5α-reductase type 2 regulates glucocorticoid action and metabolic phenotype in human hepatocytes

Maryam Nasiri¹, Nikolaos Nikolaou²,³, Silvia Parajes¹ Nils P Krone³, George Valsamakis³, George Mastorakos³, Beverly Hughes¹, Angela Taylor¹, Iwona J Bujalska¹, Laura L Gathercole², and Jeremy W Tomlinson²

Centre for Endocrinology, Diabetes and Metabolism, Institute of Biomedical Research, School of Clinical and Experimental Medicine, University of Birmingham, Edgbaston, Birmingham, UK. B15 2TT; 2. Oxford Centre for Diabetes, Endocrinology & Metabolism, NIHR Oxford Biomedical Research Centre, University of Oxford, Churchill Hospital, Headington, Oxford, UK. OX3 7LJ; 3. Endocrine Unit, second Department of Obstetrics and Gynecology and Pathology Department, Aretaieion University Hospital, Athens Medical School, Athens, Greece

Glucocorticoids and androgens have both been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD); androgen deficiency in males, androgen excess in females and glucocorticoid excess in both sexes are associated with NAFLD. Glucocorticoid and androgen action are regulated at a pre-receptor level by the enzyme 5α-reductase type 2 (SRD5A2) that inactivates glucocorticoids to their dihydrometabolites and converts testosterone to dihydrotestosterone (DHT). We have therefore explored the role of androgens and glucocorticoids and their metabolism by SRD5A2 upon lipid homeostasis in human hepatocytes.

In both primary human hepatocytes and human hepatoma cell lines, glucocorticoids decreased de novo lipogenesis in a dose-dependent manner. Whilst androgen treatment (testosterone and DHT) increased lipogenesis in cell lines and in primary cultures of human hepatocytes from female donors, it was without effect in primary hepatocyte cultures from men. SRD5A2 over-expression reduced the effects of cortisol to suppress lipogenesis and this effect was lost following transfection with an inactive mutant construct. Conversely, pharmacological inhibition using the 5α-reductase inhibitors finasteride and dutasteride, augmented cortisol action.

We have demonstrated that manipulation of 5α-reductase type 2 activity can regulate lipogenesis in human hepatocytes in vitro. This may have significant clinical implications for those patients prescribed 5α-reductase inhibitors, in particular augmenting the actions of glucocorticoids to modulate hepatic lipid flux.

The global epidemic of obesity and type 2 diabetes is tightly linked to the increasing prevalence of nonalcoholic fatty liver disease (NAFLD) which contributes significantly to increased morbidity and mortality (1). The potent role of glucocorticoids (GC) to modulate metabolic phenotype is exemplified in patients with GC excess, Cushing’s syndrome, and many of these patients develop NAFLD (2). However, in most patients with metabolic disease and NAFLD, circulating GC levels are not elevated (3). At a tissue-specific level, notably within the liver, GCs are cleared by a series of enzymes including the A-ring reductases (5α-reductase type 1 (SRD5A1) and 2 (SRD5A2) and 5β-reductase). 5α-reductase exists as 2 isoforms (SRD5A1 and 2), both have 5 exons and 4 introns, but share less than 50% homology and both isoforms are expressed in human liver (4); SRD5A1 alone is expressed...
in mouse liver. SRD5A2 is believed to be the major isoform in clearing cortisol in human studies (5), however, there is an emerging role for SRD5A1 in the pathogenesis of metabolic disease. We, and others, have shown that in a rodent model, genetic ablation of SRD5A1 increase lipid accumulation in the liver and the severity of NAFLD (6, 7).

The role of androgens in the pathogenesis of metabolic disease remains controversial. There is evidence documenting an association between hypogonadism and NAFLD (8, 9) with some evidence for improvement following androgen treatment (10, 11). SRD5A2 has an established role in the conversion of testosterone to dihydrotestosterone (DHT) and genetic mutations lead to 46XY DSD (Disorder of Sex Development). While DHT is a more potent activator of the androgen receptor (AR), testosterone binds and activates the AR. We have shown that increased global 5α-reductase activity is associated with impaired glucose tolerance and may be a future predictor of metabolic disease (12, 13). The lack of SRD5A2 expression in the mouse liver (contrasting with human liver) has limited the interpretation of data from SRD5A2 knock out mice (7) and has highlighted the importance of the use of human models. The translational importance of this not only relates to enhancing our understanding of the pathogenesis of NAFLD, but also to the widespread use of SRD5A2 inhibitors including the selective, SRD5A2 inhibitor, Finasteride, and the nonselective (SRD5A1 and 2) inhibitor, Dutasteride.

Lipid accumulation within hepatocytes is the first step in the development of NAFLD, and in some individuals can progress through inflammation (NASH), to fibrosis and eventual cirrhosis. There are multiple mechanisms that contribute to lipid accumulation in vivo including re-esterification of free fatty acids (FFA) delivered principally from intra-abdominal tissue depots, de novo synthesis of triacylglycerol from acetyl CoA (de novo lipogenesis, DNL) as well as limitation of β-oxidation and lipid export and secretion. While FFA delivery is believed to be the most important process in the development of NAFLD, the contribution of DNL increases significantly in patients with NAFLD (14). The rate-limiting step in DNL is the carboxylation of acetyl CoA to malonyl-CoA by acetyl CoA carboxylase (ACC) which is subsequently converted by a multistep reaction to palmitate by fatty acid synthase. ACC2 is predominantly and is the key regulatory step of fatty acid synthesis. ACC2 is localized to the mitochondrial membrane, and its role is to limit β-oxidation through malonyl-CoA mediated inhibition of carnitine palmitoyl transferase 1 (CPT 1).

Although it is not possible to replicate all the processes that contribute to the development of NAFLD using in vitro systems, using established cellular models, we have tested the hypothesis that SRD5A2 represents an important regulator of the metabolic actions of androgens and GCs to modulate lipid homeostasis within human hepatocytes.

**Materials and Methods**

**C3A and primary human hepatocyte culture**

The C3A human hepatocyte cell line was purchased from LGC Standards (ATCC - CRL-10 741, Middlesex, UK), and cultured in Eagle’s Minimum Essential Medium (EMEM) containing 10% fetal calf serum and glucose/penicillin/streptococcus. Cells were seeded in 24-well plates and at 70%–80% confluence were incubated with control media with or without hormonal treatments. The precise conditions for individual experiments is detailed in the results section. All reagents were supplied by Sigma-Aldrich (Dorset, UK) unless otherwise stated.

Primary human hepatocytes were purchased from Celsis In Vitro Technologies (Baltimore, Maryland, US). All donors were healthy, nondiabetic, none consumed alcohol above recommended limits (female < 14 U/wk, male < 21 U/wk), none were taking regular medications and all had negative viral hepatitis serology (male: n = 4, age 54 ± 14 years, BMI 28.4 ± 3.3 kg/m²; female: n = 4, age 56 ± 4.7 years, BMI 23.98 ± 3.1 kg/m²). Cells were cultured overnight in Williams’ Medium E without any supplements before being treated with GCs or androgens. For insulin signaling studies, media was spiked with insulin 15 minutes prior to cell harvest as described above. Lipogenesis was measured by the uptake of 1-[14C]-acetate into the lipid component (see below)

**RNA extraction and Reverse Transcription**

Total RNA was extracted from tissue and cells using the Tri-Reagent system. RNA integrity was assessed by electrophoresis on 1% agarose gel. Concentration was determined spectrophotometrically at OD$_{260}$. In a 50 μl volume, 500ng of total RNA was incubated with 250μM random hexamers, 500μM dNTPs, 20U RNase inhibitor, 63U Multiscribe reverse transcriptase, 5.5 mM MgCl$_2$ and 1 x reaction buffer. The reverse transcription reaction was carried out at 25°C for 10min, 48°C for 30min and then the reaction was terminated by heating to 95°C for 5min.

**Real-Time PCR**

mRNA levels were determined using an ABI 7500 sequence detection system (Perkin-Elmer Applied Biosystems, Warrington, UK). Reactions were performed in 10μl volumes on 96-well plates in reaction buffer containing 2 x TaqMan Universal PCR Master mix (Applied Biosystems, Foster City, CA, USA). All primers and probes were supplied by Applied Biosystems ’assay on demand’ (Applied Biosystems) and reactions normalized against the housekeeping gene 18S rRNA, provided as a preoptimized control probe. All target genes were labeled with FAM and the housekeeping gene with VIC. The reaction conditions were as follows: 95°C for 10-minutes, then 40 cycles of 95°C for 15-seconds and 60°C for 1min.

Data were obtained as ct values (ct=cycle number at which logarithmic PCR plots cross a calculated threshold line) and used
to determine Δct values (Δct = (ct of the target gene) – (ct of the housekeeping gene)). Data were expressed as arbitrary units using the following transformation [expression = 10000.(2−ΔΔct)] arbitrary units (AU).

**Protein extraction and Immunoblotting**

Total protein was extracted from cells using RIPA buffer (50 mM Tris pH 7.4, 1% NP40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF and protease inhibitor cocktail (Roche, Lewes, UK) dissolved in 10 mL of distilled water) and freeze thawing. Protein concentrations were measured using a commercially available assay (Bio-Rad Laboratories Inc., Hercules, CA). 15 μg of protein was resolved on a 12.5% SDS PAGE gel and transferred onto nitrocellulose membrane, Hybond ECL (GE Healthcare, Chalfont St Giles, UK). Primary (PKB/akt, Biosource, Nivelles, Belgium and anti phosphoPKB/akt (serine 473), R&D Systems, Abingdon, UK) and secondary antibodies (Dako, Glostrop, UK) was used at a dilution of 1/1000. Membranes were reprobed for β-Actin. Primary and secondary antibodies were used at a dilution of 1/5000 (Abcam plc, Cambridge, UK). For antibody characteristics see Supplemental Table 1. Bands were quantified with Gensnap by Syngene (Cambridge, UK) and expressed relative to β-actin to normalize for gel loading.

**De novo lipogenesis (DNL)**

DNL was measured by the uptake of 1-[14C] -acetate into the lipid component of hepatocytes as has been described previously (15). Cells were cultured in a 24-well plate, washed 3 times with serum free media and then incubated with 500μL of serum free media with 4.44kBq/L 1-[14C]-acetate acid with cold sodium acetate to a final concentration of 10 μM acetate and treated with or without insulin (0.5ng/ml). The cells were incubated at 37°C for 6-8 hours. After incubation activity was terminated by washing the cells 3 times with cold PBS and scraping into 250μL of PBS. The lipid fraction was recovered in Folch solvent, the solvent was evaporated and the radioactivity retained in the cellular lipid was determined by scintillation counting and expressed as disintegrations per minute (dpm)/well. To account for variability between experimental replicates, data are presented as percentage change from control.

**β-oxidation**

Rates of β-oxidation were measured by the conversion of [1H]-palmitate to 3H2O. Cells were cultured in a 24-well plates and were washed 3 times with serum free media. Cells were then incubated with 300μL of low glucose serum free media with 4.44kBq/L [1H]-palmitic acid with cold palmitate to a final concentration of 10 μM. The cells were incubated at 37°C for 24hours. After incubation medium was recovered and precipitated with an equal volume of 10% trichloroacetic acid. The aqueous component of the supernatants was extracted with 2:1 chloroform methanol solution. Radioactivity was determined by scintillation counting, expressed as disintegrations per minute (dpm)/well and finally calculated as percentage change from control.

**Transfection studies**

The Androgen receptor (AR) and SRD5A2 cDNAs were cloned into the pcDNA3.1 vector (Invitrogen, Paisley, UK) and transiently transfected into C3A cells. Prior to transfection, cells were seeded into a 24-well plate. Cells were ~60%-70% confluent in order to obtain the most efficient transfection results. Transfection mixture comprised 1.5 μg of DNA diluted in 50 μL of OptiMEM serum free media (Invitrogen, UK) and 2 μL of Lipofectamine 2000 (Invitrogen, Paisley, UK) diluted in 50 μL of OptiMem serum free media. 100 μL of transfection mixture was added drop-wise to each well. The plates were rocked gently and left for incubation at 37°C. Transfection duration was 48h and its efficiency was determined by applying a plasmid containing the Green Fluorescent Protein (GFP) gene. Changes in AR and SRD5A2 mRNA expression level were confirmed by real-time PCR.

**Site directed mutagenesis**

The R246Q mutant was inserted by site directed mutagenesis into the SDR5A2 cDNA using the Quikchange II site-directed mutagenesis kit (Agilent Technologies UK Limited, Cheshire, UK) as per the manufactures guidance. In a 50μl reaction the following components were added: 5μl of 10X reaction buffer, forward and reverse primers (125ng) 10ng of double-strand DNA template, 1μl of dNTP mix, 3μl of QuikSolution and ddH2O to a final volume of 50μl. Using a thermal cycler (Biometra, Göttingen, Germany) samples were incubated at 95°C for 1min and then cycled 18 times at 95°C for 50 seconds, 53.4 to 60°C for 50 seconds, and 68°C for 60 seconds. Samples were then incubated for 68°C for 7min. 1μl of DpnI was added to PCR reaction, vortexed and incubated for 1 hour at 37°C. 1ml of ethanol (100%) was then added to each tube and incubated for 1 hour at –80°C. The mixture was centrifuged at 16 000 XG for 20 minutes at 4°C and the supernatant aspirated. The DNA pellet was washed with 75% ethanol, centrifuged, aspirated, air dried for 10 minutes, resuspended in 10μl of RNase free water. Finally, the DNA vector containing the R246Q mutation was transformed to XL10-Gold ultracapentent cells.

**Gas and Liquid chromatography/Mass spectrometry (GC/MS and LC/MS)**

Cortisol was extracted from cell media after addition of the internal standard cortisol-d4. Briefly, transfected cells were incubated with 200 nM of cortisol for 24h. 1ml of media was collected, extracted by SPE and the samples were derivatized overnight to form methylxime-trimethylsilyl ethers (MO-TMS). The final derivative was dissolved in 55ul cyclohexane, which was transferred to an autosampler vial for GC/MS analysis. An Agilent 5973 instrument (www.agilent.com) was used in a selected ion monitoring mode.

5α-reductase activity was measured using LC/MS. Briefly, cells were incubated with 100 nM Testosterone for 30min. Media was removed and transferred into glass tubes. 5ml dichloromethane was added to each tube, vortexed for 30 seconds and then centrifuged for 10 minutes at 1600 rpm. The aqueous phase was removed and the steroid-containing organic solvent phase evaporated in air to dryness. The steroid extract was analyzed using LC/MSMS (Xevo TQ mass spectrometer combined with an acquity uPLC system) with an electro-spray ionisation source in positive ion mode. Steroid hormones were eluted from a BEH C18 2.1 × 50 mm 1.7 μm column using a methanol/water gradient system, solvent A was water 0.1% formic acid, and B was methanol 0.1% formic acid. The flow rate was 0.6 mL/min and
starting conditions were 45% Bincreasing linearly to 75% B over 5 minutes. Steroid hormones were positively identified by comparison of retention times and mass transitions to steroid standards.

Statistical analysis
Data are presented as mean ± standard error. Where data were normally distributed, t-tests (paired or unpaired where appropriate) were used to compare single treatments to control. If normality tests failed, nonparametric tests were used. ANOVA was used to compare multiple doses and / or treatments. Statistical analysis on real-time PCR data was performed on mean Δct values and not fold changes. All analysis was performed using the GraphPad Prism 6.0 software package (GraphPad Software, Inc. La Jolla, CA)

Results

Regulation of lipogenesis human hepatocytes by androgens
Fatty acid synthase (FASN), ACC1, ACC2 and CPT1 mRNA expression increased after treatment with testosterone in a dose-dependent manner (Figure 1a-d) in C3A human hepatoma cells. Observations were similar following DHT treatment (Figure 1a-d). Absolute changes in mRNA expression levels are presented in Supplemental Table 2. Lipogenic gene expression changes were mirrored by functional assays of 1-[14C]-acetate incorporation into lipid; both testosterone and DHT increased lipogenesis Lipogenic gene expression changes were mirrored by functional assays of 1-[14C]-acetate incorporation into lipid; both testosterone and DHT increased lipogenesis (ctrl 100% vs. T (50 nM, 24h) 124.9 ± 6.2%, DHT (10 nM, 24h) 128.1 ± 4.7%). AR overexpression was confirmed by real-time PCR (ctrl 0.02 ± 0.003 vs. AR 30.04 ± 0.018ASU, P < .05). Even in the absence of testosterone or DHT, AR overexpression alone caused a significant increase in 1-[14C]-acetate incorporation into lipid (ctrl vector only 100% vs. AR 202.7 ± 10.8%, P < .05). Treatment with both testosterone and DHT did not further enhance lipid accumulation (ctrl 202.7 ± 10.8% vs. T (50 nM, 24h) 209.6 ± 16.5%, DHT (10 nM, 24h) 224.6 ± 8.6%) (Figure 1e). In addition, AR overexpression increased lipid metabolism gene expression compared to cells transfected with vector alone (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 < .05).

Studies were also performed in primary cultures of human hepatocytes from both male and female donors. Both testosterone and DHT were without effect in cultures from male patients, however, in samples from female donors, testosterone increased lipogenesis (139.6 ± 17.6% (Testosterone, 5 nM, 24h) vs. 100% (control), P < .05). Interestingly, DHT decreased lipogenesis in hepatocytes from female donors only (Figure 1f).

Regulation of lipid flux in human hepatocytes by glucocorticoids and insulin
GC receptor, IRS1/2, Insulin receptor and AKT1/2 were all expressed in primary cultures of human hepatocytes from male donors. Incubation with cortisol alone or in combination with insulin did not alter gene expression levels (Supplemental Table 3). Cortisol decreased 1-[14C]-acetate incorporation into lipid in a dose-dependent manner (Figure 2a). In male hepatocytes (123.6 ± 10.7% (insulin, 5 nM, 24h) vs. 100% (control), P < .05) (Figure 2b). Interestingly, coincubation with increasing doses of cortisol increased the ability of insulin to stimulate lipogenesis suggesting that insulin and glucocorticoids may work synergistically to promote lipid storage in human hepatocytes (43.9 ± 12.7% [250 nM], 66.13 ± 9.8% [1000 nM] vs. control (23.61 ± 10.7%), P < .05) (Figure 2c).

Cortisol treatment did not alter total PKB/akt levels. However, insulin-stimulated phosphorylation of PKB/akt at serine 473 increased following cortisol pretreatment in a dose dependant manner (1.23-fold [100 nM], 1.68-fold [250 nM], 2.44-fold [1000 nM] vs. control n = 4 P < .05) (Figure 2d and e).

Cortisol treatment did not alter rates of β-oxidation of free fatty acid uptake in C3A cells (data not shown)

SRD5A2 regulates lipogenesis in human hepatocytes
The effects of testosterone were similar to that of DHT upon lipogenesis in our hepatocyte models and we therefore postulated that SRD5A2 may have a more important role to regulate GC exposure in this context.

SRD5A2 overexpression was confirmed using real-time PCR (Figure 3a). Functional activity was assessed through increased DHT generation following incubation with T (Figure 3b) and clearance of cortisol (Figure 3c) as measured by LC/MSMS and GC/MS respectively. The mutant SRD5A2 construct, R246Q, was without activity. Conversion of testosterone to DHT was similar to that observed in the vector only transfection control (Figure 3b).

As observed previously, cortisol decreased lipogenesis in a dose dependent manner in C3A cells transfected with vector construct alone and in the absence of cortisol, SRD5A2 overexpression had no effect. However, in the presence of cortisol, SRD5A2 restored lipogenesis to levels observed in untreated controls (eg, 61.9 ± 7.6%[cortisol] vs. 103.8 ± 8.8% [SRD5A2+cortisol], P < .05, control =
Complementary experiments using the R246Q SRD5A2 construct that is devoid of functional activity did not alter cortisol-mediated suppression of DNL (Figure 4b). To further endorse these findings, experiments were undertaken using pharmacological inhibitors of 5α-reductase isoforms in primary cultures of human hepatocytes. Consistent with our transfection studies, both finasteride (selective SRD5A2 inhibitor) and...
dutasteride (nonselective SRD5A1 and 2 inhibitor) augmented the action of cortisol to suppress DNL (eg, 88.3 ± 5.3 vs. 76.9 ± 5.2%, cortisol vs. cortisol + finasteride, P = .05) (Figure 4c).

Discussion
In this study, we have shown that androgens are able to increase lipid accumulation within human hepatocytes. Cross-sectional clinical studies have shown that low testosterone concentrations are associated with increased hepatic steatosis in men (8, 9) and are consistent with findings in rodent models suggesting that DHT treatment can decrease hepatic lipid accumulation (16). In contrast, women with polycystic ovarian syndrome (PCOS), a condition characterized by androgen excess as well as insulin resistance, are at an increased risk of developing NAFLD although the precise contribution of each of these processes (insulin resistance and androgen excess) to the development of NALFD remains unclear (17, 18).

Figure 2.  

**a.** Cortisol decreases 1-[^14]C-acetate incorporation into lipid in primary human hepatocytes from male donors. b. While cortisol (black bars) decreases lipogenesis, under hyperinsulinemic conditions (white bars), cortisol increases acetate incorporation into lipid. c. Cortisol treatment increases the ability of insulin to stimulate lipogenesis in a dose-dependent manner. d. Pretreatment of primary human hepatocytes from male donors with cortisol increases insulin-stimulated phosphorylation of AKT at residue ser473. The formal quantification of Western blot densitometry relative to β-actin (n = 4 experiments) is presented (total AKT (black bars) and pAKT ser473 (white bars)). e. Representative Western blot. Data presented are mean±SE, *P < .05.
Although in vitro cell models are not able to replicate all the processes that contribute to the development of NAFLD in vivo, in C3A cells, testosterone and DHT increased lipid accumulation, but interestingly, in primary cultures, we observed sexually dimorphic effects. In cells derived from male donors, androgen treatment failed to have a...
significant impact upon lipogenesis, however in female samples, 5 nM testosterone increased DNL. It is interesting to note that DHT did not alter lipogenesis in hepatocytes from male donors, but decreased lipogenesis in female hepatocytes. The mechanisms underpinning this observation and the physiological relevance (the concentrations of DHT used far exceed those seen in the female circulation) are not clear. The discrepancy between the effects of androgens on C3A cells and in primary cultures may reflect origin of C3A cells from human hepatoma (and the well described impact of androgens upon their pathogenesis (19)) and serves to emphasize the importance of endorsing in vitro observations in additional models including human primary cultures.

Enhancing androgen action through androgen receptor overexpression increased DNL providing further evidence as to the potent ability of this pathway to regulate lipid accumulation. Interestingly, we observed no additional effects of providing additional AR ligand, perhaps suggesting maximal stimulation with receptor overexpression alone. Furthermore, AR over expression alone in the absence of ligand was able to increase lipogenesis. While, it is possible that this may reflect existing intracellular androgen availability, ligand-independent activation of the AR remains plausible. This has been identified as a potential mechanism that might be crucial in regulating cell growth in the context of malignancy (20) notably in prostate cancer (21) although the precise mechanisms that underpin ligand-independent AR activation remain unclear. Importantly, not all actions of androgens upon the liver may be mediated by classical AR signaling. AR-independent regulation of lipogenesis in the liver has been observed in testicular feminized mice that lack a functional androgen receptor, with a reduction in lipogenesis following testosterone treatment (22).

The impact of GCs to regulate carbohydrate metabolism in particular gluconeogenesis in the fasting state is well described. However, their impact on lipid metabolism remains relatively poorly understood in human models. We have previously shown in adipose and skeletal muscle that GCs decrease lipogenesis in the absence of insulin consistent with their role to mobilize fuel in the fasting state (23, 24). In the fed state, however, GCs and insulin act synergistically to drive lipid accumulation. Studies performed in rodent hepatocytes have demonstrated this relationship (25) and we have now shown this in primary cultures of human hepatocytes. In our in vitro model, the ability of GCs to enhance the ability of insulin to drive lipogenesis was associated with increased activation of the insulin signaling cascade as demonstrated by increased phosphorylation of PKB/akt, similar to our published observations in human adipose tissue (26, 27). Although augmentation of insulin action by GCs has been observed in rodent hepatocytes (25), these in vitro data may not be reflective of more complex in vivo physiology. Clinical studies that have administered GCs have shown evidence of increased hepatic insulin resistance in most cases (28).

There is now an emerging role for the 5α-reductase isoforms in the regulation of metabolic phenotype. The ability of testosterone to regulate lipid metabolism does not appear to be dependent upon the presence of SRD5A2. The C3A cell line does not express SRD5A2, and yet both Testosterone and DHT were able to stimulate lipogenesis to a similar extent. Our experiments therefore focused on the role of SRD5A2 to regulate the effects of GCs upon liver metabolism. While genetic ablation of SRD5A1 in rodent models increases lipid accumulation and fibrosis, the precise mechanisms that underpin this are not clear (6, 7). In a recently published clinical study, nonselective 5α-reductase inhibition with Dutasteride was associated with peripheral insulin resistance and it has been suggested that this may reflect a specific role for SRD5A1 in skeletal muscle (29). The precise impact upon liver fat accumulation could not be determined as pre and post intervention assessment of hepatic lipid content was not performed.

The role of SRD5A2 in clearing cortisol is well established through the examination of urinary steroid metabolite profiles in patients with proven SRD5A2 mutations (5). Detailed metabolic studies in patients with mutations SRD5A2, have not been performed. Increasing 5α-reductase activity is associated with an adverse metabolic phenotype (12, 30). This may reflect a compensatory mechanism to clear active GCs, in particular from the liver in an attempt to protect it from lipid accumulation. With more severe disease activity decreases and this may increase GC exposure and may serve and a local anti-inflammatory measure to try to limit the progression of nonalcoholic steatohepatitis, fibrosis and scarring (31).

In conclusion, we have demonstrated the potent actions of androgens and GCs to regulate lipid metabolism in human hepatocytes in vitro and shown that prereceptor regulation through that expression and activity of SRD5A2 is able to modify their action. This has important implications not only in terms of predisposing individuals to the development of hepatic steatosis, but also to the large numbers of patients prescribed 5α-reductase inhibitors. While the role of these compounds in the treatment of prostate-related disease is established, the long-term met-

**Legend to Figure 4 Continued. . .**

Suppress lipogenesis. Data presented are mean±SE of n = 3–5 experiments performed in triplicate, *P < .05, **P < .01 vs. control and § P < .05, §§ P < .01 vs. cortisol)**
abolic consequences of these medications have not been assessed.

Acknowledgments

This work was supported by the Medical Research Council (Senior Clinical Fellowship to JWT Ref. G0802765) and through the NIHR Oxford Biomedical Research Centre.

Address all correspondence and requests for reprints to: Jeremy W Tomlinson PhD FRCP, Oxford Centre for Diabetes, Endocrinology & Metabolism University of Oxford, Churchill Hospital, Headington, Oxford, OX3 7LJ, Tel 0.44 (0)1865 857 359, E-Mail. jeremy.tomlinson@ocdem.ox.ac.uk.

This work was supported by .

Disclosure: The authors have nothing to disclose.

References

18. Gathercole LL, Bujalska IJ, Stewart PM, Tomlinson JW. Glucocor-


