors.

Conclusion Summarizing, our data suggest that the composition of circulating microvesicles changes with age and that they deliver factors impacting on osteogenic differentiation. Among other factors microvesicular Galectin-3 was shown to enhance osteogenesis and to be enriched within microvesicles isolated from young human plasma. Furthermore endothelial cells were identified as a potential source for circulating microvesicles containing Galectin-3.

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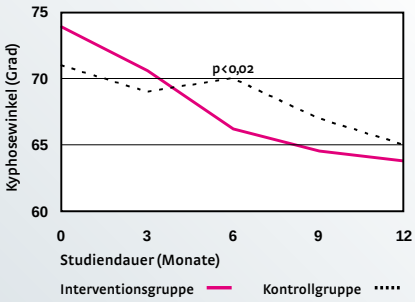

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* Spinomed®: Pfeifer M, Begerow B, Minne HW: „Die Wirkungen einer neu entwickelten Rückenorthese auf Körperhaltung, Rumpfmuskelkraft und Lebensqualität bei Frauen mit postmenopausaler Osteoporose." Am J Phys Med Rehabil 2004; 83: Seite 177-186.

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