Background: An inguinal hernia occurs when an intestinal loop or fat pushes through a weak spot in the lower abdominal muscle (LAM), causing a painful bulge that has the potential to cause bowel obstruction. Despite a high prevalence in men (~25%), non-surgical approaches are not available to treat this disease. We recently found a critical role of estrogen and estrogen receptor alpha (ERα) in inguinal hernia formation. To examine this further, we use a humanized aromatase mouse model (*Arom*^{hum}) where all of the male mice develop scrotal hernias as a pre-clinical model to test the first pharmacological intervention for inguinal hernias. These mice are utilized because their skeletal muscle tissue contains aromatase and produces estradiol (E2), which acts via ERα in the LAM stromal fibroblasts and leads to fibrosis and muscle atrophy. **Hypothesis:** E2-ERα modulation can inhibit and reverse the formation of inguinal hernias in *Arom*^{hum} mice by reducing LAM fibrosis and atrophy. **Results:** We tested three types of treatments to inhibit E2-ERα signaling: letrozole, fulvestrant, and raloxifene. Letrozole, an aromatase inhibitor, was shown to inhibit hernia formation and reversed small (150-175 mm^2) scrotal hernias (n = 10-15/group, p<0.0001). The LAM tissues also showed a reduction in fibrosis (n = 5-8/group, p = 0.0004) and a concurrent increase in myofiber cross-sectional area (n = 5-8, p=0.0356) compared to placebo-treated mice. Similarly, fulvestrant and raloxifene, E2-ERα antagonists, also inhibited hernia formation (n = 10-15/group). Most interestingly, both drugs reversed large and severe hernias (>200 mm^2, n = 10-15/group), accompanied by a decrease in muscle fibrosis and increase in myofiber cross-sectional area (ongoing study, n = 10-11, p<0.0001) compared to placebo mice. The drug-treated mice had lower expression of pro-fibrotic genes such as *Mmp3*, *Emb*, *Spon2*, *Timp1*, and *Tgfb1* in the LAM tissues compared to placebo-treated LAM. Furthermore, we analyzed the differences in extracellular matrix producing genes and muscle regeneration markers between the placebo and drug-treated muscle tissues. **Conclusion:** We find that inhibition of the E2-ERα signaling pathway can reverse mild or severe inguinal hernias. Successful treatment is accompanied by decreased skeletal muscle fibrosis and reversal of myocyte atrophy. These interventions are promising non-surgical treatment options for patients suffering from severe and recurrent inguinal hernias.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Investigating the Role Intestinal-Specific FXR and SHP in Regulating Lipid Metabolism in Mice

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Dysregulation of lipid metabolism is a causal factor that can lead to a variety of disorders, such as obesity and metabolic syndrome. Dietary fats are digested in the small intestine by the physiological detergents known as bile acids. They emulsify the fats and break them down into smaller molecules in order for the enterocytes to absorb the nutrients through simple diffusion or through the utilization of specific lipid transporters. Interestingly, the nuclear receptors farnesoid X receptor (FXR) and small heterodimer partner (SHP) not only regulates bile acid synthesis and circulation, but also lipid metabolism. Although many studies have examined the role of FXR in hepatic and intestinal lipid metabolism, studies investigating the role of SHP in the intestine are still lacking. Although FXR and SHP cooperate to regulate many metabolic pathways, FXR or SHP knockout models exhibit different lipid phenotypes. These data indicate there are FXR-dependent and -independent pathways of SHP that controls lipid metabolism. To delineate these two interconnecting yet separate pathways, we will utilize intestine-specific *Shp* knockout (*IShpKO*) and intestine-specific *Fxr* knockout (*IFxrKO*) mice model and place them on high fat diet to investigate their intestinal intestinal absorption and transportation of lipids. We will also monitor the bile acid pool in the intestine, serum, and liver in these knockouts to evaluate the consequence of intestinal deletion of *Fxr* as well as *Shp* on bile acid homeostasis and how this may affect lipid absorption. These experiments will identify how FXR and/or SHP regulates intestinal fat digestion and absorption and if this is secondary to the alterations in bile acid concentration and lipid transporters. In addition, we will also investigate the intestinal *Fxr-Shp* double knockout (*IDKO*) mice model to determine their combined contribution in intestinal lipid metabolism. Overall, the results obtained from this research will elucidate if intestinal FXR and SHP cooperate or can independently regulate lipid metabolism and homeostasis.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Investigating the Role of Farnesoid X Receptor in Heme Biosynthesis and Ductular Reaction

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Bile acids (BAs) have gained traction not just as emulsifiers of fat, but also as hormones. Nuclear receptor Farnesoid X receptor (FXR) is the master regulator of BAs and can also control glucose and lipid metabolism. We examined if FXR contributed towards heme biosynthesis and induction of a ductular reaction. Male and female whole body *Fxr* knockout (*Fxr*KO) mice, as well as liver- and intestine-specific knockouts (L*Fxr*KO and I*Fxr*KO, respectively) were treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC, a ferrochelatase inhibitor) for two weeks. At the end of the two weeks, mice were fasted for four hours and euthanized. All groups of mice had lost a similar percentage of body weight when fed the DDC diet. However, female *Fxr*KO mice had significantly increased liver to body weight ratio, while male *Fxr*KO mice had significantly decreased liver to body weight ratio when fed the DDC diet compared with their wild type counterparts. Serum liver injury markers were analyzed and liver histology and changes in genes involved in the heme biosynthesis pathway were examined. Both male and female whole body *Fx*rKO livers had decreased ductular reaction with minimal bile plugs

(porphyrin accumulation) compared with their wild type counterparts. L*Fxr*KO mice mimicked diminished ductular reaction, while I*Fxr*KO mice exhibited severe ductular reaction similar to that of wild type mice, indicating that the ductular reaction is dependent on hepatic FXR. ChIP-Seq for FXR revealed binding peaks in the heme biosynthesis genes, *Alas1*, *Alad*, *Uros*, and *Fech*, suggesting that FXR may act as a transcription factor for these genes. Further investigation revealed that *Pbgd* gene expression was increased, while *Fech* gene expression was decreased in female FxrKO mice compared to wild type mice. In male mice, *Pbgd*, *Uros*, *Urod*, and *Cpox* gene expression was increased in the absence of *Fxr*. In conclusion, *Fxr* is necessary to mount a ductular reaction and plays a key role in heme biosynthesis in the liver.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Maternal Total Cortisol Levels in Early Pregnancy Depends on Fetal Sexual Dimorphism. But Finally No Association With Birth Weight

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Enhanced maternal cortisol levels may have a negative impact on fetal development with a higher risk for diseases later in life, e.g. premature cardiovascular disease and type 2 diabetes. Prior studies do assume even a sex specific impact. Currently, it is unknown whether sexual dimorphism in the fetus could display a different maternal cortisol level that is associated with intra uterine growth. In the present study (performed in the Amsterdam Born Children and their Development (ABCD) –cohort), we evaluated in 3049 pregnant women (in early pregnancy) whether fetal sex is related to the level of maternal serum total cortisol and whether this contributes to fetal growth. Maternal serum total cortisol levels increased along early pregnancy from on average 390±22 nmol/L (at 5th week of pregnancy) to 589±15 nmol/L (at 20th week of pregnancy). The presence of a female fetus was associated with higher maternal total cortisol level in a distinctive time interval along early pregnancy; before 11th week of pregnancy, no difference, and from the 12th week of pregnancy a difference of 15 (SE:7) nmol/L between mothers carrying a male vs female fetus was found and that difference increased to 45 (22) nmol/L at 20th week of pregnancy (p-for-interaction=0.05). Maternal serum total cortisol levels were negatively associated with maternal age, pBMI, smoking and parity, the last one also increasing with pregnancy duration. After adjusting for these factors, the association between fetal sex and maternal cortisol remained Maternal serum total cortisol levels were significantly associated with birth weight, standardized for pregnancy duration (ß -.22; SE:0.06; P < 0.001). Girls had a significantly lower birth weight (-132 SE:16 gram) compared to males, however, maternal cortisol did not alter the association between fetal sex and birth weight to a relevant degree indicating no mediation by maternal cortisol. In early pregnancy, the maternal total cortisol levels are related to fetal sex. However this difference in maternal total cortisol level was finally not related to birth weight.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Measurement of Steroid Fatty Acyl Esters in Blood and Brain

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Steroid fatty acyl esters (FAEs) are a class of steroid conjugates that are abundant in circulation, have long half-lives, and are stored in lipid-rich tissues. Steroid-FAEs are present in many species, but their functions are poorly understood. They can be metabolized to active, unconjugated steroids and therefore may act as a reservoir of steroids. Dehydroepiandrosterone (DHEA) is an androgen precursor that can be conjugated to various fatty acids. DHEA also modulates aggression in several species, including songbirds, rodents and humans. Recent studies suggest that DHEA-FAEs might be present in songbird blood and/or brain, in part, to regulate aggression. Here, we (1) investigated the abundance of multiple fatty acids in songbird blood and (2) developed an indirect method to measure DHEA-FAEs in songbird blood and brain. First, preliminary work demonstrated high circulating levels of total (esterified and non-esterified) fatty acids, especially oleic, linoleic, and palmitic acids. These data, in conjunction with previous research, suggest that these fatty acids might be conjugated to steroids, including DHEA. Second, we successfully developed a saponification technique to indirectly measure DHEA-FAEs. Saponification cleaves the bond between the steroid molecule and the fatty acid, and we then measure the unconjugated steroid. DHEA-FAEs were incubated in 0.5M potassium hydroxide in ethanol for 30 min at room temperature, and steroids were subsequently extracted twice with dichloromethane. Unconjugated DHEA was quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS), the gold standard in steroid measurement. DHEA recovery was 88% using reference standards in neat solution. We validated this method with song sparrow plasma and chicken serum and obtained recoveries of 94-105% with intra-assay variation of 2.6%. Future research will directly measure specific DHEA-FAEs (e.g. DHEA-oleate) in blood and brain using LC-MS/MS. This research will elucidate the possible roles of steroid-FAEs in brain function and the regulation of steroid-dependent behavior. This work may also clarify the identities, levels and functions of steroid-FAEs in other species, including rodent models and humans. These data have implications for basic and clinical neuroendocrinology, offering insights into a possible storage system for steroids that may influence social behaviour.