Regulation of lipogenesis in human hepatocytes by androgens, glucocorticoids and 5α-reductase.

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Background

Non-alcoholic fatty liver disease [NAFLD] is rapidly becoming the commonest cause of liver cirrhosis and leading indication for liver transplant worldwide. It is tightly associated with obesity and type 2 diabetes, yet the precise mechanisms that drive its aetiology are not fully defined. Dysregulation of both glucocorticoid and androgen metabolism has been implicated in its pathogenesis. The availability of these hormones to bind and activate their receptors is regulated by 5α-reductase type 2 [5αR2] that inactivates glucocorticoids and converts testosterone [T] to dihydrotestosterone [DHT]. We have therefore explored the role of androgens and glucocorticoids and their metabolism by 5αR2 upon lipid homeostasis in human hepatocytes.

Methods

C3A human hepatoma cells and primary human hepatocytes were cultured and treated with Testosterone [T] (5nM, 50nM) or the more potent androgen, Dihydrotestosterone [DHT] (1nM, 10nM) for 24h. Lipid accumulation was measured by C14 acetate incorporation into triglyceride and gene expression by real-time PCR. As an additional model of androgen excess, cells were transfected with an androgen receptor (AR) construct (pcDNA3.1+AR) or vector alone as a control. In addition, C3A cells were treated with Cortisol and transfected with a 5α-reductase (SRD5A2) construct and lipid accumulation was measured as previously. Finally, pharmacological inhibitors of 5α-reductase isoforms were used in primary cultures of human hepatocytes. Between-group comparisons were made with T-Test and ANOVA.

Results

Despite androgen receptor (AR) expression being undetectable in C3A cells, FAS, ACC1, ACC2 and CPT1 mRNA expression was significantly increased after treatment with testosterone and DHT in a dose-dependent manner (figure 1) suggesting a receptor independent action. Endorsing these data, both testosterone and DHT increased *de novo* lipogenesis [DNL] as measured by C14-acetate incorporation into triglyceride (ctrl 100% vs. T (50nM, 24h) 124.9±6.2%, DHT (10nM, 24h) 128.1±4.7%), p<0.05) (figure 2). Following AR transfection, even in the absence of ligand, lipogenic gene expression increased (figure 3) as did *de novo* lipogenesis (figure 4) suggesting a ligand-independent action (ctrl 100% vs. AR 202.7±5.3%, p<0.05). Similar observations were made in primary human hepatocytes from female, but not male donors (figure 5). Glucocorticoids decreased DNL, an effect that was abrogated by overexpression of 5αR2 and augmented by pharmacological inhibition of 5αR2 activity (e.g. 88.3±5.3 vs. 76.9± 5.2%, cortisol vs. cortisol + finasteride, p=0.05) (figures 6,7,8).

Impact of androgens on lipid metabolism in C3A human hepatoma cells.

Gene expression.

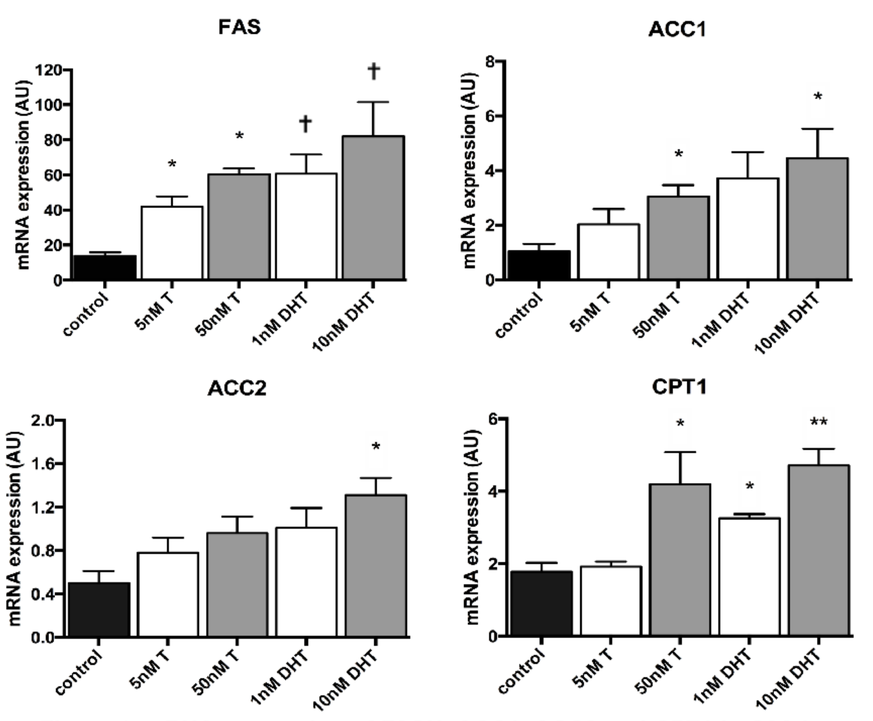

***Figure 1:* mRNA expression of FASN, ACC1, ACC2 and CPT1 in C3A human hepatoma cells increased across dose dependent treatment with testosterone and DHT.** *Data shown as the mean of arbitrary units (AU).*

Lipogenesis.

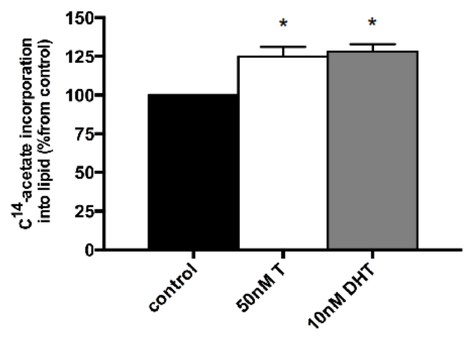

***Figure 2: De novo* lipogenesis in C3A cells increased across treatment with testosterone and DHT measured by scintillation counting.** *Data shown as % change from control.*

Impact of AR over expression on lipid metabolism.

Gene expression.

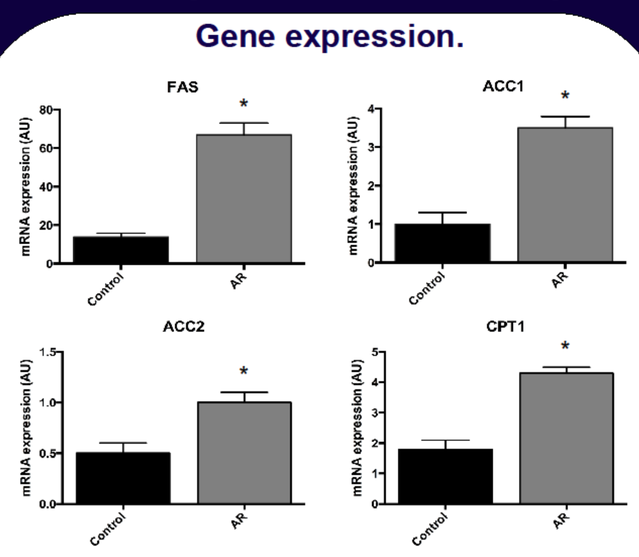

***Figure 3*: mRNA expression in C3A human hepatoma cells increased across transfection with AR measured by real time PCR.** *Data shown as the mean of arbitrary units (AU).*

Lipogenesis.

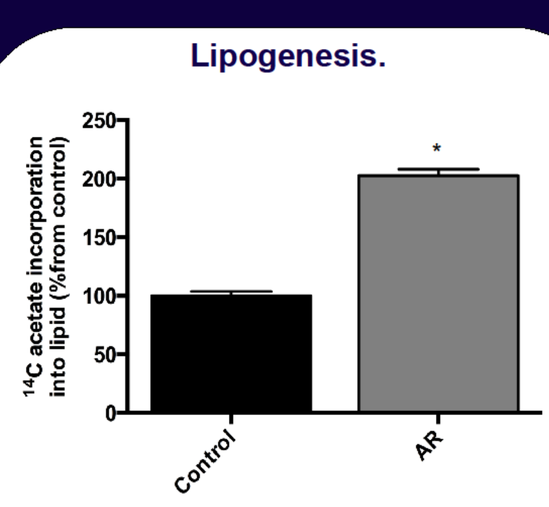

***Figure 4: De novo* lipogenesis increased across transfection in C3A cells.** *Data shown as % change from control.*

Impact of androgens on lipid metabolism in primary human hepatocytes.

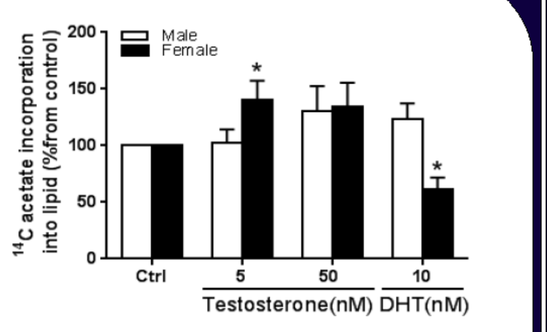

***Figure 5: De novo* lipogenesis in primary human hepatocytes increased across treatment with testosterone but not with DHT measured by scintillation counting.** *Data shown as % change from control.*

Impact of glucocorticoids on lipid metabolism in primary human hepatocytes.

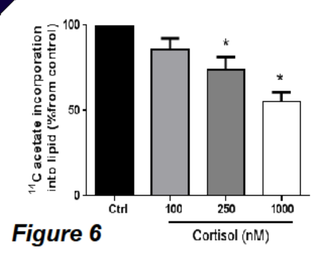

Figure 6

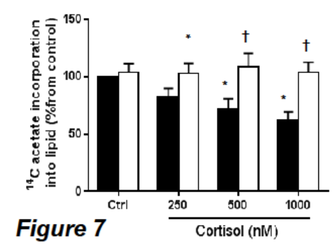

Figure 7

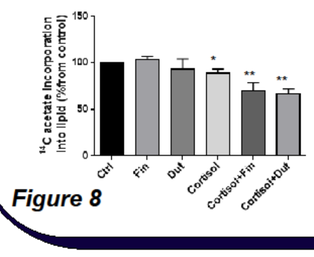

Figure 8

De novo* lipogenesis decreased across treatment with cortisol (*figure 6*), but was restored by SRD5A2 overexpression (*figure 7*) and augmented by 5aR2 inhibitors *(figure 8). *Data shown as % change from control.*

Conclusion

Increased mRNA expression of FAS, ACC1 and ACC2 as well decreased CPT1 mRNA expression contribute to the increase in *de novo* lipogenesis that is observed with testosterone and DHT treatment. Surprisingly, we also observed that AR overexpression alone, in the absence of ligand, also regulates hepatic lipid metabolism by increasing both the expression of key components of the lipogenic pathway (FAS, ACC1, ACC2) and functional lipid accumulation. We have shown that glucocorticoids decrease *de novo* lipogenesis in a dose-dependent manner and manipulation of 5αR2 activity can regulate lipogenesis in human hepatocytes *in vitro*. These data demonstrate that androgens and glucocorticoids are able to stimulate lipid accumulation in human hepatocytes and this may be crucial in understanding the association between PCOS and NAFLD.

17 ECE
4--GP-04
Steroids, developmental and paediatric endocrinology
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DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY