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

N Nikolaou¹, M Nasiri², LL Gathercole¹, S Parajes², N Krone², G Valsamakis³, G Mastorakos³, JW Tomlinson¹

¹University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism, United Kingdom
²University of Birmingham, Centre for Endocrinology, Diabetes and Metabolism, United Kingdom
³Endocrine Unit, Aretaieion Hospital, University of Athens, Medical School, Athens, Greece

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).

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.