Bone complaints and secondary hyperparathyroidism in an 11-year-old boy with a late diagnosis of celiac disease

G. Viterbo, C. Tau
Metabolismo Cálculo y Óseo, Endocrinología, Hospital de Pediatría J.P. Garrahan, Buenos Aires, Argentina

Here we report a clinical case of an 11-year-old boy who was referred for fractures. Two months previously, celiac disease had been diagnosed and a gluten-free diet (GFD) was started. His calcium intake from dairy products was very poor. Physical examination showed short stature: Z-Score: −3.9, weight: Z-Score: −3.2, Tanner stage 1, severe genu valgum, and bone deformations due to previous bone fractures. Laboratory showed serum calcium: 10.6 mg/dl, serum phosphate: 3.6 mg/dl, 25-hydroxyvitamin D: 22 ng/ml, high alkaline phosphatase: 1872 IU/l, and increased PTH: 260 pg/ml (normal value 12–72). X-rays showed osteopenia, rickets, and bone deformations in the legs and left forelimb, spine osteopenia, and reduced vertebral height. Lumbar bone densitometry was within normal values: bone mineral density (BMD): L2-L4: 0.77 g/cm2, Z-score: −1.3, and bone mineral content (BMC): 17.4 g. Rickets and secondary hyperparathyroidism were explained by intestinal malabsorption due to celiac disease. In addition to the GFD, he began plentiful milk intake, calcium supplement 1 g/day, and vitamin D 4800 IU/day. PTH normalized and alkaline phosphatase decreased to 772 IU/l after 6 months of treatment. Two years later, X-rays showed complete resolution of rickets and improvement of osteopenia and vertebral height. At age of 14 years and 11 months, after 3 years of therapy, he had normal height and weight (Z-Score: −1.7 and −1.6, respectively), and Tanner stage 4. Bone densitometry showed increases in BMD (38%, L2-L4: 1.062 g/cm2, Z-score: +0.9) and BMC (107%, 36.1 g). In summary: we report a clinical case of rickets, fractures, and secondary hyperparathyroidism due to celiac disease in an 11-year-old boy with a late diagnosis. He improved on the GFD with added calcium and vitamin D.

doi:10.1016/j.bone.2014.03.011

Changes in extracellular calcium and phosphorus concentration affect vascular homeostasis

M. Sandoval, B. Rauschemberger, A. Dorronsoro, P. Cutini, G. Santillán, V. Massheimer
Departamento de Biología, Biogéqueda y Farmacia, Universidad Nacional del Sur, INIBIUSOR, CONICET, Argentina

Changes in Ca or P serum promote vascular calcification (CaV) through independent mechanisms and/or by common pathways. We studied the effect of Ca and P on critical cellular processes of vascular homeostasis. The metabolic and phenotypic changes of endothelial cells (EC) and smooth muscle cells (VSMC) in an unfavorable environment represented by high P or Ca/P concentrations (10 mM glycerophosphate (GP); 4 mM Ca plus GP 5 mM) were assed. Osteoblastic VSMC transdifferentiation was evaluated using cell monolayers exposed GP 10 mM for 25 days. Alkaline phosphatase (ALP) activity was represented by high P or Ca/P concentrations (10 mM glycerophosphate (GP) 4 mM Ca). After 25 days, ALP activity levels and calcium content were signiﬁcantly higher compared to native VSMC (FAL: 280 ± 31 vs 34 ± 1.8 IU/mg and Ca: 45 ± 6.6 vs 28 ± 1.9 mg/mg protein). A synergistic calcifying effect was detected using high Ca and P containing medium (FAL: 438 ± 35 IU/mg Ca: 71 ± 8.8 mg/mg protein). Calcium deposits were conﬁrmed by alizarine red staining. Since endothelium nitric oxide (NO) production inhibits CaV, we studied the impact of a calcifying environment on NO production. High Ca concentrations (3; 5 mM) reduced NO bioavailability (48%; 44% reduction). Significant impairment in NO production was detected at all calcium concentrations tested (2, 3, 4 and 5 mM Ca). High calcium also affects the tissue’s ability to respond to its natural agonist acetylcholine (ACh). The stimulatory action of ACh on NO production at physiological conditions (1 mM) was suppressed when extracellular calcium increases (58% vs 2%). These results suggest that a procalcifying environment triggers detrimental events on the vascular system, prompting cardiovascular pathogenesis.

doi:10.1016/j.bone.2014.03.013

Glucocorticoid induced osteoporosis: Analysis of guidelines for prevention and treatment

M.L. Brance
Centro de Reumatología, Rosario, Argentina

Glucocorticoid-induced osteoporosis (GIO) is the second cause after postmenopausal osteoporosis, and the most frequent of secondary osteoporosis. Different scientific
societies (ACR, RCP, ASBMR, IOF-ECTS) established guidelines for the treatment of GIO considering different dose and time of glucocorticoid (GC) treatment and degrees of bone loss. The aim of this study was to evaluate the clinical characteristics of patients who were treated with GC and the percentage of the population that met requirements to consider treatment according to guidelines. An observational, retrospective, descriptive study of 60 patients with rheumatic diseases was performed. Results are shown as means ± SD. Age (years): 49.9 [15–87]; BMI (kg/m²): 26.2 ± 4.9; smoking: 27.3%; alcoholism: 3.4%; sedentary: 95%. The 89.65% were women and 79.31% of them were premenopausal. Among men, 43% were ≤ 50 years. Prednisone was the GC most used (93.1%). The daily dose of prednisone the first month was 166 ± 149 mg, at 3th month 143 ± 118 mg and at 1 year was 99.8 ± 88.2 mg. 62% of patients had rheumatoid arthritis, 17.2% SLE and the remainder had vasculitis and other connective tissue diseases. 10% of patients had vertebral fractures, of which 50% had more than one vertebral fracture. 50% of fractured patients had developed a new fracture in the first 12 months. Levels of vitamin D were 20.4 ± 5.9 ng/mL. The 54.5% had vitamin D levels < 20 ng/mL, 40.9% between 20-30 ng/mL and 4.5% > 30 ng/mL. About patients who had fracture, the annual dose of GC was 13.1 ± 11.0 mg/day (p < 0.07, Mann–Whitney U test). The percentage of patients who met criteria for considering treatment for osteoporosis according to different guidelines was: ACR 2003 = 80%, RCP = 70%, ACR 2010 = 48%, ASBMR 2011 = 58%, IOF-ECTS 2012 = 27%. 20% did not meet criteria neither ACR 2001 nor CPR. Although a high percentage of patients had GC ≥ 7.5 mg/day, 42% did not meet criteria for considering treatment according to current guidelines.

doi:10.1016/j.bone.2014.03.014

Coated stainless steel permanent implants with bioactive surface: Bone quality as success parameter
J. Ballarre, M. Desimone, M.R. Katunari, M. Baca, J.C. Orellano, S.M. Ceré
*Instituto de Ciencia y Tecnología de Materiales (INTEMA), Universidad Nacional del Mar del Plata-CONICET, Mar del Plata, Argentina
†Traumatología y Ortopedia, Hospital Interzonal General de Aguadó “Oscar Alende”, Mar del Plata, Argentina

Surface modification of surgical stainless steel implants by sol gel coatings has been proposed as a tool to generate a surface that besides being protective could also create a “bioactive” interface to generate a natural bonding between the metal surface and the existing bone. The aim of this work is to analyze the quality and bone formation around hybrid bioactive coatings containing glass–ceramic particles, made by sol–gel on 316L stainless steel used as permanent implant in terms of mineralization, calcium content and bone maturity with micro Raman, X-ray fluorescence and X-ray absorption techniques. Uncoated implants seem to generate a thin bone layer at the beginning of osteointegration process and then this layer being separated from the surface with time. The hybrid coatings without glass-ceramic particles generate new bone around implants, with high concentration of Ca and P at the implant/tissue interface. This fact seems to be related with the presence of silica nanoparticles in the layer. The addition of bioactive particles promotes and enhances the bone quality with a homogeneous Ca and P content and a low rate of beta carbonate substitution and crystallinity, related with a young and mechanical resistant bone.

doi:10.1016/j.bone.2014.03.015

Correlation between femoral robustness and pattern of bone mineral density loss in postmenopausal women
H. Claus-Hermberg, M.P. Lozano Bullrich, M. Rey, M.J. Pozzo
Servicio de Endocrinología Hospital Alemán, Buenos Aires, Argentina

We analyse if changes in bone mineral density (BMD), bone mineral content (BMC) and area (A) in postmenopausal women around 4–4.5 years with DXA could identify patterns of the behavior of these variables associated to morphological traits of the femoral neck (FN). In a retrospective cohort we evaluated 41 healthy women (52.7–79.1 years since spontaneous menopause). We analysed repeated BMD of the FN with DXA with an interval of 4–4.5 years. We normalized B by neck axis length as a measure of FN robustness. Results: Mean difference in FN BMD between the first study and the second showed a significant reduction (p < 0.001 T-test). This difference did not correlate with age and years since menopause. Mean difference of FN BMD correlate with robustness only in women ≥ 61 y (n: 26, R: −0.59, p = 0.002, Spearman’s Rank Test). Robustness mean was 4.58 mm and used as cutoff to categorize femora as robust (R) or slender (S). We observed: only in the group > 61 y a significant reduction in bone mass (BMD) in the FN: T-score (FS): −2.017 (−0.027 –0.1) vs. −2.010 (−0.028 –0.014) (p = 0.003) (median (quartiles), Mann–Whitney U test) in robust femora associated to a trend in higher increase in A (p = 0.06). Conclusions: 1) In the first years after menopause FN BMD decreases due to loss in BMC independently of robustness. 2) In women older than 61 years FN BMD decreases as a consequence of reduction in BMC and a trend in A increase, partially due to some FN morphological traits. 3) These changes in BMD with DXA in more robust femora are comparable to that seen with more sophisticated methodology, which correlate them to greater fracture risk.

doi:10.1016/j.bone.2014.03.016

dl-Buthionine-S,R-sulfoximine enhances anti-tumoral effects of calcitriol on neoplastic intestinal cells
A.C. Liaudat, L.P. Bolli, N.G. Toloza de Talamoni, G. Picotto
Lab “Dr. F Cañas”, Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-UNC), Argentina

Colon cancer is one of the most leading causes of death in the entire world. Prognosis and incidence of colorectal cancer are closely connected to 25(OH)D3 serum levels. Besides, oxidant drugs like dl-buthionine-S,R-sulfoximine (BSO) increase tumour cell sensibility, especially in resistant cancers. The aim of this study was to evaluate the mechanisms involved in the effects of calcitriol and BSO on colon cancer cell growth. Caco-2 human colon cancer cells were treated with calcitriol (D3), BSO, both or vehicle (ethanol) at different times. Cell proliferation was evaluated by crystal violet staining. Catalase (CAT), superoxide dismutase (SOD), alkaline phosphatase (FAL) activities and glutathione levels (GSH) were analysed by spectrophotometry. Mitochondrial membrane potential (ΔΨm) and cell cycle were measured by flow cytometry. Nuclear morphology was evaluated by DAPI staining and DNA fragmentation by TUNEL assay. Results were statistically analysed by one way ANOVA and Bonferroni as a post-hoc test. BSO and D inhibited Caco-2 growth and this effect was time and dose-dependent. Total GSH levels decreased at 6 and 48 h with either BSO or BSO + D and CAT activity was modified only at 96 h with combined treatment. BSO plus D produced cell cycle arrest in S/G2 phase at 48 h and reduced mitosis cell division at 96 h. BSO and BSO + D augmented DNA fragmentation. D and BSO + D modified ΔΨm; and increased FAL activity at 96 h. In conclusion, BSO increases the antiproliferative effect of D on Caco-2 cells via oxidative stress induction, cell cycle arrest and DNA fragmentation. FAL activity increment suggests cell differentiation induction.

doi:10.1016/j.bone.2014.03.017

Effect of sodium fluoride (F) on rat growth plate cartilage (GPC)
B.L. Fina, S.M. Roma, F. Bues, V.E. Di Loreto
Bone Biology Laboratory, School of Medicine, Rosario, Argentina

The use of F for therapeutic purposes has remained controversial for a long time and it is still under health problems. Changes in the exposed population at fluoride (F) level may be the result of F in toothpaste. The growth of long bones occurs associated to GPC and can be disturbed by chemical agents affecting new bone formation. The aim of this study was to evaluate the effect of different doses of F on the process of endochondral ossification in growing rats. Eighteen Sprague–Dawley rats of 21 days were divided into 3 groups (n = 6/group): NaF0; NaF20: 20 μg/kg body weight; and NaF40: 40 μg/kg body weight. In 30 days and control that received water. After treatment left tibiae were removed. Histopathological features of GPC and primary spongiosa were analyzed on sections of the metaphysis. The following parameters were measured: total thickness of the GPC (GPC.th), thickness of proliferative zone (PZ.th) and hypertrophic zone (Htz.th) which was expressed as% of GPC.th. The histopathologic examination revealed only hyperplasia of the cartilaginous proliferative zone. In the interzone with bone, some apoptotic bodies and hematic extravasation existed. In primary spongiosa some immature trabeculae, inflammation and sinusoidal dilatation were detected. There was an increase in ±SEM, *p < 0.05 vs control, Kruskal–Wallis, Dunn’s post test) without changes in GPC.th and a non significant decreased of Htz.th. The results suggest that chronically administered F as a single daily dose alters endochondral ossification increasing chondrocyte proliferation and delaying the maturity of new bone.

doi:10.1016/j.bone.2014.03.018

ERK signaling: A key event in the antiapoptotic actions of 17β-estradiol in skeletal muscle
A.C. Ronda, R. Boland
Departamento de Biología, Bioquímica & Farmacia, Universidad Nacional del Sur, San Juan 670, Bahía Blanca 8000, Argentina

We have previously shown that 17β-estradiol (E2) protects skeletal muscle cells from apoptosis induced by H2O2 through the mitochondrial pathway. Specifically, it was found that the hormone preserves the integrity and the potential of the mitochondrial membrane