UNIVERSITY OF BIRMINGHAM
CEDAM
MRC Medical Research Council

Androgen receptor over expression drives lipid accumulation in human hepatocytes.

N Nikolaou[1], M Nasiri[1], LL Gathercole[1], S Parajes[1], N Krone[1], G Valsamakis[2], G Mastorakos[2], JW Tomlinson[1]

[1]University of Birmingham, Centre for Endocrinology, Diabetes and Metabolism, United Kingdom
[2]Endocrine Unit, Aretaieion Hospital, University of Athens, Medical School, Athens, Greece

National and Kapodistrian UNIVERSITY OF ATHENS

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.

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

Gene expression.

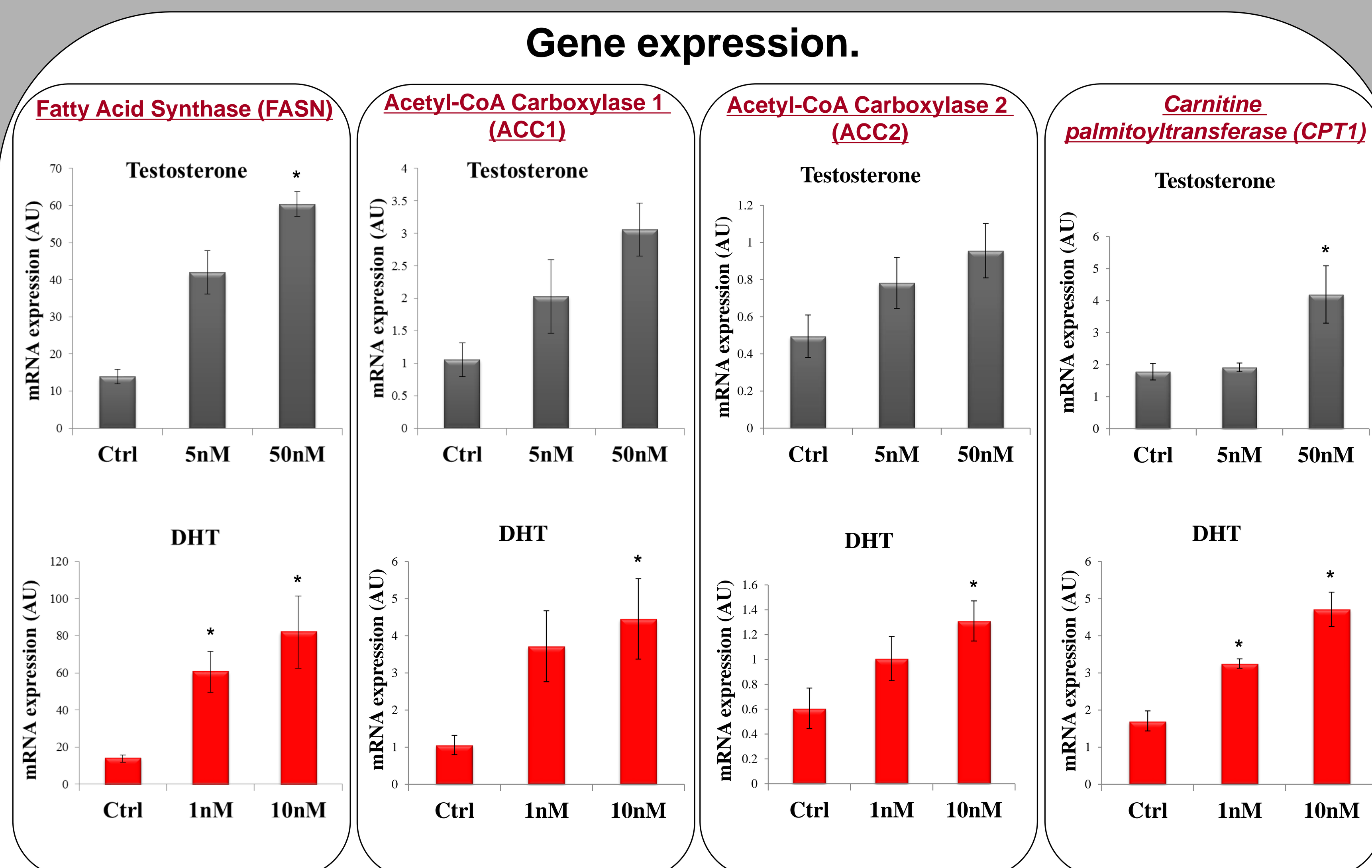

Figure 1: mRNA expression of FASN, ACC1, ACC2 and CPT1 in C3A human hepatoma cells increased across dose dependent treatment with testosterone and DHT. *Data shown as the mean of arbitrary units (AU).*

Lipogenesis.

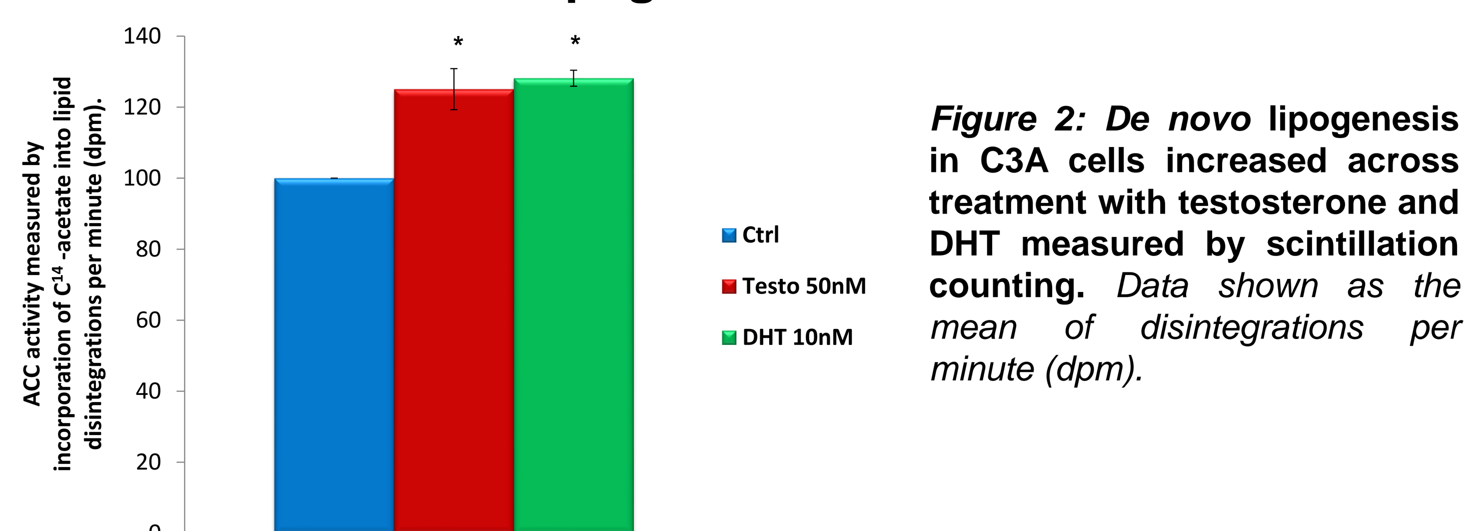

***Figure 2: De novo* lipogenesis in C3A cells increased across treatment with testosterone and DHT measured by scintillation counting.** *Data shown as the mean of disintegrations per minute (dpm).*

AR over expression as a model of androgen excess.

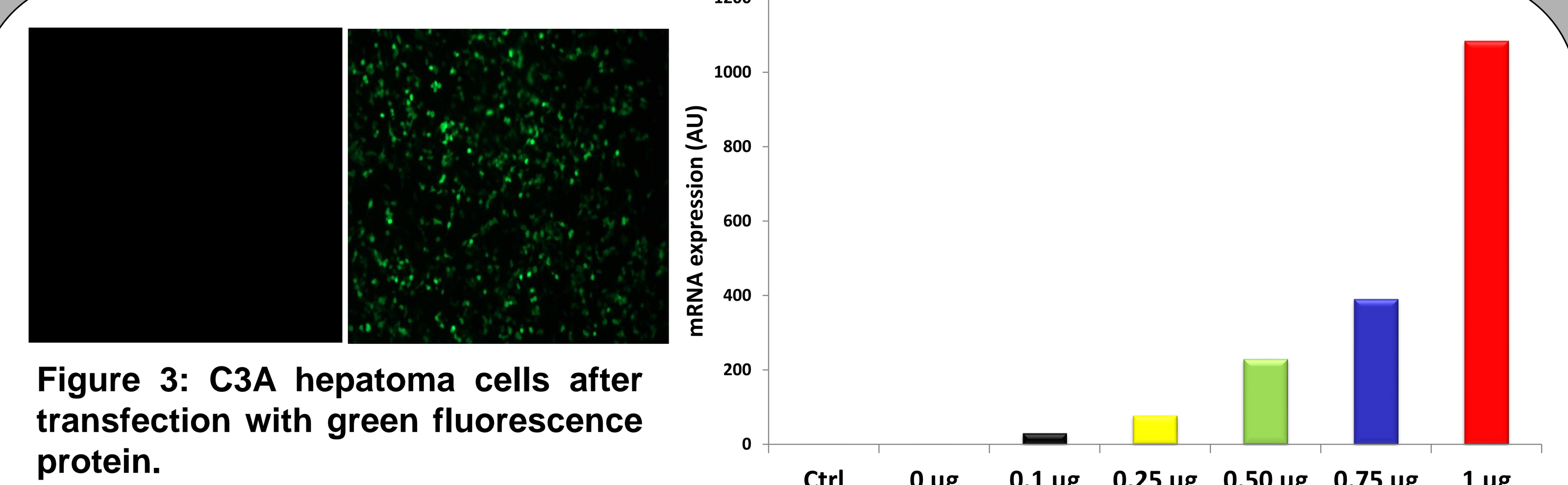

Figure 3: C3A hepatoma cells after transfection with green fluorescence protein.

Figure 4: AR mRNA expression increased across increasing concentrations measured by real time PCR. *Data shown as the mean of arbitrary units (AU).*

Impact of AR over expression on lipid metabolism.

Gene expression.

Lipogenesis.

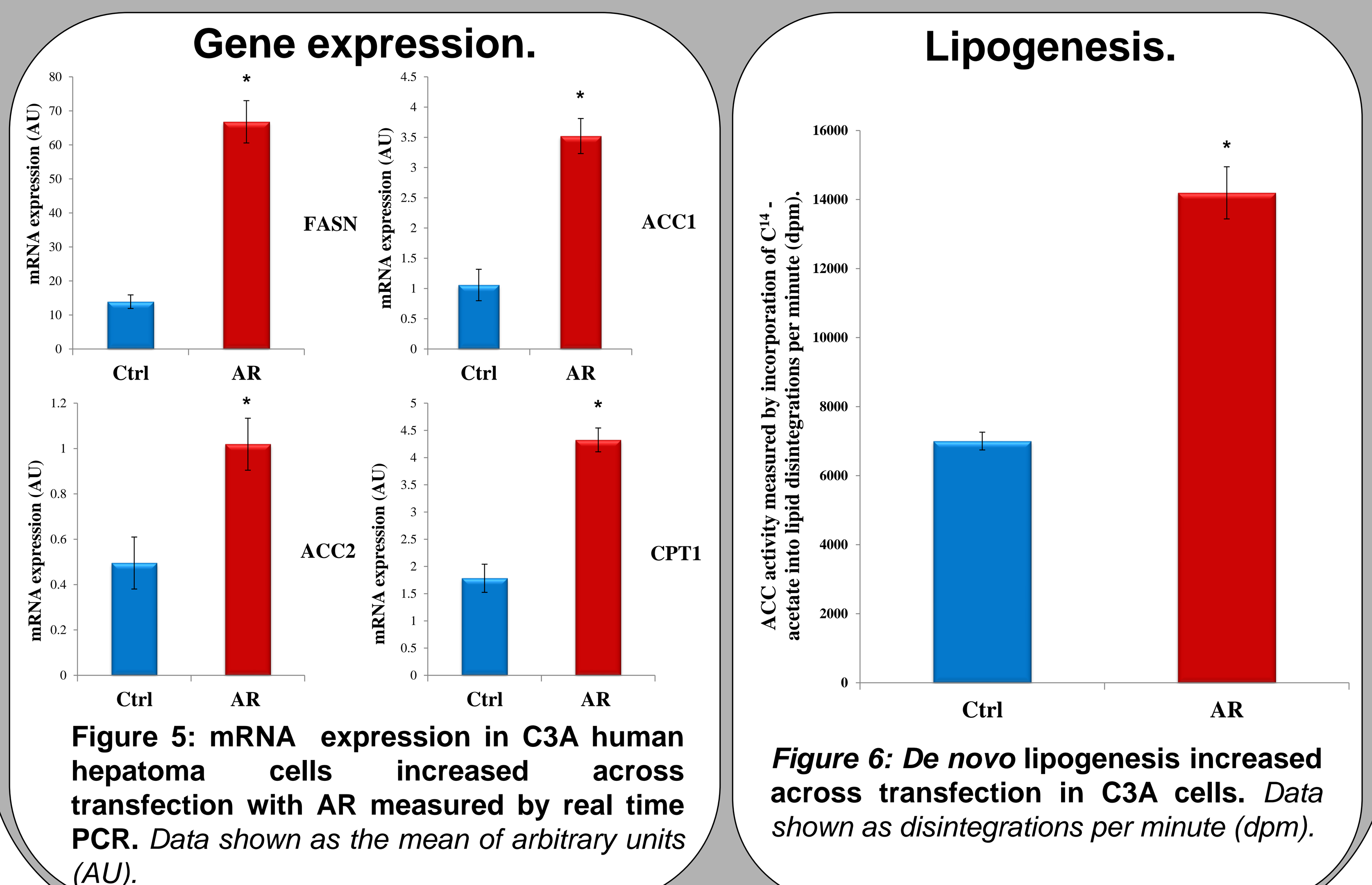

Figure 5: mRNA expression in C3A human hepatoma cells increased across transfection with AR measured by real time PCR. *Data shown as the mean of arbitrary units (AU).*

***Figure 6: De novo* lipogenesis increased across transfection in C3A cells.** *Data shown as disintegrations per minute (dpm).*

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.