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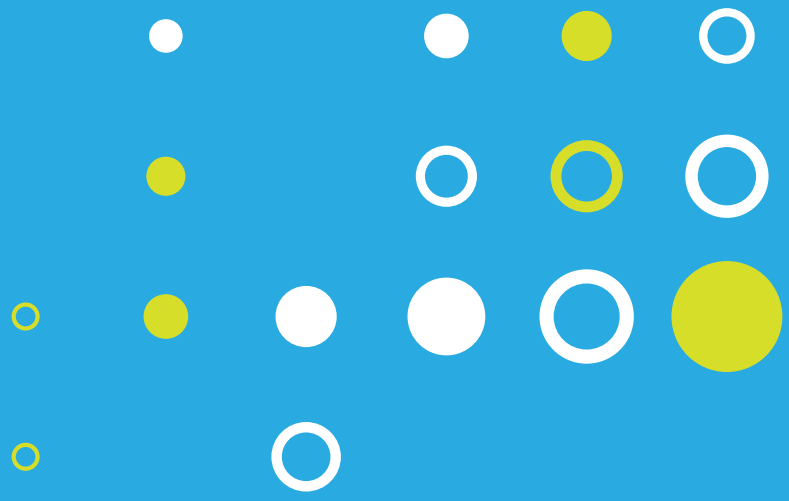

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LI-P05.
MOLECULAR MECHANISMS INVOLVED IN COX-2 EXPRESSION UNDER HYPERTONIC STRESS

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Renal medullary cells are exposed to variable and high concentrations of NaCl as part of the urinary concentrating system. Despite such adverse conditions, renal cells still survive and function by activating the transcription of various osmoprotective genes, among them, cyclooxygenase 2 (COX2). It was reported that PI3K and ERK1/2 are signaling pathways involved in cell survival and activated by osmotic stress. Therefore, in the present work we studied their role in the induction of COX2 expression reported as cytoprotective protein. With this purpose cultures of the renal cell line MDCK were grown during 5, 15, 30, 60 min and 1.5, 3, 6, 12 and 24 h in isotonic (298 mOsm/Kg H_2O) and NaCl-hypertonic (500 mOsm/Kg H2O) conditions, in the absence or presence of different specific inhibitors (LY294002, U0126). After the treatments cells were collected and submitted to westernblot for COX2 analysis. Hypertonic medium induced COX2 expression after 12 h of NaCl treatment. Surprisingly, LY294002, a PI3K inhibitor, did not prevent NaCl-induced COX2 expression but caused an over-expression of the protein from 6 h of treatment. ERK1/2 inhibitor U0126 did not affect COX2 protein in 60 min of treatment but a slight decrease in COX2 was observed in long-term treatment. Our data show that PI3K and ERK1/2 signaling pathways counterbalance NaCl-induced COX2 expression contributing to renal cell survival.

LI-P06.
OSMOTIC REGULATION OF LIPID METABOLISM IN RENAL EPITHELIAL CELLS

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Due to the urine concentration mechanism, the renal papillary interstitium has the highest osmolality of the body. To survive and work in such harmful environment, renal cells have protective mechanisms. We showed that papillary cells possess the highest phospholipid (PL) synthesis and turnover of the kidney, which contribute to preserve membrane homeostasis and cell viability. We also showed that renal phospholipid metabolism is regulated by osmolarity. Considering that PL synthesis require an adequate supply of fatty acids, in this work we characterized the relationship between PL and triacylglycerol (TG) in renal cells.The levels of PL and TG were determined in the different zones of the kidney and in cultures of the renal cell line MDCK grown during 24, 48, 72 and 96 h in media with osmolalities from 298 to 570 mOsm/kgH2O. Renal cortex (isosmolarity) showed the highest PL content but the lowest TG content and PL synthesis. In opposite, papilla showed the lowest PL content but the highest TG content and PL synthesis. In MDCK cells, we found that hyperosmolarity significantly increases PL and TG content. Such increase is dependent on the time of incubation and the osmolarity of the medium. These results demonstrate the relationship between renal PL synthesis and TG content and the role of environmental osmolarity as a distinctive regulatory factor of renal lipid metabolism.

LI-P07.
ETHER-LINKED TRIGLYCERIDES OF RAT EPIDIDYMIS

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Long-chain n-9 polyunsaturated fatty acids (PUFA), particularly 22:4n-9, are normal components of the plasmenylcholines of rat epididymal spermatozoa. Since these uncommon PUFA are absent from lipids of testicular cells, their accretion is expected to occur during the passage of the gametes through the epididymis. In this work we focused on the neutral lipids that are present in the epididymal caput, corpus, and cauda regions. In addition to cholesteryl esters, the three areas contained ether-linked triglycerides (1-O-alkyl-, and 1-alk-1´enyl- diacylglycerols, ADG). Interestingly, the latter were characterized by high proportions of n-9 PUFA. The amounts of these ADG gradually increased during development: they were detected in rat epididymis on day 14 after birth (P14), their amounts increased markedly at P49, in association with the complete differentiation of all epididymal cells, while reaching maximum levels at P55, the time at which the first spermatozoa appeared in the epididymal lumen. In adult rats, the smallest but metabolically most active portion of the epididymis, the corpus, had relatively more 22:4n-9-rich ADG than the other two regions. Our data suggest that epididymal ADG may be involved as lipid intermediates during the active membrane remodeling that spermatozoa are known to undergo during their extra-testicular maturation

LI-P08.
GERANIOL REGULATES HMG COA REDUCTASE AT A TRANSCRIPTIONAL LEVEL IN HepG2

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Plant isoprenoids, a broad class of plant products derived from the mevalonate pathway are widely distributed in fruits and vegetables. It has been suggested that these compounds suppress the growth of cancer cells and development through multiple effects on mevalonate pathway. We have reported that geraniol (G), a natural monoterpene, inhibits cholesterol synthesis and cell growth at concentration greater than 10 µM and 100 µM respectively in HepG2 cells. We also reported that 50 µM G inhibit the conversion of lanosterol into cholesterol. The aim of this study was to analyze the mechanism through which G exerts these effects on the mevalonate pathway in human liver cells. We studied the expression of the HMGCoA reductase and SREBP2 genes in cells incubated at different times with 50µM and 200µM G. HMGCoA reductase levels were determined by western blot and enzyme activity was measured. At 4 h G treatment, real time PCR revealed that the expression of genes was reduced in a dose dependent manner. Consequently, a decrease in quantity and activity of the enzyme and a reduction in cholesterol levels were observed at 24 h. This induces an increase in the enzyme expression. These results suggest that geraniol decreases cholesterol synthesis by transcriptional inhibition of HMGCoA reductase in a direct or indirectly way, and by inhibiting the conversion of lanosterol to cholesterol.