A novel microscopy tool to study *in vitro* lipid droplet dynamics in human primary myotubes.

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Background: High levels of intramyocellular lipid droplets (LDs) are associated with insulin resistance. Insulin sensitive athletes, however, have similar intramyocellular lipid levels as type 2 diabetes (T2DM) patients. LDs are dynamic organelles which release and store fatty acids (FAs) depending on energy demand. LD dynamics may be an underlying factor explaining this athlete's paradox. We aimed to develop an *in vitro* fluorescent microscopy tool to study LD dynamics in human primary myotubes (HPM) from athletes and T2DM patients.

Methods: HPM were incubated overnight with 50 µM oleate and trace amounts of Bodipy-FL (green)-labeled C12-FA, followed by a 6-hour 50 µM oleate incubation with trace amounts of Bodipy 558/568 (red)-labeled C12-FA. ImageJ scripts were developed to analyze the Bodipy labeled-FA incorporation into LDs. To examine if the combined use of both Bodipy-labeled FAs resulted in visualization of all LDs, we stained LDs with MDH.

Results: All LDs were labeled as indicated by Manders coefficients M1 and M2 of MDH with any of the Bodipy's approaching 1.000 (0.998 and 0.979 for Bodipy 558/568; 0.976 and 1.000 for Bodipy-FL). Bodipy-FL to Bodipy 558/568 content permitted to make the distinction between three LD pools (pre-formed, incorporating and new). Live-cell experiments showed that LD formation in HPM from an athlete occurred more rapidly compared to HPM from a T2DM patient and plateaued after 10 hours.

Discussion/conclusion: The use of two fluorescently-labeled FAs in combination with our developed ImageJ scripts is a promising tool to study LD dynamics in HPM. This methodology permits the detection of differences in LD formation rate in HPM from an athlete vs. a T2DM patient. More studies are needed to confirm this observation and to examine if regional differences within the cell exist with respect to FA incorporation rate into LDs and if this is different in HPM from athletes vs. T2DM.