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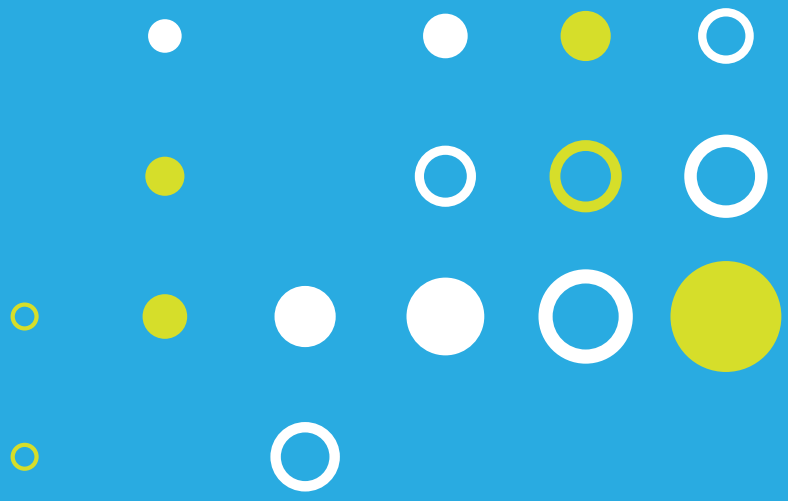

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LI-C05.
IMPLICATION OF SPHINGOSINE KINASE IN MDCK CELL TRANSITION FROM POLARIZED TO DIFFERENTIATED PHENOTYPE

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We have demonstrated that sphingolipid biosynthesis is essential for hypertonicity-induced MDCK cell differentiation. Sphingolipids regulates several aspects of cell behavior, being sphingosine 1-phosphate (S1P) one of most important. Thus, we study the importance of S1P synthesis in the acquisition of the polarized-differentiated MDCK cell phenotype. In epithelial cell polarization/differentiation transition, cell-cell adhesion is an early event characterized by the establishment of adherents junctions. To evaluate the importance of S1P in this process, confluent MDCK cells were subjected to hypertonicity and concomitantly treated or not (control) with different concentrations of D-threo-dihydrosphingosine (Sphingosine Kinase inhibitor). After 48 h, cell phenotype was visualized by fluorescence microscopy of actin cytoskeleton and cell-cell adhesion structures (E-Cadherin, ß-catenin and a-catenin). As inhibitor concentration rise the cell-cell adhesions were impaired, and the characteristic polarized phenotype of the cells was lost. First, cells appeared lengthened, and thereafter acquired a fibroblast-like phenotype. Interestingly, ß-catenin suffered redistribution from plasmatic membrane to cytoplasmic and nuclear localization. In the present work we demonstrate that SK activity is essential in the MDCK cell differentiation process induced by hypertonic stress.

LI-C06.
NUCLEAR LIPID DROPLETS ARE DYNAMIC STRUCTURES

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The cell nucleus (N) is a highly compartmentalized organelle characterized by several dynamic domains. We have demonstrated that nuclear neutral lipids are organized and stored in lipid droplets (nLD), composed of few and small droplets randomly distributed. The nLD could thus be involved in nuclear-lipid homeostasis and serve as an endonuclear buffering system that can rapidly provides or incorporates lipids involved in signaling paths and transcription factors in the nucleus.
The aim of this work was to determine if nLD are a dynamic domain. With this purpose HepG2 were incubated with 18:1n-9 as an external stimulus since it increases cytosolic lipid droplet (cLD) number and size. Both LD (c and n) were stained with BODIPY493/503 and N with DAPI and viability controls were done. Due to 18:1n-9 treatment cellular shape was modified and both cLD and nLD increased (number and size). nLD increments were smaller than cLD (4 and 10 times respectively). If 18:1n-9 was excluded from the incubation mixture, LD increments were reverted. Under all conditions tested, nLD corresponded to a small pool (3-5%) with respect to total (nLD + cLD).
In conclusion, nLD are dynamic structures; their size and number can vary in response to external signals that influence TAG pools by a reversible mechanism.

LI-C07.
LIPID CHANGES IN RAT SPERMATOZOA DURING ISOLATION AND FUNCTIONAL ACTIVATION *IN VITRO*

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Hydrolysis of glycerophospholipids (GPL) and sphingomyelin (SM) are calcium-dependent reactions that are significantly stimulated in rat epididymal spermatozoa during incubations leading to capacitation (Cap) and particularly after acrosomal reaction (AR). In comparison with gametes whose contact during isolation with tissue divalent cations was avoided (C1), or kept to a minimum (C2), those used as controls in these incubations (C3) already had their glycerophospholipids (GPL) and sphingomyelin (SM) partially hydrolyzed. Using C1 as controls, the C3 gametes had less GPL per cell and more free fatty acids. The latter were released with cholesterol from cells to media during albumin-dependent capacitation. Significant decreases in membrane GPL occurred gradually in the order C1 > C2 > C3 > Cap > AR. Diminution in the major choline GPL specifically targeted phosphatidylcholine in the presence of a virtually unchanged amount of plasmenylcholine, the acrosome-reacted cells thus becoming relatively rich in plasmalogens. The unique SM with very long chain PUFA located on sperm heads was intact in C1 cells and maximally hydrolyzed into ceramides after completion of AR. Using fluorescent probes, liposomes prepared from the 5 conditions showed that C1 gametes had the minimun degree of order of the lipid bilayer, this increasing significantly as Cap and AR proceeded.