5β-reductase (AKR1D1) is a regulator of glucose homeostasis in human hepatocytes and development of model systems to define its role in metabolic liver disease.

Nikolaos Nikolaou*, James Dunford, Wenhwa Lee, Charlotte Green, Laura Gathercole, Leanne Hodson, Jeremy Tomlinson

University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism, United Kingdom

**E-mail address: nikolaos.nikolaou@keble.ox.ac.uk*

Background & Aims

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. Steroid hormones, including glucocorticoids and sex steroids, regulate metabolic phenotype; additionally bile acids have been identified as potent metabolic regulators. 5β-reductase (AKR1D1), is predominantly expressed in the liver, and is a crucial regulator of steroid hormone clearance as well as bile acid synthesis. Its role in the pathogenesis of metabolic disease has not been examined. Therefore, we developed systems to define the enzymology of human AKR1D1 in cell free assays, to determine the impact of manipulation of AKR1D1 expression and activity in human hepatocyte models.

Methods

- B21 bacteria cells were transformed with an AKR1D1 construct and the recombinant protein extracted and purified. A high throughput assay was developed to determine AKR1D1 activity, substrate specificity and enzyme kinetics, using fluorimetric analysis through NAPDH utilization.
- AKR1D1 expression was genetically manipulated in HepG2 cells through over-expression and siRNA silencing assays and confirmed using qPCR. Changes in gene expression were paralleled by functional activity as measured by cortisone clearance and tetrahydrocortisone generation using Gas Chromatography/ Mass Spectrometry (GC/MS).
- mRNA, intracellular triglyceride (TAG) and cell culture media were collected and gene expression, intracellular triglyceride, media glucose and media 3-hydroxybutyrate (3-OHB) levels were determined, using an iLAB 600 Clinical Analyser.

Results

- AKR1D1 enzyme kinetics were determined for 7 steroid substrates and 2 bile acid intermediates *(Table 1)*. AKR1D1 activity was inhibited by Finasteride (selective 5α-reductase type 2 inhibitor), but not Dutasteride (non-selective 5α-reductase type 1 inhibitor) *(Figure 1a,1b)*
- AKR1D1 mRNA expression was increased after over-expression as measured by qPCR; cortisone clearance was also increased in a time-dependent manner. *(Figure 2a, 2b)* Furthermore, mRNA expression was decreased after successful gene silencing as measured by qPCR and decreased cortisone clearance. *(Figure 2c, 2d)*
- AKR1D1 knockdown increased the mRNA expression of the glucose transporters GLUT1 and GLUT9, as measured by qPCR *(Figure 3a, 3b)*. In addition, AKR1D1 knockdown decreased the extracellular glucose concentration in the cell culture media *(Figure 4)*.
- AKR1D1 knockdown increased the mRNA expression of ACC1, the rate-limiting enzyme of fatty acid synthesis, as measured by qPCR *(Figure 5a)*. In addition, AKR1D1 knockdown increased the intracellular triglyceride content and decreased fatty acid oxidation, as measured by decreased 3-hydroxybutyrate levels in the cell culture media *(Figure 5b, 5c)*.

Substrate specificity and enzyme kinetics

Substrate	Vmax (FU/min)	Km	Kcat	Kcat/Km
Aldosterone	15.429	6.65	0.83	0.124
Cortisone	13.577	5.44	0.73	0.134
Testosterone	10.5	7.2	0.73	0.101
Cortisol	8.571	25.42	0.46	0.018
Androstenedione	6.546	3.1	0.35	0.113
Corticosterone	5.634	4.36	0.30	0.069
Progesterone	4.743	3.29	0.25	0.077
7a hydroxy cholestenone	4.737	25.77	0.24	0.009
7a,12a dihydroxy cholestenone	2.789	15.82	0.15	0.009

Table 1: *Enzyme kinetic analysis (Km, Kcat and Kcat/Km) for AKR1D1 substrates, as measured by high throughput screening assay.*

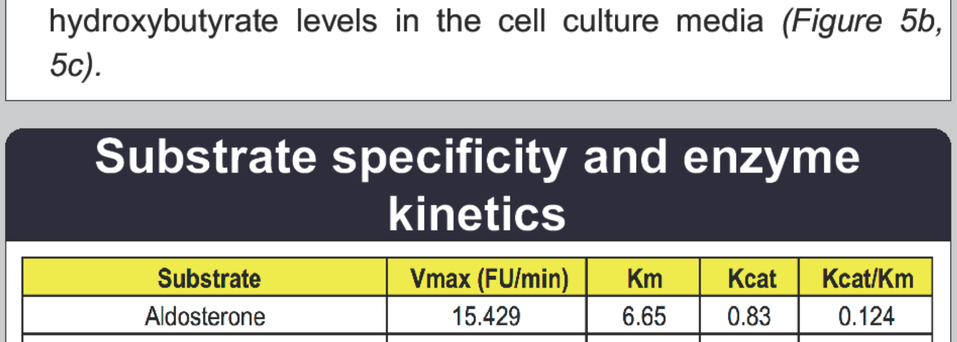

Figure 1: *AKR1D1 activity (a) and inhibition by Finasteride and Dutasteride (b) as measured by NAPDH reduction (AU), using cortisone as a substrate.*

AKR1D1 genetic manipulation

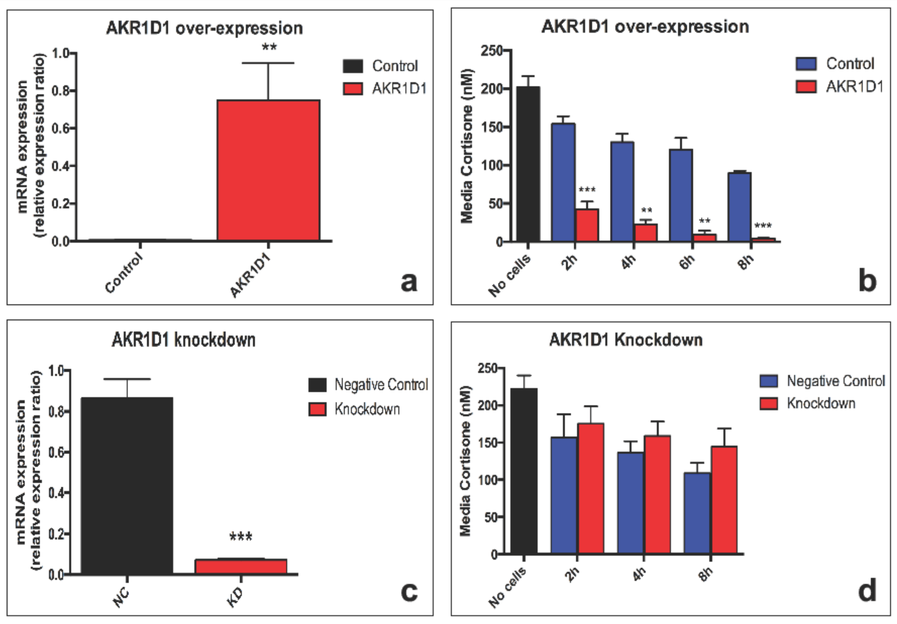

Figure 2: *AKR1D1 over-expression, as measured by qPCR (a) and cortisone clearance (b) and AKR1D1 knockdown, as measured by qPCR (c) and cortisone clearance (d) in HepG2 cells, in a time-dependent manner. Cortisone clearance was measured by GC/MS.*

AKR1D1 regulates glucose flux

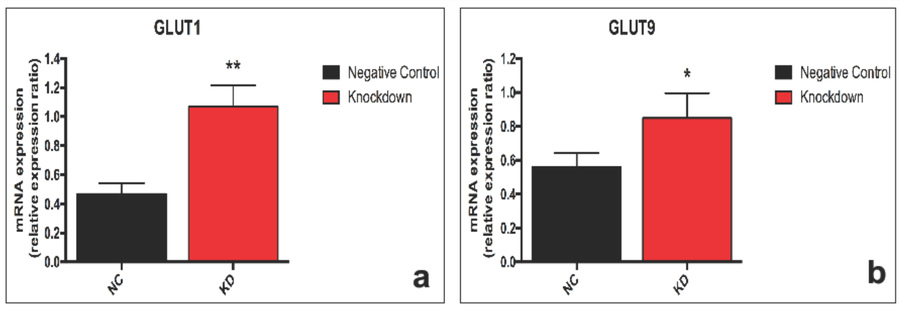

Figure 3: *AKR1D1 silencing increased GLUT1 (a) and GLUT9 (b) mRNA expression, as measured by qPCR.*

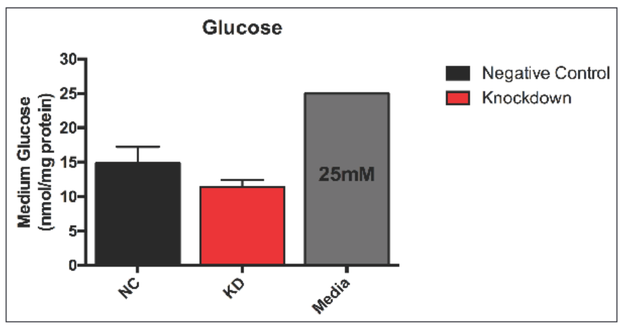

Figure 4: *AKR1D1 knockdown decreased glucose levels in the cell culture media, as measured by iLAB Clinical Analyser.*

AKR1D1 regulates lipid metabolism

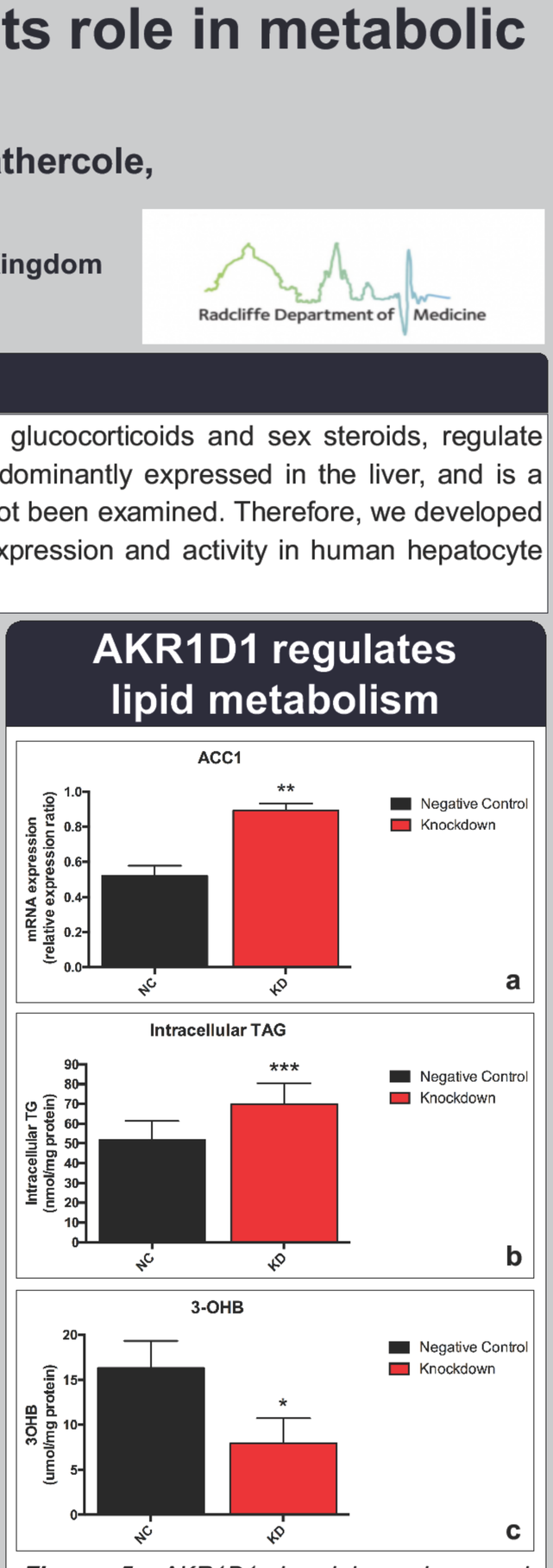

Figure 5: *AKR1D1 knockdown increased ACC1 mRNA expression (a) and intracellular triglyceride (b) and decreased 3-hydroxybutyrate levels in the cell culture media (c).*

Proposed role of AKR1D1 in hepatic glucose and lipid metabolism

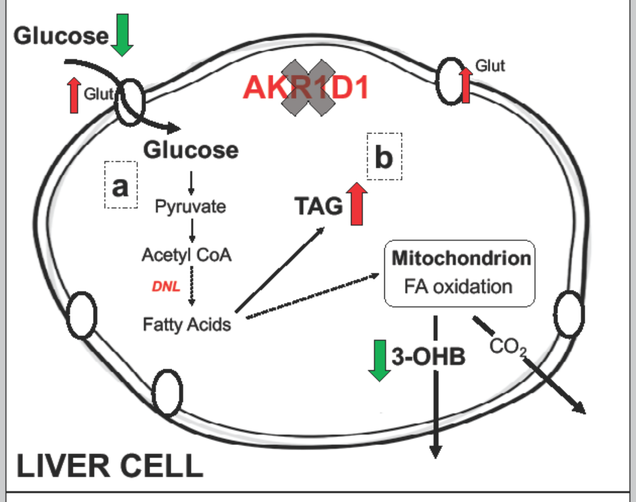

Figure 6: *Overview of the proposed role of AKR1D1 silencing in (a) glucose and (b) lipid metabolism within human hepatocytes.*

Conclusion and future studies

We have successfully characterised human AKR1D1 in a cell-free system and determined its activity and enzyme kinetics. Our data demonstrate that Finasteride, but not Dutasteride, inhibits AKR1D1 activity, although the inhibition was modest. In addition, we have genetically manipulated AKR1D1 activity in a hepatocyte model; although AKR1D1 over-expression was without effect (possibly relating to existing high basal levels of expression), we demonstrate that AKR1D1 knockdown regulates glucose homeostasis, triglyceride accumulation and fatty acid oxidation in human hepatocytes and therefore is likely to have an important role in the control of metabolic phenotype within the liver *(Figure 6)*.

Future studies will include determination of the rate of *de novo* lipogenesis within hepatocytes, along with unbiased "omic" approaches in order to further elucidate the phenotype associated with this genetic manipulation.

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Receptors & Signaling

Nikolaou Nikolaos

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