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tomography revealed that administration of genistein increased trabecular bone numbers and improved the bone thickness and volume. This study showed that genistein can improve bone healing via triggering ERα-mediated osteogenesis-associated gene expressions and subsequent osteoblast maturation.

***Disclosures: Jui-Tai Chen**, None*

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Progesterone (Pg) and Medroxyprogesterone acetate (MPA) regulate same events with opposite actions in bone and vascular cells *Pablo Hernan Cutini[1], Adrián Esteban Campelo[2], Virginia Massheimer[1]. [1]Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), Universidad Nacional del Sur (UNS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Buenos Aires, Argentina., Argentina, [2]Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), Dpto. de Matemática, Universidad Nacional del Sur (UNS), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Buenos Aires, Argentina., Argentina

Bone and cardiovascular diseases are multifactorial clinical entities that often coexist in postmenopausal women. Clinical and epidemiological studies have shown an interesting relationship between high bone turnover and cardiovascular disease mortality. Disorders in bone metabolism reversely correlate with vascular calcification (VCa). Hormone replacement therapy including natural Pg or synthetic progestins such as MPA emerged as a therapeutic option, although the risk/benefit of its use is controversial. VCa developed within atherosclerotic plaque is partly due to the osteogenic transdifferentiation of vascular smooth muscle cells (VSMC). The aim of this work was to investigate the effect of Pg and MPA on cellular/molecular events involved in VCa and in osteoblastogenesis. Primary cultures of calvarial osteoblasts (OB) and aortic VSMC, were in vitro exposed to 10 nM Pg or 10 nM MPA. Measurements of matrix extracellular calcium content and alkaline phosphatase (ALP) activity were employed as osteoblastic differentiation markers.In order to promote osteoblastic differentiation, VSMC were cultured for 21 days in osteogenic medium (10 mM β-glycerophosphate and 4 mM CaCl2). When VSMC were cultured in osteogenic medium (VSMC-OB), treatment with Pg for 21 days significantly reduced ALP activity (15% below control, $p<0.02$) and calcium content (32% below control, $p<0.02$). Similar treatment with the synthetic progestin also showed a significant reduction in ALP activity (239.9 +/- 21.0 vs. 159.6 +/- 19.1 x103 IU/mg protein, control vs MPA, $p<0.02$), as well as in the extracellular calcium deposition (365.1 +/- 38.2 vs 253.6 +/- 21.9 µg/mg protein, control vs MPA, $p<0.02$). Conversely, exposure of OB cells to Pg or MPA significantly increased ALP activity (59%; 290% above control, Pg; MPA, $p<0.02$) and matrix calcium levels (12%; 55% above control, Pg; MPA, $p<0.02$). The mechanism of action of Pg and MPA on both cells involves the participation of Pg receptor (PgR), since pre-treatment of cells with RU486, a PgR antagonist, completely reversed the hormonal action. However, we ruled out the involvement of the androgen receptor (AR), since in the presence of the AR antagonist flutamide, the effect of the progestogens was sustained. In conclusion, although Pg and MPA exert opposite effects on OB and VSMC-OB, both steroids would exhibit a potential beneficial effect by promoting osteoblastic differentiation and inhibiting VCa.

***Disclosures: Pablo Hernan Cutini**, None*

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Mass spectrometry profiling of estrogens in bones derived from a rat model to identify potential targets for the prevention of postmenopausal osteoporosis *Tine Albrecht[1], Daniela Pemp[1], Sebastian Müller[2], Lukas Häfner[1], Günter Vollmer[2], Leane Lehmann[1]. [1]University of Würzburg, Germany, [2]University of Dresden, Germany

The impact of exogenous factors, as xenobiotics, on bone density and the risk of postmenopausal osteoporosis, is often tested using an animal model based on ovariectomy with or without additional oral exposure to the estrogen 17beta-estradiol (E2) to simulate the postmenopausal situation. Although it is well established that estrogens significantly contribute to the maintainance of the bone homeostasis, the estrogen profile in bone has not been determined in this model before.Thus, female mature Wistar-Hannover rats were either sham operated (sham group, n=18) or subjected to ovariectomy and were either fed a diet containing E2 benzoate (0.825 ppm to mimick menopausal estradiol levels; ovx+E2B group, n=15) or unsupplemented diet (ovx-E2B group, n=15) for 8 weeks. Then, the estrogen profile was analyzed in femur specimens by GC (estrone (E1), E2, 2- and 4-methoxy (MeO)-E1 and -E2) and UHPLC (E1-sulfate, E1-glucouronide), coupled with mass spectrometry and using deuterated internal reference standards for identification and quantification.Only E2, E1 and 2-MeO-E1 were detected and also quantified (E2 <16-124 fmol/g; E1 <16-104 fmol/g; 2-MeO-E1 <19-118 fmol/g). As expected, frequencies of detection of both E2 and E1 were significantly higher in bones derived from the sham group than from the ovx-E2B group ($p<0.05$, Fischer´s exact test). Frequency of detection of E2 did not differ between bones derived from ovariectomized animals with or without oral exposure to E2B. In contrast, frequencies of detection of E1 did not differ between bones derived from the sham and the ovx+E2B groups. Despite comparable levels of E1 were observed after in sham group and ovx+E2B group, 2-MeO-E1 was detected exclusively in specimens derived from the ovx+E2B group (27%). Since direct ingestion of 2-MeO-E1 was excluded (<0.05% of E2 benzoate), differences in biotransformation between endogenous and exogenously administered E2 seem to be reflected in the bone.In conclusion, the oxidative metabolite 2-MeO-E1 seems only be present above limit of detection in the bone under the conditions of the animal model. Ovariectomy decreases levels of E2 within the bone which cannot be recovered by oral exposure to 0.825 ppm E2 benzoate which instead seems to restore E1 levels. The characterization of the estrogen profile in the ovariectomized rat model, allows to understand and target the estrogen profile as a potential key factor related to changes in bone density.

***Disclosures: Tine Albrecht**, None*

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In vivo effects of 1α25(OH)2D3-glycosides from Solanum glaucophyllum leaves in mice and skeletal muscle cells *Paula Irazoqui[1], Veronica Gonzalez Pardo[1], Claudia Buitrago[1], Kathrin Bühler[2], Ana Russo de Boland[1]. [1]Universidad Nacional del Sur-INBIOSUR, Argentina, [2]Herbonis Animal Health GmbH, Switzerland

We have previously shown that Solanum glaucophyllum leaves extract (SGE) enriched with 1a,25(OH)2D3-glucosides exhibits at least equal or greater effects on early myoblast differentiation as synthetic 1a,25(OH)2D3 thus being an effective substitute to promote muscle growth. Recently, we reported the effects of SGE compared to synthetic 1a,25(OH)2D3 in the regulation of mitogen activated protein kinases (MAPKs), genes involved on the differentiation of murine skeletal muscle cells C2C12, and their role on myotube formation. Since MAP kinase phosphatases (MKPs) deficiency impairs skeletal muscle regeneration and exacerbates muscular dystrophy, we further studied the effects of SGE on MKP1 and MKP5 which function to specifically dephosphorylate and inactivate MAPKs. SGE did not statistically modified MKP-1 and MKP-5 protein levels although basal changes were observed. MKP-1 protein levels remained unchanged at 1, 24 and 48 hours and decreased at 6 and 72 hours. MKP-5 protein levels increased at differentiation onset (24-72 h). We found that SGE significantly induced biphasic changes in phosphatase activity, decreased at 1 and 24 hours (which correlates with the pro-differentiation cell arrest previously reported), and increased at 6 hours. We also investigated the effects of SGE on Akt-mTOR signaling during the differentiation phase. SGE, decreased Akt phosphorylation after 5-day and increased its phosphorylation after 7-day of treatment. However, SGE did not induced changes in mTOR phosphorylation during time (3-7-day). Following with this approach, we investigated in vivo effects of SGE on skeletal muscle function in mice. Results from the first in vivo pilot test, have evidenced that 2 mg SGE/kg BW for 30 days did not show statically significant differences in phosphorus, calcium and glucose parameters from SGE groups compared to control. However, SGE leads to an increase in soleus muscle VDR protein expression in line with a significant decrease in 25(OH)D3 in blood serum while lower SGE doses did not change biochemical parameters measured in serum or protein content in mice muscle. These results contribute to elucidate the mechanism of action of the herbal active form of vitamin D when used as substitute to promote muscle growth

***Disclosures: Paula Irazoqui**, None*

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Osteocyte Ca2+ Responses to in vivo Mechanical Loading in Mice Expressing Genetically Encoded Calcium Indicators with Different Sensitivities *James Boorman-Padgett[1], David Spray[2], Mia Thi[2], Jelena Basta-Pljakic[1], Karl Lewis[3], Robert Majeska[1], Mitchell Schaffler[1]. [1]City College of New York, United States, [2]Albert Einstein College of Medicine, United States, [3]Cornell University, United States

PURPOSE: Lewis+ in our laboratory recently reported the first ever studies using a genetically encoded calcium indicator (GECI) targeted to osteocytes (Ot) to measure Ca2+ responses to mechanical loading in vivo1. We discovered that increasing strain levels recruited more Ca2+ responding Ot following a well-defined response curve. These studies used an early GECI, GCaMP31. New GECIs, like GCaMP6f, have increased dynamic range and sensitivity compared to GCaMP32-4 but we do not know if Ot Ca2+responses in vivo are modified by the GECI used. Here, we examined how Ot Ca2+ responses to loading in vivo differ with GCaMP3 vs GCaMP6f. METHODS: Ot-targeted GCaMP3 or GCaMP6f mice were obtained from crossing DMP1-Cre with Ai38 or Ai95 mice, yielding OtGP3 and OtGP6 mice respectively [16-18 week old females, IACUC approved]. Under anesthesia, 3rd metatarsals (MT3) were cyclically loaded to strains between 250 and 3000 µstrain1. Multiphoton microscopy was used to visualize Ot fluorescence in the mid-diaphyseal cortex during loading. For each Ot, fluorescence intensity was measured (ImageJ) at baseline and during loading; cells >25% intensity increases were counted. Linear regression was used to model strain vs Ot responses.RESULTS: Within the physiological strain range (250-2000 µstrain), Ca2+ signaling Ot in both OtGP6 and OtGP3 mice rose monotonically ($r^2>0.9$ for both) (Fig). Signal intensity in OtGP6 was higher than OtGP3 cells, with ~twice as many responding Ot/strain level. Critically, loading-response curve slopes were equivalent (ANCOVA, $p>0.4$).DISCUSSION: OtGP6 mice show more Ot responding to a given mechanical strain level than OtGP3 mice consistent with higher fluorescence intensity reported in GCaMP6f. However, the Ot-loading-Ca2+ response curve slopes were equivalent, revealing that the recruitment relationship between Ca2+ responding Ot number vs strain magnitude is the same in both reporter systems, and thus represents a fundamental characteristic of the system. Finally, we note that linear extrapolation of the OtGP6 response curve