cardiovascular disease (CVD) is the main complication of DM. DM patients usually have increased levels of atherogenic subfractions of low-density lipoproteins (LDL), such as aggregated LDL (agLDL). This latter lipoprotein is responsible to induce anomalous intracellular lipid accumulation in cardiomyocytes in a low-density lipoprotein receptor-related protein-1 (LRP1)-dependent manner. LRP1 is an endocytic and signaling receptor, belonging to the LDL receptor family. Moreover, LRP1 regulates the intracellular trafficking of membrane proteins and vesicles, including insulin receptor (IR), GLUT4 and GSVs (GLUT4 storage vesicles). Thus, it has been suggested that a functional deficiency of LRP1 would be directly associated with insulin resistance disorders. In the present work, we evaluate whether agLDL via its interaction with LRP1 may affect the insulin-induced intracellular signaling and GLUT4 trafficking to the plasma membrane (PM) in HL-1 cardiomyocytes. By quantitative PCR and Western blot analysis we determined that LRP1 and GLUT4 expression were unaffected after agLDL treatment for 8 h at 37 °C. By confocal microscopy we found a substantial modification of the intracellular localization of LRP1 and GLUT4 in agLDL-stimulated cells. Also, after insulin stimulus (100 nM) in agLDL-preincubated cells, a decreased level of Akt and AS160 phosphorylation was observed compared to unpreincubated cells. By immunoprecipitation we found that agLDL-preincubated cells showed a weakly molecular association between IR and LRP1 with respect to control conditions. Finally, by biotin-labeling protein assay we demonstrated that agLDL reduced the GLUT4 translocation to the plasma membrane in insulin-stimulated cells. Thus, we conclude that LRP1 is a key regulator of insulin response and glucose metabolism in cardiomyocytes, being its action affected by agLDL.

Palabras Clave: endocytosis, atherosclerosis, metabolism, heart.

(1036) **ALPHA-1-ANTI-TRYPSIN INCREASED NA⁺/K⁺-ATPASE AND CX43 EXPRESSION IN AN *IN VITRO* DIABETIC RETINOPATHY MODEL**

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Purpose: The ophthalmic therapy for diabetic retinopathy is focused on severe stages of the disease. Previous results obtained in our group show that Alpha-1-Anti-Trypsin (A1AT) acts like an anti-inflammatory agent that could play a role on diabetic retinopathy treatment. It is important to evaluate A1AT impact on cellular components that are essential to retina function like Na+/K+-ATPase (NKA). This protein is involved in synaptic activity and action potentials in this tissue. It is known that NKA activity and expression is diminished in diabetic retinopathy. Cx43, part of gap junction channels, play an essential role for maintenance of retinal homeostasis. High glucose or diabetes has been shown to reduce Cx43 expression in retinal cells.

A1AT may stimulate NKA and Cx43 expression through different cellular mechanisms. For this reason, we aimed at evaluating NKA and Cx43 with A1AT treatment in an in vitro diabetic retinopathy cell model.

Methods: Mouse retinas were obtained from freshly enucleated eyes incubated with collagenase I and Trypsin. Retinas were desegregated and incubated with DMEM for 5 days to allow the enrichment of Müller cells population. Müller cells obtained, were incubated 24h with DMEM 30mM glucose (Control), DMEM 30mM glucose + 4.5mg/ml A1AT (Control + A1AT), DMEM 100mM glucose (Diabetic), DMEM 100mM glucose + 4.5mg/ml A1AT (Diabetic + A1AT). Cells were harvested with RIPA buffer for Western Blot Assay or Fixed for Immunohistochemistry.

Results: Alpha subunit of Na^+/K^+-ATPase and Cx43 expression were increased in A1AT treated cells. NKA: Diabetic (46.0±4.3)%, Diabetic+A1AT(69.9±17.7)%, $P<0.05$; Cx43: Diabetic (75.3±3.7)%, Diabetic+A1AT(98.6±1.2)%, $P<0.01$.

Conclusions: Results support the hypothesis that A1AT promotes Na+/K+-ATPase and Cx43 expression. This is a novel aspect about Na+/K+-ATPase and Cx43 expression modulation. Although molecular mechanisms involved remained unknown, A1AT might play a new role in diabetic retinopathy treatment.

Keywords: Alpha-1-Anti-Trypsin, Na+/K+-ATPase, Cx43, Müller cells, diabetic retinopathy

(1085) **RESVERATROL, LIPID METABOLISM AND CELL SURVIVAL**

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Resveratrol (trans-3,4′,5-trihydroxystilbene, Rsv) is a small polyphenol molecule present in a large variety of plant species such as mulberries, peanuts and grapes. Rsv was largely utilized by Asian medicine for treatment of fungal, inflammatory, hypertensive, allergic, cancer and lipid diseases. Nowadays it is used in "natural medicine" and widely consumed by public as dietary supplementation due to its antioxidant properties. Despite various molecular targets have been proposed, AMP-activated protein kinase (AMPK) and NAD^+-dependent histone deacetylase sirtuin 1 (SIRT1) are considered as the main effectors for Rsv actions. Rsv-induced SIRT1 activation would mediate anti-inflammatory by downregulation of NF-kB and COX2 expression and activity. Rsv-induced AMPK would decrease lipogenic genes expression. We have previously shown that both, COX2 expression and activity and remodeling of cell membranes are key factors for adaptation and survival of renal cells subjected to abrupt changes of environmental osmolality. In the present work, we evaluated whether Rsv affects such survival pathways. To do this, lipid profile and synthesis, determined by incorporation of [U-^{14}C]-glycerol to lipid molecules, and COX2 expression were evaluated in cultures of the renal epithelial cell line MDCK incubated in NaCl-hyperosmotic media (~550 mOsm) without or with 100 μM Rsv for 0, 6, 12, 24 48 and 72 h. After treatment, cells were collected, counted and viability determined. Lipids were extracted by Bligh-Dyer and separated by TLC. COX2 expression was assessed by western blot. Both lipid synthesis and COX2 expression were induced after 24, 48 and 72 h of hyperosmolar treatment, and both processes were harmed by Rsv from early time of treatment. Cell number recovered after Rsv was significant lower. These preliminary results suggest that in renal cells Rsv abolished protective mechanism against osmotic stress.

Keywords: resveratrol; osmoprotection; renal cells.

(1408) **ALTERING MICROTUBULE POLYMERIZATION IN SERTOLI CELLS STRONGLY AFFECTS THEIR LIPID METABOLISM.**

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The aim of this work was to study the biological consequences of altering microtubule polymerization in Sertoli cells, with a special focus on their lipid metabolism. For this purpose, the effect of long-term exposures to nocodazole (NCZ) (0.01 μM) was studied on TM4 cells in culture. In addition to the expected effect of NCZ on the depolymerization of tubulin microtubules, a remarkable reorganization was observed in the intermediate filament network (vimentin), which showed a more peripheral distribution than in untreated cells. As compared to cells cultured in control conditions, those exposed to NCZ displayed an increased mitochondrial membrane depolarization ($p<0.001$). Their plasma membrane integrity was concomitantly altered, as shown by a considerable increase in the leakage of lactate dehydrogenase ($p<0.01$) released into the culture medium. In addition, the cell proliferation was significantly reduced ($p<0.003$) in NCZ-exposed cells. That the drug induced a major alteration on lipid biosynthesis was clearly apparent from a significant increase in triacylglycerol (TAG) levels ($p<0.0002$) and a massive accumulation of lipid droplets in the cytoplasm of cells. This observation was sustained by following the incorporation and distribution of labeled arachidonic acid ([^{3}H]AA). After 1 hour of incubation, the most highly [^{3}H]-labeled lipid class were TAG in control cells, but choline glycerophospholipids in drug-treated cells. Interestingly 72 hours later, the percentages of label in TAG and cholesterol esters were about twice higher in NCZ-treated than in control cells. We conclude that the cytoskeletal alterations that Sertoli cells undergo after being ex-

posed to NCZ, through their impact on mitochondrial functions and cytoplasmic architecture, are closely involved in the deranged lipid metabolism induced by the drug.

Keywords: microtubules; nocodazole; lipid droplets

(1430) **COORDINATION BETWEEN SPHINGOLIPIDS AND PROTEINS REQUIRED FOR PRIMARY CILIOGENESIS**

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The final stage in the process of epithelial cell differentiation is the outgrowth of a primary cilium from the apical surface. We have previously described that extracellular hypertonicity induces the differentiation of MDCK cells by the regulation of the sphingolipid metabolism. The aim of this work was to investigate the ciliogenesis induced by hypertonicity in MDCK cells and the role of glycosphingolipids in the process. Confluent MDCK cells were cultured under isotonicity (150 mM NaCl, control) or hypertonicity (300 mM NaCl) for 48 h in the presence or absence of D-PDMP, a glucosylceramide synthase inhibitor. The presence of primary cilium was detected in MDCK cells stable expressing α-tubulin–GFP subjected to hypertonicity. For the immunofluorescence assays, cells were stained with acetylated tubulin to visualize primary cilium, and co-stained with IFT20 or Rab8, proteins required for vesicle-mediated trafficking pathway from the Golgi to the base of the cilium. Isotonicity-cultured cells did not show cilium formation and IFT20 and Rab8 appeared diffusely distributed. Cells cultured under hypertonicity displayed primary cilium and the number of ciliated cells significantly decreased when the cells were subjected to hypertonicity in the presence of D-PDMP. Cells cultured under hypertonicity showed IFT20 with a Golgi – like distribution and perinuclear Rab8, but when cells were treated with D-PDMP the distribution of these proteins was diffuse. A proteomic analysis performed by mass spectrometry of IFT20+ vesicles immune-isolated from cells cultured under hypertonicity revealed the presence of galectin 3, a protein with an important role in apical sorting that associates with the primary cilium. In conjunction, these results suggest that hypertonicity induces ciliogenesis by displaying a protein machinery that is correctly assembled in the presence of glycosphingolipids.

Keywords: primary cilium, cell differentiation, renal epithelial cells, sphingolipids

(1246) **14-3-3γ AND β HAVE OPPOSITE ROLES IN ADIPOGENIC DIFFERENTIATION**

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The 14-3-3 protein family regulates several cellular processes including differentiation. Regarding adipogenic differentiation, this family binds to regulatory proteins involved in adipogenesis as TAZ, PPARγ2, Lipin-1 and Seipin. 14-3-3 proteins bind their targets through specific phospho-Serine/Threonine residues. Among the seven highly conserved mammalian family members, specifically 14-3-3γ and β mRNA and protein levels increase during adipogenic differentiation of 3T3-L1 preadipocytes. To investigate the role of these paralogs in adipogenesis, we decreased their levels by using lentiviral transduction particles containing short hairpin RNAs (shRNAs) specific to silence the expression of both of these paralogs, and the ZsGreen protein as a marker of transduced cells. 3T3-L1 cells were infected with these vectors, and then induced to the adipogenic linage during 7 days. Adipogenic differentiation was evaluated by Oil Red O staining of lipid droplets followed by confocal microscopy analysis. The results revealed an increment of number and size of lipids droplets after 14-3-3γ silencing compared to neighboring cells that were not infected. The opposite effect was observed upon 14-3-3β silencing, where a pronounced decrease in lipid droplets number and sizes was evident in transduced cells. Taken together our findings suggest that 14-3-3γ and β differentially regulate 3T3-L1 adipogenesis.

Keywords: 14-3-3, 3T3-L1, adipogenesis, differentiation.

(1175) **IDENTIFICATION OF PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) INTERACTORS INVOLVED IN CELL MOTILITY**

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PTP1B is an ER-bound protein tyrosine phosphatase implicated in the dynamics of cell-matrix adhesions. This regulation relates to a positive role of PTP1B in directional cell migration and attenuation of cell contractility. The aim of the current study is to identify and characterize potential substrates and interactors of PTP1B involved in cell motility. To this end we used a Bimolecular Fluorescence Complementation (BiFC) approach for direct visualization and analysis of PTP1B interactors in intact cells. BiFC is based on complementation and restoration of fluorescence when two non-fluorescent fragments of a native fluorescent protein are a few nanometers apart. These fragments were fused to PTP1B and the potential substrates/interactors, chosen on the basis of previous phosphoproteomic data and their relevance in cell motility. We observed BiFC between PTP1B and epidermal growth factor receptor (EGFR), the scaffold protein Mena and the focal adhesion tyrosine kinase FAK. PTP1B/EGFR interaction was revealed as puncta with increasing size and density in internal locations compared to the cell periphery. The colocalization with cell-matrix markers was not evident. In contrast, PTP1B/FAK and PTP1B/Mena interactions were prominent at peripheral adhesions. FAK BiFC fluorescent signal was attenuated when using a FAK mutant (Y4-9F-FAK) in which tyrosine phosphorylation sites Tyr 407, 576, 577, 861, and 925 were mutated to phenylalanine. The role of individual mutations on FAK, as well as on putative interacting motifs on EGFR and Mena are currently investigated. Our results suggest a dual role of PTP1B on integrin and EGFR signaling driving the cell motility machinery. Supported by CONICET and ANPCyT.

Keywords: Adhesion, Motility; Ptp1b; Bifc

IMMUNOLOGY (INNATE IMMUNITY) 4

(48) **HISTOLOGICAL STUDY OF THE ADVENTITIAL LAYER OF FERTILE, INFERTILE AND SMALL HYDATID CYSTS REVEALS HALLMARK INFLAMMATORY FEATURES**

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Introduction: Cystic Echinococosis (CE), a worldwide-distributed zoonosis caused by the cestode Echinococcus granulosus, is an endemic disease in Chile in both humans and animals, with cattle being the most affected of the intermediate hosts. CE can manifest as either fertile or infertile cysts, the latter unable to continue the biological cycle of the parasite. Small hydatid cysts (smaller than 1 cm in diameter) usually don't fall in neither category, because they are still developing. The following work studies the adventitial layer of bovine hydatid cysts, to better understand the host-parasite interplay.

Material and Methods: Through routine abattoir visits, fertile, infertile and small hydatid cysts samples were collected, all of which were paraffin embedded, cut in 5 μm thick sections and stained with hematoxylin-eosin. The adventitial layer was examined by a seasoned pathologist

Results: Fertile hydatid cysts present an adventitial layer consisting mainly of collagen fibers and fibroblast whereas infertile and small hydatid cysts have hallmark inflammatory features: palisading foamy macrophages in direct contact with parasite tissue, lympho-