

adipose-specific LXR β ^{-/-} mice (Ad β KO). Dex-dependent increases in lipolysis in gonadal WAT and plasma free fatty acids (FFA) were also attenuated by GSK co-treatment. With GSK co-treatment, Dex-induced liver steatosis was diminished suggesting that LXR β antagonism attenuated FFA shuttling to the liver. The lipolytic and lipotoxic effects of Dex in AT and liver were largely abrogated in Ad β KO along with improved systemic insulin sensitivity. Overall, our data suggest that LXR β antagonism prevents disruption of BAT and WAT (and indirectly liver) function caused by GC treatment in an *in vivo* model, highlighting the potential role of LXR β antagonists in combating the negative effects of excessive GC exposure on the development of diabetes and obesity. The identification of this novel mechanism of interrupting GC adipose tissue action suggests therapeutic targeting of LXR β with an antagonist could improve the health of patients currently taking GCs to control inflammation but suffer the detrimental side effects of drug treatment.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

LBD Dimerization of the Androgen Receptor but Not N/C Interaction Is Crucial for Normal Male Development in Mice

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The androgen receptor (AR) is a nuclear receptor with a main role in the development and maintenance of the male phenotype. To execute its role as transcription factor, the AR forms homodimers. Three dimerization modes have been described for the AR: one via the DNA binding domain, a second via the ligand binding domain (LBD) and a third via interactions between the LBD and the aminoterminal of the AR (N/C). Based exclusively on *in vitro* data, all three dimerization modes seem to contribute to full AR activity, albeit to a different extent. The *in vivo* role of the dimerization modes, however, remains unknown. To study the physiological relevance, we generated two mouse models using a CRISPR/Cas9 approach, in which either the N/C interaction (AR^{NoC}) or LBD dimerization (AR^{Lmon}) was disrupted. Surprisingly, the male AR^{NoC} mice have a normal phenotype, indicating that the N/C interaction is not crucial for male development. In contrast, AR^{Lmon} males have an external female phenotype with cryptorchid testes and high levels of circulating testosterone (T), androstenedione and luteinizing hormone (LH) (6-, 13- and 45-fold higher, respectively). They have no prostate, seminal vesicles or epididymis, illustrating the importance of LBD dimerization during male development. Phenotyping the AR^{Lmon} model furthermore provided evidence of a crucial role for the AR in bone homeostasis as well as steroidogenesis. The AR^{Lmon} males display a severe bone phenotype, similar to that of complete AR knockout (ARKO) mice. The bone phenotype of ARKO was postulated to be mainly due to lower estrogen levels. However, in contrast to ARKO mice, AR^{Lmon} mice

have high circulating levels of T, which can still function as prohormone for estradiol and support bone function via the ER α . Immunohistological analysis of AR^{Lmon} testes showed hyperplasia of the Leydig cells and residual spermatogenesis. Analysis of the steroidogenic pathway revealed that while the expression of most genes is increased, the expression of *Hsd17b3*, encoding the enzyme responsible for conversion of androstenedione into T, is low in AR^{Lmon} testis. Reporter assays confirmed that the promoter of this gene is indeed upregulated by the AR itself. In conclusion, our work uncovers the physiological role of the N/C interaction and LBD dimerization of the AR. It furthermore demonstrates a direct role for AR in male bone development independent of T aromatization into estrogens. Finally, we show that the AR controls the final step in the synthesis of its own ligand. In contrast to the *in vitro* data, N/C interaction is not crucial for male development *in vivo*. The AR^{Lmon} model illustrates that LBD dimerization could be an excellent new therapeutic target for inhibiting AR activity for example in advanced prostate cancer that has developed resistance to the current AR-targeting therapies.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

Mapping of Corticosteroids in Murine Kidneys Using Mass Spectrometry Imaging

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Renal sodium reabsorption is important for blood pressure homeostasis and is physiologically regulated by aldosterone; glucocorticoids may also contribute. Abnormal steroid hormone activity within the kidney contributes to hypertension but the mechanisms are not fully defined. Molecular profiling of receptors and metabolising enzymes indicates that steroid hormone action is compartmentalised within the kidney. Ambient steroid concentrations are a critical factor governing bioactivity at a cellular level, but this is largely unknown, and the kidney remains a "black box". Mass spectrometry imaging (MSI) was applied recently to localise steroids in brain and testes, and here is applied to kidney. Image reconstruction permits characterisation and co-registration of kidney histological regions based on regional markers detectable by MSI. Our aim was to map and quantify glucocorticoids and aldosterone in different histological zones (cortex, medulla) of murine kidneys, using an optimised MSI method. This approach has the potential to map steroids within functional zones of the kidney, providing fundamental new information relevant to hormone action in health and in disease. Cryosections of male C57BL6 mouse kidneys (age 12 weeks, n=6) were subject to MSI following derivatisation using Girard T reagent and α -cyano-4-hydroxycinnamic acid matrix application. Images were reconstructed, and methods optimised to enhance signal and limit diffusion of analytes of interest. Matrix assisted laser desorption/ionisation (MALDI) was used as a sampling method, coupled to Fourier Transform Ion cyclotron mass spectrometry. Ions with m/z 458.3010,

460.3166 and 474.2957 were detected, using MALDI, in renal sections, close to the predicted masses of 458.3013 ($\Delta\text{ppm}=0.65$), 460.3169 ($\Delta\text{ppm}=0.65$), and 474.2962 ($\Delta\text{ppm}=1.05$), for derivatives of 11-dehydrocorticosterone, corticosterone and aldosterone respectively. Untargeted evaluation of ions was conducted to find regional markers that would allow definition of kidney histological zones. The Heat maps generated indicated that corticosterone intensity was higher in the inner cortex area close to the corticomedullary junction than the rest of the kidney. In contrast 11-dehydrocorticosterone was detected mainly in medulla and aldosterone signal was equally strong in medulla and outer cortex. Thus, MSI can be used map the sites where glucocorticoid and mineralocorticoids are most active in regulating renal tubular function. Co-localisation of steroids of interest with zonal markers by MSI permits steroid mapping in functional renal zones of the kidney. This approach provides fundamental new insights into the physiological control of sodium transport by steroids and opens doors to understanding changes in disorders of blood pressure. The project was supported and funded by Kidney Research UK.

Thyroid

FROM HYPO- TO HYPERTHYROIDISM

A Randomised, Controlled Trial of Adjunctive Cholestyramine or Prednisolone Compared to Standard Therapy for the Treatment of Uncontrolled Graves' Disease (the Chops Study)

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Introduction: Antithyroid drugs (ATDs) are recommended effective treatment for uncontrolled Graves' disease but achieves maximal antithyroid effects between 6 to 12 weeks. Cholestyramine sequesters thyroid hormones in the intestine and enhances its fecal excretion. Steroids inhibits the conversion of thyroxine to triiodothyronine peripherally and blocks thyroid hormone production. We hypothesize that adjunctive cholestyramine or prednisolone to ATDs may reduce circulating thyroid hormones and improve biochemical control.

Methods: In this multicenter, open labelled, parallel-group trial, we randomly assigned in a 1:1:1 ratio, adult Graves' disease patients with moderate to severe hyperthyroidism (FT4 levels > 40 pmol/L) to receive either adjunctive cholestyramine 4g twice daily or prednisolone 30 mg daily in tapering down doses in addition to standard treatment or standard treatment alone for 4 weeks. Standard treatment was carbimazole 30mg daily and propranolol 40mg BD for 4 weeks. The primary endpoint was change from baseline for FT4 and FT3 levels at the end of 2 and 4 weeks of intervention. Safety endpoints including gastrointestinal

adverse events, hypokalemia, hypothyroid and hyperglycemia were recorded.

Results: A total of 107 patients were screened and 97 patients randomised. Baseline demographics, clinical and biochemical characteristics were similar between the groups. The baseline median FT4 levels were 51.6 pmol/L (42.2-71.1) and FT3 levels 22.5 pmol/L (5.7-30.8). Both FT4 and FT3 declined at two and four weeks from baseline but were no different between the three groups. At week 2, median FT4 levels declined by 43.3% (25.8-53.3), 39.8% (19.1-55.1) and 33.4% (20.1-62.0) ($p=0.988$) and at 4 weeks, 50.9% (33.3-63.8), 57.8% (39-70.9), 55.8% (36.2-72.0) ($p=0.362$) in the cholestyramine, prednisolone and standard treatment group respectively. Median FT3 levels reduced by 51.2% (22.8- 58.9), 59.9% (38.9-69.3) and 50.9% (26.9-63.9) ($p=0.084$) at week 2 and 60% (39.2-67.9), 67.5% (38.4-78.4), 63.1% (45.7-69.3) ($p=0.387$) in the corresponding cholestyramine, prednisolone and standard treatment only group. A higher number of gastrointestinal adverse events: constipation, bloating, diarrhea, abdominal pain and vomiting were observed in the cholestyramine group in the first 2 weeks of treatment and no difference in the incidence of hypokalemia between groups.

Conclusion: Adjunctive cholestyramine or prednisolone did not improve the biochemical control of uncontrolled moderate to severe Graves' disease when added to ATDs. The additional use of cholestyramine resulted in a higher number of gastrointestinal adverse events but were mild and self-limiting.

Thyroid

FROM HYPO- TO HYPERTHYROIDISM

A Tale of Two Therapies- A Comparison of Armour Thyroid and Levothyroxine

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Treatment of hypothyroidism is predominantly with levothyroxine due to its ability to generate stable T3 levels and its long half-life. Many patients report continued hypothyroid symptoms despite normal TSH values on levothyroxine and request to switch to desiccated animal thyroid extract. Desiccated thyroid extract is less used for fear of side effects and risks. There are only a handful of studies available comparing desiccated animal thyroid extract to levothyroxine. We conducted a retrospective study on 250 hypothyroid patients over the age of 18 who presented to our clinic from 2008-2018. We excluded patients who had a history of thyroid cancer and documented non-adherence. We analyzed 125 patients on levothyroxine (males=43, females=82) and 125 patients who were on levothyroxine but chose to switch to Armour Thyroid (males=7, females=118). We examined the following variables; when comparisons of proportions were made between the two groups, N-1 chi square test was used to determine significance.

1. Reason for change to Armour Thyroid from levothyroxine: Top reasons were fatigue (n=51/125, 40.8%), inability to