duration. The use of zoledronate (Z) in children is a matter of concern because the studies are insufficient with respect to efficacy and safety. The aim of this study was to determine the significant influence of the different factors and their interactions on the action of Z on the structural characteristics and mechanical properties of rat growing bone. The factors studied and their levels were: dose of Z (D:0–2.5–12.5–25 μg Z/kg bw/week s.c.), duration of treatment (T:15–30 days) and sex (S:male–female). A $4 \times 2 \times 2$ factorial design was applied and 16 Sprague-Dawley rats of 21 days were assigned to a different level combination of each factor. At the end of the experiment both femurs were removed and length (L), cross sectional area (CS.A) and cortical area (Ct.A) were measured. The mechanical properties, maximum load (ML) and breaking energy (BE), were evaluated by 3-point bending test. The ANOVA t-test and p-values were used to evaluate the significance of factors and interactions, assuming $p < 0.05$. Results showed a significant interaction between D and T on L, Cs.A and Ct.A with lower values with D12.5 and D25 at T30 ($p < 0.05$). Concerning mechanical properties, the T was significant for ML (T30 > T15, $p < 0.05$) regardless of the dose of Z used. BE was significantly increased by T and D with the exception of D25 that negatively influenced BEf ($p < 0.05$). These preliminary findings show that the Z would not impact negatively on the mechanical and structural properties of growing bone at least at low doses. However, it is necessary to carry out further studies with the higher doses to better know the effects of Z when used during growth.

doi:10.1016/j.bone.2014.12.052

Effect of pre- and post-natal exposure to fluoride on fluorosis indicators in offspring of rats

V. Interlandi[a], P. Fontanetti[a], R.H. Ponce[a], G. Tirao[b], A. Rigalli[c], R. Gallará[a], V. Centeno[a]
[a]*Cátedra "A" de Química Biológica, Facultad de Odontología, UNC, Argentina*
[b]*LAMARX, Facultad de Matemática, Astronomía y Física, UNC, Argentina*
[c]*Laboratorio de Biología Ósea, Facultad de Medicina, UNR, Argentina*

Controversy exists over the maternal–fetal passage of fluoride (F-). This work sought to explore different aspects of F-metabolism during pregnancy and lactation in mothers and offspring. The specific aim was to study F-accumulation in bones and teeth and growth morphometric parameters in the offspring of mothers exposed to high levels of F- in drinking water. Pups aged 10-, 15- and 21-day-old from 2 groups of mothers were used: a) control mothers (0.3 mg/L F-); b) treated mothers (50 mg/L F-). The treatment was performed during the period of pregnancy and lactation. All animals were euthanized and the upper and lower jaw and tibia of mothers were removed for the determination of F-. Incisor diameter, mandibular growth and tibia length were determined on RX digitalized images. Results were analyzed by Student t test. Accumulated F-content was significantly higher in the jaws ($p < 0.05$), incisor ($p < 0.01$) and tibia ($p < 0.05$) of mothers exposed to F- compared to control. In 10- and 15-day-old pups born to treated mothers, F-content in jaws did not differ from the control. However, in 21-day-old pups, accumulated F- was higher ($p < 0.05$). No differences were found in F-content or growth parameters in tibia of the offspring in all studied groups. Lower incisor diameter was lower in 21-day-old pups born to mothers exposed 50 mg/L F-compared to their controls ($p < 0.01$). The results suggest that F-accumulation in calcified tissues of the offspring is lower than in maternal tissues. The passage of F- mother-offspring could occur through milk during lactation. Further studies would help to elucidate the mechanism of this process.

doi:10.1016/j.bone.2014.12.053

Bone mineral density evaluation in children with Gaucher disease

M.S. Larroude, H.R. Cassinelli, D. Drelichman, L. Richard, Grupo Argentino para el estudio de la enfermedad de Gaucher
Hospital de Niños R Gutierrez, Argentina

Bone involvement is described as a relevant sign in patients suffering Gaucher disease (GD). To analyze the long-term effect of enzyme replacement therapy on bone mineral density (BMD), a retrospective observational study was conducted in a cohort of 34 GD pediatric patients (14 males, 20 females, median age 11.3 years). Lumbar spine (LS) (L2–L4, $n = 34$) and total body (TB) ($n = 24$) BMD (determined by dual-energy X-ray absorptiometry (DXA, GE Lunar), were performed and expressed as Z-scores. According to the International Society of Clinical Densitometry guidelines, a Z-score <2.0 was considered pathological. Results were expressed in X ± SD. Patients received imiglucerase infusions (57.7 ± 17.4 IU/kg), during a period of 7.7 ± 4.5 years. They were divided in two groups: prepubertal (G1) and pubertal (G2), both for boys (G1B–G2B) and girls (G1G–G2G). Results: Mean BMD were normal in the whole group: −0.26 ± 1.4 and 0.11 ± 1.3 in LS and TB respectively. Only two patients had LS BMD below −2.0 z-score, but none in TB BMD. BMD was: G1B: LS −0.6 ± 1.28; G2B: −0.15 ± 1.4, TB −0.07 ± 1.4 and 0.58 ± 1.2; and G1G: LS: −0.23 ± 1.4, G2G −0.56 ± 1.9; TB G1G −0.08 ± 0.9, G2G 0.05 ± 1.9 respectively. Conclusions: Patients with GD have normal BMD during therapy with imiglucerase. It seems that the pubertal groups are better than the prepubertal, reflecting the action of sexual steroids on bone. A longitudinal study is needed to confirm this.

doi:10.1016/j.bone.2014.12.054

Preparation of an inexpensive milk with high calcium and low lactose content

B.L. Fina, L.R. Brun, A. Rigalli
Laboratorio de Biología Ósea, Facultad Cs. Médicas, Universidad Nacional de Rosario, Argentina

Dairy products are the main source of calcium (Ca). However the high cost and gastrointestinal disorders such as lactose intolerance contribute to the low-Ca intake. Commercial products such as yogurt are associated with less gastrointestinal disorders but they are expensive. Kefir is a group of lactose-fermenting microorganisms, easily obtained and maintained. Furthermore, the eggshell is a natural source of Ca, which is discarded. The aim of this work was to develop milk with low lactose content, enriched in Ca from eggshell and easily prepared at home. pH, lactose and Ca were measured in: 1 — milk (M), 2 — enriched-Ca milk with eggshell (MCa), 3 — milk with kefir (MK) and 4 — enriched-Ca milk with eggshell and kefir (MCaK). Samples of homemade MCaK were taken over a month to measure Ca and lactose. Finally, Ca was measured in 24 h urine in volunteers that consumed MCaK and compared with the basal calciuria of the same volunteers when consumed M. The analysis was performed using Two-way ANOVA (posttest LSD) and Student's t test. Significant difference was considered when $p < 0.05$ (*). After 730 min a decrease in pH was observed in MK and MCaK groups compared to M and MCa. The lactose content decreased significantly in the groups treated with kefir (MK: −12.7 ± 2.5%; MCaK: −15.9 ± 3.3%). The Ca content of M, MCa and MK did not change over time, while MCaK showed a significant increase of it (42.1 ± 16.2%). Ca content (mg/dl) in MCaK (120 ± 18.2*) prepared at home was significantly higher compared with M (98 ± 5.9) and the lactose content (mg/dl) was significantly lower (MCaK: 3.1 ± 0.4*, M: 4.2 ± 0.4). In volunteers, the basal urine Ca was significantly lower compared with MCaK ingestion. We conclude that kefir and eggshell can be easily used at home to obtain a low lactose and high content milk.

doi:10.1016/j.bone.2014.12.055

The antiapoptotic effect of 17ß-estradiol in skeletal muscle cells involves PKCδ, JNK and p66Shc

A.B. La Colla, L. Pronsato, L.M. Milanesi, R.L. Boland, A.A. Vasconsuelo
Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina

The hormone 17β-Estradiol (E2) acts on several non-reproductive tissues, including skeletal muscle. We have shown that E2 at physiological concentrations prevented apoptosis induced by hydrogen peroxide (H_2O_2) in skeletal myoblasts. The present work further characterizes the signaling mechanisms modulated by E2, responsible for apoptosis inhibition in skeletal muscle cells. We found that H_2O_2 induces activation of PKCδ; and JNK in C2C12 cells. By TUNEL assays using specific inhibitors, we demonstrated that the H_2O_2-induced activation of PKCδ and JNK are necessary to trigger apoptosis in skeletal muscle cells. Moreover, immunological assays support the data that PKCδ acts upstream JNK. We observed that E2 inhibits the activation of these kinases, resulting in the inhibition of phosphorylation and translocation to mitochondria of the adaptor protein p66Shc associated to oxidative stress. Additionally, we found that E2 diminishes the H_2O_2-induced p66Shc messenger RNA (mRNA) level. Tetramethylrhodamine methyl ester (TMRM) staining showed that pretreatment with E2 conduces to protection of the mitochondrial membrane potential ($\Delta\psi m$) in line with the inhibition of p66Shc translocation to mitochondria. In agreement, by qRT-PCR we demonstrated that E2 diminishes the H_2O_2-induced mRNA levels of the apoptotic proteins PERP and Puma associated to $\Delta\psi m$ loss, and increases those of the antiapoptotic protein Bcl-2. Our results provide basis for a putative mechanism by which E2 exerts beneficial effects on mitochondria, against oxidative stress, in skeletal muscle cells, helping to find new targets for the development of therapies for myophaties associated to deregulated apoptosis by hormonal deficits.

doi:10.1016/j.bone.2014.12.056