DHT promoted gene expression in TN2 (673 up-regulated genes versus192 down-regulated genes). TNBC subtyping analyses based on RNA-Seq data predicted distinct molecular subtypes of TN1 and TN2: TN1 correlated to a basal-like 1 (BL1) subtype, and TN2 correlated to a basal-like 2 (BL2) subtype. These analyses suggest that TN1 and TN2, which both express functional AR, are two molecularly distinct PDX models that expand our current knowledge of AR-positive TNBC. Our results do not support that AR is a suitable therapeutic target in TNBC. To our best knowledge, the molecular mechanisms of AR in TNBC are equivocal and should be evaluated using clinically relevant models, considering both the heterogeneous expression of AR in TNBC and the general complexities of AR signaling.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Association of Maternal-Neonatal Steroids With Early Pregnancy Endocrine Disrupting Chemicals and Pregnancy Outcomes

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Steroids are important for fetal development and parturition, and aberrant exposure during gestation can lead to abnormal fetal outcome. Gestational exposures to endocrine disrupting chemicals (EDCs) have the potential to alter pregnancy steroidal milieu. Most studies to date have focused on individual EDCs, when in real life humans are exposed to a host of EDCs in parallel, emphasizing the need to consider cumulative impact. To meet this goal, 121 pregnant women (18-42 years of age) were recruited between 8 and 14 weeks of gestation from Southeastern Michigan, and maternal samples at recruitment and delivery were collected, as well as neonatal cord blood.

Maternal and neonatal steroids were measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS) in blood samples collected from 121 mother-infant dyads of spontaneously conceived singleton pregnancies. A total of 41 EDCs encompassing commonly encountered environmental EDCs (phenols and phthalates), metals and metalloids were quantified in early pregnancy maternal urine from a subset (56 dyads) via LC-MS/MS.

Maternal and neonatal steroid levels from all 121 subjects were related to pregnancy outcomes and, in the subset, individual and uniquely weighted EDC mixtures were related to steroid milieu. Additionally, the influence of BMI, maternal age, and offspring sex in modulating the EDC associations with steroids were determined. To determine the association of steroids at each time point with pregnancy outcomes or individual EDCs, multiple linear regression was used. Correlations between the steroids and potential confounding variables were analyzed using Spearman correlation. The cumulative effect of EDC mixtures generated by Principal Component Analysis on steroid measures was determined using Principal Component regression. The Benjamini-Hochberg False Discovery Rate procedure was employed to account for the multiple outcomes in each analysis.

The findings showed 1) steroid-specific positive or negative associations with pregnancy measures; (2) many maternal first trimester EDCs were negatively associated with estrogens

and positively with androgen/estrogen ratios; (3) EDC-steroid associations were influenced by maternal age, pre-pregnancy BMI, and fetal sex and (4) EDCs individually and as mixtures showed direct and inverse fetal sex-dependent associations with maternal and neonatal steroids. These findings indicate that maternal and neonatal steroids influence pregnancy outcomes depending on maternal age, pre-pregnancy BMI, and fetal sex and that the effects of EDCs on steroids differs when considered individually or as mixtures. Our results suggest that steroid measures might serve as biomarkers for the impact of EDC exposures on fetal outcomes during pregnancy, but these measures must be corrected for maternal factors. (Supported by P01 ES022844/RD 83543601, 1U2C ES026553, P30 ES017885)

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Calcineurin-NFATc4 Pathway Is Activated Upon K^+-stimulation of Adrenal Aldosterone Production

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The mineralocorticoid aldosterone secreted by the adrenal zona glomerulosa (ZG) cells promote renal K^+ secretion and Na^+ reabsorption; thereby it is critical for the control of ion homeostasis and blood pressure. While the Ca^{2+}/calmodulin-dependent protein kinase (CAMK) pathway regulating K^+ stimulated aldosterone production is well studied, little is known about the potentially involved phosphatases. Interestingly, immunosuppression therapy of transplanted patients with protein phosphatase 3 (calcineurin) inhibitors often results in rather low plasma aldosterone levels despite a concomitant hyperkalemia and hyperreninemia. Calcineurin (Cn) is a calcium and calmodulin-dependent protein phosphatase expressed in the adrenal cortex. We tested the hypothesis that Cn participates in the signal transduction pathway mediating the K^+-dependent stimulation of aldosterone production. To address this question, we used the adrenocortical cell model NCI-H295R, mouse and human ex vivo adrenal preparations and a ZG-specific and inducible Cn knockout mouse model (ZG-CnB1-KO). Inhibition of Cn with tacrolimus abolished the K^+-stimulated expression of CYP11B2 in NCI-H295R cell line as well as mouse and human adrenal pieces, *ex vivo*. Using a phosphoproteomics analysis, we identified *n*uclear *f*actor of *a*ctivated *T*-cells, *c*ytoplasmic 4 (NFATc4) as a critical downstream factor mediating Cn function. In support of

this result, genetic deletion of NFATc4 reduced the basal expression of CYP11B2 and impaired the K^+-stimulated expression of this gene. Conversely, the expression of a constitutively active form of NFATc4 drastically increased the expression of CYP11B2 in NCI-H295R cells which remained unaltered upon treatment with K^+ or tacrolimus. Finally, preliminary experiments using ZG-CnB1-KO mice suggest that Cn deletion in the ZG blunts the increase in aldosterone excretion triggered by high K^+ diet. Altogether, our data indicate that Cn function is indispensable for the physiological regulation of aldosterone production. Moreover, Cn may represent a novel molecular target for the pharmacological treatment of primary aldosteronism.

Steroid Hormones and Receptors
STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Carbonyl Reductase 1 Overexpression in Adipose Amplifies Local Glucocorticoid Action and Impairs Glucose Tolerance in Lean Mice

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Glucocorticoids play a critical role in metabolic homeostasis. Chronic or excessive activation of the glucocorticoid receptor (GR) in adipose tissue contributes to metabolic disorders such as glucose intolerance and insulin resistance. Steroid-metabolising enzymes in adipose, such as 11β-HSD1 or 5α-reductase, modulate the activation of GR by converting primary glucocorticoids into more or less potent ligands. Carbonyl reductase 1 (CBR1) is a novel regulator of glucocorticoid metabolism, converting corticosterone/cortisol to 20β-dihydrocorticosterone/cortisol (20β-DHB/F); a metabolite which retains GR activity. CBR1 is abundant in adipose tissue and increased in obese adipose of mice and humans[1] and increased Cbr1 expression is associated with increased fasting glucose[1]. We hypothesised that increased Cbr1/20β-DHB in obese adipose contributes to excessive GR activation and worsens glucose tolerance. We generated a novel murine model of adipose-specific *Cbr1* over-expression (R26-Cbr1Adpq) by crossing conditional knock-in mice with Adiponectin-Cre mice. CBR1 protein and activity were doubled in subcutaneous adipose tissue of male and female R26-Cbr1Adpq mice compared with floxed controls; corresponding to a two-fold increase 20β-DHB (1.6 vs. 4.2ng/g adipose; P=0.0003; n=5-7/group). There were no differences in plasma 20β-DHB or corticosterone. Bodyweight, lean or fat mass, did not differ between male or female R26-Cbr1Adpq mice and floxed controls. Lean male R26-Cbr1Adpq mice had higher fasting glucose (9.5±0.3 vs. 8.4±0.3mmol/L; P=0.04) and worsened glucose tolerance (AUC 1819±66 vs. 1392±14; P=0.03). Female R26-Cbr1Adpq mice also had a worsened glucose tolerance but fasting glucose was not altered with genotype. There were no differences in fasting insulin or non-esterified fatty acid between genotypes in either sex. Expression of GR-induced genes *Pnpla2*, *Gilz* and *Per1*, were increased in adipose of R26-Cbr1^{Adpq} mice. Following high-fat diet induced obesity, no differences in bodyweight, lean or fat mass, with genotype were observed in male and female mice, and genotype differences in fasting glucose and glucose tolerance were abolished. In conclusion, adipose-specific over-expression of *Cbr1* in lean male and female mice led to increased levels of 20β-DHB in adipose but not plasma, and both sexes having worsened glucose tolerance. The influence of adipose CBR1/20β-DHB on glucose tolerance was not associated with altered fat mass or bodyweight and was attenuated by high-fat diet-induced obesity. These metabolic consequences of Cbr1 manipulation require careful consideration given the wide variation in CBR1 expression in the human population, the presence of inhibitors and enhancers in many foodstuffs and the proposed use of inhibitors as an adjunct for cancer treatment regimens. **Reference:** Morgan et al., Scientific Reports. 2017; 7.

Steroid Hormones and Receptors
STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Chronic Dexamethasone Treatment Leads to Less Weight Gain and Ameliorated Glucose Tolerance in Mice; Role of the Cytoprotective Nrf2 Pathway

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Introduction: Chronic glucocorticoid administration is necessary in a variety of conditions including but not limited to autoimmune, inflammatory and cancer-related diseases in order to relieve symptoms and sustain disease progression. However, there are adverse effects that include increase in glucose levels and others whose severity depends on the dose and duration of glucocorticoid exposure. It has been described that dexamethasone induces oxidative stress in cells by increasing reactive oxygen species (ROS) and this is one of the causes of insulin resistance at the cellular level. Nrf2 is a transcription factor which co-ordinates the antioxidant response and its activation has been shown to ameliorate insulin resistance in murine models. **Hypothesis**: We hypothesized that deletion of Nrf2 will lead to a more glucose intolerant insulin resistant phenotype in mice chronically treated with dexamethasone as cells would be exposed to higher ROS levels. **Methods:** To this end, 3-months old wild-type (WT) and Nrf2 knockout (KO) C57BL6J mice were treated intraperitoneally with 2 mg/kg dexamethasone or saline 3 times per week for 3 months. 5-10 mice were included per genotype per treatment and both male and female mice were used. Weekly measurements of body weights were performed and intraperitoneal glucose tolerance tests were done on the second and third month of treatment. Mice were sacrificed