dependent. The gene expression of the Ca2+pump decreased after 30 and 60 min, whereas that of Na+/Ca2+exchanger and CB rema ined unchanged. CB protein expression decreased after 30 min. Besides, NDXC triggered oxidative stress, evidenced by the appeareance of OH. and a transient reduction in GSH. The activity of antioxidant enzymes increased. Mitochondria inner membrane permeability was altered depending on NDXC dose. Apoptosis increased after NDXC treatment. To conclude, NDXC inhibits intestinal calcium absorption by oxidative stress, which affect mitochondrial inner membrane integrity, Ca2+transcellular pathway and cell survival. Although the antioxidant system activity increases, it is insufficient to compensate the oxidative stress caused by NDXC.

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Vitamin D and mitochondrial malate dehydrogenase: Molecular mechanisms of modulation in the intestine

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Previous studies from our laboratory have shown that cholecalciferol or 1,25(OH)2D3 treatment increases the activity of Krebs cycle oxidoreductases such as oxoglutarate, isocitrate and malate dehydrogenases in D-deficient chicks. The aim of the present study was to elucidate the mechanisms through which cholecalciferol or 1,25(OH)2D3 regulates malate dehydrogenase (MDH-NAD) activity in D-deficient chicks. In order to evaluate non-genomic effects of 1,25(OH)2D3, intestinal epithelial cells were isolated from D-deficient chicks and treated "in vitro" with this hormone, whereas the genomic mechanisms were assessed by the injection of cycloheximide (CHX) to both untreated and treated with cholecalciferol or 1,25(OH)2D3 D-deficient chicks. MDH-NAD activity was determined by spectrophotometry in supernates of mithochondrial extracts, and the MDH-NAD mRNA expression analyzed by RT-PCR. MDH-NAD activity remained unchanged after 15 minutes of incubation with 1,25(OH)2D3 (-D: 0,132 µm ol NAD/min. mg protein;+1,25D3: 0,124 µmol NAD/min. mg protein); however, the activity tended to increase after 90 minutes of incubation. CHX reduced MDH-NAD activity both in untreated and treated with cholecalciferol D-deficient chicks (-D: 100,4%,-D+CHX: 62%,+D:143%,+D+CHX: 78%;+D: $p<0,01$ vs –D,-D+CHX, +D+CHX; one way ANOVA and Duncan "post hoc" test). 1,25(OH)2D3 did not modify MDH-NAD gene expression. According to these results, the increase in MDH-NAD activity in intestinal mitochondria induced by cholecalciferol or 1,25(OH)2D3 treatment in D-deficient chicks would not be the consequence of a rapid non genomic effect, but possibly the result of a genomic mechanism of regulation at the translation or post-translation level.

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Protein tyrosine phosphatases are involved in Bisphosphonate binding and signalling in bone cells

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It has been previously reported that bisphosphonates (BPs) inhibit osteocyte and osteoblast apoptosis via opening of connexin (Cx) 43 hemichannels and activation of the extracellular signal regulated kinases ERKs, although the events elicited by BPs upstream of hemichannel opening remain unknown. We hypothesized that BPs bind to Cx43 leading to ERK activation. However, using whole cell binding assays with 3H-alendronate, we demonstrated the presence of saturable, specific and high affinity binding sites in the Cx43-expressing ROS 17/2.8 osteoblastic cells and rat calvaria-derived osteoblasts, as well as in HeLa cells lacking Cx43 expression. Remarkably, substrates of protein tyrosine phosphatases (PTPs) displaced bound 3H-alendronate. Moreover, both BPs and the PTP inhibitor Na3VO4 increased proliferation of cells expressing Cx43 or not. Furthermore, like Na3VO4, alendronate inhibited PTP activity in ROS 17/2.8 cells. These results suggest that BPs bind and inhibit PTPs. Cons istent with this, PTPmu, PTPalpha and PTP1B are highly expressed in ROS 17/2.8, MLO-Y4 osteocytic cells and HeLa cells, as evidenced by Western blot analysis and real time RT-PCR. Moreover, co-immunoprecipitation assays showed that Cx43 physically interacts with PTPmu and PTPalpha, as well as with the non-receptor tyrosine kinase c-Src, in ROS 17/2.8 and MLO-Y4 cells. These results support the hypothesis that BPs bind to PTPs, which are associated with Cx43, leading in turn to activation of intracellular signalling in osteoblasts and osteocytes.

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Magnetic Resonance studies and clinic evidence of the effect of Human Recombinant Erythropoietin (RHEPO) antigen induced arthiritis treatment

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Rheumatoid arthritis (RA) is an autoimmune pathology where a high level of pro-inflammatory cytokines, like IL-1 and TNF, are produced at the sinovia, changing levels of nitric oxide (ON). Preliminary results showed that rhEPO treatment to rabbits with antigen induced arthritis (AIA) affected the progression of the pathology. Our aim was to evaluate the potential effect of these treatments. We studied the knees of 3 month-old female New Zealand rabbits: control (C) and AIA, both treated with rhEPO or not (p: placebo doses) during 30 days (n=4 per group; 1000 U rhEPO *kg-1 * 24 h-1). ON in serum was analyzed with the Griess method and by magnetic resonance (RM) to anaesthetized rabbits with small bones software:, with the following sequence: Inversion Recovery TR 2000, TE 60, NEX 3, 6 min acquisition time. No differences were detected among C-EPO and Cp treatments. Arthritic groups had higher transverse diameters: AIAp> AIA-EPO> C ($p<0.01$). AIAp had level III to V bone h yperintensities with slight alterations of the soft parts (+to+). AIA-EPO showed level II to III bone hyperintensities, with very slight alterations of the soft parts (+). NO differences were detected: (AIAp>AIA-EPO>C ($p<0.01$). The synovium of the AIAp animals were characterised by higher hyperplasia of the synovial lining cells and infiltration by lymphocyte, and plasmocytes than AIA-EPo($p<0.01$). Our results stress the need to carry out further studies on the action mechanisms of this pleiotropic hormone and of emergent therapies which promote the re-establishment of internal balance of affected individuals and maybe avoiding the use of chronic treatments with undesired side effects, such as corticoids

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Immunohistochemical expression of RANKL in osteoclasts

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RANKL is a member of the TNF superfamily. One of its functions is to stimulate osteoclast (Oc) recruitment and differentiation and inhibit Oc apoptosis. RANKL expression has been described in osteoblasts and bone marrow, and there is one report on RANKL coding RNAm in Ocs.

Bisphosphonates inhibit Oc recruitment and adhesion, and stimulate Oc apoptosis. However, previous works performed at our laboratory showed a significant increase in Ocs induced by olpadronate (OPD, Gador SA); although the rate of apoptosis was higher in treated animals, it was not sufficient to counteract the increased Oc recruitment and lifespan.

The aim of the present work was to evaluate RANKL expression in Ocs and Ocs presenting apoptotic morphology in OPD treated and sham animals. Female Wistar rats, aged 60±10 days were divided into two groups and received i.p.: