and metastasis. An important feature of this metabolic flexibility is conversion of glutamine to glutamate, an amino acid integral to protection of cells from oxidative stress. Consistent with this, we show multiple cellular models of ER+, endocrine resistant breast cancer cells markedly increase glutamate release and upregulate expression of essential glutamine/glutamate metabolic enzymes and transporters, including the glutamate/cystine antiporter xCT, glutamate dehydrogenase (GLUD1/2), and/or the glutamine importer SLC1A5. Riluzole (RIL) is FDA-approved for the treatment of amyotrophic lateral sclerosis (ALS), and has several proposed mechanisms of action, including suppression of glutamate release and increased glutamate uptake. We show ER+, endocrine responsive and resistant breast cancer cells are growth-inhibited by RIL. This is due to an increase in cell death, particularly in endocrine resistant breast cancer cells, and cell cycle arrest. Interestingly, histologic subtype confers a different cell cycle arrest profile, with invasive ductal cancer (IDC) models arresting in G1 but invasive lobular cancer (ILC) models arresting in G2/M. Isobologram analysis of RIL plus SERMs or SERDs shows additive-to-synergistic activity in a subset of ER+ cell line models, and preliminary studies show combination activity in patient-derived explants (PDEs). Mechanistically, we tested whether signaling through metabotropic glutamate receptors (mGluRs, GRMs) and/or cystine import contribute to RIL's growth-inhibitory phenotype. Antagonists of mGluRs/GRMs don't phenocopy the effects of RIL, suggesting extracellular glutamate signaling through these receptors is not a key mechanism. Rescue experiments with β-mercaptoethanol to promote cystine uptake through transporters other than xCT show partial reversal of RIL-mediated cell cycle arrest in some cells, suggesting xCT may contribute to RIL-induced growth inhibition. In summary, we show RIL may be a viable addition to endocrine therapy in ER+ breast cancer. Ongoing studies will test additional mechanism(s) by which RIL may attenuate the growth of ER+ breast cancer models *in vitro*, including inhibition of protein kinase C and casein kinase 1 delta. We are further testing RIL efficacy alone and in combination with a SERD in primary tumors and lung metastases in a ER+ patient-derived xenograft (PDX) model.

Diabetes Mellitus and Glucose Metabolism

GESTATIONAL DIABETES, DIABETES IN PREGNANCY, AND IN UTERO EXPOSURES

Profiling of Activation Patterns of Placental mTOR in Pregnancies Complicated by Gestational Diabetes Mellitus

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SUN-635

The mammalian target of rapamycin (mTOR) couples' energy and nutrient abundance to cell growth and is critically involved in the onset and progression of diabetes, cancer and ageing. Placental mTOR is involved in nutrient sensing and transfer to the fetus; animal models suggest that placental mTOR is upregulated in pregnancies complicated by hyperglycaemia (1). In this study we investigated expression patterns and activation of placental mTOR and possible effects of gestational diabetes (GDM). Our study consisted of GDM-mothers (n=28) and their offspring and ii) mothers (n=33) with normal pregnancies (non-GDM) and their infants. Total and phospho-mTOR (Ser2448) expression were determined in placental biopsies using either immunoblotting and immunohistochemistry (IHC) analysis. Newborn anthropometric parameters were also determined at delivery. GDM pregnant women presented with higher fasting glucose levels than non-GDM (98.12±22.82mg/dL; 73.61±9.89mg/dL; $p<0.001$). No significant difference was found in birth weight or baby length between GDM and non-GDM infants. IHC analysis showed that both total and activated mTOR were predominantly expressed in trophoblasts and to a lesser extend in syncytiotrophoblasts, in both GDM and non-GDM placentas. GDM placentas exhibited a higher mTOR H-score (2) compared to non-GDM ($p<0.012$), and WB analysis showed a higher phosphor-mTOR signal intensity ($p=0.047$) in the same group, most likely due to increased total mTOR expression. mTOR expression was also increased in both GDM syncytiotrophoblasts and endothelial cells compared to non-GDM ($p<0.001$) whereas a reduced signal was detected in stromal phospho-mTOR ($p=0.004$). No difference was found in trophoblasts or endothelial cells between the 2 study groups suggesting that activation of this kinase is tightly regulated and is relatively independent of changes in total kinase levels. Interestingly bivariate correlation analysis identified an extensive network of significant associations in the expression levels of total, phosphor-mTOR and P/T mTOR between trophoblasts, stroma, endothelial and syncytiotrophoblastsin control placental biopsies; this network was significantly disrupted in GDM placentas, identifying a disheveled regulation of placental mTOR activity. In conclusion, placental mTOR/PmTOR expression is differentially regulated across different placental cell types and is sensitive to hyperglycaemia associated with gestational diabetes mellitus.(1)M. Castillo-Castrejon and TL. Powell. Front Endocrinol (Lausanne). 2017; 8: 306. (2) E. Lakiotaki, et al., Scientific Reports 2016; 6, 21252.

Adrenal

ADRENAL - CORTISOL EXCESS AND DEFICIENCIES

The Burdens of Adrenal Insufficiency: A Survey Study from Two Tertiary Care Centers in the United States

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MON-160

Background: Adrenal insufficiency (AI) is a chronic disorder necessitating life-long replacement. Patients' quality of life and health outcomes depend on knowledge and comfort level with self-management.

Objective: To determine patients' knowledge in regards to diagnosis and management, estimate burden of disease and to identify predictors of adverse outcomes in patients with AI

Methods: Survey study of patients with AI evaluated at two tertiary medical centers between 2015 and 2019. Collected variables included data on circumstances of AI diagnosis, symptoms, management, burden of disease, and overall well-being.

Results: Among 785 patients (mean age at diagnosis 44.2 ± 18.0, 64% women, and 92% Caucasian), 310 (40%) had primary AI (PAI), 255 (33%) had secondary AI (SAI) not related to glucocorticoid use, and 211 (27%) had steroid-induced AI (SIAI). Patients were diagnosed with AI after presenting with symptoms for a median of 1 year (0-6), 28% with symptoms lasting >2 years, 44% visiting emergency room (ER) at least once prior to diagnosis. A third of patients reported a discordant diagnosis from their medical record.

Baseline glucocorticoid replacement therapy included hydrocortisone (HC) in 447 (59%), median of 20 mg (IQR 15 - 25mg), prednisone in 190 (25%), median of 5 mg (IQR 4 - 7.5mg), other regimens in 38 (5%), and no steroids in the remainder (85, 11%); 197 (26%) patients reported daily equivalent HC dose of >25 mg. Overall, 549 (73%) of patients reported use of stress dose steroids at least once per year, higher in patients taking HC >25 mg/day (3.2 vs 2.7 times per year if HC<25mg/day, p=0.01). Improper use of stress steroids was reported in 193, 25% patients. Patients taking HC>25 mg /day reported a higher number of adrenal crises (1.6 vs 1.3 in patients on HC<25 mg/day, p=0.04). Among 314 (41%) patients who reported ER visits due to adrenal crisis, only a third received prompt glucocorticoids.

One third of patients described their general health as fair or poor. Predictors of negative perceptions of overall health included SIAI (OR 6.2 and 2.5, vs PAI and SAI respectively), poor understanding of diagnosis (OR 2.6), daily HC>25 mg (OR 2.1), and presence of at least one adrenal crisis (OR 2.3) (p<0.001 for all).

Conclusion: Patients with AI experience delay in diagnosis, and a third do not fully understand their diagnosis. In addition to patient education, interventions to improve general health and outcomes may include selecting a physiological glucocorticoid replacement therapy, prevention of adrenal crisis, and improving ER care.

Adipose Tissue, Appetite, and Obesity

ADIPOSE TISSUE BIOLOGY AND OBESITY II

27-Hydroxycholesterol Triggers the Whitening of Brown Adipose Tissue

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SUN-590

27-Hydroxycholesterol (27HC) is the most abundant oxysterol in circulation and metabolized by a P450 enzyme CYP7B1. Its levels closely correspond to those of cholesterol in the body. In addition, previously it was found that 27HC is an endogenous selective estrogen receptor modulator (SERM), which links cholesterol metabolism to estrogen receptor actions (1). Brown adipose tissue (BAT) is the primary source of energy expenditure and energy homeostasis, as well as body temperature maintenance. While previously it was believed that BAT activity is limited to neonates and young children, it is now recognized that BAT is also active in adult humans and its function is impaired by metabolic diseases such as obesity. BAT is also a secretory organ and produces brown adipokines, although the exact function of BAT and adipokines from this tissue in obesity has not been completely understood. Recently, it was reported that 27HC plays an important role in obesity and augments body weight gain in response to a high fat, high cholesterol (HFHC) diet by increasing pre-adipocyte population in the white adipose tissue. 27HC mimics the effects by HFHC diet-feeding on white adipose tissue, such as promoting the inflammation and macrophage infiltration (2). In this study, we explored the effect of 27HC on BAT morphology and function. First, we compared the morphology of BAT from wild-type mice and *Cyp7b1*$^{-/-}$ mice that have elevated levels of 27HC using H&E staining. Interestingly, brown adipocytes from *Cyp7b1*$^{-/-}$ mice were larger in cell size than those from wild-type mice, and the cells were mostly unilocular compared to the multilocular cells from wild-type mice, indicating the transition toward a "whitening" phenotype. Next, We treated mice fed a normal chow or a HFHC diet with 27HC or vehicle control for 8 weeks to examine the direct effect by 27HC on BAT. Similar to the phenotype in *Cyp7b1*$^{-/-}$mice, 27HC increased the "whitening" of BAT regardless of the diet. We also determined the gene expression of brown adipocyte markers such as UCP1, PGC1a, and DIO2, and found that 27HC significantly decreased the expression of the BAT markers regardless of the diet, confirming the "whitening" observed in the morphology. Moreover, the energy expenditure in mice treated with 27HC was decreased compared to the vehicle control on a HFHC diet, suggesting that 27HC also alters BAT function. These results show that 27HC causes the whitening of BAT, and shed light on the important role of 27HC in brown adipose tissue function. Future experiments will be warranted toward further understanding of the role of 27HC in BAT function. **Reference:**(1) Umetani, Michihisa, et al. *Nature medicine* 13.10 (2007): 1185. (2) Asghari, Arvand, et al. *Endocrinology* 160.10 (2019): 2485-2494.

Reproductive Endocrinology

CLINICAL STUDIES IN FEMALE REPRODUCTION I

A New Concept for the Endocrinology of Pre-Eclampsia: The Role of Spiral Steroids

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SAT-006

Background: In 1987, Graves observed that during the 3rd trimester, some patients with pre-eclampsia had high levels of unknown materials that could be detected with