UNRAVELING LIPID DROPLETS FORMATION MECHANISMS BY CONFOCAL DUAL-CHANNEL IMAGING OF INTRACELLULAR HYDROPHOBICITY

Flavio Di Giacinto[1], Giuseppe Maulucci[1], Gabriele Ciasca[1], Massimiliano Papi[1], Bareket Daniel[2], Ofir Cohen[2], Shlomo Sasson[2], Marco De Spirito[1]
[1]Istituto di Fisica, Università Cattolica del Sacro cuore, Largo Francesco Vito 1, 00168, Rome Italy
[2] Department of Pharmacology, Institute for Drug Research, Faculty of Medicine, the Hebrew University of Jerusalem, Israel
Email: digiacinto.flavio@gmail.com

KEYWORDS: Metabolic imaging, Lipid droplets, size distribution, hydrophobicity, Palmitic acid

ABSTRACT:
Lipid Droplets (LDs) are intracellular organelles that have a fundamental role in lipid metabolism. In the previous years, several mechanisms that underlie their formation and growth in different metabolic conditions were discovered and quantified, as homotypic fusion, de novo triacylglycerol synthesis in situ and membrane ripening [1]. Nevertheless, the role and the influence of each of these different mechanisms in the regulation of LD size in normal and nutrient overload conditions are not fully investigated since an extensive quantification of LD kinetics is still lacking.

Here we analyzed by a dual-channel confocal microscopy approach beta cells labeled with the Nile Red fluorescent lipophilic dye and exposed to different glucose and fatty acids (palmitic acid and palmitoleic acid) concentrations, in order to quantify the formation and growth of LD and simultaneously characterize their degree of hydrophobicity [2]. An image analysis algorithm was developed to determine the Lipid Droplets Size Distribution (LDSD) and the Lipid Droplet Hydrophobicity Distribution (LDHD). The contextual determination of LDSD and LDHD in different nutritional settings enabled the distinction of the several mechanisms involved in LD formation allowing a fine-tuned and comprehensive real time characterization of LD kinetics. [3].

[1] Thiam, A.R., R.V. Farese Jr, and T.C. Walther, *The biophysics and cell biology of lipid droplets.* Nature reviews Molecular cell biology, 2013. **14**(12): p. 775-786.

[2] Diaz G. et al, *Hydrophobic characterization of intracellular lipids in situ by Nile Red red/yellow emission ratio*. Micron 2008 Oct;39(7):819-24

[3] Di Giacinto et al. *Biochimica et Biophysica Acta*, submitted