Development of a diet-induced disease-mimicking *in vitro* model of non-alcoholic steatohepatitis / fibrosis

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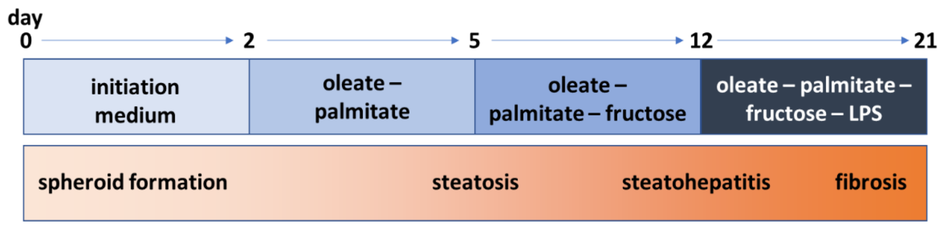
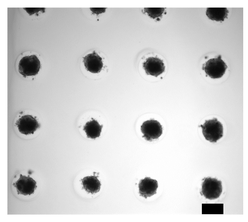
Background

Non-alcoholic fatty liver disease (NAFLD), characterized by hepatocyte steatosis, is the most common form of chronic liver disease and may progress towards development of non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma. Currently no effective therapeutic treatment is available to halt or reverse progression of NAFLD, partly due to the absence of translational cell models.
We present data on induction of steatosis, modulation of steatosis by prototype compounds and profibrotic cell activation in a 3D liver spheroid model using primary human cells.

Methods

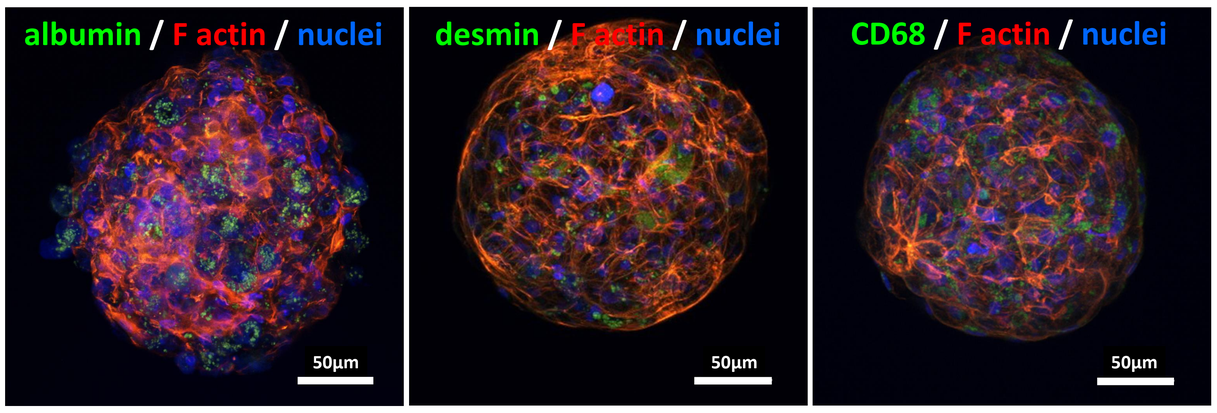
Culture and treatment scheme:





- Primary human hepatocytes, stellate and Kupffer cells were cultured in matrix-free molds (above right, bar = 200µm), resulting in formation of liver spheroids;
- Steatosis was induced by adding BSA-conjugated fatty acids, fibrosis was induced by adding fructose and LPS to the steatotic spheroids, controls are treated with culture medium only;
- Immunostainings and steatosis (LipidTOX labelling) were imaged by confocal microscopy;
- YKL-40, TIMP1, IL-6 and human albumin were measured in medium by ELISA, CYP3A4 activity using a fluorimetric assay;
- Cells were treated with pioglitazone (5µM), fenofibrate (30µM) from day 9 of culture and collected on day 12 for further analysis.

Liver spheroid morphology

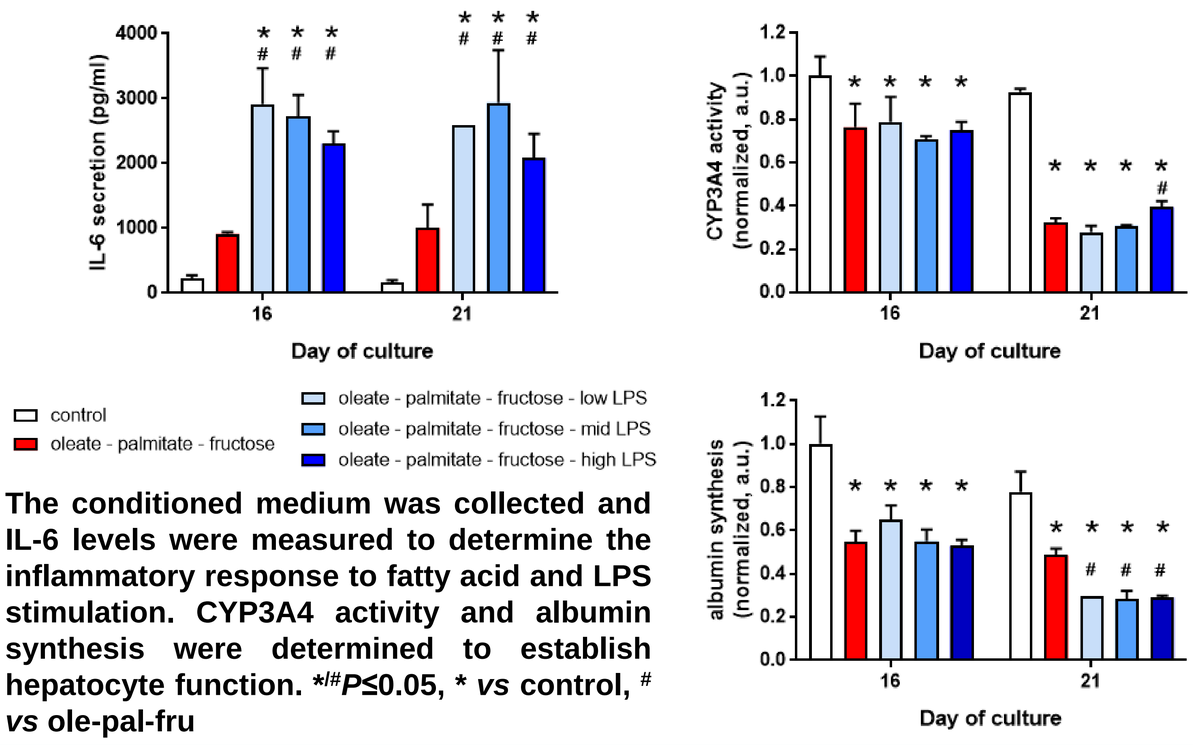


Spheroids cultured for 5 days in culture medium. Immunofluorescent labeling and imaging by confocal microscopy of hepatocytes (left), stellate cells (middle) and Kupffer cells (right) using cell markers as indicated.

Pro-fibrotic cell activation by LPS in steatotic liver spheroids

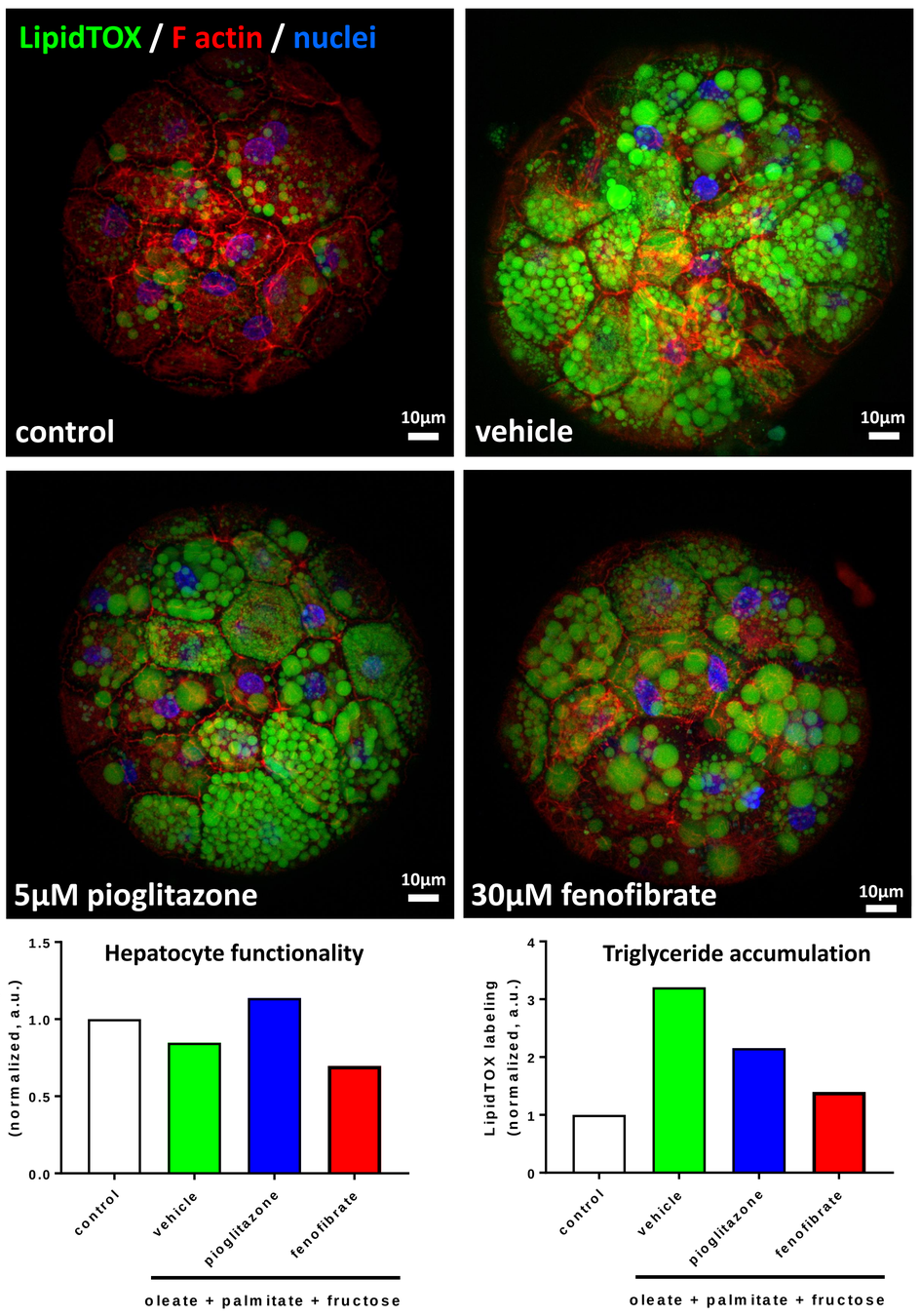
YKL-40 and TIMP1 protein was measured in conditioned medium samples by specific ELISA. n.d. not detected; *$P \leq 0.05$

Inflammation and hepatocyte function



The conditioned medium was collected and IL-6 levels were measured to determine the inflammatory response to fatty acid and LPS stimulation. CYP3A4 activity and albumin synthesis were determined to establish hepatocyte function. */#$P \leq 0.05$, * *vs* control, # *vs* ole-pal-fru

Therapeutic modulation of hepatocyte triglyceride accumulation in spheroids



Spheroids were cultured up to day 9 according to scheme in methods and then treated with pioglitazone or fenofibrate until day 12. LipidTOX labeling of triglycerides was measured by confocal microscopy and fluorometric analysis. CYP3A4 activity was determined to assess hepatocyte functionality.

Conclusions

- A diet-induced disease-mimicking 3D *in vitro* model, closely resembling the pathophysiology of liver steatosis and early fibrosis was developed;
- Liver spheroids composed of primary hepatocytes, stellate and Kupffer cells exhibit expected cell function for at least 21 days;
- The steatosis and steatohepatitis induced by fatty acids and fructose can be modulated by model drugs;
- Additional validation experiments on-going.

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