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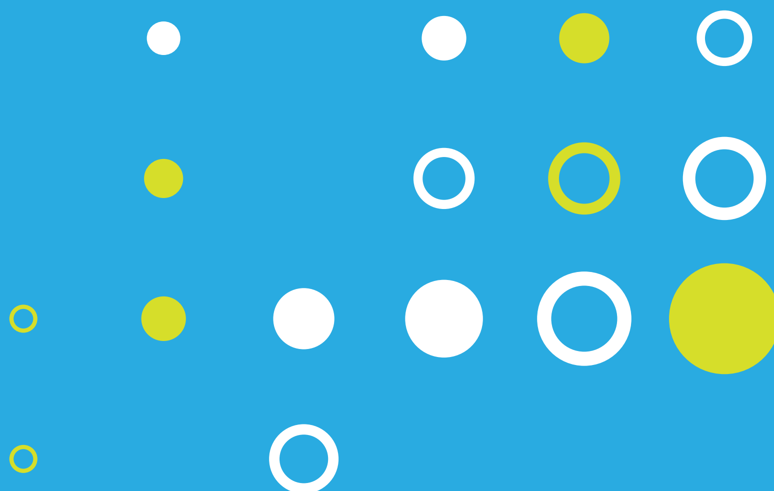

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LI-P05.
SPHINGOLIPID METABOLISM IS A SHARP REGULATOR OF A RENAL EPITHELIAL CELL PROLIFERATION

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Sphingosine Kinase (SK) is a key regulator enzyme in lipid metabolism that regulates the balance between sphingosine and sphingosine-1-Phosphate (S1P), being S1P a lipid involved in several cellular processes. We have demonstrated that SK is strongly expressed in renal epithelial cells and is a key regulator in cell survival. In the present work we evaluated the importance of the tight regulation of SK activity in renal epithelial cell cycle. For this purpose, MDCK cells cultured at low density were seeded with 10% BFS for 24 hs, to allow cell cycle progression. Then, cell were treated or not with D,L-threo-dihydrosphingosine (DHS), a selective SK1 inhibitor at different concentrations(1,5 and 1,75μM). SK inhibition induced a decrease in cell number in a concentration dependent manner after 24 hs of incubation (257,200, 129,650 and 85,768 cells, control, 1.5 and 1.75 μM respectively) with no alteration in cell viability. Cells were arrested in G1 phase (57.9%, 72.7% and 76.1% for Control, 1.5 and 1.75 μM, respectively) and decreased of mitotic cell/field (4.5, 1.4 and 1.3 for Control, 1.5 and 1.75 μM, respectively) showing a cell cycle arrest. In summary we proposed that depending on SK rate activity, the enzyme can be, not only a regulator of the cell survival, but also of the cell cycle progression.

LI-P06.
EFFECT OF FATOSTATIN ON HYPEROSMOLARITY-ACTIVATED LIPID METABOLISM IN RENAL EPITHELIAL CELLS

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In the renal cell line MDCK, we demonstrated that the increase in environmental osmolarity up-regulates phospholipid (PL) and triglycerides (TG) metabolism. The aim of the present work was to evaluate whether or not the increase in lipid metabolism requires the activation of the transcription factor sterol response element binding protein (SREBP). With this purpose, MDCK cells were incubated in isosmolar and hyperosmolar medium (containing 125 mM NaCl) alone or in the presence of different concentrations (10-25 μM) of fatostatin (FATO), a SREBP inhibitor. To evaluate lipid synthesis 0.4 μCi [^{14}C(U)]-glycerol was added to the incubation medium 3 h before cell collection. After 24 h of treatment with NaCl, cells were collected and counted and lipid content and biosynthesis were determined. FATO decreased the number of cells. Hyperosmolarity increased total PL and TG content by 60 and 100%, respectively, unexpectedly FATO increased PL and TG accumulation. FATO (10, 15 and 20 μM) decreased [^{14}C]-glycerol incorporation into DAG (by 60, 70 and 80%) but not change on PL and TG synthesis was observed. These results suggest that hyperosmolarity would activate SREBP which in turn would modulate the up-regulation observed in lipid synthesis under hyperosmotic conditions.

LI-P07.
TRIGLYCERIDE SYNTHESIS DYNAMICS UNDER HYPEROSMOLAR STRESS

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Renal medullary cells are immersed in a hyperosmolar environment due to their function in the urine concentrating system. In order to survive in these conditions, cells must develop protective mechanisms. We showed that the renal papilla is the zone with the highest content and synthesis of phospholipids (PL), which helps to preserve the membrane structure and cell viability. Triglycerides (TG) are a possible source of fatty acids for the synthesis of PL. In this work we study the dynamics of TG and PL synthesis in renal epithelial cells subjected to hyperosmolarity. We studied lipid synthesis in renal medullary slices and in MDCK cells submitted to different hyperosmolar media (from 298 to 579 mOsm/kgH2O) by using different precursors [^{14}C(U)]-glycerol, [^{14}C]-arachidonic acid (AA), [^{14}C]-palmitic acid and [^{3}H]-oleic acid. After treatment lipids were extracted, separated by TLC and quantified by liquid scintillation. We observed that high osmolarities increase TG and PL synthesis in all the samples assayed. All precursors were incorporated to both TG and PL molecules except for the AA which was found only in PLs. Cerulenin, a FAS inhibitor used to suppress fatty acid *de novo* synthesis, decreases TG synthesis and content while PL synthesis remains active. These results show that molecular machinery involved in lipid metabolism is differentially regulated by hyperosmolarity.

LI-P08.
HYPERTHERMIA IMPAIRS SERTOLI CELL FUNCTIONS. EFFECTS ON LIPID METABOLISM

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Spermatogenesis is known to be vulnerable to temperature. Previous work from our group showed that *in vivo* testicular hyperthermia selectively damages germ cells at specific developmental stages and rapidly induces a seminiferous epithelium involution. This concurred with a considerable buildup of lipid droplets in Sertoli cells (SC). The aim of this study was to examine the effects of heat exposure on isolated SC in culture. Warming to 43ºC disrupted SC microtubule network and actin microfilaments. This SC cytoskeleton perturbation was accompanied by increased ROS production, mitochondrial depolarization, and accumulation of triacylglicerols (TAG). The heat exposure-dependent TAG accumulation could be explained by an impairment of mitochondrial fatty acid oxidation and a re-direction of fatty acids into TAG synthesis and lipid droplet formation. The impaired mitochondrial physiology also reduced the *de novo* synthesis of cardiolipin, as observed after incubating SC cultures with [^{3}H]arachidonic acid. The hyperthermia-induced cytoskeletal and mitochondrial alterations could in part respond for the lipid metabolic disorder that SC undergo. Since these cells provide support to germ cells, these changes might be one of the underlying causes of the temporal impairment of spermatogenesis observed *in vivo* during testicular hyperthermia.