

APPLICATION: REGULATION OF LIPID ACCUMULATION

INTRODUCTION

Retinoic acid and Caffeine are reference molecules known for their inhibitory activity on lipogenesis. Retinoic acid, a derivative of vitamin A, inhibits adipogenesis by blocking adipogenic factors such as PPAR γ 2 and C / EBP α , via the Retinoic acid receptor (RAR) ligand (1). Caffeine, a non-selective adenosine antagonist receptor, is used for in vitro studies as well as in obesity animal models. This molecule reduces lipid accumulation by adipocytes and attenuates dysfunctions related to lipid metabolism (2).

Lipolysis allows functional adipocytes to provide energy to other organs through hydrolysis of triglycerides stored in lipid vesicles, releasing fatty acids and glycerol. This process can be regulated by hormonal and biochemical signals acting on lipolytic enzymes (3). Isoprenaline and Caffeine are well-known regulators of lipolysis (4-6).

Materials required

- Mouse cell-line 3T3-F442A, HWP and mouse pre-adipocyte cell-line 3T3-F442A grown in BIOMIMESYS® *Adipose tissue*
- Retinoic acid (Promokine), Isoprenaline and Caffeine (Sigma-Aldrich)
- AdipoRed™ Assay Reagent (Lonza)
- Oil Red O staining (Sigma-Aldrich)
- Glycerol detection Kit (Sigma-Aldrich)
- DNA Quantitation Kit, Fluorescence Assay using bisBenzimide (SIGMA-Aldrich)

Hydro scaffold properties

Porous and Translucent

Method

For lipogenesis study:

- AdipoRed™ kit allows lipid quantification, normalized by DNA quantitation kit, follow manufacturer's protocol
- Adipocytes in culture are subjected to retinoic acid chronic treatment at concentrations from 10⁻⁶M to 10⁻⁵M (day 0 to day 28), by renewing the medium every two days.
- Oil Red O staining, to assess appearance of lipid vesicles and maturation, in HWP cultured with or without Caffeine (500 μ m and 2 mM), during nutrition period.

For lipolysis study:

- Glycerol release assays: 3T3-F442A were cultured in BIOMIMESYS® *Adipose tissue* until 12 days of nutrition, and then incubated for 90 minutes in the presence of increasing concentrations of Isoprenalin (1 and 10µM) or Caffeine (5 and 10 mM).
- Released glycerol in the supernatants is then measured.

RESULTS

1. Inhibition of lipogenesis

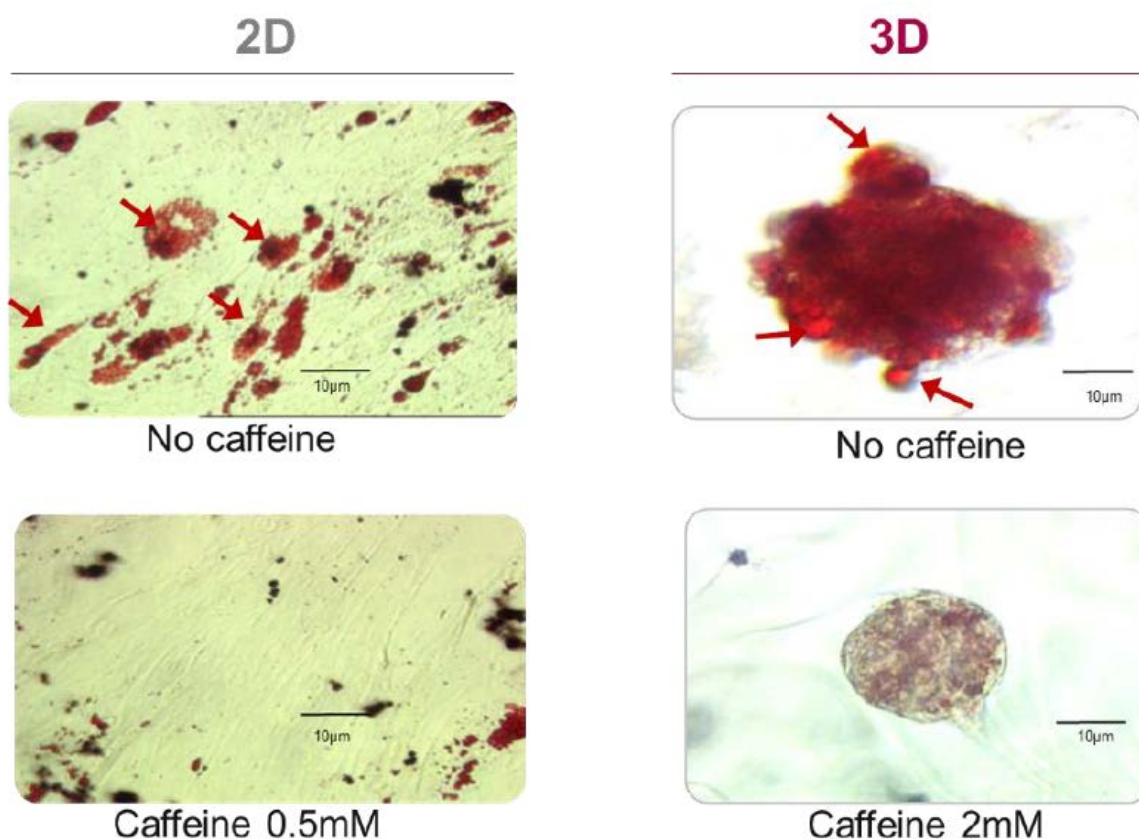


Figure 1: Oil Red O lipids staining in HWP at day 10 of nutrition with or without chronic caffeine at 0.5mM and 2mM

To inhibit the accumulation of lipids with Caffeine in 3D in the same way as in 2D, the concentration must be increased four-fold in 3D (Fig 1).

To quantify the inhibition of lipogenesis, human pre-adipocytes and 3T3-L1 cells cultured in BIOMIMESYS® *Adipose tissue* and in 2D culture are treated with Retinoic acid, a well-known regulator. Lipid accumulation is measured using the AdipoRed™ kit, normalized by DNA level.

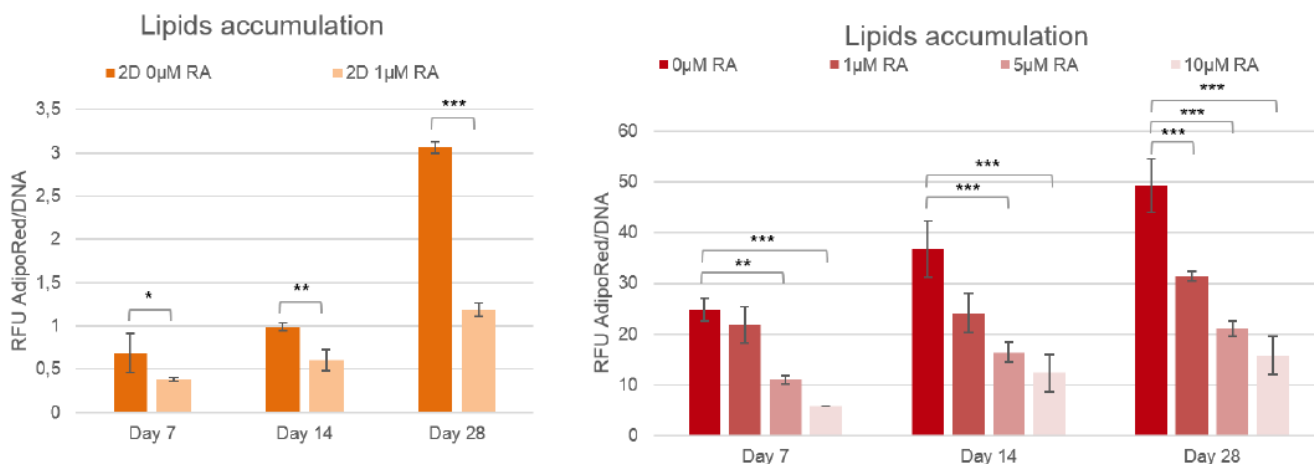


Figure 2: Lipid accumulation in 3T3-L1 with or with Retinoic acid, compared to 2D cultures (n=3, two-ways ANOVA statistics with Tukey post-hoc ; * : p<0,05 ; ** : p<0,01 ; *** : p<0,001)

As low as 1µM of Retinoic acid lead to a significant lipids accumulation inhibition in 2D, whereas at least 5µM of this molecule was needed to significantly inhibit the lipogenesis of adipocytes grown in BIOMIMESYS® Adipose tissue.

For an in-depth understanding of the inhibition mechanisms of lipogenesis, cellular parameter such as reactive oxygen species (ROS) accumulation and mitochondrial potential can be monitored, as shown in the following poster.

2. Induction of lipolysis

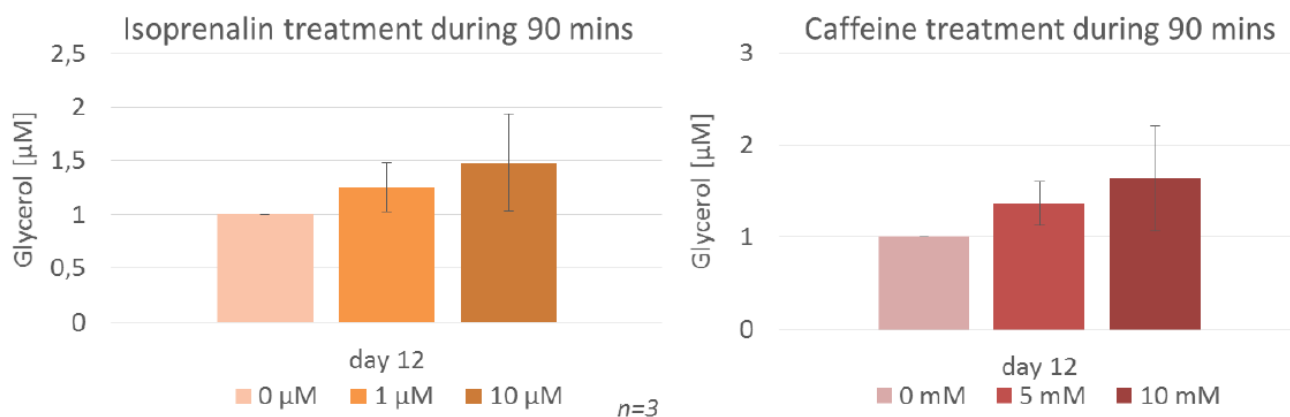


Figure 3: Effects of Isoprenalin and Caffeine acute treatment on lipolysis in 3T3-F442A

Treatments of adipocytes in 3D cultures with 1µM and 10 µM of Isoprenalin at day 12 of nutrition show 25-50% increase of lipolysis compared to untreated cells (Figure 3).

This increase of lipolysis is also observed with Caffeine treatments at 5 and 10mM (40-60% increase compared to untreated control).

CONCLUSION

BIOMIMESYS® *Adipose tissue* can be used as a model for screening of molecules that regulate lipogenesis and lipolysis in adipocytes.

Reference:

1. Distinct Stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPAR γ , XUE JC., Schwartz EJ. Et al., Molecular and cellular biology, 16,4:1567-1575, 1996.
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