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# Glucocorticoids Enhance Insulin Sensitivity in Human Hepatocytes

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## Background

Glucocorticoid (GC) excess (Cushing's syndrome) is characterized by central obesity, insulin resistance and in up to 20% of cases, non-alcoholic fatty liver disease (NAFLD) (Rockall *et al*., 2003). NAFLD is a progressive spectrum of disease ranging from hepatic steatosis to steatohepatitis, fibrosis and cirrhosis (Fig. 1).

In patients with simple obesity and insulin resistance, circulating cortisol is not increased (Fraser *et al*., 1999). However, intracellular GC metabolism in insulin target tissues including liver, adipose tissue, and muscle might be important in regulating insulin action. Many processes contribute to lipid accumulation within heptocytes including *de novo* lipogenesis which includes the rate-limiting carboxylation of acetyl CoA to malonyl-CoA by acetyl CoA carboxylase (ACC) and conversion to palmitate by fatty acid synthase (FAS) (Fig. 2). We have previously shown that GCs decrease lipogenesis in muscle and adipose tissue and we have hypothesized that this may also occur in hepatocytes. Endogenous GCs are inactivated via a series of enzymes including isoforms of 5α-reductase (type 1 [5αR1] and type 2 [5αR2]) (Fig. 3a) . In addition, 5αR1 and 2 generate dihydrotestosterone (DHT) from testosterone (T) and importantly both isoforms are highly expressed in human liver (Fig. 3b) . We propose that GCs modulate lipid homeostasis within the human liver and that their action is regulated by 5αR expression and activity.

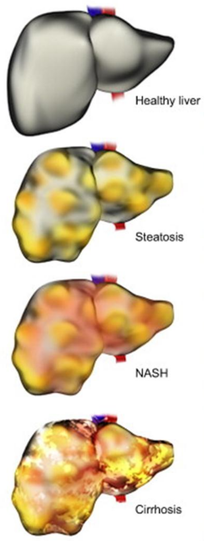


Figure 1. Stages of Liver Damage in NAFLD. Taken from Bechmann et al. 2011

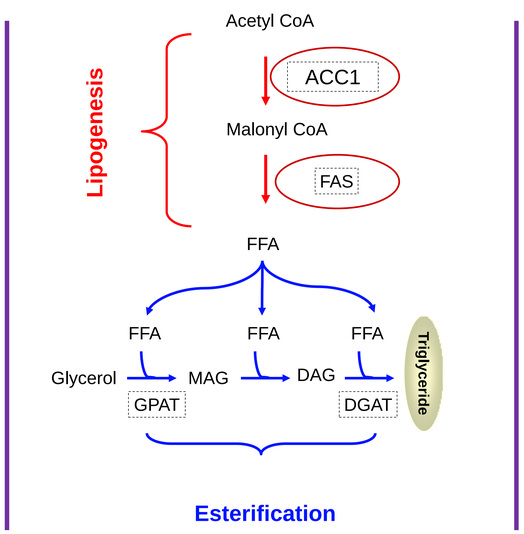


Figure 2. *de novo* lipogenesis which includes the rate-limiting carboxylation of acetyl CoA to malonyl-CoA by ACC and conversion to palmitate by FAS.

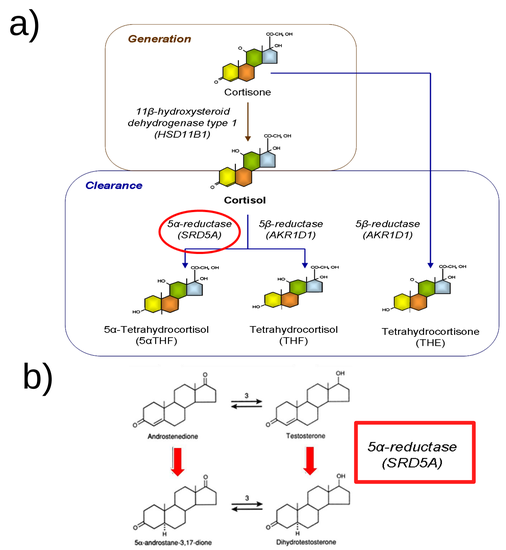


Figure 3. (a) Cortisol clearance with 5α-reductase activity. (b) 5α-reductase type 1 and 2 convert testosterone (T) into dehydrotestosterone (DHT).

## Materials and Methods

- Cryopreserved human hepatocytes were purchased from Celsis in Vitro Technologies (Baltimore, USA) 5αR2(Fig. 4a) and incubated with variable doses of cortisol (0-1000 nM) for 24h in the presence and absence of insulin(5 nM).
- Insulin signalling gene expression levels were quantified by real-time PCR and western blotting was performed to determine total and phospho PKB/akt protein expression levels.
- C3A cells are a subclone of the hepatoma-derived HepG2 cell line which expresses 5αR1, but not 5αR2(Fig. 4b). 5-αR2 has been successfully overexpressed in C3As using the pcDNA3.1+5-αR2 construct. Expression levels were quantified by real-time PCR.
- After transfecting C3As with 5-αR2, these cells were treated with cortisol (dose range 100, 250, 1000 nM) in presence or absence of insulin (5nM) for the final 24h of transfection.
- To measure lipid accumulation in both primary and hepatocyte cell line , for the final 6 hr of treatment 1-[14C]-acetic acid [0.12μCi/L] with cold sodium acetate to a final concentration of 10uM acetate was added to each well. The lipid content of the cells was recovered in Folch solvent and radioactivity was measured by scintillation counting.

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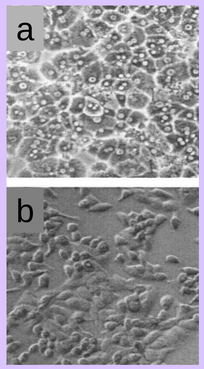



Figure 4. (a) Primary Human Hepatocytes, (b) C3A Cell Line.

## Results

- Cortisol decreased functional lipogenesis in a dose dependent manner in C3A cells (85.6±6.6% [100nM], 73.5±7.9% [250nM], 55.04±5.6% [1000nM], p<0.05) (Fig. 5a) and primary hepatocyes (97.7% [100nM], 85.1% [250nM], 67.08% [1000nM], p<0.05) (Fig. 5b and that was paralleled by an increase in inactivating ser-79/218 phosphorylation of ACC (Fig. 5c).
- In primary hepatocyes, insulin (5nM) was able to reverse the effect of cortisol. This stimulatory effect of insulin upon lipogenesis was augmented in a dose dependant manner (130.3% [INS + 100nM cortisol],139.9% [INS + 250nM cortisol], 152.37% [INS + 1000nM cortisol] vs. Insulin (127.8%), p<0.05) (Fig. 5b).
- GC receptor, IRS1/2, Insulin receptor and AKT1/2 were all expressed in primary cultures. Incubation with cortisol alone or in combination with insulin did not significantly alter gene expression levels.
- Whilst cortisol treatment did not alter total PKB/akt levels, insulin stimulated phosphorylation of PKB/akt at serine 473 increased following cortisol pre-treatment in a dose dependant manner (1.23-fold [100nM], 1.68-fold [250nM], 2.44-fold [1000nM] vs. control n=4 p<0.05) (Fig. 6a) .
- In the absence of cortisol, 5αR2 transfection did not alter rates of DNL. However, in the presence of cortisol, 5αR2 completely restored rate of lipogenesis to those of untreated controls (e.g. 61.9±7.6% [1000nM cortisol] vs. 103.8±8.8% [5αR2+1000nM cortisol], p<0.05, untreated control=1 (Fig. 6b).

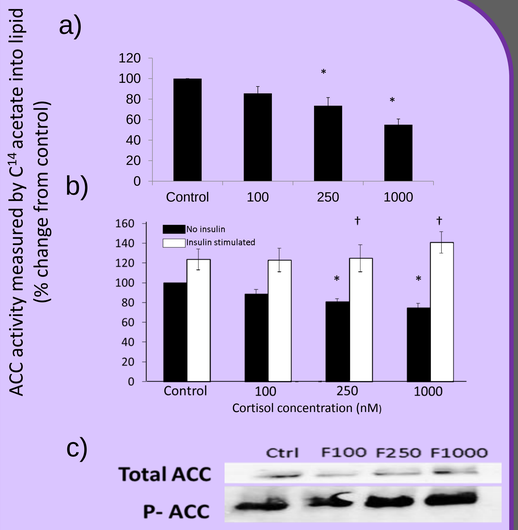
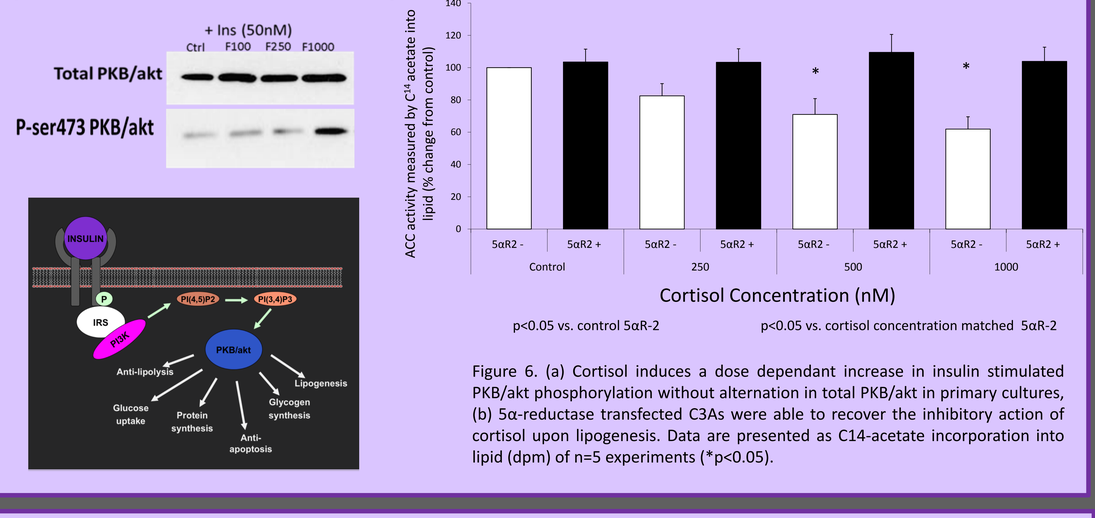



Figure 5. Cortisol decreases functional lipogenesis in a dose dependent manner in C3A cells and primary hepatocytes (b) (*p<0.05 vs. control) and this was confirmed by increasing ser-79/218 phosphorylation of ACC (c). Plus, In primary hepatocytes, with increasing doses of cortisol, the incremental response to insulin (5nM) increased (b) (†p<0.05 vs. cortisol).




p<0.05 vs. control 5αR-2 p<0.05 vs. cortisol concentration matched 5αR-2

Figure 6. (a) Cortisol induces a dose dependant increase in insulin stimulated PKB/akt phosphorylation without alternation in total PKB/akt in primary cultures, (b) 5α-reductase transfected C3As were able to recover the inhibitory action of cortisol upon lipogenesis. Data are presented as C14-acetate incorporation into lipid (dpm) of n=5 experiments (*p<0.05).

## Conclusions

- GCs decrease DNL in C3As and primary hepatocytes in the absence of insulin.
- In primary hepatocytes, insulin and cortisol act synergistically promoting functional lipogenesis.
- In primary human hepatocytes GC treatment enhances insulin signalling through increased serine phosphorylation of PKB/akt
- 5αR2 transfection ameliorates the functional impact of cortisol to decrease lipogenesis.
- 5αR2 activity has the potential to modulate the metabolic phenotype in human hepatocytes.

**References;** Rockall,AG *et al.* Eur.J.Endocrinol. 149:543-548, 2003 Fraser,R *et al*. Hypertension 33:1364-1368, 1999 Bechmann,LP *et al*. J Hepatol. 2011