Androgen receptor over expression drives lipid accumulation in human hepatocytes.

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Background
Non alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common cause of hepatic dysfunction in the western world. It encompasses a spectrum of disease ranging from simple lipid accumulation within hepatocytes to steatohepatitis (NASH) with fibrosis and leading finally to cirrhosis and liver failure. Recent studies have shown an association between NAFLD and androgen deficiency, yet in the majority of patients with NASH, androgen levels are normal. In contrast, in patients with polycystic ovarian syndrome (PCOS) which is characterised by androgen excess, hepatic steatosis is prevalent. Our hypothesis is that androgen exposure may be a critical regulator of lipid flux within human hepatocytes.

Methods
C3A human hepatoma cells 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. Between-group comparisons were made with T-Test and ANOVA.

Results
Despite androgen receptor (AR) expression being undetectable in C3A cells, FASN, ACC1, ACC2 and CPT1 mRNA expression was significantly increased after treatment with testosterone and DHT in a dose-dependent manner (figure 1) suggesting a non-genomic action. Endorsing these data, both testosterone and DHT increased de novo lipogenesis as measured by C14-acetate incorporation into triglyceride (ctrl 7002±259 vs. T 8748±433, DHT 8970±330, p<0.05). Following AR transfection (figure 3, 4), even in the absence of ligand, lipogenic gene expression increased (FASN: ctrl 13.9±2.0 vs. AR 66.8±6.2, ACC1: ctrl 1.0±0.3 vs. AR 3.5±0.3, ACC2: ctrl 0.5±0.1 vs. AR 1.0±0.1, CPT1: ctrl 1.8±0.3 vs. AR 4.3±0.2, p<0.05) (figure 5) as did de novo lipogenesis (figure 6) suggesting a ligand-independent action ( ctrl 7002±259 vs. AR 14193±755, p<0.05). Lipogenesis was further increased following incubation of AR over-expressing C3A cells with testosterone and DHT.

Conclusion
Increased mRNA expression of FASN, 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 over-expression alone, in the absence of ligand, also regulates hepatic lipid metabolism by increasing both the expression of key components of the lipogenic pathway (FASN, ACC1, ACC2) and functional lipid accumulation. In conclusion, these data demonstrate that enhanced androgen action is able to stimulate lipid accumulation in human hepatocytes and this may be crucial in understanding the association between PCOS and NAFLD.