TRT not requiring dose titration, demonstrated improvement in sexual and mental PROs, a significant unmet need in hypogonadal males. Further placebo-controlled studies are warranted to better elucidate these improvements.

Endocrine Disruption

ENVIRONMENTAL ENDOCRINE DISRUPTION IN DEVELOPMENT AND DISEASE

Disruption of Estrogenic and Androgenic Bioactivities in Human Fetuses Exposed to Maternal Smoking

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Endocrine disruptors (EDs) interfere with hormonal signalling and, given that multiple developmental processes are hormone-driven, the prenatal period is a window of increased sensitivity. Maternal smoking is a real-life model of *in utero* exposure to a complex mixture of EDs. Cigarette smoke contains of >7,000 pollutants, including polycyclic aromatic hydrocarbons (PAHs), which are AhR ligands and cross-talk with the estrogen receptor (ER) system. Prenatal exposure to cigarette smoke is associated with adverse outcomes, including intrauterine growth restriction and increased risk of metabolic syndrome later in life. We aimed to evaluate ED effects associated with smoke exposure in human fetuses. Fetal tissues (plasma, n=48; placenta, n=30; liver, n=29) from elective terminations of normally progressing pregnancies, ranging from 10 to 20 gestation weeks, were collected (SAFeR and FEGO studies: REC 15/NS/0123, REC 04/S0802/21). PAHs and PAH-like compounds were extracted from placenta and fetal liver. Bioactivity levels in plasma, placenta and liver extracts were determined using ER and androgen receptor (AR) transactivation reporter gene assays. PAH burden was evaluated using the AhR-responsive DR_{hp}-CALUX assay. Smoke exposure was associated with a 1.3-fold increase in plasma estrogenic activity. The developmental trajectory of androgenic activity was altered in plasma of smoke-exposed fetuses, with significant anti-androgenic activity in older fetuses (>16 weeks of gestation). In males, plasma androgenic activity was positively associated with testes weight and anogenital distance. In contrast, placentas from smoking mothers had significantly increased androgenic potential. Furthermore, AhR-like activity was 2.9-fold higher in smoke-exposed placentas compared to controls, and 2.3-fold higher in female compared to male fetal livers. Overall, all bioactivity levels were higher in placentas compared to fetal liver. Prenatal exposure to cigarette smoke is associated with higher placental AhR activation, indicative of increased xenotoxicants burden. We also report that smoke-exposed fetuses showed increased circulating estrogenic activity and disrupted androgenic potential, across 10-20 weeks of gestation, in both fetal plasma and placenta. This demonstrates that EDs present in cigarette smoke are able to interfere with hormonal signalling and alter dynamic endocrine activity profiles, which are critical to ensure appropriate, sex-specific, development. These ED effects are likely to disturb placental function and reprogramme fetal development and thus impacting on life-long health.

Endocrine Disruption

ENVIRONMENTAL ENDOCRINE DISRUPTION IN DEVELOPMENT AND DISEASE

In Utero Exposure to 17α-Hydroxyprogesterone Caproate May Contribute to Increasing Incidence Rates of Early-Onset Cancer

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Background: 17α-hydroxyprogesterone caproate (17-OHPC) is a synthetic progestogen introduced in the 1950s to treat habitual and threatened abortion in pregnant women. Although 17-OHPC is still available (tradename Makena), little is known about its effects on health of adult offspring, and questions concerning safety and effectiveness remain. For example, progestogens have been implicated in cancer, and trends in the use of 17-OHPC in early pregnancy during the 1950s and 60s parallel increasing incidence rates of certain cancers in young adults, such as early-onset colorectal cancer, born during that time. **Methods:** We examined the effect of 17-OHPC exposure in utero on risk of cancer in adult offspring in the Child Health and Development Studies, a cohort of women receiving prenatal care between June 1959 and September 1966, with deliveries through June 1967 (n=18,751 live births excluding neonatal deaths among 14,507 mothers). Diagnosed conditions and prescribed medications were abstracted from mothers' medical records beginning 6 months prior to pregnancy through delivery. We identified mothers who received 17-OHPC (tradenames Delalutin and Proluton) in early pregnancy, defined as day 1 - 140 of gestation. Incident cancers diagnosed in offspring through 2018 were ascertained by linkage with the California Cancer Registry. **Results:** Among 18,751 live births, 954 cancers were diagnosed at ages 18 - 58 years. The most frequent cancers were breast (20.9%), cervical (10.9%), colorectal (7.1%), and prostate (5.9%) cancer and melanoma (9.2%). Although few mothers (n=181, 1.0%) received 17-OHPC in early pregnancy, in utero exposure was more common in offspring diagnosed with cancer (n=18, 1.9%) compared to those without cancer (n=163, 0.9%). Conditions indicating 17-OHPC included threatened abortion (54.0%), amnionitis (9.4%), and incompetent cervix (3.0%). 17-OHPC increased risk of any cancer in offspring (OR 2.08, 95% CI 1.27, 3.40), with particularly striking associations for colorectal (OR 4.78, 95% CI 1.49, 15.41) and prostate (OR 3.83, 95% CI 0.93, 15.83) cancer. There was no association between conditions indicating 17-OHPC and risk of any cancer in offspring (threatened abortion: n=1,891 mothers, OR 1.07, 95% CI 0.87, 1.32), or with use of other progestogens within 6 months prior to pregnancy (medroxyprogesterone acetate: n=50 mothers, OR 0.38, 95% CI 0.05, 2.76). **Conclusions:** Findings support susceptibility of multiple organ systems to endocrine disruption during early development and risk of cancer decades later - and may partly explain increasing rates of

early-onset colorectal cancer. Even before mechanisms of carcinogenesis are elucidated, caution using 17-OHPC and other endocrine-active pharmaceuticals in early pregnancy is warranted, especially in the absence of a clear short-term benefit, and given the possible effect on risk of cancer in adult offspring.

Endocrine Disruption

ENVIRONMENTAL ENDOCRINE DISRUPTION IN DEVELOPMENT AND DISEASE

Novel Assay for Detection of Progesterone Receptor-Interacting Endocrine Disruptors

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The presence of progesterone receptor (PR)-interacting compounds in the environment may have serious health consequences for humans and wildlife, but the methods for their detection and monitoring are limited. Here we report the development and testing of a cell line expressing a chimeric construct containing ligand-binding domain of progesterone receptor and green fluorescent protein-tagged domain of the glucocorticoid receptor (GFP-GR-PR) under tetracycline regulation. Unlike the constitutively nuclear PR, this chimera is cytoplasmic in the absence of the ligand and translocates to the nucleus in response to the hormone or its analogues. The GFP-GR-PR chimera maintains specificity for binding to progesterone and does not cross-react with GR-activating hormones. A concentration- and time-dependent translocation in response to progesterone confirmed picomolar sensitivity for detecting PR ligands. Importantly, the assay can detect both agonist and antagonist activities and thus can be used for screening environmental samples for contamination with endocrine disruptors and for drug development. Using this approach, we screened water samples collected at 23 sites along 2 major rivers in Virginia: Mattaponi and Rappahannock Rivers. We detected a low, but reproducible PR-binding activity in 34.8 % of the sites tested. The calculated progesterone equivalent concentration (EQ) in some of these sites reached ~ 0.8 ng/L. The assay provides an effect-based approach for screening PR-interacting endocrine disrupting chemicals regardless of whether they exert agonist or antagonist activities. Either one could be seriously disruptive for the health of humans and wildlife.

Endocrine Disruption

ENVIRONMENTAL ENDOCRINE DISRUPTION IN DEVELOPMENT AND DISEASE

Perchlorate and Nitrate Treatments Disrupt the Endoderm and Thyroid Development Through Epigenetic Mechanisms

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Nitrate and perchlorate competitively inhibit iodide uptake in thyrocytes and disrupt thyroid function in rodents and humans. Our previous data indicated that the intrauterine exposure to perchlorate or nitrate induced thyroid dysfunction in the offspring rats during adult life. Therefore, this study aimed to investigate the effects of these endocrine disruptors during the embryonic period on the endoderm and thyroid development. Additionally, it was also investigated the role of epigenetic modifications in the programming of gene expression of the evaluated tissues. For this purpose, CD1 pregnant female mice received filtered water (control) or filtered water supplemented with sodium perchlorate (0.3 or 1 ppm) or sodium nitrate (20 or 50 ppm). At gestational day 16.5 (GD16.5), the embryonic thyroid lobes were collected and processed for molecular analysis. Besides the *in vivo* model, the effect of these thyroid disruptors was also evaluated during the differentiation of mouse embryonic stem cells (mESc) into endoderm and thyroid cells. Endoderm cells differentiation was achieved through the treatment of mESc with several growth factors. During the entire protocol the cells were exposed or not to sodium perchlorate or sodium nitrate (10^{-5} or 10^{-7} M). The effects of perchlorate and nitrate were also evaluated during the differentiation of mESC into thyroid cells. For this purpose, mESC-derived endoderm cells were transiently transfected with Pax8/Nkx2.1 expressing vectors. During the endoderm-to-thyrocytes differentiation protocol, the cells were also exposed or not to perchlorate or nitrate (10^{-5} or 10^{-7} M). The results demonstrated that both thyroid disruptors reduced the mRNA and protein expression of several endoderm markers (Foxa1, Gata4, Sox17) in the mESc-derived endoderm cells. Moreover, perchlorate or nitrate treatment also reduced the expression of thyroid transcription factors (*Pax8, Nkx2.1, Foxe1*) and thyroid differentiation markers (*Slc5a5, Tpo, Tshr, Tg*) both in the embryonic thyroid lobes and in the mESc-derived thyrocytes. Epigenetic mechanisms related to transcription repression seem to be involved in the gene expression downregulation both *in vivo* and *in vitro*, since perchlorate and nitrate increased the mRNA expression of *Dnmt1, Dnmt3, Hdac* and reduced the expression of *Hat*. Additionally, the methylation of histone H3 was increased, and the acetylation status of this histone was decreased in perchlorate- or nitrate-exposed thyroid lobes and mESc-derived endoderm/thyroid cells. In conclusion, our data strongly suggest that the programming of thyroid dysfunction induced by intrauterine exposure to perchlorate or nitrate involve the disruption of the endoderm and thyroid development during embryonic life through epigenetic mechanisms.

Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

A Novel Algorithm for Rare Disease Gene Prediction Based on Phenotypic Similarity

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Genetic studies have yielded only a limited number of genes clearly implicated in endocrine disorders, in large part due to two current knowledge gaps. First, genome