# The changing 'steroid metabolome' across the spectrum of non-alcoholic fatty liver disease

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## INTRODUCTION & OBJECTIVES

Dysregulated glucocorticoid (GC) metabolism has been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). NAFLD extends from simple steatosis, to inflammation (steatohepatitis / NASH), fibrosis and cirrhosis. It is the hepatic manifestation of the metabolic syndrome and is independently associated with liver and cardiovascular mortality. Liver biopsy – which is both invasive and resource intensive – remains the gold standard diagnostic tool, and lifestyle induced weight loss achieved through diet and exercise remains the current mainstay of treatment for NAFLD.

GC availability to bind and activate the GC-receptor is controlled at the pre-receptor level by a series of enzymes that are able to regenerate or inactivate cortisol. Specifically, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive cortisone to cortisol and thus amplifies local GC action. In contrast, the A-ring reductases (5α-reductase type 1 and 2 and 5β-reductase) clear cortisol to its inactive dihydro-metabolites.

The liver represents the major site of steroid hormone metabolism within the body. Previous studies have suggested dynamic changes in NAFLD, but have only been performed in very small numbers of patients within defined histological groups[1-2].

Better characterisation and understanding of these steroid hormone pathways may well yield novel targets for intervention and improved non-invasive diagnostic markers, and help to define better staging and treatment strategies for NAFLD.

## METHODS

To investigate changes to the pathways which regulate the bioavailability of cortisol, we recruited 39 subjects with biopsy proven NASH, 44 subjects with biopsy proven cirrhosis and 58 healthy controls without known liver disease.

All subjects provided a spot urine sample for analysis alongside collection of clinical data. Urinary steroid metabolites were analysed using gas chromatography / mass spectrometry (GC/MS). Steroid metabolite data were corrected for urinary creatinine.

Computational analysis of the steroid metabolome was performed employing generalized matrix learning vector quantitisation (GMLVQ) to identify steroids that have the ability to discriminate between the different groups.

## RESULTS

Demographic data are presented in Table 1. Mean age in those with NASH was lower ($p<0.0001$) in comparison to the other groups. There were more females in the control group compared to the other groups. Those with NASH had a significantly higher BMI ($p<0.0001$).

**Table 1:**

| | Control | NASH | Cirrhosis |
|---|---|---|---|
| **Sample size** | 58 | 39 | 44 |
| **Age** | 60 (12) | 47 (11) | 59 (11) |
| **Gender m:f** | 24:34 | 22:17 | 23:21 |
| **BMI (kg/m²)** | 27 (6) | 39 (5) | 28 (5) |

Age and BMI data expressed as mean (s.d)

Total cortisol (F) metabolites were significantly raised in those with NASH compared to controls. In contrast, total cortisol (F) metabolites in those with cirrhosis were significantly reduced when compared to controls (Fig. 1).

11β-HSD1 activity – reflected by the (THF+5αTHF) / THE ratio – was similar in those with NASH and controls but was significantly increased in those with cirrhosis ($p<0.0001$) (Fig. 2). 5α-reductase activity – reflected by the THF/5αTHF ratio – did not differ across the 3 groups ($p=0.09$) (Fig.3).

Generalised machine learning vector quantitisation (GMLVQ) analysis allowed complete separation of control and cirrhosis groups (Fig.4a) with an AUC ROC of 0.99 (Fig.4b).

**Figure 1. Total Cortisol Metabolites**

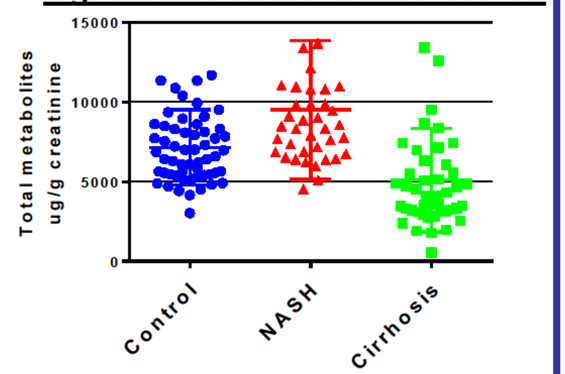



**Figure 2. THF+5α-THF/THE ratio (11β-HSD1 Activity)**

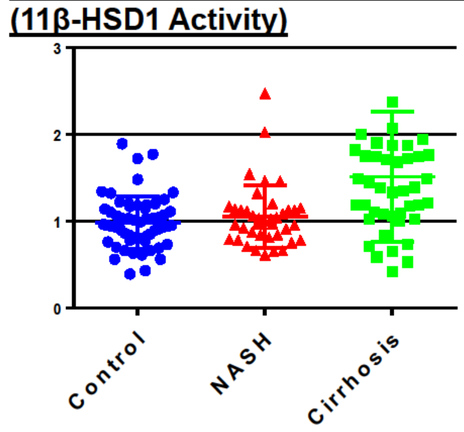



**Figure 3. THF/5α-THF ratio (5α-reductase Activity)**

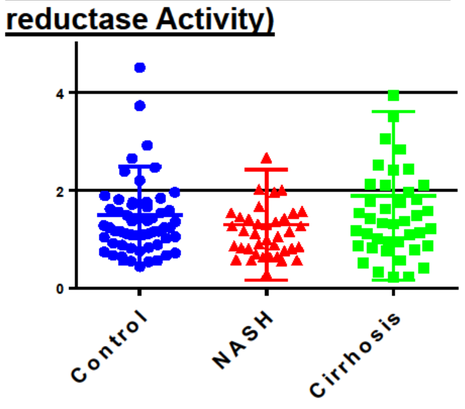



**Figure 4a. GMLVQ 2D Analysis**

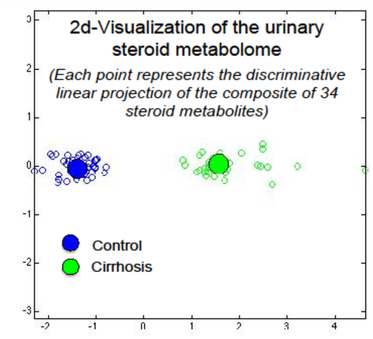
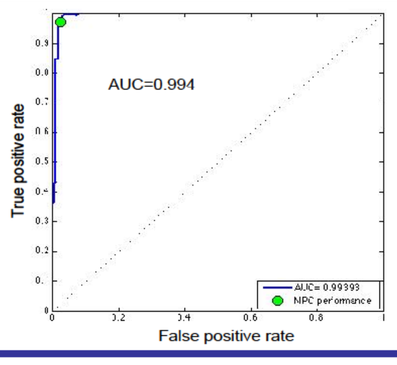



**Figure 4b. GMLVQ ROC Analysis**




## CONCLUSIONS

Glucocorticoid metabolic pathways are complex systems which share substrates and products. Through this work, we have identified specific dysregulated pathways in cortisol metabolism that appear differentially regulated across the spectrum of non-alcoholic fatty liver disease, which provides an insight into its pathophysiology.

Examining steroid metabolism using an unbiased 'whole system' model, offers considerable potential in providing a non-invasive assessment of staging and monitoring liver disease alongside the identification of novel discrete targets for intervention in non-alcoholic fatty liver disease.

## References

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2. Westerbacka J. et al. Body fat distribution and cortisol metabolism in healthy men: enhanced 5beta-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. J Clin Endocrinol Metab. 2003 Oct;88(10):4924-31.

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