on fasting serum levels of appetite-regulating hormones (leptin, insulin, adiponectin, GIP, PP, PYY, CCK, FGF21) were available. Hormone levels were correlated to BMI at baseline (T0) and compared across three time points: T0, T1 (after 10 weeks; initial weight loss) and T2 (after 75 weeks; weight loss maintenance). T0-T1 hormone changes were correlated to BMI changes between T1 and T2 to investigate whether hormonal alterations during initial weight loss are associated with weight regain. At T0, hormone levels were not associated with BMI. BMI decreased significantly from T0 (40.13 kg/m^2 ± 5.7) to T1 (38.2 ± 5.4, p < .001) which was maintained at T2 (38.2 kg/m^2 ± 5.9, p < .001). There were no significant changes in GIP, PP, PYY, CCK and FGF21. Leptin decreased from T0 (44.9 ng/nl ± 15.3) to T1 (33 ng/nl ± 14.8, p < .001) and T2 (38.6 ng/nl ± 16.0, p < .01), just like insulin which was significantly decreased at T1 (123 pmol/l ± 65, p < .05) and T2 (128 pmol/l ± 64, p < .05) compared to T0 (160 pmol/l ± 80). Adiponectin did not change between T0 (3.36 ug/ml ± 2.1) and T1 (3.2 ug/ml ± 2.1), but was increased at T2 (3.7 ug/ml ± 2.9, p < .01) compared to T1. T0-T2 BMI decrease correlated positively with T0-T2 decreases in leptin (r = .667, p < .001), insulin (rho = .535, p < .001) and increases of adiponectin (r = .412, p < .01), but no other hormone. T0-T1 hormone changes did not predict T1-T2 BMI changes. Thus, a 75-week CLI was associated with beneficial changes in the long-term energy regulators adiponectin, leptin and insulin, but no changes in short-term appetite-regulating hormones were observed despite significant weight loss. Initial changes in appetite-regulating hormones were not associated with subsequent weight regain. Overall, our data suggest that a CLI does not lead to adverse changes in appetite regulation, but rather long-term improvements such as e.g. increased leptin and insulin sensitivity.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

In-Vitro Exposure to Endocrine Disruptors Alters Inflammatory Markers in Whole Hypothalami and Immortalized GnRH Neurons

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Bisphenol A (BPA), a monomer of polycarbonate plastics, and Benzophenones (BPs), used as UV-filters, are endocrine disrupting chemicals (EDC) found in everyday products. Previously, we showed that the in-vitro exposure to BPA decreased Kiss-induced GnRH expression in GN11 cells (donated by Dr. Susan Wray, NIH), immature GnRH neurons, and that exposure to all the EDC decreased Kiss-induced GnRH gene expression in GT1-7 cells (donated by Dr. Pamela Mellon, UCSD), mature GnRH neurons. In this study, we analyzed the effect of in-vitro exposure to the same EDC (BPA, BP2 or BP3, Sigma, 1x10^{-9} M) or medium as control (C) in mature GnRH neurons (GT1-7 cells), and isolated hypothalami from adult Balb/c males on Glial fibrillary acidic protein (GFAP) and cytokine gene expression. Cells were exposed to the compounds for 12 or 24 h, in DMEM (high glucose) with charcoal-stripped FBS, and the hypothalami for 6 h to the EDC, in Krebs-Ringer buffer. After the incubations, RNA was extracted using Tri-Reagent (Molecular Research Center, OH, USA), 1-2 µg RNA was reverse transcribed and Real-Time PCR performed using specific primers. Results were expressed as Mean±SE and analyzed by T-test or ANOVA using Statistica v12 (StatSoft Inc, USA) Twenty-four hour BPA exposure increased *il18* in GT1-7 cells (C=1.0±0.04, BPA=1.2±0.1, T-test p<0.05, n=7), whereas neither BP2 nor BP3 had an effect on *il18* expression (ANOVA: ns, n=7). When *il6* was analyzed, 24-hour BPA decreased its expression relative to C and to 12-hour BPA, whereas BP3 had a dual effect depending on the time-point analyzed, increasing the expression at 12-hour stimulation and decreasing it after 24-hour stimulation (DMSO-12h=0.82±0.08, DMSO-24h= 1.01±0.13, BPA-12h=1.00±0.11, BPA-24h=0.68±0.06, BP2-12h=0.75±0.12, BP2-24h=0.82±0.18, BP3-12h=1.20±0.10, BP3-24h=0.83±0.05; Repeated Measures Two-way ANOVA: BPA-24h different from DMSO-24h and BPA-12h p<0.05, BP3-12h different from DMSO-12h and from BP3-24h p<0.05, n=4). In the hypothalami, 6-hour BPA exposure increased *gfap* gene expression (C=0.8±0.2, BPA=1.7±0.4; T-test p<0.05, n=9), whereas neither BP2 nor BP3 had any significant effect (C=0.8±0.2, BP2=1.4±0.3, BP3=1.0±0.3, ANOVA ns, n=9). There was no significant change in *il18, il6 or il1b* gene expression in whole hypothalami with any of the EDC tested (ANOVA ns). Our results show that the EDC herein studied have the potential to alter the inflammatory state of mature GnRH neurons and to activate astrocytes in the hypothalamus. The pattern for cytokine expression in the whole tissue could be different from the one observed in GnRH neurons, as the hypothalamus contain multiple cell types, and effects can be different in the different cells. More experiments are needed to dissect the mechanisms involved in the effects observed.
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Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

PFOA Exposure Prior to Hepatocyte Differentiation Leads to Gene Expression Changes Implicated in Non-Alcoholic Fatty Liver Disease

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Background: Perfluorooctanoic acid (PFOA), is a persistent fluorinated compound with oil and water repelling

properties found in cookware, food packaging and municipal water systems. Adult animals exposed to PFOA develop hepatomegaly, fatty liver, peroxisome proliferation, and immunotoxicity. Rodents exposed to PFCs *in utero* have altered hepatic lipid metabolism, increased hepatic *de novo* lipogenesis and susceptibility to non-alcoholic fatty liver disease (NAFLD), but underlying molecular mechanisms remain unknown. With increasing rates of obesity, diabetes, and NAFLD it is critical to examine the mechanisms by which *in utero* exposure to PFOA contributes to the development of metabolic syndrome in offspring. **Objective:** To determine mechanism by which PFOA alters gene expression in undifferentiated hepatic progenitor cells. Design/methods: HepaRG cells, a human derived hepatocyte progenitor cell line, was treated with 0.5uM PFOA or vehicle for 48 hours followed by differentiation into hepatocytes. Total RNA was extracted using the RNeasy (Qiagen) [total RNA A260/280>2 and RNA integrity number >7 (Agilent Bioanalyzer)] to generate libraries with the Illumina TruSeq stranded total RNA kit. RNA-Seq was performed using 85 bp single-end read sequencing to generate >20 million reads per sample. RNAseq data was aligned to hg38 using STAR v2.6.1a and then quantified with featureCounts v1.6.2. DESeq2 identified differentially expressed genes via FDR (false discovery rate) after Bonferroni correction. Differentially expressed gene lists were used for Ingenuity Pathway Analysis (IPA) to identify pathways of biological significance.
Results: PFOA treatment resulted in increased expression of transcription factors EGR1 (early growth response protein 1), NR4A1 (nuclear receptor Nur77), EGR2 (early growth response protein 2), KLF10 (Krueppel-like factor 10) and FOSL1 (Fos-related antigen 1), key genes linked to impaired hepatic insulin signaling, hepatic lipid metabolism, steatosis and fibrosis (fold change > 1.5; q <0.05). IPA identified enrichment of canonical pathways with biological relevance including hepatic fibrosis signaling, stellate cell activation, VDR/RXR/TR activation, and Type 2 diabetes mellitus signaling (p<0.01). **Conclusion:** Hepatocyte progenitor cells exposed to low dose PFOA for 48 hours prior to differentiation results in changes in expression of key metabolic genes linked to the development of NAFLD and enrichment of biologically relevant pathways associated with hepatic fibrosis and hepatocellular carcinoma. These results suggest that PFOA exposure *in utero* may have lasting effects on hepatic glucose and lipid metabolism after differentiation. Further studies are needed to characterize the longstanding metabolic effects of *in utero* PFOA exposure in offspring and the mechanisms driving the persistence of these changes.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Prenatal Exposure to the Endocrine Disrupting Chemical DEHP Impacts Reproduction-Related Gene Expression in the Pituitary

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Phthalates are chemicals used in various common products including plastics and medical devices, leading to widespread contact. Phthalate exposure during embryonic development can cause changes in puberty timing, reduced fertility and genital abnormalities. Previous studies on prenatal exposure to Di-(2-ethylhexyl) phthalate (DEHP) in mice indicated that it disrupts pituitary-gonadal feedback and alters reproductive performance in the offspring, however, the mechanism behind this is unknown. We hypothesize that prenatal exposure to DEHP during a critical period of embryonic development (e15.5 to e18.5) will cause sex-specific disruptions in reproduction-related functions in the pituitary in offspring due to interference with androgen and aryl hydrocarbon receptor (AhR) signaling. In order to discover the direct effects of DEHP on the reproduction-related functions in the pituitary, we performed both in vivo dosing and in vitro pituitary culture experiments. First, we dosed pregnant CD-1 mice with corn oil, the antiandrogen flutamide or DEHP from gestational day 15.5 to 18.5, then collected the pituitaries of the offspring on postnatal day 0. We found that prenatal DEHP exposure caused a significant increase in *Fshb* specifically in males, and flutamide caused significant increases in both *Lhb* and *Fshb* in males. Besides, DEHP exposure significantly increased AhR pathway related gene *Cyp1b1* in both males and females. In the in vitro experiment, we took whole pituitaries from e16.5 embryos and cultured them in media containing DEHP, MEHP and/or AhR antagonist for 72hrs. We found that the DEHP metabolite MEHP was actually the chemical that exerted the effects directly at the level of the pituitary. Similar to in vivo experiments, *Cyp1a1* and *Cyp1b1* mRNA level were increased in pituitaries treated with MEHP in both sexes and the induction could be reduced by co-treatment with AhR antagonist. The mRNA level of *Lhb*, *Fshb* and *Gnrhr* were significantly decreased in both sexes by MEHP and co-treatment with AhR antagonist did not restore mRNA levels. The induction of *Cyp1a1/Cyp1b1* gene in both in vivo and in vitro experiments indicates the possible activation of AhR by DEHP/MEHP. The in vitro experiment with AhR antagonist further proved that the induction of *Cyp1a1/Cyp1b1* was indeed due to AhR activation directly at the level of the pituitary. The difference between *in vivo* and *in vitro* experiments in terms of gonadotropin gene expression indicates multiple mechanisms should be involved in the regulation of gonadotropin gene expression in vivo including androgen-related pathways and possibly AhR-related pathways. In summary, our data suggest that phthalates can directly affect the function of the pituitary in terms of regulation of reproductive- related genes. This indicates that pituitary impacts of phthalates could contribute to reproductive dysfunction observed in exposed mice and humans.

Endocrine Disruption

ENDOCRINE DISRUPTING COMPOUNDS: MECHANISMS OF ACTION AND CLINICAL IMPLICATIONS

Protocol Based Standardized Endocrinological Evaluation of Children With Traumatic Brain Injury: A QI Initiative

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