Optimising human hepatocyte models for metabolic phenotype: effects of treatment with dimethyl sulfoxide (DMSO)

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Background & Aims

Primary human hepatocytes are considered the "gold standard" *in vitro* cellular model to explore hepatic metabolic phenotype, however they come with limitations, such as donor variability, lack of proliferation and rapid phenotype loss. The human hepatoma cell line, HepG2, has been used extensively in cell-based metabolic studies but they have limitations including their malignant origin and inherent low rates of triglyceride secretion. The aim of this study was to investigate whether dimethyl sulfoxide (DMSO) supplementation in the cell media could enhance HepG2 cell metabolic functionality leading to development of an improved model that may more closely resemble primary human hepatocytes.

Methods

HepG2 cells were cultured in cell media containing 1% DMSO for 7,14 and 21 days and gene expression, protein levels, intracellular triglyceride content and triglyceride, urea and 3-hydroxybutyrate concentrations in the cell medium were measured.

Results

- mRNA expression of 4 markers of hepatocyte liver function (albumin, HNF4A, transthyretin and α1-antithrypsin) changed with DMSO treatment to levels that were similar to those seen in primary cultures of human hepatocytes. *(Figure 1a-1d)*
- mRNA expression of the tumor marker alpha-fetoprotein decreased, suggesting a less malignant phenotype. ER stress marker expression (CHOP, IRE) was also decreased, as shown by western blotting experiments. *(Figure 1e,1f)*
- DMSO treatment decreased intracellular triglyceride content while media triglyceride and 3-hydroxybutyrate levels increased in a time-dependent manner. (*Figure 2)* DMSO also significantly decreased the mRNA expression of genes involved in lipid metabolism (ACC1, ACC2, DGAT1, DGAT2, FAS, SCD). *(Figure 3)*
- DMSO treatment significantly increased the mRNA expression of genes involved in glucose metabolism (PEPCK, G6PC). Changes in mRNA expression were mirrored by changes at the protein level as measured by western blotting experiments. *(Figure 4)*

Liver function and ER stress markers

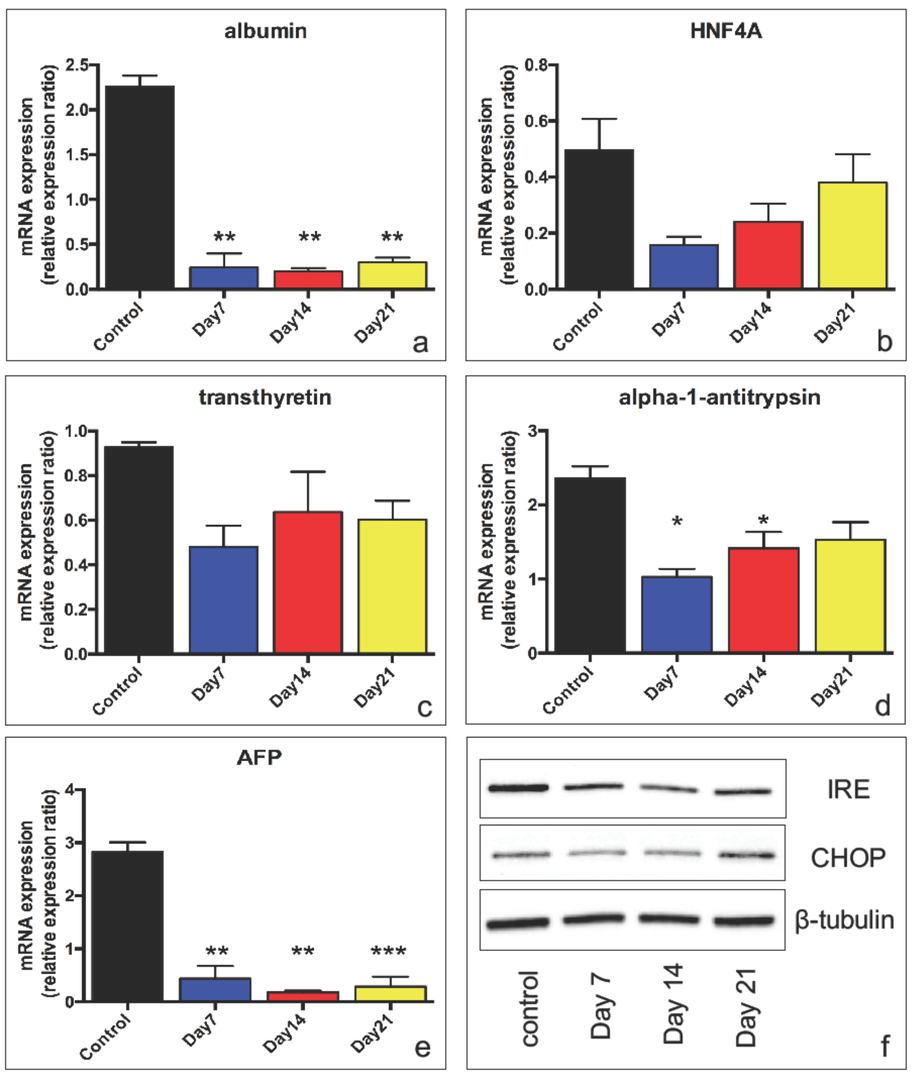

***Figure 1**: mRNA expression of the hepatic differentiation markers a. albumin, b. HNF4A, c. transthyretin and d. α1-antithrypsin were decreased with DMSO treatment to levels that were similar to those from primary human hepatocytes. Also, AFP mRNA expression (e) was decreased, as well as the protein levels of the ER stress markers IRE and CHOP (f), suggesting a less malignant phenotype.*

Lipid metabolism

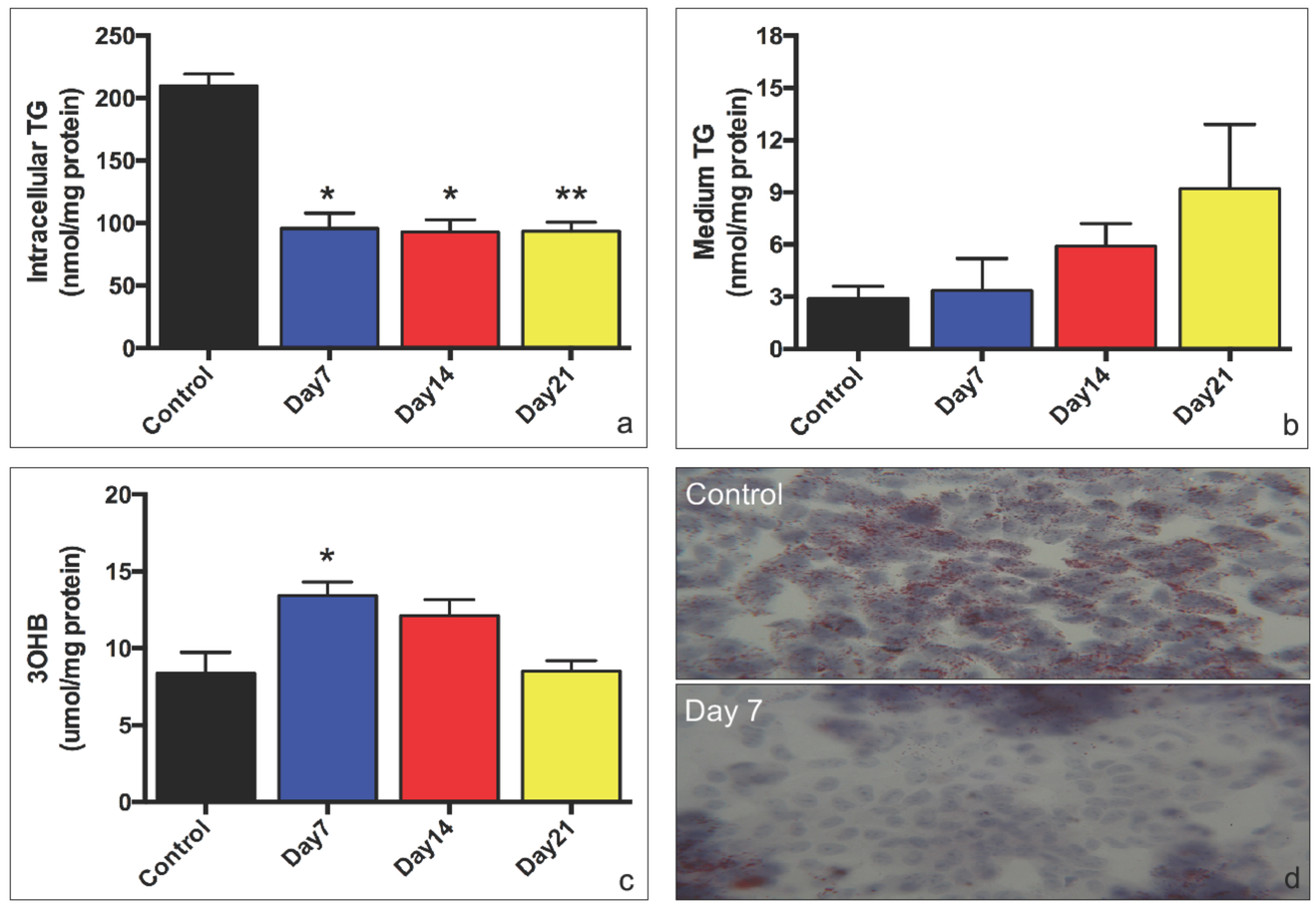

***Figure 2:** DMSO treatment decreased intracellular triglyceride content (a, d) and increased medium triglyceride (b) as well as 3-hydroxybutyrate levels (c) in a time-dependent manner.*

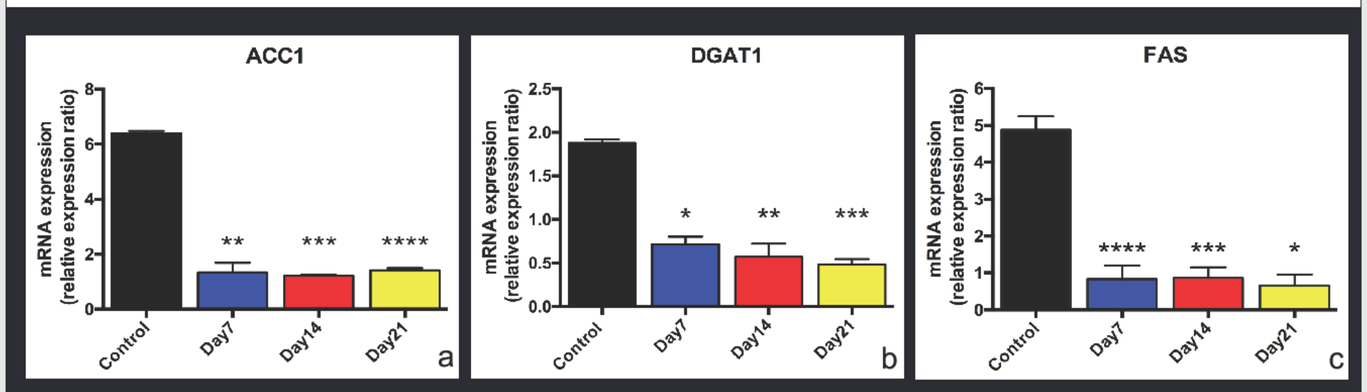

***Figure 3:** DMSO treatment decreased the mRNA expression of genes involved in lipid metabolism: a. ACC1, b. DGAT1 and c. FAS, in a time-dependent manner, as measured by qPCR.*

Glucose metabolism

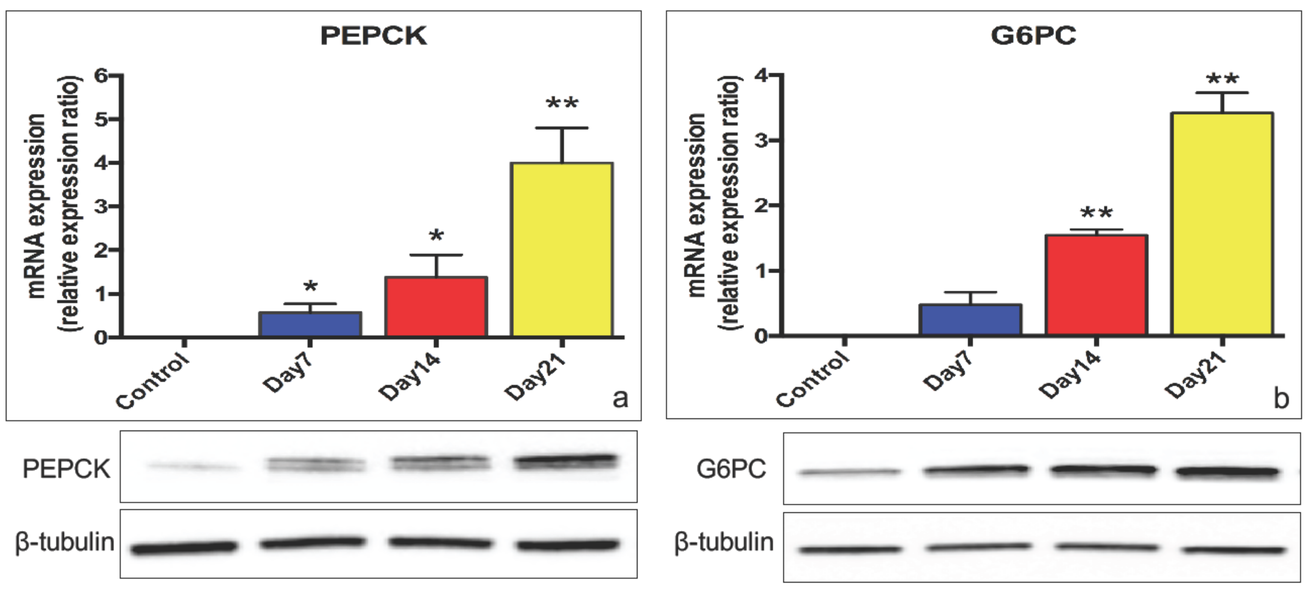

***Figure 4:** DMSO treatment increased a. PEPCK and b. G6PC mRNA expression in a time-dependent manner, as measured by qPCR. Changes in mRNA expression were mirrored by changes at the protein level as measured by western blotting.*

Conclusion

Our data demonstrate that DMSO treatment changes the metabolic phenotype of HepG2 cells such that they more closely resemble primary human hepatocytes and has the potential to significantly enhance currently available cell systems to study liver biology.

ECE 2016 208--EP

Cardiovascular Endocrinology and Lipid Metabolism

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Poster presented at: ECE2016 28 – 31 May 2016 MUNICH, GERMANY 18th EUROPEAN CONGRESS OF ENDOCRINOLOGY

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