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**054** (603) **REMOTE ISCHEMIC PRECONDITIONING ACTIVATES ADENOSINE A1 RECEPTOR AND ATTENUATES MITOCHONDRIAL DAMAGE DURING MYOCARDIAL REPERFUSION**

Diamela Paez[1], Martin Donato[1, 2], Mariana Garces[2], Timoteo Marchini[2], María Ailín Goyeneche[1], Pablo Evelson[2], Ricardo J Gelpi[1,]

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Introduction: Remote ischemic preconditioning (rIPC) reduces infarct size through the activation of pre-ischemic muscarinic pathway. However, the mechanism activated during reperfusión remains unclear.

Objective The first aim was to evaluate whether $A_1$ adenosine receptor is part of the rIPC cardioprotective mechanism during early reperfusion. A second objective was to evaluate the effect of rIPC on ischemia/reperfusion mitochondrial damage.

Methods: Isolated rat hearts were subjected to 30 minutes of global ischemia and 60 minutes of reperfusión (I/R, n=9). In a second (n=10) group, before the isolation of the heart, a rIPC protocol (three cycles of left femoral artery ischemia/reperfusion) was performed, followed by I/R protocol. In a third group (n=5), the above protocol was repeated but, during the first 5 min of reperfusion, an adenosine A1 receptor blocker was administered (DPCPX). We evaluated infarct size using triphenyl tetrazolium chloride staining and oxidative damage to macromolecules by TBARS and carbonylated proteins. Additionally, we measured mitochondrial respiration and $H_20_2$ production rate in freshly isolated mitochondria.

Results: rIPC significantly decreased infarct size (50,33±2,74 vs 31,29±2,52%, p< 0,05 Vs I/R) and this effect was abolished by DPCPX administration (55,74±6,41%). rIPC significantly decreased carbonylated proteins without any modification in TBARS content. Finally, 30 min of global ischemia followed by 60 min of reperfusion induced an impairment of mitochondrial respiration and decreased $H_2O_2$ production rate, which was attenuated in the rIPC group.

Conclusions: rIPC reduces infarct size by activation of adenosine $A_1$ receptors at reperfusion, and attenuates oxidative stress, preserving mitochondrial function.

**055** (680) **THYROID DESEASE: CARDIOVASCULAR NITRIC OXIDE TO HYPOVOLEMIA**

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We previously demonstrated that activation of nitric oxide (NO) pathway is involved in the restoration of vascular volume and blood pressure following bleeding. Cardiovascular function is influenced by the autonomic nervous system and numerous endocrine hormones in which thyroid hormones have relevance. Additionally, a functional relation involving thyroid hormones, endothelial cells and NO has been extensively described in the past several years. This study aimed to investigate whether NO participates in the cardiovascular function and haemodynamic adaptation to acute haemorrhage in animals with thyroid disorders. Sprague-Dawley rats aged 2 months old treated with T3 (hyper, 20 µg/100 g body weight) or 0.02% methimazole (hypo, w/v) during 28 days were pre-treated with NG nitro-l-arginine methyl ester (L-NAME) and submitted to 20% blood loss. Heart function was evaluated by echocardiography. Measurements of arterial blood pressure, heart rate, nitric oxide synthase activity and protein levels were performed. Hypothyroid animals had decreased fractional shortening and ejection fraction and increased left ventricle internal diameter. However, hyperthyroid rats had decreased ventricle diameter and no changes in cardiac contractility. Haemorrhage elicited a hypotension of similar magnitude within 10 min. Then, this parameter was stabilized at about 30–40 min and maintained until finalized, 120 min. L-NAME rats showed that the immediate hypotension would be independent of nitric oxide. Nitric oxide synthase inhibition blunted the changes of heart rate induced by blood loss. Animals with thyroid disorder had lower atrial enzyme activity associated with a decreased enzyme isoform in hypothyroid group. In ventricle, thyroid abnormalities were associated with higher enzyme activity, which was not correlated with changes in protein levels. Haemorrhage induced an increased heart nitric oxide production. We concluded that thyroid disorders were associated with hypertrophic remodelling which impacted differently on cardiac function and its adaptation to a hypovolemia. Hypovolemia triggered a nitric oxide synthase activation modulating the heart function to maintain haemodynamic homeostasis. This involvement depends on a specific enzyme isoform, cardiac chamber and thyroid state.

**056** (686) **ANALYSIS OF ANTICOAGULANT AND ANTIPLATELET CAPACITY OF NITINOL-POLYPYRROLE DEVICES DOPED WITH DRUGS**

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Hemostasis plays an active role in atherosclerotic disease and arterial stenosis. Nitinol (NiTi) is used in several biomedical applications such as stents. Despite its biocompatibility, elasticity and corrosion resistance, some $Ni^{2+}$ and $Ti^{2+}$ ions may be released, resulting in local and systemic unwanted effects. The use of polypyrrole (PPy) coating, protects from ion release and, allows doping with drugs designed such as heparin (Hep) and sodium salicylate (NaSa). This work evaluates the effect on blood coagulation and platelet aggregation (PA) of NiTi-PPy devices (slices of $1cm^2$) doped with Hep (0.2; 0.3; 0.4 g/L), NaSa (0.1 and 0.5 M) or the combination of NaSa plus Hep (0.5M + 0.2 g/L). The slices were incubated 35 min with human plasma, and immediately after aliquots were taken for coagulation tests. Platelet poor plasma (PPP) was used for thrombin time (TT) and fibrinogen (F) measurements, and platelet rich plasma (PRP) for PA assays. TT was prolonged by Hep device. The anticoagulant effect was proportional to Hep concentration (25 - >120 sec; 0.2 to 0.4 g/L). F content was unchanged compared to PPP (316±16.5 vs 322±35.7 mg/dL). The 0.1 M NaSa slice inhibited PA (42% IPA). TT time was enlarged (34.8±9.9 sec.), F content decreased (15% vs PPP) and IAP was higher (90% IAP, p<0.05) using 0.5 M NaSa. Both drugs doping showed an anticoagulant additive effect (TT: 60±20.5 sec.) with significant F diminution (49% vs PPP). Scanning electron microscopy showed that slices that contained 0.5 M NaSa or NaSa plus Hep, exhibited a complex microtubular structure. The anticoagulant effect of NaSa slices could be due to the hiding of F in these three-dimensional structures. Platelet deposition was rule out since platelet counting in the remaining PRP was not changed respect to basal count. The results suggest that, these devices are able to produce an *in situ* beneficial regulation of hemostasis, and could represent a potential design to prevent restenosis of blood vessels.

**057** (778) **THE EXPOSURE TO AIR POLLUTION PARTICULATE MATTER AGGRAVATES EXPERIMENTAL MYOCARDIAL INFARCTION IN MICE BY POTENTIATING CYTOKINE SECRETION FROM ALVEOLAR MACROPHAGES**

Timoteo Marchini[1,2], Dennis Wolf[2], Nathaly Anto Michel[2], Maximilian Mauler[2], Natalia Magnani[1], Deborah Tasat[3], Silvia Alvarez[1], Ingo Hilgendorf[2], Andreas Zirlik[2], Pablo Evelson[1].

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Excess body Fe has been related with alterations in glucose homeostasis.To study relationship between Fe status and increased risk of prediabetes, 135 male blood donors, attending, Hospital de Clínicas José de San Martín, Universidad de Buenos Aires (2012-2014) were enrolled. Total Fe intake (FeI), including Fe from mandatory wheat flour fortification (FeIf), was estimated (ARGENFOODS and USDA National Nutrient Database on Standard Reference). Serum ferritin (SF) (IMMULITE Ferritin, DPC); transferrin saturation (TS) (%) (serum Fe/total iron binding capacity x 100) (IRON2 and Tina-quant Transferrin, Cobas) and HbA1c (COBAS, Roche) were determined in blood samples negative for infectious diseases and C-reactive protein (PCR-latex, Wiener lab). The characteristics of the studied population (mean±SD) were: age (y): 34.9±10.4 (older than 40y: 30%); body mass index (BMI): 27.1±3.9 Kg/m$^2$, overweight (OW) 44% and obesity (Ob) 20% ; FeI and FeIf (mg/d) (range): 21.9 ± 9.2 (7.6-58) and 10.4±6.8 (1.5-45) respectively; SF (ng/mL): 230±205 (4.9-1403); TS(%): 29.7±10.6 (6-77.7) and HbA1c (%): 5.3±0.5 (4.3-7.7) (ref. range: 4.5 to 5.9%). To determine interaction between HbA1c and FeI, SF and/or TS, data were divided into FeI quartiles (Q) (mg Fe/d) (mean±DS): 12.5±1.8; 17.6±1.7; 23.4±1.4 and 34.4±8.2, respectively (RDA: 8 mg Fe/d, FNB, 2001). Between FeI Q, HbAc1(%) values were not different (mean± SD):5.4±0.8, 5.2±0.3, 5.2±0.2, 5.3±0.3 (p=0.8878); and no subject showed criteria of Fe overload (TS and SF higher than 50% and 300 ng/mL, respectively). However, 7 subjects from different FeIQ, showed HbA1c values compatible with increased risk for prediabetes (5.7%-6.4%). As FeI was higher than Fe RDA in 157, 220, 292 and 430%, Q1 to Q4, and the FeIf accounts for 50% of FeI, increased risk of chronic diseases with age may be possible in people healthy and unaware of any family history of Fe overload. *Universidad de Buenos Aires, Programación Científica 2016, UBACyT 20720150100004BA.*

**085** (248) **TIME AND DOSE PROFILE OF CLINICAL SYMPTOMS, MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE DAMAGE BY COPPER TOXICITY IN RAT LIVER AND BRAIN**

Juan Manuel Acosta[1], Rosario Natalia Musacco Sebio[1], Christian Martín Saporito Magriñá[1], María Victoria Tuttolomondo[2], Martín Desimone[2,3], Alberto Boveris[1,3], Marisa Gabriela Repetto[1,3],

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The epidemic increase in obesity causing insulin resistance and type 2 diabetes has become a worldwide problem. To better understand the mechanisms that lead to metabolic disorders, it is crucial to develop a better underlying knowledge of the molecular events that regulate adipocyte differentiation. Many positive modulators of this process have been identified, such as the CCAAT/enhancer binding protein (C/EBPβ) and peptide hormones like insulin.

The insulin receptor (IR) is transmembrane tyrosine kinase receptor, encoded by a single gene composed of 22 exons. Due to alternative splicing of exon 11, the gene gives rise to two protein isoforms that differ by a 12-amino-acid insertion: the IR lacking exon 11 (IR-A) and the IR containing exon 11 (IR-B). We hypothesize that IR plays an important role in regulating adipocyte differentiation process.

To address this issue, we have generated two IR KO clones from the 3T3-L1 cell line, using the CRIPSR/Cas9 system, and tested it as an adipocyte differentiation model. We verified accurate genome editing through sequencing and IR protein lacking through WB. These clones were unable to differentiate under standard differentiation protocols.

In order to recover the differentiation capacity in these KO clones, we re-expressed IR-A or IR-B through retroviral infection. Correct isoform expression was determined at mRNA level through RT-PCR. Preliminary results show that they recapitulated some of the molecular events typical of adipocyte differentiation. This was evaluated at the level of early adipogenic markers such as C/EBP and in late adipocyte-specific genes, such as the gene encoding aP2, a lipid-binding protein.

Taken together, these results suggest that IR could be modulating important steps of adipocyte differentiation. Further studies are necessary to elucidate the targets of IR action.

**086** (265) **BONE CELLULAR ACTIONS OF GENISTEIN (GEN) INVOLVES ENDOTHELIAL CELLS ACTIVATION VIA NITRIC OXIDE PATHWAY**

Sabrina Cepeda[1], Carla Crescitelli[1], María Belén Rauschemberger[1], Marisa Sandoval[1], Virginia Massheimer[1].

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Previously we showed that, Gen enhances endothelial cells (EC) proliferation and nitric oxide (NO) synthesis, through a mechanism of action that involves the estrogen receptor. In this work, we studied the effect of Gen on bone-vascular axis and, its relationship with osteoblastic (OB) differentiation. To that end, primary cultures of murine (Wistar rats) endothelial cell (EC) or calvarial preosteoblast cell (OB) were used. OB monolayers were incubated (24 h) with culture medium obtained from EC (conditioned medium C). Thus, OB proliferation was measured (MTT assay and cell counting). Medium C stimulated OB proliferation (13%, $p<0.01$), mitogenic action that was enhanced (1-5 fold above control, $p<0.01$) when EC were exposed to different concentrations of Gen (10nM-5uM). Similar results were observed at different time intervals of Gen treatment. Medium C obtained from EC cultures pre-incubated with 10 uM L-NAME (nitric oxide synthase inhibitor), diminished OB proliferation. When OB were directly exposed to sodium nitroprusside, an exogenous nitric oxide (NO) donor, stimulation of OB proliferation (48% above control, $p<0.01$) was detected. Direct treatment of OB with Gen or estrone, a natural estrogen ER agonist, did not modified cell growth. The effect of Gen on OB differentiation was studied using two markers: alkaline phosphatase activity (AP) and extracellular calcium deposition (HCl leaching of calcium assay). Both markers were enhanced after 12 days of culture (0.42±0.1 vs 0.25±0.08 IU/mg prot. AP activity, $p<0.05$; 144±43 vs 80±19 ugCa/mg prot., $p<0.001$, Gen vs. C). Similar results were obtained after 15 days of culture. Using red Alizarin staining, an increase in the number and size of calcification nodules was also observed. These results suggest that Gen promotes bone cells growth and differentiation through its action at endothelial level. Nitric oxide is a potential biochemical messenger involved in this close link between bone and vascular systems.

**087** (303) **COMPARISON OF REDOX STATUS IN LIVER AND BRAIN IN AN EXPERIMENTAL MODEL OF GLUTAMATE MEDIATED EXCITOTOXICITY**

Fabiana Lairion[1,2], Ailen Hvozda Arana[1], Valeria Calabro[2], Claudia Reides[1], Nathalie Weichsler[1], Susana Llesuy[1,2], Sandra Ferreira[1,2].

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Increased levels of glutamate, main excitatory neurotransmitter in the central nervous system, have been associated with various neurological disorders, such as epilepsy, cerebral ischemia, Parkinson and glaucoma. The aim of this study was to assess changes in the redox status in the liver and the brain of rats subject to glutamate mediated excitotoxicity.

**453** (934) **VALIDATION OF THE PHARMACOGENETIC DOSE ADJUSTMENT ALGORITHM FOR THE INITIAL DOSE OF ACENOCOUMAROL**

Esteban Jauregui[1], Paula Scibona[1], Carolina Vazquez[1], María Orlova[1], Jorge Arbelbide[1], Ventura Simonovich[1], Waldo Belloso[1]
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Introduction: Coumarin anticoagulants require a strict dosing control through INR (International Normalized Ratio) in order to prevent lack or excess of pharmacological effects. Usually fixed initial dose is prescribed to adults. Previous reports have shown pharmacogenetic approaches towards an initial adjustment of warfarin dose. We have previously developed an algorithm including clinical and pharmacogenetics data to individualize initial dosing of acenocoumarol, the most extensively used oral anticoagulant in our country. Objectives: To validate the acenocoumarol initial dosing adjustment algorithm previously derived in a different cohort of patients requiring chronic oral anticoagulation. In addition we analyzed the behavior of the algorithm when a new polymorphism (CYP4F2 alleles) was added. Methods: Nested cross-sectional study that included patients under anticoagulation with stable doses of acenocoumarol (3 consecutives INR values between 2-3 at time of enrollment). Genotyping of CYP2C9 (1/1 normal, 1/2 and 1/3 intermediate, 2/3, 2/2 and 3/3 low expression alleles), CYP4F2 (rs2108622 vs any other genotype) and VKORC-1 (A haplotype > susceptible to OAC, no A haplotypes considered normal) were conducted through PCR/RFLP techniques. Algorithm (with and without CYP4F2) was analyzed in its ability to predict the actual dose required for anticoagulation. Results: 40 patients were included in the validation cohort. Median age, weight and amiodarone use were similar to the observed in the derivation cohort. The algorithm was applied to each patient and compared with the actual acenocoumarol dose required. All patients had a stabilized dose of oral anticoagulant. Conclusions: Initial dose of acenocoumarol can be individualized through an algorithm including both clinical and pharmacogenetic data. This “a priori” adjustment may prevent the risks of adverse effects of acenocoumarol during the initial weeks of oral anticoagulation, being subsequently complemented by INR.

## NANOMEDR POSTER PRESENTATION / NANOMEDICINE SESSION VI POSTERS: PRIZE TO THE BEST POSTER IN NANOMEDICINE
## I. Drug Delivery & Targeting

**454** (262) **BIOLOGICAL AND TOXIC EFFECTS OF BILIARY SALTS USED IN THE DEVELOPMENT OF NANOPARTICULATE SYSTEMS FOR DRUG DELIVERY.**

Gándola, Yamila B.[1,2], Luschnat, Tania T.[1]; Fontana, C.[1]; Gonzalez, L.[1, 2].
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Biliary acids (BA) are bioactive molecules, mainly implicated in fat diet absorption and digestion, which have multiple metabolic actions. Salts derived from BA are used for the design of severalpharmaceutical nanoparticulated systems. However, high levels of BA have been associated with tumorogenesis of the colon, oesophagus, stomach, pancreas and breast tissue. Considering the multiple biological actions of BA, their association with cancer pathology and their use in pharmaceutical technology, it is highly relevant to study their biological and toxic effects over cancer cells. The objective of this work was to analyze the molecular and cellular effects ofsodium cholate and deoxycholate salts (SC and SDC) over the epithelial breast cancer cells MCF-7. For this purpose, the effects of bile salts over cell proliferation and apoptosis, and the activation of signaling molecules involved in cell cycle and survival promotion were studied. MCF-7 cells were incubated with different concentrations of each bile salt during 24 and 48 hours and cell viability wasassed by a colorimetric assay. Results showed that both biliary salts induced an increase in cell viability at low doses, but citotoxic effects were evidenced when cells were incubated with high SC and SDC concentrations. Cleavage of PARP, which is a hallmark of apoptosis, was found to increase 24hours after incubation with the highest SC concentration tested. Kinetic assays of Akt and Erk1/2 phosphorylation evidenced that citotoxic concentrations of SC and SDC induced a strong and sustained activation of both signalling mediators, while low SC and SDC concentrations only promoted a slight activation of Akt. In conclusion, biliary salts have a dosage-dependent effect over the MCF-7 cells viability. Citotoxicity induced by high bile salts concentrations could be associated to the marked and sustained activation of signalling mediators involved in cell proliferation and survival.

**455** (381) **SOLID SILICA-FUNCTIONALIZED MAGNETIC NANOPARTICLES AS DRUG TARGETING DEVICES: DRUG INCORPORATION AND ENDOTHELIAL CYTOTOXICITY.**

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Introduction: Solid silica (Si) coated magnetic nanoparticles (MNPs) are potential devices for drug targeting. Stability and biocompatibility of silica turn these systems feasible forbiomedical applications althoughdrug loading is a challenge due to silica inertia. Objective: Synthesis, characterization and Diclofenac (Dic) incorporation onto citric acid(CA)-functionalized magnetite(MG) coated with Siand 3-aminopropyltriethoxysilane(APTES). Evaluation of cytotoxicity on rat aortic endothelial cells (EC). Design: MG/CA NPs were obtained by co-precipitation. Si and APTES functionalization was performed bya modified Stöber process, prolonged for 12h at room temperature, using ratios MG/CA:TEOS:APTES=(1:0.5:2; 1:1:2; 1:2:2), named 1, 2, and 3. They were characterized by FTIR, DLS, z potential and HR-TEM microscopy. Dic was bonded by N,N´-dicyclohexylcarbodiimideand studied by UV-Vis spectroscopy. Primary cultures of EC were exposed for 48h to final concentrations of 1, 10 and 100µg/mL of MNPs and Dic-loaded MNPs. Cell viability was studied by MTT assay and by the capacity to produce NO (DAN assay). Results: 1 and 2 presented Dh of 400.0±10.0nm and 520±10.0nm; z of 27.9±5.78mV and21.9±6.02mV in aqueous dispersions. 3 was polydisperse with z 32.6±5.81mV. HR-TEM micrographs showed matrix dispersed structures for the MNPs. Formulation 2 incorporated approx. 40% of Dic. Cell viability was not affected at 10µg/mL or below for all the samples ($p<0.001$). Basal NO production was not altered at any of the assayed concentrations for allMNPs except for Dic loaded nanocarrier at 100µg/mL ($p<0.001$). Conclusion: Synthesis of Si/APTES functionalized MNPs rendered aqueous dispersible formulations dependant on precursors´ ratio and concentrations. Diclofenac was covalently incorporated. EC cytotoxicity was dose and drug dependent. No adverse effects for unloaded MNPs were observed. These novel nanocarriers may be suitable for drug targeting.

**456** (383) **BETA-CYCLODENTRIN COATED MAGNETIC NANOPARTICLES: NEW INSIGHTS FOR THE LOCALIZED TREATMENT OF DIVERSE PATHOLOGIES.**

Agotegaray, Mariela[1]; García, Elba [1]; Campelo, Adrián[2]; Massheimer, Virginia [2]; Lassalle Verónica[1]
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Introduction Magnetic nanoparticles (MNPs) provide new insights for targeted drug delivery to aspecific site in the organism by an external magnetic field. Coating of MNPs improvesbiocompatibility and provides a platform to attach drugs for diverse purposes.

Objective Synthesis, characterization and evaluation of endothelial cytotoxicity of nano-systemscomposed of magnetite (MG),

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Thyroid dyshormonogenesis due to thyroglobulin (TG) gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. The clinical spectrum ranges from euthyroid to mild or severe hypothyroidism. The majority of patients have congenital goiter or goiter appearing shortly after birth. Human TG gene is a single copy gene, 270 kb long which maps on chromosome 8q24 and contains an 8.5-kb coding sequence divided into 48 exons. Up to now, 62 inactivating mutations in the TG gene have been identified in patients with congenital goiter.

The 180 bp of the promoter region and all 48 exons of the TG gene, including splicing signals and the flanking intronic regions were amplified using the primers and PCR conditions reported elsewhere. TG PCR fragments were sequenced using sense and antisense specific primers or M13 universal primers.

The purpose of the present study was to identify and characterize new mutations in the TG gene. We report 7 patients from 6 unrelated families with goiter, hypothyroidism and low levels of serum TG. All patients underwent clinical, biochemical and imaging evaluation. Molecular analyses revealed three novel inactivating TG mutations: c.5560G>T [p.E1835X, exon 30], c.7084G>C [p.A2343P,exon 41] and c. 7093T>C [p.W2346R, exon 41], and four previously reported mutations: c.378C>A [p.Y107X] c.886C>T [p.R277X], c.1351C>T [p.R432X] and c.7006C>T [p.R2317X]. One patient was homozygous for p.W2346R mutation, four were compound heterozygous mutations and the remaining two siblings from another family with typical phenotype had a single p.E1835X mutated allele. p.E1835X includes regions I, II and only a part of region III of TG. Lacks all the carboxyl-terminal hormonogenic sites.

In conclusion, our results confirm the genetic heterogeneity of TG defects and the pathophysiological importance of altered TG folding as a consequency of truncated TG proteins and missense mutations located in ACHE-like domain.

**493** (305) **OPPOSITE ACTIONS OF MEDROXYPROGESTERONE ACETATE (MPA) ON BONE AND VASCULAR CELLS**

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A high bone turnover isassociated with increased cardiovascular mortality in aging. Hormone replacement therapy combined with progestins is proposed as an alternative choice in prevention of cardiovascular diseases. Vascular calcification (VCa) mainly involves vascular smooth muscle cells (VSMC) transdifferentiation to bone lineage. The aim of this work was to investigate the role of MPA on osteoblasts and osteoblasts derived from VSMC, as well as its impact on vascular calcification. The following murine cell cultures were used: a) VSMC; b) VSMC induced to osteoblastic transdifferentiation (VSMC-OB) in osteogenic medium (β-glycerophosphate 5 mM; CaCl2 and 4 mM); c) calvarial osteoblasts (OB). VSMC proliferation and migration, and inducible muscle nitric oxide (NO) synthesis are early events that conduct to VCa. We found that 96 h treatment with the progestin (10 nM) inhibited the VSMC growth (17.6% vs control, $p<0.05$, MTT assay) and does not alter VSMC migration (wound healing assays). On bone cells, treatment of OB with MPA (10 nM) induced a significant increase in both calcium levels (HCl leaching of calcium) and alkaline phosphatase (ALP) activity (426±61 vs 659±14 mg calcium/g protein, control vs MPA, $p<0.02$; ALP: 41±4 vs 158±26 IU/g protein, control vs MPA, $p<0.02$). VSMC and VSMC-OB were characterized by measurement specific bone markers (RT-PCR). RUNX2 and TNAP (tissue non-specific alkaline phosphatase) were detected only in VSMC-OB but not in native VSMC. Long treatment of VSMC-OB with MPA showed a significant reduction in ALP activity with respect to control (6.50±0.19 vs 14.05±0.15 x10-3 IU, control vs MPA 10nM, $p<0.001$) as well as in extracellular calcium deposition (alizarin staining). Indeed 24 h treatment with MPA (10 nM) markedly inhibited NO production (37% vs control, $p<0.01$). In conclusion, although MPA exerts opposite effects on OB and VSMC-OB, their impact could represent favorable actions in order to promote bone growth and vessel remodeling.

**494** (313) **NEUROESTRADIOL IS INVOLVED IN GNRH MODULATION DURING PREGNANCY IN THE SOUTH AMERICAN PLAINS VIZCACHA, LAGOSTOMUS MAXIMUS**

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Neuroendocrine areas involved in the reproductive control have shown aromatase (ARO) expression and activity suggesting local synthesis of estradiol (E2). Gonadotropin-releasing hormone (GnRH) surge is affected by E2 availability. The South American plains vizcacha, Lagostomus maximus, shows ovulation up to 800 oocytes per reproductive cycle and ovulation at mid-gestation. The aim of this work was to analyze hypothalamic expression of ARO in the vizcacha at different gestational time-points, and its relationship with GnRH expression and delivery. Hypothalami of non-pregnant non-ovulating (NPNO), early-pregnant (EP), mid-pregnant (MP), and term-pregnant (TP) female vizcachas (n=6 per group) were used to study ARO and GnRH mRNA by qPCR and protein expression by Western-blot or RIA respectively; hypothalamic colocalization of ARO and GnRH was evaluated by immunofluorescence and confocal microscopy, and GnRH pulsatility under ARO action using letrozole (ARO specific inhibitor) by RIA. A significant increment in ARO expression ($p<0.05$) was detected in MP vs other groups, and these results were correlated with GnRH expression. Cytoplasmic localization of ARO and GnRH was observed in neurons of preoptic area (POA) and supraoptic nucleus (SON). All GnRH neurons showed ARO expression and the quantification of neurons co-expressing both proteins did not show statistical differences among groups. To investigate neuroE2 action on GnRH pulsatility, hypothalamic explants were treated with letrozole and GnRH secretion was evaluated. Total GnRH secretion was significantly decreased by letrozole vs. control; however, pulsatile frequency did not change. The correlation between GnRH and aromatase expression at mid-gestation, and their co-localization in the hypothalamic neurons suggests the modulation GnRH expression by neuroE2 as part of a reproductive strategy of the vizcacha to assure GnRH synthesis during pregnancy.

**495** (338) **RELATIOSHIP BETWEEN UNDERCARBOXYLATED OSTEOCALCIN AND MENOPAUSAL STATUS AND/OR BODY MASS INDEX IN NORMOGLUCEMIC ADULT WOMEN**

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Evidences from our group demonstrated the emergence of cellular senescence process as a growth control mechanism during the progression of estrogen-induced pituitary tumors. Also, estrogen exerts a modulatory action on pituitary cells proliferation. Considering the preponderant role of NF-kB (p65) in cellular senescence and points of convergence between ERa and NF-kB signaling pathways in cell cycle control, we evaluate the contribution of the interaction between these two proteins in the pituitary senescence in experimental pituitary tumors. Wistar adult male rats were implanted subcutaneously with silastic capsules containing estradiol benzoate (30mg) for 10, 20, 40 and 60 days (E10-60). The control group was implanted with empty capsules. Subsequently, ERa:NF-kB immunoprecipitation was performed at the different stages of tumoral development. NF-kB and IkBa levels were also determined from nuclear and cytosolic fractions by Western blot. The ERa:NF-kB co-localization was analyzed by immunofluorescence (IF) and transmission electron microscopy (TEM). Statistical analysis: ANOVA-Fischer test ($p<0.05$). During the course of estrogen-induced pituitary tumoral development, a significant ERa:NF-kB association was detected, with a marked interaction at E10 and E60, result that was corroborated by IF and TEM. Also, a significant increase in NF-kB and IkBa protein levels in the cytosolic compartment was detected. Interestingly, a substantial increased in the NF-kB nuclear levels was evidenced at E20 and E40 compared to those observed at E10 and E60. Probably, ERa recruits NF-kB at the cytoplasmic compartment in order to inhibit their function as a transcription factor and thereby modulate cell senescence-associated molecular mechanisms during the progression of the experimental pituitary tumor. These results suggest a cross-talk between NF-kB and ERa signaling pathway that may lead to the emergence of cellular senescence, thus contributing to the control of the cell growth.

**718** (813) **SIGNS OF ALTERATIONS IN THE MITOCHONDRIAL DYNAMIC AND OXIDATIVE STRESS IN THE SENESCENCE PROCESS DURING THE DEVELOPMENT OF ESTROGEN-INDUCED PITUITARY TUMORS**

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Evidence of cellular senescence process during in vivo estrogen-induced pituitary tumor development was recently described in our laboratory. Since mitochondrial metabolism and dynamic are targets of estrogen action and senescence is considered a stress response triggered by different factors including oxidative stress; we evaluate the effects of estrogen in vivo on mitochondrial function and dynamics in experimentally

induced proliferative lesions. To induce pituitary tumoral development, Wistar male rats were exposed to estradiol benzoate (30mg) implanted subcutaneously in silastic capsules for 10, 20, 40 and 60 days (E10-60). Control group: animals treated with empty capsules. The morphological and morphometric mitochondrial analysis was evaluated by transmission electron microscopy; ROS production and mitochondrial membrane potential was determined by flow cytometry. Mfn1, Mfn2, OPA-1, Drp1; 8OHdG and Nrf2 protein expression were assessed by immunohistochemistry and western blot. Statistical analysis: ANOVA-Fischer test ($p <0.05$). Increases in mitochondrial number accompanied by a circular and less elongated morphology was observed at E10. The gradual increase of mitochondrial fusion proteins expression: Mfn1, Mfn2 and OPA-1 and the reduction of Drp1 fission protein levels, suggested the prevalence toward the mitochondrial fusion. A significant increase in ROS production and changes in mitochondrial membrane polarity, were signs of oxidative stress. The increase of nuclear 8OHdG expression at the beginning of tumoral development and increases in Nrf2 levels revealed the activation of defense mechanisms against the estrogen-induced proliferative injury. These data suggest that alterations in the mitochondrial dynamic and oxidative stress detected in early stages of estrogen-induced pituitary tumor development could be responsible for the emergence of senescence as a regulatory mechanism of cellular growth.

**719** (881) **DEHIDROEPIANDROSTENEDIONE MODULATES CELLULAR EVENTS INVOLVED IN VASCULAR REPAIR**

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In recent years dehidroepiandrostenedione (DHEA) has emerged as a promising alternative for hormone replacement therapy due to its ability to act as a precursor for local formation of active steroids. Maintenance of vascular health depends mainly on the prevention of vascular injury and the promotion of vessel remodeling (angiogenesis). Endothelial cells (EC) migration and proliferation, and the expression of endothelial factors that enhance EC adhesion to subendothelium (uPA and tPA) are crucial events in new vessel formation. In this study we evaluated the effects of DHEA on processes involved in the initiation of vascular lesions (platelet adhesion and aggregation) and in angiogenesis. We demonstrated that EC treatment with 20nM DHEA produces an inhibition on platelet adhesion to endothelium (24h - 25%below Cont $p<0.05$), and decreases endothelium dependent platelet aggregation (60min - 15%below cont $p<0.05$) in a nitric oxide dependent manner, since preincubation with NAME annulated this effect ($p<0.01$). EC proliferation studies (MTT assay) showed that 24h treatment with DHEA stimulates cell growth (32, 22 and 12% above Cont 2, 20 and 200nM DHEA $p<0.05$). Indeed, using wound healing assays, we found that the steroid also promotes cell motility (9±2, 25±8 Cont, 20nM DHEA migrating cells/field $p<0.01$). The expression of uPA, tPA and androgen receptor (AR) was measured by inmunoblot. To that end, EC were treated for 12 to 48h with 20 or 200nM DHEA. The steroid enhances the expression of both factors (30-80% above control $p<0.05$). The androgen receptor expression was also increased, suggesting that DHEA mechanism of action could involve AR. Finally, in rat aortic ring angiogenesis assays, we observed that DHEA treatment promotes EC sprouting and capillary like tube formation (30% above control, $p<0.05$). The presented results show that DHEA exerts a direct action on EC, contributing to the prevention of vascular injury and promoting angiogenesis

**720** (897) **IMPORTANCE OF HORMONAL OVARIAN FOLLICULAR FLUID LEVELS IN AN ASSISTED FERTILIZATION PROGRAM: ROLE OF THYROID HORMONES AND ESTRADIOL**

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Follicular fluid (FF) is the microenvironment in which the follicles develop and the oocytes mature. Hormonal composition influences oocyte quality and maturity, main parameters for assisted fertilization outcome. Thyroid hormones in the FF would have a positive role during folliculogenesis and ovulation.

Aim: to analyze the role of thyroid hormone and estradiol in the FF in relation to oocyte maturation rate (OMR) in women recruited for assisted fertilization procedure.

Subjects and methods: 51 women (29 to 42 years) without autoimmunity or medication affecting thyroid function were evaluated after a controlled ovarian stimulation protocol. In the remnant FF