nuclear atypia in endometrial glands. We hypothesized that hyperinsulinemia and unopposed estradiol have a synergistic effect on inducing abnormal architecture and DNA damage in the endometrium, than either alone.
At 8-10 weeks old, cohorts of MKR (n=20) and WT (n=20) mice underwent ovariectomy and placement of either an estradiol (E2) or placebo (P) pellet. Metabolic profiling included insulin tolerance testing and MR for body composition. At 3 months post-implantation, mice received a partial hysterectomy and second pellet replacement. At 6 months, the remaining uterus was bisected into pieces. A blinded histological analysis was conducted by a gynecology pathologist. A marker of DNA damage due to oxidative stress, 8-oxoguanine-DNA-glycosylase (8-OHdG), was quantified by ELISA. Data was analyzed using Kruskal-Wallis test with multiple test correction, or Fischer's exact test.
By 6 months, MKR-E2 treated mice had a 27% lower body weight than MKR-P mice ($p<0.05$), and 31% lower than WT-E2 mice ($p<0.01$). WT-E2 and WT-P had similar weight, and were similar to MKR-P (p=ns). Percent body fat was similar across all 4 cohorts of mice (p=ns). Since placebo-treated mice had small, atrophied uteri with minimal gland formation, E2 pellet failure was determined by the presence of small, atrophied uteri and occurred in 4 MKR and 3 WT mice at either 3 or 6 months. All other MKR and WT E2 treated mice had enlarged uteri. The frequency of endometrial gland dilation was similar in MKR-E2 and WT-E2 uteri (p=ns), but all MKR mice had moderate-severe dilation, whereas WT mice had 50% mild and 50% moderate-severe dilation (p=0.07). Focal hyperplasia was present in one MKR-E2 mouse, and nuclear atypia was present in one WT-E2 mouse. MKR-P uteri had a 7-fold higher mean 8-OHdG relative to MKR-E2 uteri (5.0±3.7 vs 0.7±1.6, $p<0.002$). WT-E2 and WT-P uteri had similar 8-OHdG (1.6±0.8 vs 1.8±0.6, p=ns), as did MKR-E2 and WT-E2 uteri (p=ns).
Our findings show that hyperinsulinemia exacerbates the cystic dilation induced by chronic unopposed estradiol, indicating a synergy of insulin and estradiol in promoting abnormal glandular growth in the endometrium. Surprisingly, uterine DNA damage was highest in the setting of hyperinsulinemia alone, in a hormonal state mimicking post-menopause. Further work is needed to understand the effect of estradiol on intrauterine oxidative stress-induced damage.

Reproductive Endocrinology
IMPLANTATION AND PREGNANCY: IMPACT ON MATERNAL AND FETAL HEALTH

Long-Term Effects of Late Gestation** in Utero **Hypoxic Stress on Mood Disorders: Sex and Age Differences

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Introduction: *In utero* insults have been linked with increased fear and anxiety in progeny. I*n utero* hypoxic stress is associated with a multitude of gestational complications such as pregnancy-associated hypertensive disorders and intrauterine growth restriction. Maternal hypertension during pregnancy is also associated with increased mood and anxiety disorders in progeny. However, it is unknown if these associations are due to *in utero* hypoxic stress. We hypothesized that exposure to late gestational hypoxia will have a long-term impact on anxiety in progeny. **Methods:** Timed pregnant female Long-Evans rats were exposed to five days (gestational days: 15-20) of chronic intermittent hypoxia (CIH) or room air (normoxia - 21% O_2) for 8 hours during their sleep phase. Each CIH cycle was 6 min of 3 min hypoxia (10% O_2) and 3 min normoxia (21% O_2) for a total of 10 CIH cycles/hour. At weaning (PND 28), progeny was pair-housed with a conspecific of same sex and similar weight. To examine mood and anxiety disorders, we quantified anxiety-related behaviors (time spent in the center of open field arena, marble burying test, social and anti-social behaviors with conspecifics) along with quantifying food intake and circulating sex hormone levels during puberty (postnatal day, PND 40-45) and young adulthood (PND 60-65) in male and female progeny. **Results:** Gestational CIH did not impact circulating sex hormones or food intake, regardless of sex or age of progeny. However, gestational CIH increased anxiety related behaviors in pubertal females. These effects of gestational CIH on anxiety in pubertal females were not maintained, as these behaviors resolved in young adulthood. Gestational CIH did not impact male progeny, regardless of age. **Conclusion:** Exposure to CIH during gestation resulted in increased anxiety related behaviors in pubertal female progeny. *In utero* hypoxia during late gestation may temporarily increase the risk for mood and anxiety disorders in pubertal females.

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IMPLANTATION AND PREGNANCY: IMPACT ON MATERNAL AND FETAL HEALTH

Long-Term Effects of Late Gestation** in Utero **Hypoxic Stress on Neurodegeneration: Sex and Age Differences

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Introduction: *In utero* insults have been proposed to lead to the onset of neurodegenerative diseases later in life, such as Parkinson's disease (PD). I*n utero* hypoxia is associated with a multitude of conditions, such as maternal sleep apnea, preeclampsia, gestational diabetes, and maternal hypertension. Exposure to *in utero* hypoxia may impact male progeny more than female progeny, which may underlie the male biased sex differences in PD. It is currently unknown whether late gestational hypoxic stress has a long-term effect on brain regions associated with PD, such as the nigrostriatal pathway. We hypothesized that exposure to late gestational hypoxia will result in nigrostriatal impairment in adult male progeny compared to adult female progeny. **Methods:** Timed pregnant female Long-Evans rats were exposed to five days (gestational days: 15-20) of chronic intermittent hypoxia (CIH) or room air (normoxia - 21% O_2) for 8 hours during their sleep phase. Each CIH cycle was 6 min of 3 min hypoxia

(10% O_2) and 3 min normoxia (21% O_2) for a total of 10 CIH cycles/hour. Gestational age at delivery was recorded and neonate's body weights were measured within 12-16 hours from birth. At weaning (postnatal day, PND 28), progeny was pair-housed with a conspecific of the same sex and similar weight. To examine PD, we focused on PD associated characteristics of oxidative stress in the nigrostriatal pathway and behavioral impairments of motor (open field activity and ultrasonic vocalizations) and cognitive (spatial memory) function during puberty (PND 40-45) and young adulthood (PND 60-65). **Results:** Gestational CIH had no effect on the duration of gestation, litter size, and neonatal weight at birth. Gestational CIH did not impact circulating oxidative stress, regardless of sex or age of progeny. Offspring gross motor function (open field activity) and cognitive (Morris Water maze) function were unaffected by gestational CIH. In contrast, gestational CIH impaired ultrasonic vocalizations in adult male progeny. Gestational CIH increased the latency to vocalize and decreased the loudness of the vocalizations in adult male progeny. **Conclusion:** Exposure to CIH during gestation resulted in nigrostriatal impairment in adult male progeny, as evidenced by impaired ultrasonic vocalizations that require a functional nigrostriatal pathway. *In utero* hypoxia during late gestation may increase the risk for PD in males.

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IMPLANTATION AND PREGNANCY: IMPACT ON MATERNAL AND FETAL HEALTH

Obesity Induces Elevated Oxidative Stress in Uteri of Reproductive Age Mice

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Obesity is an independent risk factor for endometrial cancer. We hypothesize that obesity changes endometrial physiology, increasing cellular vulnerability to cancer development. We previously found widespread increases in cytochrome P450 (CYP) expression in the uteri of diet-induced obese mice. CYP enzymatic activity is associated with oxidative stress and altered intracellular estrogen metabolism, which increases the potential for oncogenesis. To assess oxidative stress and related DNA damage in uterine tissue, we measured protein carbonyl (PC) and 8-oxo-2'-deoxyguanosine (8-OHdG) levels, respectively, in obese and lean mice.

Post-pubertal C57BL/6 female mice were fed high-fat chow (HF; 45% fat; n=20) or normal chow (NC; 18% calories from fat; n=10) for either 10 or 22 weeks. The mice underwent insulin tolerance testing and MRI for body composition. Vaginal smears were analyzed over 10 days for estrous cycling (n=5 per group). PC and 8-OHdG levels were quantified from uterine tissue lysates and DNA fractions using colorimetric ELISAs and analyzed by 2-tailed t-tests. The HF mice had 64% higher body fat than NC mice after 10 weeks of diet (24±2% vs. 15±1%; p<0.003) and 176% higher body fat after 22 weeks of diet (35±2% vs. 13±2%; p<0.0001). Additionally, compared to NC mice, insulin levels in HF mice were 1.6-fold higher after 10 weeks (0.89±0.05 vs. 0.55±0.03 ng/mL; p<0.0008) and 2.8-fold higher after 22 weeks (3.56±0.49 vs. 1.28±0.21 ng/mL; p<0.005). Estrous cycle analysis showed obesity-related disruptions by 22 weeks, with HF mice spending 30±3% of their time in the proestrus phase versus 18±2% in NC mice (p<0.03). After 10 weeks of diet, HF and NC mice had similar levels of PC (p=NS). By 22 weeks of diet, however, the HF mice had a 2.3-fold increase in PC compared to the NC mice (1.11±0.2 vs. 0.49±0.09 nmol/mg; p<0.02). While HF and NC mice had similar levels of 8-OHdG for each diet group (p=NS, respectively), 8-OHdG levels increased with age between 10 and 22 weeks of diet in both HF (7.51±1.25 vs. 12.41±1.87 ng/mL; p<0.03) and NC (6.87±1.28 vs. 15.16±2.88 ng/mL; p<0.01) mice.

We show that the uteri of reproductive age mice are altered by chronic diet-induced obesity and hyperinsulinemia. Uterine oxidative stress was higher with increased duration and severity of obesity, and may also be affected by the disrupted HPO axis. In contrast, obesity did not appear to promote DNA damage, suggesting that DNA repair mechanisms remain intact under conditions of increased oxidative stress. The age-dependent increase in 8-OHdG levels demonstrates an expected accumulation of DNA damage over time, which reinforces C57BL/6 mice as an adequate model for human endometrial disease. In sum, this study suggests that chronic high-fat consumption and long-term obesity increase oxidative stress over time and may contribute to the development of a pre-cancerous phenotype in human endometrium.

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Paternal Obesity and SGLT2 Inhibition Alter Expression of Placental Regulatory Genes

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We previously demonstrated that paternal obesity is associated with offspring metabolic risk during later life, and that paternal SGLT2i treatment improves offspring metabolic phenotypes. Since the placenta is a key determinant of prenatal growth and development, we hypothesized the placenta could mediate the impact of paternal obesity and paternal SGLT2i treatment. Male C57BL/6J mice were fed standard chow (Purina 9F) or 60% high-fat diet (HFD, D12492, Research Diet), or 60% HFD plus the SGLT2 inhibitor canagliflozin (CANA, 25 mg/kg/d) for 4 weeks before mating with chow-fed females. Placenta were collected on E16.5, and RNA-seq was performed on placenta from male offspring (paternal chow, pChow, n=4, pHFD, n=5, and pHFD+CANA, n=4), and differentially expressed genes were identified using Limma. Placenta weight was significantly lower in pHFD (0.089±0.004 g, 7 litters from 6 fathers) vs. both pChow (0.108±0.011 g, 4 litters, 4 fathers) and pHFD+CANA (0.107±0.013 g, 5 litters, 5 fathers) (p<0.05). Litter size, fetal or liver weight, or fetal/placental