Establishing Human Liver Cell Models to Investigate the Effects of Exogenous Metabolic Substrates on Fatty Acid Partitioning

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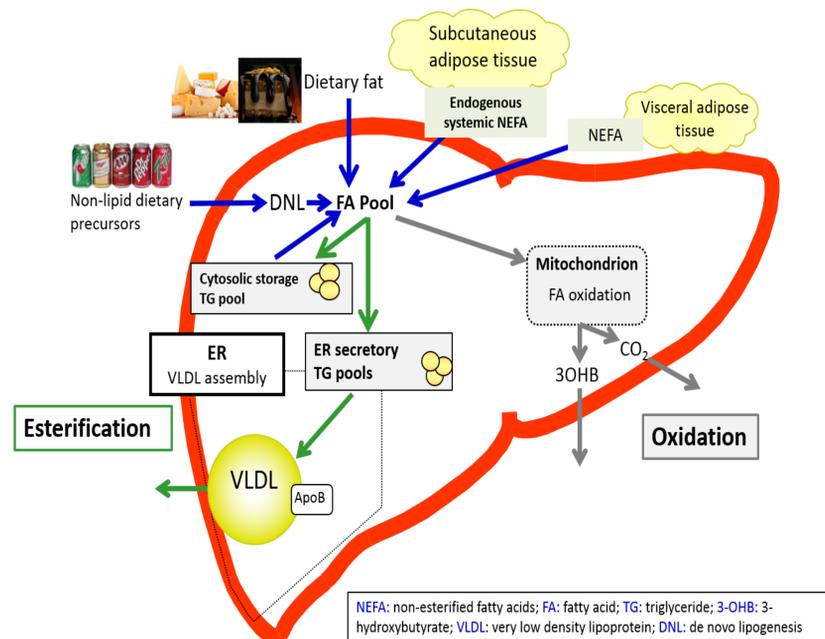UNIVERSITY OF OXFORD

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Introduction

- Hepatic steatosis, accumulation of intracellular triglyceride (TG) (> 5% of hepatic tissue), is the prerequisite for the development of non-alcoholic fatty liver disease (NAFLD).
- NAFLD is associated with obesity and obesity-related metabolic diseases.
- Steatosis occurs due to an imbalance between fatty acid input and removal (fatty acid partitioning) which may be influenced by genetics, diet, hormones and drugs; making the cause of NAFLD difficult to elucidate.
- Hepatocyte cell models that represent fatty acid partitioning within the human liver would be beneficial for the study of the development and progression of NAFLD.

OBJECTIVE: we used a well characterised human liver cell line (LIV0APOLY) and human primary hepatocytes to investigate the effect of specific nutrients (glucose, fructose and fatty acids) on hepatic lipid metabolism
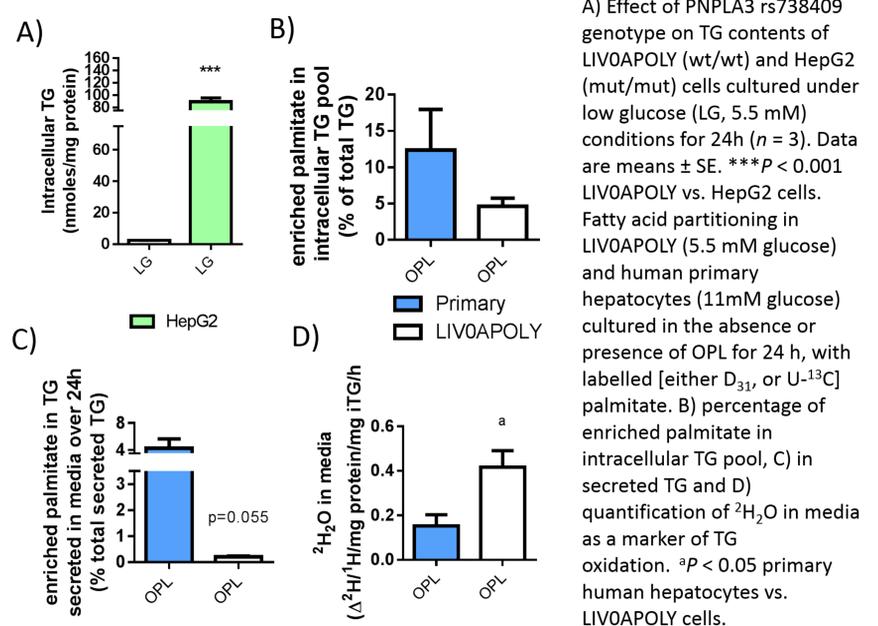
Hepatic fatty acid partitioning

NEFA: non-esterified fatty acids; FA: fatty acid; TG: triglyceride; 3-OHB: 3-hydroxybytrate; VLDL: very low density lipoprotein; DNL: de novo lipogenesis
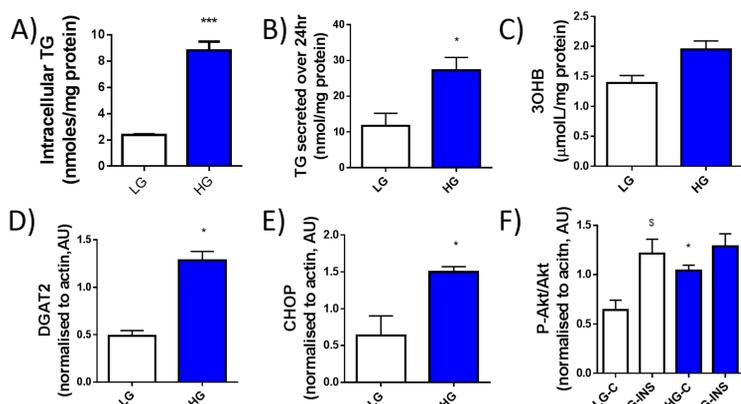
Methods

- LIV0APOLY cells were grown for 48 h on low-glucose (LG, 5.5 mM) or high-glucose (HG, 22.5 mM) DMEM. Primary human hepatocytes cultured for 24hrs (11mM glucose).
- Cells were treated with 0.25% fatty acid-free BSA alone or conjugated to 49.5 μM oleic, 33 μM palmitic, and 27.5 μM linoleic acid (OPL; physiological ratio 45:30:25%).
- Palmitate was labelled [50% D_{31}, 50% U-^{13}C] for 24 h.
- Cells and media were collected for analysis by GC-Ms and biochemical analyser

LIV0APOLY cells effects of exogenous substrates on lipid partitioning

A) Effect of PNPLA3 rs738409 genotype on TG contents of LIV0APOLY (wt/wt) and HepG2 (mut/mut) cells cultured under low glucose (LG, 5.5 mM) conditions for 24h (n = 3). Data are means ± SE. ***$P < 0.001$ LIV0APOLY vs. HepG2 cells. Fatty acid partitioning in LIV0APOLY (5.5 mM glucose) and human primary hepatocytes (11mM glucose) cultured in the absence or presence of OPL for 24 h, with labelled [either D_{31}, or U-^{13}C] palmitate. B) percentage of enriched palmitate in intracellular TG pool, C) in secreted TG and D) quantification of ^2H_2O in media as a marker of TG oxidation. ^{a}$P < 0.05$ primary human hepatocytes vs. LIV0APOLY cells.

Effects of high glucose on lipid portioning and cell health

A) Effect of high glucose (HG, 22.5mM) on LIV0APOLY cells. LIV0APOLY cells treated with either LG or HG for 48h A) intracellular TG storage, B) secreted TG into media over 24h, C) appearenace of 3-hydroxybytarte (3OHB) in the media, D) DGAT2 protein expression, E) CHOP protein expression. LIV0APOLY cells treated with either LG or HG for 48h in the absence or presence of 100nM insulin (10min) F) protein phosphorylation of Akt (S473). *$P < 0.05$ LG vs HG, *** $P < 0.001$. $ $P < 0.05$ C vs Ins.

Conclusions

- LIV0APOLY cells represent a human liver cell line that can be used to investigate lipid partitioning independently of genotype.
- Culturing liver cells in high glucose (22.5mM) promotes ER stress, steatosis and insulin resistance.

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