PERINATAL EXPOSURE TO ORGANOPHOSPHATE FLAME RETARDANTS: EFFECTS ON GENE EXPRESSION, METABOLISM, FEEDING AND EXPLORATORY BEHAVIOR

By

SABRINA NICOLE WALLEY

A thesis submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science (M.S.)

Graduate Program in Toxicology

Written under the direction of

Troy Adam Roepke

And Approved by

_____

_____

_____

New Brunswick, New Jersey

May, 2019

ABSTRACT OF THE THESIS

EFFECTS OF FLAME RETARDANTS ON ARCUATE GENE EXPRESSION AND ENERGY HOMEOSTASIS IN MICE

By SABRINA NICOLE WALLEY

Thesis Director:

Troy Adam Roepke

Following the phase out of polybrominated diphenyl ethers (PBDEs) due to their persistence in the environment and endocrine disrupting properties, organophosphate flame retardants (OPFR) massively increased in production. Because of this persistence, flame retardants (FR) are ubiquitous in the environment and can interact with multiple nuclear receptors, including the estrogen receptors (ERs) and peroxisome proliferating activated receptors (PPARs). While many studies have assessed the effects of developmental exposure to EDCs on energy homeostasis, little is known about FR like OPFRs, individual food intake, metabolic parameters and their effects on metabolic syndrome. Therefore, we investigated maternal OPFR exposure followed by adult high-fat diet (HFD) or low-fat diet (LFD) challenge results in sexually dimorphic differences in behavior, activity and neuropeptides through interactions with steroids and nuclear receptors (experiment 1) and if maternal OPFR exposure with a HFD or LFD challenge results in sexually dimorphic changes in gene expression and higher susceptibility to symptoms

of metabolic syndrome (experiment 2).

In experiment 1, WT C57Bl/6J dams were orally dosed with vehicle (oil) or an OPFR mixture {1mg/kg combination each of tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate} from gestation day 7 to postnatal day 14. During maternal exposure, anogenital distance (AGD) was measured in pups as an early sign of maternal influences on progeny. Males had a reduced AGD, exhibiting estrogenic or anti-androgenic effects. After weaning, pups were challenged with a high fat (HFD) or low fat diet (LFD). In order to evaluate anxiety- like behavior, we used the elevated plus maze (EPM) and open field test (OFT) and the comprehensive lab animal monitoring system (CLAMS) for general locomotor activity. EPM results found males exhibited more anxiogenic behavior, while females had the same effect in OFT. CLAMS showed a reduction in activity for males. Individual food intake, and meal patterns were quantified with the Biological Data Acquisition System (BioDAQ), which found that OPFR and HFD males ate more during acrophase. Increased energy intake was observed in OPFR HFD female mice. Arcuate (ARC) neuropeptide and hormone receptor expression were measured to assess changes in gene expression, which showed only females had an increase in ARC expression genes. Showing that OPFR, or an interaction of OPFR and diet had a sexually dimorphic effect.

In experiment 2, WT C57Bl/6J dams were orally dosed with vehicle (oil) or an OPFR mixture {1mg/kg combination each of tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate} from gestation day 7 to postnatal day 14. After weaning, pups were challenged with a high-fat (HFD) or low-fat diet (LFD). As symptoms of metabolic syndrome are not exclusive to obese individuals, we not only analyzed

bodyweight and body composition, but other metabolic activity parameters. OPFR altered substrate utilization in both sexes, altered carbon dioxide and oxygen consumption in females following CLAMS use. OPFR altered fasting glucose in females, and glucose and hepatic glucose homeostasis in males. Plasma leptin was reduced in males while liver enzymes and receptors were reduced in both sexes. These data suggest that OPFRs alter ARC and liver homeostatic gene expression and energy balance in a sex-dependent manner.

Glossary: 17-β estradiol (E2), 2,3,7,8-tetrachlorodibenzodioxin (TCDD), agouti-related protein (AgRP), α-melanocyte stimulating hormone (α-MSH), anogenital distance (AGD), arcuate nucleus (ARC), aryl hydrocarbon receptor (AhR), Biological Data Acquisition (BioDAQ), bisphenol A (BPA), bisphenol F (BPF), bisphenol S (BPS), cholecystokinin (CCK), chlorpyrifos (CPO), cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH), dichlorodiphenyltrichloroethane (DDT), diethylstilbestrol (DES), dorsomedial hypothalamus (DMH), endocrine-disrupting compound (EDC), Environmental Protection Agency (EPA), estrogen receptor (ER), flame retardants (FR), gestational day (GD), high-fat (HFD), lateral hypothalamus (LH), low-fat diet (LFD), melanin-concentrating hormone (MCH), neuropeptide Y (NPY), nucleus tractus solitarius (NTS), organochlorines (OC), organophosphate FR (OPFR), parabrachial nucleus (PBN), parathion (PTN), paraventricular nucleus (PVN), perfluorinated compounds (PFC), perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS),

peroxisome proliferating activated receptor (PPAR), persistent organic pollutant (POP), polybrominated diphenyl ether (PBDE), polychlorinated biphenyls (PCB), postnatal day (PND), proopiomelanocortin (POMC), steroidogenic factor 1 (SF1), respiratory exchange ratio (RER), thyroid hormone (TH), thyrotropin-releasing hormone (TRH), tricresyl phosphate (TCP), triphenyl phosphate (TPP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP), ventromedial nucleus of the hypothalamus (VMH), wastewater treatment plants (WWTPs), women of child bearing potential (WOCBP)

Acknowledgement

I would like to thank my mentor Dr. Roepke for his guidance and support and my sisters and parents encouragement throughout the process. Special thanks to my committee members, Drs. Keith Cooper and Phoebe Stapleton for their time and valuable research input. Furthermore, I would like to thank Roepke members – Ali Yasrebi, Gwyn Vail, Kristie Conde, and Dr. Kim Wiersielis for their contribution to my thesis. I would also like to thank Dr. Storch for the use of EchoMRI and Oxymax CLAMS system for the project.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER 1:

INTRODUCTION AND BACKGROUND

1. INTRODUCTION

Polybrominated diphenyl ethers (PBDE) were the successor to the earliest flame retardant (FR), polychlorinated biphenyls (PCB), after they were shown to have serious toxic effects around 1977. Similar to PCBs, PBDEs have many congeners, but the most commonly used are: tetra-, penta-, octa- and decaBDE. Marine sponges are also a contributor to worldwide PBDE concentrations but are not the major contributors (Carté *et al.*, 1981; Fu *et al.*, 1995). PBDEs are an additive FR that is applied to many consumer products like furniture, textiles, building materials and plastic products. The main feature of additive FRs is that they are not chemically bound to the polymer material, and thus, have the ability to leach into the environment. Currently, there are no studies to determine the migration rate of PBDEs, but estimates are 0.038%, 0.054%, and 0.39% for deca-, octa- and penta-BDEs, respectively (EU, 2001). Although the previous estimates are for worst-case emission factors, the cause for concern is raised when the main routes of elimination are incineration and landfills. Specifically, when higher brominated PBDEs can degrade to lower, and more harmful, PBDEs upon combustion and landfills can contaminate water via runoff.

Scrutinous testing found that PBDEs were also neurotoxic, leading to alterations in learning, increased ADHD and autism on the DSM IV scale and overall cognitive function in exposed young children and toddlers. This as well as high levels found in biota led to a manufacture ban in 2004 and eventually its overall use in the US by 2013. As a result, organophosphate flame retardants (OPFR) started replacing PBDEs in 2006 and are now the main FR used today. However, recent studies have also shown similar responses in children from exposure to OPFRs.

The impacts of developmental endocrine disrupting compounds (EDC) exposures on energy homeostasis may be contributing to the increase in metabolic syndrome and its sequelae, type II diabetes and obesity, in children and adults. Presence of EDCs are widespread and found in the work and home environment at concentrations potentially harmful to the developing fetus and neonate. EDCs exert their effects by interacting with nuclear receptors including steroid receptors and xenobiotic receptors or by altering the production of steroid hormones. Exposure to EDCs such as diethylstilbestrol (DES) and bisphenol A (BPA) can lead to metabolic disruption in rodent models (Golden *et al.*, 1998; vom Saal and Myers, 2008) and these effects are dependent on the concentration, duration, route, and developmental stage of exposure. Many studies have reported that a variety of EDCs including BPA, polychlorinated biphenyls (PCB), dioxins, and dichlorodiphenyltrichloroethane (DDT) cause disruption of energy or glucose homeostasis. These effects include elevated adult body weights, fat accumulation, triacylglycerol and cholesterol levels, and altered glucose and insulin homeostasis in both male and female adult offspring (Belcher *et al.*, 2014; Kojima *et al.*, 2013; La Merrill *et al.*, 2014; Miyawaki *et al.*, 2007; Newbold *et al.*, 2007; Pillai *et al.*, 2014; Rashid *et al.*, 2013; Rubin *et al.*, 2001; Suvorov *et al.*, 2009; Xi *et al.*, 2011; Xu *et al.*, 2011). However, very few studies fully characterize the effects of perinatal EDC exposure on feeding behaviors and meal pattern (size, frequency, duration) opting instead to examine simple crude food intake over the course of the experiment or for a short period as adults.

The control of energy homeostasis and feeding behavior has been extensively reviewed (Cowley *et al.*, 2001; Williams *et al.*, 2001) and will be described briefly herein. Many of the central and peripheral regulators of energy homeostasis and feeding behavior are

known. Food intake is controlled centrally through communication between the hindbrain and hypothalamus with inputs from the emotion and reward centers of the brain (Berthoud, 2002). The hypothalamus is regarded as the key center that regulates feeding behavior. Discrete hypothalamic nuclei project numerous reciprocal neural connections between each other and to other brain regions including the hindbrain. The hypothalamic nuclei involved include the arcuate nucleus (ARC), paraventricular nucleus (PVN), lateral hypothalamus (LH), the dorsomedial hypothalamus (DMH), and ventromedial nucleus of the hypothalamus (VMH) (Saper *et al.*, 2002).

ARC neurons are in a unique position because their axonal terminals have direct contact with peripheral circulation (incomplete blood-brain barrier) and thus are controlled by peripheral satiety factors such as glucose, insulin, and leptin (Schwartz *et al.*, 2000). ARC neurons integrate those peripheral signals with inputs from other brain regions regulating sensory attributes, reward expectancies, and emotional aspects of food while orexin neurons primarily control sleeping behavior and arousal (Cowley et al., 2001; Elmquist *et al.*, 1999; Kalra *et al.*, 1999; Schlingemann *et al.*, 2003; Schwartz *et al.*, 2000). At least two distinct ARC neuronal populations act in opposition to each other to control energy homeostasis. Neurons expressing neuropeptide Y (NPY) and agouti-related protein (AgRP) are orexigenic while neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) are anorexigenic (Schwartz *et al.*, 2000). Specifically, the posttranslational POMC product, α-melanocyte stimulating hormone (α-MSH), reduces food intake via activation of the melanocortin receptors (MC-3/4) expressed in other hypothalamic nuclei such as the PVN. NPY and AgRP also act on

the same neurons to increase food intake with AgRP acting as an antagonist to melanocortin receptors, thus exerting an orexigenic influence (Saper *et al.*, 2002).

The VMH is a satiety center of the hypothalamus (Williams *et al.*, 2001). VMH neurons have direct connections with other nuclei such as the PVN and the DMH (Williams et al., 2001) and ablation of VMH steroidogenic factor 1 (SF1) neurons leads to an age-dependent increase in food intake in mice (Kinyua *et al.*, 2016). The DMH expresses both the orexigenic peptide, NPY (Bi, 2007), and the anorexigenic peptide, CART (Elias *et al.*, 2001; Williams *et al.*, 2001). This nucleus controls thermoregulation (Dodd *et al.*, 2014) and food intake through cholinergic neurons (Jeong *et al.*, 2017), suggesting that it functions as an integrator of energy homeostasis and thermoregulation (Dimicco and Zaretsky, 2007). The PVN is a command center upon which the multiple signals from the LH and ARC converge to control energy expenditure and intake. The PVN is also the site where the hypothalamic control of stress (corticotropin-releasing hormone (CRH)) and metabolism (thyrotropin-releasing hormone (TRH)) intersects to control energy homeostasis and feeding (Arora and Anubhuti, 2006; Lechan and Fekete, 2006; Mastorakos and Zapanti, 2004; Williams *et al.*, 2001). The LH, a downstream target of ARC POMC and NPY neurons, is also a feeding center of the hypothalamus given that stimulation of the LH induces food intake. The primary LH neurons that control feeding are melanin-concentrating hormone (MCH) and orexin neurons (Arora and Anubhuti, 2006; Horvath, 2006; Nahon, 2006; Williams *et al.*, 2001). Activation of MCH neurons induces hyperphagia and MCH neuron deficiency causes hypophagia (Mystkowski *et al.*, 2000).

The other brain region involved in feeding behaviors is the hindbrain, specifically the nucleus tractus solitarius (NTS) and parabrachial nucleus (PBN). These two regions control ingestive or consummatory behaviors such as chewing, licking, and swallowing and have been extensively reviewed (Grill and Hayes, 2012; Riediger, 2012; Williams and Schwartz, 2011). Briefly, the NTS receives hypothalamic (PVN, ARC, LH) and gastrointestinal vagal inputs to integrate central and peripheral signals of energy status and meal ingestion (satiety). Neurons from the rostral NTS then project to the PBN and the parvocellular reticular formation leading to the control of feeding behaviors. One peripheral gut hormone that is a major satiety signal is cholecystokinin (CCK) (Schwartz and Moran, 1996) produced after gut distension. CCK triggers satiation and the cessation of feeding simultaneously with other signals such as serotonin (Hayes and Covasa, 2006; Mazda *et al.*, 2004). Interestingly, 17β-estradiol via activation of ERα potentiates the NTS response to CCK and lipid ingestion (Asarian and Geary, 2007), opening the door to disruption by estrogenic EDC exposure in females.

Because they elicit their effects through steroid and nuclear receptors that control feeding circuits, EDCs may alter the hypothalamic hindbrain circuits and disrupt normal feeding behavior. Development of this area of the brain is formed during the early stages of development (E12) and, therefore, can be altered by adverse conditions like EDC exposure. The exposure window to EDC is critical as the central control of feeding behaviors develops both in utero and neonatally (Toda *et al.*, 2017; Zhu *et al.*, 2016). A previous review in this journal described the potential interplay between EDC and maternal programming on the control of energy homeostasis (Schneider *et al.*, 2014). The authors also described the importance of sexual dimorphism that is programmed, in part, through

steroid production at discrete developmental time periods during gestation, lactation, and puberty. In particular, the organization of the hypothalamic and extrahypothalamic centers that control feeding, reward, and motivation are key targets for the hormonally-driven programming of energy homeostasis that may be impacted by EDC exposure. However, few studies have directly examined the hypothalamic-hindbrain circuits after perinatal EDC exposure. Furthermore, there is little data on EDC's effects on meal patterns (size, frequency, duration), the feeding response to peripheral peptides (leptin, ghrelin, cholecystokinin, etc.) after refeeding, or other feeding behavior paradigms.

2. PBDEs and Environment

It is well known that PBDEs are a persistent organic pollutant (POP) and can accumulate in many environmental areas, like sediment and sludge (Kovner, 2009). This excessive accumulation is due to slow biodegradation under aerobic or anaerobic conditions since photolysis can only occur within a few millimeters from the surface. Although there is little information on transformation and degradation of PBDEs, researchers from the Netherlands showed a time-dependent increase of PBDEs in soil (de Boer *et al.*, 2003). This observation paralleled the appearance of penta- and decaBDE 30 and 20 years before, respectively. Similar results were also found in soil samples from Eastern China and the Italian Alps (Dong *et al.*, 2014; Parolini *et al.*, 2012).

In developing countries like South Africa, consumer products are not properly disposed of leading to massive growth of municipal landfill sites composed of domestic and household waste (Daso *et al.*, 2013; Odusanya *et al.*, 2009; Sibiya *et al.*, 2017 . In addition, business and commercial waste products also have extensive use of PBDEs in products and increase

the likelihood of PBDE accumulation (Sibiya *et al.*, 2017). Similar findings were observed in North America, with moderate PBDE contamination (0.2-2.5 ng/g) observed in Mexico, and high concentrations in India (15.98 - 132.72 ng/g) (Ontiveros-Cuadras *et al.*, 2019; Tiwari *et al.*, 2018). Though PBDE use was actively used in Australia, mean PBDE concentrations were relatively low, even when samples were distinguished between up-, mid-, and downstream collection areas (< 0.013 ng/g-1.98) (Anim *et al.*, 2017). In some nations, like China, explanation for the rapid increase in PBDE congeners in sediment is due to swift urbanization, industrialization, and absence of strict environmental policies (Da *et al.*, 2019). In areas of China and Ethiopia that enacted strict PBDE laws, though it is still present in sediment, it is not in the top layer nor in high concentrations. This shows following strict regulation of POPs can lead to reduced exposure, occurrences, spatial distribution, and ecological effects (Da *et al.*, 2019; Dirbaba *et al.*, 2018).

High concentrations of PBDE congeners is not just limited to sediment but has also been observed in water and sludge. In various countries, like China, freshwater lakes and other bodies of water were highly polluted (0.11-4.48 ng/L) (Li *et al.*, 2015; Li *et al.*, 2016; Liu *et al.*, 2018). Higher levels were observed in other bays north of China's Yellow Sea (0.16 – 49.9 ng/g) (Zhen *et al.*, 2016). Alarmingly, countries like Taiwan have not banned all PBDE products. Due to their persistent use of deca-BDE, PBDE concentrations have risen to an alarming 2.3 to 10,490 ng/g in the Danshui River basin, one of the largest rivers of Taiwan (Cheng *et al.*, 2018).

German sewage sludge samples were found to have tri- to heptaBDE samples while US Mid-Atlantic publicly owned treatment workers found varying concentrations of pentaBDE congeners (Andrade *et al.*, 2010; Hagenmaier *et al.*, 1992). In comparison,

German wastewater treatment plants (WWTP) between June 2009 and March 2010 had levels similar to those found in Taiwanese basins and Latvia, 158.3 to 9427 and 78 to 714 ng/g, respectively, with decaBDE playing the biggest role (Aigars *et al.,* 2017;Cincinelli *et al.*, 2012). Even higher levels were found in Canada ranging from 230-82,000 ng/g, with decaDBE dominating (Kim *et al.,* 2013). In contrast, low PBDE levels in Denmark were observed during high production and use (Christensen *et al.*, 2004). These studies show that residential waste, not atmospheric washout, during and after extensive PBDE use, persist and potentially cause adverse effects.

PBDE Animal Models

Numerous studies have found high levels of PBDEs in aquatic biota, with the highest levels corresponding to lower BDEs (Alaee *et al.,* 1999; Ikonomou *et al.,* 2002). In other words, a lower, and thus smaller, hydrophobic BDE congener can pass biological membranes more easily. European studies also found PBDE concentrations in aquatic biota were positively correlated with their location in the trophic levels and age (Evenset *et al.*, 2004; Olsson *et al.,* 2000). Similar results in the US supported these findings with the highest concentrations (57 ugBDE-47/kg lipid weight) found in bottom feeding fish (Hale *et al.*, 2001). In contrast, lower concentrations were found in harbor seals in San Francisco Bay, dolphins in the Gulf of Mexico, oysters in South Korea, and fish from the Great Lakes and Latvia (Aigars *et al.*, 2017; Crimmins *et al.*, 2012; Kuehl *et al.,* 1995; Lee *et al.*, 2018; She *et al.*, 2002). A recent study analyzed the same regions in fish from the Great Lakes and still found higher levels of PBDEs even after their ban on production and use (Gandhi *et al.*, 2017). Effects of behavior were found to be altered in zebrafish when exposed to the highest concentration. Specifically, zebrafish larvae exposed to 1,000 ug/kg 120 hours

post fertilization escaped but did not travel as far compared to control (Jin *et al.*, 2018). During this time there was also the first example of PBDEs causing adverse effects on neurodevelopment in zebra fish larvae (Chen *et al.*, 2012). Bioaccumulation in aquatic animals led to increased research, providing examples of PBDEs causing endocrine disrupting effects and developmental neurotoxicity.

Discovery of PBDE bioaccumulation prompted analysis of the location. Findings demonstrated a 300% increase in accumulation of PBDEs in adipose tissue than other areas of the body, with around 90 days to clear in rat adipose tissue (Siddiqi *et al.*, 2003). It was also noted that prenatal oral exposure can lead to increased weight gain or behavioral aberration, while newborn exposure caused learning and motor deficits that worsened with increasing age (Kuriyama *et al.*, 2007). In addition, endocrine disrupting effects from PBDE exposure altered thyroid hormone levels at low doses, with the LOAEL at 1ug/kg/day (Alaee *et al.*, 1999; Darnerud *et al.*, 2001; Smyth *et al.*, 1997); a NOEL has not been found. Together these studies show that bioaccumulation of PBDE congeners is higher in aquatic organisms compared to murine models orally dosed with PBDEs, but it is unknown why. These effects have spoken to other systemic effects like issues with neuropyscholoigcal development, enlarged kidneys, reduced hematocrit and red cell count in rodent models (EPA, 2014; Norris *et al.*, 1975). A possible explanation could be higher metabolic debromination in fish compared to murine models. PBDEs interact with steroid and nuclear receptors that control energy homeostasis and feeding behaviors including ERα, androgen receptors (AR), and PPARγ (Kojima *et al.*, 2013; Lu *et al.*, 2014; Pillai *et al.*, 2014). However, there are few studies that record food intake or feeding behavior from perinatal exposures to PBDE. One adult exposure study focused

on the effects of PBDE-99 exposure by oral gavage on exploratory behaviors, locomotor activity, and spatial learning in male rats. The authors report finding no effects on food or water consumption (Daubie *et al.*, 2011). OPFR, the successors to PBDE, interact with a range of nuclear and steroid receptors including ER, AR, and PPARs (Belcher *et al.*, 2014; Kojima *et al.*, 2013; Pillai *et al.*, 2014), which are all involved in the control of energy homeostasis and the melanocortin neurocircuitry (Barros and Gustafsson, 2011; Long *et al.*, 2014).

Human Exposure

Humans can be exposed to PBDEs through inhalation, dermal absorption, and oral ingestion. PBDE concentrations in air in Taiwan and Japan ranged from 23-53 pg/m^3 and 7.1-21 pg/m^3 (Watanabe, 1992). The most common BDEs found in air samples were tetra- to hexaBDE because of their low vapor pressure, with tetraBDE traveling the furthest. While higher brominated BDEs, like deca- and octaBDE, are less likely to travel, in the presence of UV light can degrade to lower brominated BDEs. The distance traveled for PBDE can be vast after studies found concentrations of PBDEs in areas with no known consumption or production (Stern, 2000). The highest exposure of the three exposure pathways in the US is through indoor dust. Studies have found PBDE concentrations ranging from 1,300 - 106,624 ng/g, 0.68 -38 ng/g, and 0.59 – 260 ng/g in office space, vehicle and domestic dust, respectively (Batterman *et al.*, 2010; Muenhor *et al.*, 2018) Watkins *et al.*, 2011). Additionally, the most present PBDE congener present were tetra- and pentaBDE, compounds that were phased out in most countries, but not Thailand. Studies assessing PBDE dust concentrations in US residential homes are not well characterized. This poses a serious problem because the US has the highest levels of

PBDEs in indoor environments, and overall higher levels in tissue compared to other nations. Moreover, higher risk groups can spend more time at home potentially leading to adverse effects.

Toddlers are one of the most at risk groups because of multiple routes of exposure in addition to the previously mentioned. During pregnancy PBDEs, and their metabolites, can be transferred through cord blood or placental tissue with concentrations from 1 to about 400 ng/g in lipid (Frederiksen *et al.*, 2009; Gómara *et al.*, 2007; Leonetti *et al.*, 2016). Specifically, breast milk can transfer PBDEs, and their metabolites, from the mother to the child. While there are a few epidemiological PBDEs studies, the amount is still not sufficient. However, what is understood is maternal prenatal PBDE concentrations were associated with impaired attention at 5 years, incidences of cryptorchidism in newborn boys, in addition to other adverse effects increased with higher PBDE levels in breast milk (Chen *et al.*, 2015b; Eskenazi *et al.*, 2013; Lignell *et al.*, 2013a). A more in-depth study found pregnant mothers with higher levels of serum PBDE had a higher incidence of pregnancy loss (Choi *et al.*, 2019). Prevalence of impaired fetal growth in late pregnancy, reduced birth size and the potential to act as a developmental neurotoxicant later on in life are all increased from PBDE exposure, with some of the possible neurological targets being the prefrontal cortex and hypothalamus (Ding *et al.*, 2015; Lema *et al.*, 2008; Lopez-Espinosa *et al.*, 2015; Sagiv *et al.*, 2015).

Mechanism of Toxicity

The thyroid hormone (TH) mediated pathway appears to be one of the targets within the endocrine system. Important for its crucial role in normal brain development, PBDE

exposure can mimic T3 and T4 hormones and induce liver CYP1A1 and 2B (Legler *et al.*, 2003). This structural feature allows PBDEs to bind with high and low affinity to transthyretin and hormone receptors, TR-α1 and TRβ, respectively (Chen *et al.*, 2003; Marsh, 1998). Binding of PBDEs to transthyretin can explain some of the fetal effects seen as well as show its potential to play a role in T4 transportation to fetus and across the BBB.

Although studies showed TetraBDE reduced TH levels in female rats, rats may be more sensitive to TH disruption compared to humans (Hallgren, 1998; Hill *et al.*, 1998). In addition, parent and metabolite PBDEs can alter deiodinase and sulfotransferase activity and also have similar neurotoxic effects seen in PCBs (Butt *et al.*, 2013). They cause these changes in activity by preventing removal of the iodine moiety from the precursor molecule, T4, to its active compound T3, influencing gene expression in various tissues (Butt *et al.*, 2013). In comparison, sulfated THs are more readily deiodinated compared to non-sulfated analogues and have been found to play a critical role in regulating TH levels in the fetus (Chopra *et al.*, 1992).

A proposal of the estrogen receptor (ER) as a target from PBDE exposure was tested with 17 BDE congeners using human breast cell line assays (Meerts *et al.*, 2001b). Eleven of the 17 congeners showed estrogenic activity, in a dose dependent response, with the most potent being tri- to tetraBDEs. This shows that lower bromination has a greater effect on estrogenic activity, hydroxylation results in greater potency and overall can be an agonist for ERα (Meerts *et al.*, 2001b). Specifically, Yang found that hydrogen bonding with Glu353/Arg394 contributes to the estrogenic activity of hydroxylated BDEs in human

ERα (Lee *et al.*, 2017). This suggests that there are multiple endpoints by which hormone regulation may be impacted from PBDE exposure.

All of these studies led to the labeling of PBDEs as endocrine disrupting compounds (EDC) worldwide. Moreover, the Stockholm convention labeled PBDEs a persistent organic pollutant (POP) due to its stability and high lipid solubility from bromine. These properties allow PBDEs to become unreactive in hydrolytic and photolytic degradation, stably keeping them in the gas phase. Gaseous PBDE can then biomagnify by accumulating at sites where they were used or After the Organisation for Economic Co-operation and Development (OECD) recommended suspending the use of tetra- and pentaBDEs, the most problematic, and limiting exposure, the need for a replacement was urgent.

3. Organophosphate flame retardants

The replacement of PBDEs led to the production of organophosphate flame retardants (OPFRs) and within a short amount of time OPFR global consumption was well over 500,000 tons in 2011. Organophosphate flame retardants are used in upholstery, building materials, electronics, and plastics. OPFRs are structurally similar to their neurotoxic OP pesticides and PBDES in that they can be additive or reactive, with the most concern for additive OPFRs. The inability of additive OPFRs to chemically bind to their polymer material can lead to volatilization and leaching. Similar to PBDEs, OPFRs persist in air, but a definitive reason has yet to be determined. One study proposed the heterogeneous reactions between hydroxyl radicals and FR compounds as a possibility (Liu *et al.*, 2014). Phosphate (TCP), triphenyl phosphate (TPP), tris (1, 3 dichloro-2-propryl) phosphate (TDCPP) and tris (1-chloro-2-propyl) phosphate (TCPP) are the most commonly used FRs.

Additionally, these OPFRs, mixed with brominated and other non-halogen compounds, are found in Firemaster® 550 (FM550).

Sediment, Sludge, and Water

Similar to PBDEs, water is a major medium that contributes to OPFR distribution and acts as an input pathway into the marine environment. Specifically, OPFRs were found in air, snow, remote lakes and aquatic environments (Bacaloni *et al.*, 2008; Regnery *et al.*, 2009). The major entry mode into aquatic biota is atmospheric washout by precipitation and industrial wash off from factories and wastewater treatment plants (WWTPs) (Schreder *et al.*, 2014). This capability allows OPFRs to globally distribute and have ubiquitous presentation in environmental mediums.

Noted concentrations of TCPP were found in most of the European river and one of the biggest drinking water supplies for Europe, the Ruhr river (Fries *et al.*, 2001). These levels in Europe appeared to have notably increased in the past decade, ranging from 0.31 to 549 ng/g dry weight (Giulivo *et al.*, 2017). In China TCPP was the highest of nine found in airborne particles in the south sea and water samples (Lai *et al.*, 2015). Similarly, dry weight of OPFRs were for the range from 0.1 to 0.4 ng/g per 2g of sediment samples in Taiwan while higher samples (1.0 – 12.6 ng/g) were observed in marine and river sediments (Chung *et al.*, 2009). These findings are supported by a nearby country, Korea, supporting similar levels of OPFRs in their waters and sediment (Lee *et al.*, 2018). Though there are few studies examining OPFRs in these mediums, if consumers of this water do not make considerable efforts to pay the WWTPs to remove the xenobiotics from the water, it is estimated that an annual 300kg and 100kg of TCPP and TCP, respectively, would transport from the Ruhr to connecting rivers (Andresen *et al.*, 2004).

Animal Studies

OPFRs can be classified as bioaccumulative compounds, with biomagnification only calculated in aquatic food webs. The rapid replacement of PBDEs without proper assessment of their potential carcinogenic, neurological and endocrine disrupting effects leaves gaps in our knowledge of widely used chemicals. Early studies have already shown toxicity changes in neurodevelopment, similar to OP pesticides (Sprague *et al.*, 1984; Sprague *et al.*, 1981). In addition, sex specific effects led to changes in physiological responses and pup mortality, while liver and kidney weights showed no sexual dimorphism (NTP, 1994).

TDCPP was shown to affect embryonic survival, behavior and purkinje cells development in white leghorn chicken (Bradley *et al.*, 2015). Additionally, TDCPP inhibited DNA synthesis, reduced cell number and altered neurodifferentiation (Dishaw *et al.*, 2011). These findings provide evidence that the developing nervous system is vulnerable to OPFR exposure. In PC12 cells TDCPP showed signs of decreased replication and growth, increased oxidative stress and altered cellular differentiation can affect the developing brain thereby changing the overall nervous system function (Farhat *et al.*, 2013). Ta and colleagues assessed the same endpoints as well as mRNA and protein expression and found decreased cell growth and increased apoptosis as well as noticeable changes in gene expression and protein levels (Ta *et al.*, 2014). This effect has the potential to elicit neurotoxicity by disrupting the developing nervous system, leading to loss of PC12 cells, an increase in cell apoptosis, reduction of nerve nodes and failed formation of neural

network structures. High concentrations (45 μg/g) of TDCPP administered to chicken embryos led to shorter bill lengths, reduced body weight and smaller gallbladders. Low concentrations (7.64 μg/g) of TDCPP lowered free thyroxine in the blood (Farhat, et al., 2013). Hepatocyte and embryonic neural cells exposed to TDCPP and TCPP were found to alter gene expression in the chicken xenobiotic-sensing orphan nuclear receptor (CXR), the thyroid hormone (TH) pathway, lipid regulation and growth (Crump *et al.*, 2012). TDCPP alone was found to be hepatotoxic and elicit neural cell toxicity.

There is also evidence that TDCPP causes sex dependent effects on the HPG axis in zebrafish and Japanese medaka, with almost all the same effects seen in human cell lines (Liu *et al.*, 2012; Liu *et al.*, 2013c; Sun *et al.*, 2016; Wang *et al.*, 2015b). This proposes the idea that the effects seen are conserved across vertebrate species. TDCPP can adversely affect early life development in zebrafish by passing through the chorion and bioaccumulating in early stage fish tissues at levels that were not overly toxic (Dishaw *et al.*, 2014a). In addition, adult fish absorb OPFRs through gill and epithelial tissue rather than food web biomagnification (Malarvannan *et al.*, 2015; Sundkvist *et al.*, 2010). More recently, another aquatic organism, the crucian carp, was also found to show sex dependent accumulation of OPFRs in female eggs compared to male eggs (Choo *et al.*, 2018). Terrestrial birds and mammal OPFRs can be absorbed by ingestion of food, inhalation and water or dermal contact, but studies confirming this are sparse (Eulaers *et al.*, 2014). TDCPP carcinogenicity studies in rats for over two years revealed increased tumor formation in liver and brain as well as its metabolites (Biodynamics, 1981). These results eventually led to California listing TDCPP as a carcinogen. Just like TCP and TCPP, an

annual 100 kg of TDCPP is estimated to transport from the Ruhr to the Rhine river (Andresen, *et al.*, 2004).

TPP is one of many chemicals found in FM550, with similar effects to TDCPP. Zebrafish exposed to TPP for 14 or 21 days exhibited decreased fecundity, altered sex steroid levels and changes in mRNA expression of HPG axis related genes. In the nematode, *c. elegans*, it was one of the six aromatic OPFRs that had similar toxicity to PBDEs (Behl *et al.*, 2016). Furthermore, TPP exposure inhibited larval development and reproduction at similar levels to PBDEs due to their high sensitivity to mitochondrial function.

Few, if any studies characterize the effects of perinatal OPFR exposure on food intake or feeding behavior. In a recent study using a common OPFR mixture, Firemaster® 550, perinatal exposure to 100 or 1000 μg/day via food in rats increased body weight while decreasing exploratory activity in males and females without any determination of food intake or feeding behavior (Patisaul *et al.*, 2013).

Human Exposure

Just like PBDEs, OPFR exposure can occur through inhalation, dermal contact or ingestion. Building materials are a significant source of OPFRs and have been found to be higher than that of PBDEs, but have low levels in the atmosphere. The major concern is ingestion from food and drinking water. Many studies have documented levels of OPFRs in drinking water and food with OPFR packaged plastic, but these are only indoor estimates (Benotti *et al.*, 2009; Li *et al.*, 2014). The indoor studies have found that a reference dose of 22.6 μg*$(kgd)^{-1}$ was not met in adults, but was exceeded in children, including their mothers (Butt *et al.*, 2014). This finding can be altered depending on where the child spends a majority of their

time. Those that attended daycare centers were found to have higher level of TDCPP in their urine (Hoffman *et al.*, 2015). This could be due to a higher hand to mouth ratio, lower body weight and more time spent at home. However, men that are exposed to house dust containing TDCPP showed generally stable metabolites over time, reduced sperm counts and altered levels of hormones related to fertility and thyroid function while women showed high levels of TPP (Meeker *et al.*, 2013). Providing evidence that low concentrations can have an effect.

There are only a few studies concerning OPFR ingestion. Zhang is one of the few studies that assessed rice ingestion as the major route of OPFR exposure and found detectable levels in more than half of human hair samples analyzed (Zhang *et al.*, 2016). The second highest OPFR concentration was in vegetables and showed that OPFRs translocated and uptake in certain regions of plants and vegetables (Eggen *et al.*, 2013). Based on the recommended amount of fish consumption of 375 g/week an estimated 20 $\mu g*(kg\ d)^{-1}$ of OPFR is consumed according to the National Food Administration. Babies were estimated to consume 0.67 $\mu g*(kg\ d)^{-1}$ for one liter of breast milk ingested per day.

Low TCP levels have been analyzed in humans and were found to cause behavioral changes, like memory and learning, which was not seen until adulthood (Eaton *et al.*, 2008). TPP is one of many chemicals found in FM550, with similar effects to TDCPP. Although similar results were seen in human cell lines, neurotoxicity was not observed (Hendriks *et al.*, 2014; Liu, *et al.*, 2012).

Mechanism

Not much is known regarding the mechanism of OPFRs. What is known is that OPFRs can bind to the estrogen receptor (ER) when hydroxylated (Kojima *et al.*, 2016). PPARγ is also a potential target for OPFRs, with binding primarily driven by TPP. The result of this interaction in BSM2 cells is increased transcriptional activity leading to induced lipid accumulation and perilipin protein expression (Pillai *et al.*, 2014). Their affinity for lipids is not completely associated compared to PBDEs, due to limited affinity for lipid content in fish (Brandsma *et al.*, 2015; Kim *et al.*, 2011; Malarvannan, *et al.*, 2015).

4. Bisphenol A

One of the most widely studied EDCs, BPA is directly applied to metal or plastic products to prevent leeching of metals into food. The structure of BPA is similar to endogenous ligands and can activate transcription factors like peroxisome proliferator-activated receptor gamma (PPARγ), estrogen receptor (ER) α/β, and estrogen response element gamma (ERRγ). Activation of these receptors by BPA may have adverse effects on feeding behavior (Anderson *et al.*, 2013). Numerous studies have found changes in glucose homeostasis and activity, which are related to feeding behavior, but do not directly assess its connection with EDCs.

There are few experiments that specifically examine food intake and perinatal BPA exposure. The studies discussed below have reported food intake or related endpoints. California mice are frequently used in studies to assess reproductive behaviors due to their preference in monogamous mating. Following perinatal exposure to BPA (50 mg/kg in diet) in pregnant California mice, male offspring were found to have the same exploratory

behavior compared to females with no change in body weight (Williams *et al.*, 2013). An experiment using the same animal model examined the effects of periconceptional and perinatal, diet-based, BPA exposure on metabolic and voluntary physical activity. Significant sex-dependent effects were found with body weight, water consumption, drinking episodes, and voluntary activity (Johnson *et al.*, 2015). While there were no differences in overall food intake due to BPA exposure, BPA-exposed males did not exhibit a distinct diurnal food intake pattern as was observed in the controls. Conversely, BPA-exposed females consumed less food during the dark cycle compared to the light cycle, which was not observed in the positive control (ethinyl estradiol) and spent the same amount of time eating in the light and dark cycle, unlike both negative and positive control groups (Johnson *et al.*, 2015). Water consumption in BPA exposed males increased significantly in both light and dark cycles, while no effect was seen in females. BPA exposure produced an opposite effect in drinking episodes with females exhibiting a decrease in episodes during the dark cycle and no change observed in males. The authors explained their sex-dependent findings by attributing the amount of time spent in spontaneous activity was a strong predictor of adiposity and weight gain, with evidence from other papers to support their conclusion (Perez-Leighton *et al.*, 2013; Perez-Leighton *et al.*, 2012).

Rubin and Soto have written extensive reviews on the developmental effects of BPA and their own studies have also confirmed perinatal exposure to both high and low doses of BPA through drinking water increase body weight in offspring compared to control (Rubin *et al.*, 2001). They also showed females had a difference in mean body weight between high and low doses of BPA on day 28 compared to their male littermates (Rubin *et al.*,

2001). However, Anderson et al. (2013) observed the opposite effect in energy expenditure and body weight from perinatal exposure to BPA (Anderson *et al.*, 2013). Effects of maternal exposure to BPA recorded at 3, 6, and 9 months of age showed increased energy expenditure in females at all concentrations and a decrease in food intake compared to controls (Anderson *et al.*, 2013). Males also had significant changes in increased energy expenditure at 3 and 9 months, which can be explained by the increase in ambulatory activity in males and horizontal activity in both sexes. Nevertheless, no significant effect on food intake was observed.

While perinatal BPA exposure may alter the melanocortin neurocircuitry in mice, many other studies have not found a clear effect on crude food intake. Using an isogenic mouse model, perinatal BPA exposure through maternal diet increased energy expenditure due, in part, to increased horizontal and vertical activity and a decrease in food intake in females (Anderson *et al.*, 2013). In a rat study, perinatal BPA exposure by oral gavage did not alter energy intake on a low-fat diet despite an increase in body weight indicating that the primary effect was a decrease in metabolism (Wei *et al.*, 2011). A multi-generational study in Wistar rats explored the effects of low-dose perinatal BPA exposure (oral pipette) on flavor preference and food intake. In the unexposed F2 generation, body weight was higher without any clear effect on crude food or water intake (Boudalia *et al.*, 2014). The F2 generation rats also preferred sweeter water solutions, salt, and fat compared to both F1 and control (Boudalia *et al.*, 2014). Similar findings on food or flavor preference for high sucrose/saccharin solutions have also been observed after BPA (subcutaneous) injection in adolescent rats (Diaz Weinstein *et al.*, 2013). Analysis of food intake by crude measurements is a common method in many studies that find no effect on food intake after

perinatal BPA exposure (Somm *et al.*, 2009). However, crude food intake determination may not capture subtle changes to meal pattern or food preferences.

Due to potential reproductive and metabolic disturbances by perinatal BPA exposure, the use of BPA in infant bottles, toys, and adult food and liquid storage containers has been reduced in the past decade. Industry has replaced BPA with closely related chemical substitutes such as bisphenol S (BPS) and bisphenol F (BPF). BPS and BPF are currently a concern because they are detectable in foodstuffs and human serum (Liao and Kannan, 2013). A few studies have examined the metabolic effects of these compounds in rodent models. In one study, CD-1 pregnant mice were orally dosed with BPS from gestation day (GD) 8 until postnatal day (PND) 21. BPS-exposed male mice exhibited reduced bodyweight until week 20 and increased mean velocity of movement compared to controls (Kim *et al.*, 2015). In contrast, another study reported an increase in body weight in males exposed to 1.5 and 50 μg/kg bw/d BPS perinatal and chronically through adulthood when fed a high-fat diet (Ivry Del Moral *et al.*, 2016). No effect on 24 h food intake was observed. Clearly, further investigation is needed to determine if BPS/F alters adult energy homeostasis, feeding behaviors, or the hypothalamic melanocortin circuitry like BPA.

5. Phytoestrogens

Phytoestrogens are the main ingredient in soy-based products and are another well-known EDC exerting their effects through interactions with ERs. Pregnant women that consume a high soy-based diet or bottle feed their infants a high soy-based formula are potentially at risk for adverse effects on fetal and neonatal development. Few studies that have focused

on the effects of perinatal phytoestrogen exposure on feeding behaviors or crude food intake. To understand the influence of a high phytoestrogen diet on energy balance and metabolism, one study fed dams and offspring a high or low phytoestrogen diet with continuous phytoestrogen exposure from preconception (6 weeks prior to mating) through to adulthood. Continuous exposure led to lighter and more lean mice from 10 weeks onward (Cederroth *et al.*, 2007). Mice fed the high phytoestrogen diet consumed more food at 3 and 6 months compared to the low phytoestrogen diet with an increase in metabolic rate and energy expenditure, but a decrease in respiratory exchange ratio (RER). These findings correlate with a decrease in AgRP expression and an increase in orexin A, MCH, and TRH (Cederroth *et al.*, 2007). Another study found that consumption of soy-based maternal diet led to an increase in crude food intake in male and female offspring compared to a casein-based maternal diet. When administered in conjunction with BPA during development, crude food intake was also elevated leading to greater body weight gain (Cao *et al.*, 2015). Early life (PND1–22) oral doses of genistein, a common phytoestrogen, were conducted in rat pups mimicking blood levels in infants fed a soy formula. Post-weaning body weight was reduced in males and females despite an increase in energy intake. This effect was also observed when rats were challenged with a high-fat diet (Strakovsky *et al.*, 2014). Interestingly, the low levels of phytoestrogens in the maternal diet can also disrupt energy and glucose homeostasis in offspring in a sex-dependent manner (Ruhlen *et al.*, 2008). Nevertheless, no discrete measurement of food intake was collected.

6. Dioxins

Dioxins are a very potent and persistent environmental chemical that cause serious toxicity and teratogenicity in animal models. The most potent and well-studied dioxin is 2,3,7,8-tetrachlorodibenzodioxin (TCDD), which produces its effects through interactions with the aryl hydrocarbon receptor (AhR). In adult rodent models, intraperitoneal injection of TCDD reduced food and water intake and altered flavor and macronutrient preference (Pohjanvirta and Tuomisto, 1990; Pohjanvirta *et al.*, 1998; Tuomisto *et al.*, 2000). The aversion to food and water appears to involve changes in the hypothalamic-pituitary-adrenal axis, the melanocortin neurocircuitry, and the neuropeptides that control fluid intake (Moon *et al.*, 2008; Seefeld *et al.*, 1984). Interestingly, there are few reports on perinatal TCDD exposure and metabolism with little data on food intake or meal pattern despite an increase in body weight and adiposity (La Merrill *et al.*, 2009; Sugai *et al.*, 2014; van Esterik *et al.*, 2015). In a study published in 2000, Schantz and colleagues reported that oral gavage dosing of TCDD, and PCB, to dams during gestation and lactation decreased saccharin consumption and preference in female rat offspring (Amin *et al.*, 2000). These effects on saccharin consumption and preference may be due to metabolic disturbances, which were not examined by the authors, or by an impairment in the motivation and reward circuitries. While perinatal TCDD exposure disrupt energy homeostasis, the impacts on the hypothalamic-hindbrain feeding circuits and on meal patterns are largely uncharacterized and unknown.

7. Perfluorooctanoic acid and perfluorooctanesulfonic acid (PFOA/PFOS)

Perfluorinated compounds (PFC) are widely used as environmental surface protectants to reduce the occurrence of stains, friction, and waterproofing of furniture (Domingo and Nadal, 2017). Like other EDCs, PFCs are persistent in the environment due to slow degradation rates but do not bioaccumulate in adipose tissue. Two well-studied PFCs are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). When administered in adulthood, these compounds exert their effects on food intake through the PPARα (Asakawa *et al.*, 2007; Asakawa *et al.*, 2008). Again, there are few studies that record food intake or feeding behavior from perinatal PFC exposure. In one perinatal exposure study, dams were dosed by gavage to four different concentrations of PFOA/PFOA from GD1–17 to determine any obesogenic effects. The only treatment to alter body weights was the highest PFOA dose (3.0 mg/kg/day), which reduced body weight with no change in food intake (Ngo *et al.*, 2014).

8. Polychlorinated biphenyls

PCBs are mainly used as a lubricant, cooling fluid, plasticizer, and adhesive in commercial products and are persistent in the environment despite a ban in the US since the late 1970s. While there are numerous studies on the perinatal effects of PCB exposure (Boucher *et al.*, 2009), few have closely examined food intake or feeding behavior. One perinatal study investigated how social behavior in adult offspring was impacted when dams were fed a diet containing PCBs. Exposure to 12.5 or 25 ppm PCB-47 and -77 reduced the area of the periventricular nucleus of the hypothalamus in male rats; however, there were no changes

in food consumption or body weight (Jolous-Jamshidi *et al.*, 2010). In a few studies, saccharin or sweet taste preference was examined. Males exposed perinatally to PCB in the maternal diet increased the preference for sweet (saccharin) taste but without an increase in overall food consumption (Hany et al., 1999; Kaya *et al.*, 2002).

9. Organochlorines

Organochlorines (OC), like DDT, are a type of pesticide that act on the central nervous system to confer their toxicity. Their mechanism of action is through either the GABA A receptor (i.e. cyclodienes and toxaphene) or voltage-dependent potassium and sodium channels. Though DDT is labeled as moderately toxic compared to other organochlorines, it was banned by the Environmental Protection Agency (EPA) due to unintended toxicity in wildlife and humans (Li and Jennings, 2017). There are several studies investigating how perinatal DDT exposure influences metabolism in humans (de Cock and van de Bor, 2014). In animal models, there are few studies that directly measure or report food intake or feeding behavior. In mice, maternal DDT exposure from GD11.5 to PND5 via oral gavage reduced energy expenditure and core body temperature and promoted adiposity in female offspring. DDT exposure also augmented the effects of a high-fat diet challenge leading to glucose intolerance, hyperinsulinemia, and dyslipidemia (La Merrill *et al.*, 2014). No effect on crude food intake was found during the study. Methoxychlor, a pesticide employed as a replacement for DDT and subsequently banned in the US, has also been implicated as an EDC (Padmanabhan *et al.*, 2010). In a multi-generation exposure to methoxychlor, the two higher doses (500 and 1500 ppm), reduced growth rate during the

growth period and body weight in males and females prior to weaning. Methoxychlor treatment via maternal diet also reduced crude food consumption, although the data was not shown (Aoyama *et al.*, 2012). DDT, methoxychlor, or their metabolites are potential ligands for ERα (Kim et al., 2014; Wang *et al.*, 2017), which is involved in the developmental programming of energy homeostasis, especially in females (Roepke *et al.*, 2017). Perinatal treatment of another organochlorine, the insecticide chlorpyrifos (CPO), from GD7-PND21 via gavage produced heavier male offspring after puberty and increased body volume (Lassiter and Brimijoin, 2008). No measurement of food intake or feeding behavior was noted. A similar study using the pesticide parathion (PTN) subcutaneously injected into neonatal rats from PND1–4 reported that treated mice exhibited weight gain on a low-fat diet, symptoms of pre-diabetes, and impaired fat metabolism (Lassiter *et al.*, 2008). In another study by Lassiter and colleagues, neonatal PTN injection caused a reduction in weight gain in both sexes, but only in females at 0.2 mg/kg. All sexes fed a high-fat diet showed around a 37% reduction in food consumption. However, intake was found to be isocaloric since the high-fat diet has ~ 37% more calories per gram, even though mice gained more weight (Lassiter *et al.*, 2010).

10. Tributyltin

Tributyltin (TBT) is a well-known, toxic biocide primarily used to prevent the growth of marine aquatic life on the hulls of large ships, buoys, docks, and fishnets. Perinatal TBT exposure impacts offspring metabolism and adiposity via interactions with PPARγ and retinoid X receptor (RXR) (Kirchner *et al.*, 2010). One such perinatal study attempted to understand how maternal TBT dosing via gavage from GD8 until birth combined with

postweaning pup gavage until euthanasia at PND30 altered offspring homeostasis in rats. TBT exposure did not alter food consumption in females but produced a small increase in male offspring. Interestingly, feeding efficiency was augmented in females but suppressed in males (Cooke *et al.*, 2004). It is unknown if the perinatal effects on food intake by TBT exposure involve developmental programming through PPARγ and RXR.

11. Conclusions

While not exhaustive, this review has attempted to highlight the few studies that measured food or energy intake in rodent models perinatally exposed to a range of EDC and raise a call to action for more development studies on OPFRs. Despite widespread use of OPFRs in home environments, structural similarity to neurotoxic OP pesticides, human exposure, and thus health effects remain largely unknown. Vulnerable populations are of most concern, like women of child bearing potential (WOCBP) and children, specifically in California due to higher flammability standards leading to higher exposures. A few of these studies report findings that were not consistent between them. These inconsistencies may be due to differences in species, dosages, routes of exposure or administration of EDC (oral (regular chow, novel food, or gavage); subcutaneous injection; fluid intake), or the developmental timing of exposures (gestational, lactational, pubertal). Future studies should attempt to mimic human exposures, both in concentration and route, encompass all neuroendocrine developmental periods including puberty, and examine the steroidal control of feeding behaviors in adults (Schneider *et al.*, 2014).

This review is a call for further investigation into the effects of EDC on developmental programming of feeding behaviors and the hypothalamic hindbrain neurocircuitry that

controls these behaviors. Few studies go beyond simple crude measurements of food or energy intake or measurements of melanocortin neuropeptides (α-MSH, CART, AgRP) of the hypothalamus. Two recent studies by Abizaid and colleagues are excellent examples of interrogating the impacts of perinatal EDC exposure on the hypothalamic melanocortin neurocircuitry and on peripheral peptide hormone (leptin) sensitivity. More studies on the wide array of EDC should incorporate similar endpoints while also employing behavioral instruments that measure meal patterns. Aside from crude food and liquid intake, there are other methods to record feeding behavior in rodents such as monitoring of feeding behaviors over a period of 7–14 days in instruments such as Research Diets Biological Data Acquisition (BioDAQ). This automated system can record food intake or preference, liquid intake or choice, place or taste preference, intermittent access, or a combination in up to 32 individually housed rodent models. The BioDAQ system documents the date, time, and duration of changes in weight per time period (bouts), which can be converted into meal size, duration, and frequency. Finally, another aspect of feeding behavior that should be considered is incidence of eating disorders. If perinatal (or adult) exposures to EDC can alter feeding patterns, the neurocircuitry of feeding and reward, or the sensitivity to anorectic or orexigenic hormones, these compounds potentially increase the risk for anorexia nervosa or binge eating disorders. Any relation between EDC exposures and eating disorders is largely unknown.

CHAPTER 2:

INTERACTIONS OF MATERNAL OPFR EXPOSURE AND ADULT HIGH-FAT DIET ON FEEDING, ACTIVITY, AND EXPLORATORY BEHAVIOR IN A SEXUALLY DIMORPHIC MANNER

ABSTRACT

Increased production of flame retardants (FRs) like organophosphate FRs (OPFR) can accumulate in the body and interact with nuclear receptors, like the estrogen receptor (ER). There is a lack of studies researching OPFR effects on mammalian neuroendocrine functions. Therefore, we investigated if maternal OPFR exposure followed by adult high fat diet (HFD) or low-fat diet (LFD) challenges results in sexually dimorphic differences in behavior, activity and neuropeptides through interactions with steroid and nuclear receptors. WT C57Bl/6J dams were orally dosed with vehicle (oil) or an OPFR mixture

{1mg/kg combination each of tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate} from gestation day 7 to postnatal day 14. After weaning, pups were challenged with a HFD or LFD. Anxiety like behavior was evaluated with the elevated plus maze (EPM) and open field test (OFT), while general locomotor activity was assessed with the comprehensive lab animal monitoring system (CLAMS). EPM and OFT results showed more anxiogenic behavior in males and females, respectively, while CLAMS showed a reduction in activity for males. The Biological Data Acquisition System (BioDAQ) measured individual food intake and meal patterns, which found that OPFR and HFD males ate more during acrophase. Increased energy intake was observed in OPFR HFD female mice. Arcuate (ARC) neuropeptide and hormone receptor expression were measured to assess changes in gene expression, which showed only females had an increase in ARC expression genes. Showing that OPFR, or an interaction of OPFR and diet had a sexually dimorphic effect. The physiological implications are that males are more sensitive to OPFR exposure, and that these effects are mediated by nuclear receptors including ERα.

Glossary: 17-β estradiol (E2), agouti-related peptide (AgRP), anogenital distance (AGD), apolipoprotein E (ApoE), arcuate nucleus (ARC), beta-actin (*Actb*), basal hypothalamus (BH), biological data acquisition system (BioDaQ), bisphenol A (BPA), body weight (BW), cocaine- and amphetamine-regulated transcript (CART), complementary DNA (cDNA), comprehensive lab animal monitoring system (CLAMS), elevated plus maze

(EPM), endocrine-disrupting compound (EDC), estrogen receptor (ER), estrogen receptor alpha (*Esr1*), flame retardants (FR), gestational day (GD), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), growth hormone-releasing hormone (GHRH), growth hormone secretagogue receptor (*Ghsr*), hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) hepatocyte nuclear factor 4 alpha (HNF4α), high-fat diet (HFD), insulin receptor (*Insr),* kisspeptin *(Kiss1),* kisspeptin-neurokinin B (*Tac2*)-dynorphin (KNDy), knockout (KO), leptin receptor (*Lepr*) low-fat diet (LFD), neuropeptide Y (NPY), open field test (OFT), organophosphate FR (OPFR), peroxisome proliferator activated receptor (PPAR), PPAR gamma (*Pparg*) polybrominated diphenyl ether (PBDE), postnatal day (PND), proopiomelanocortin (POMC), quantitative real-time PCR (qpCR), RNA integrity number (RIN), tricresyl phosphate (TCP), triphenyl phosphate (TPP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP)

INTRODUCTION

Production and use of polybrominated diphenyl ethers (PBDE) were quickly phased out in 2004 after numerous studies discovered they bioaccumulate in the environment (Darnerud, *et al.*, 2001). This led to many human studies assessing their affects and finding that PBDEs affect the endocrine, neurological, and reproductive systems (Costa *et al.*, 2007; Herbstman *et al.*, 2014). Specifically, they have been found to target nuclear receptors involved with homeostasis, like peroxisome proliferator activated receptor (PPAR) and the estrogen receptor (ER) (Fang *et al.*, 2015; Liu *et al.*, 2015). As a result, PBDEs were labeled as an

endocrine disrupting compound (EDC) and human concentrations have steadily declined since their phase out.

As a consequence, the production of another class of flame retardants (FR) known as organophosphate flame retardants (OPFR) was increased in many products. It is well known that OPFRs are similar in structure to PBDEs and are not chemically bound to most products. Because of this property, OPFRs can be released into dust, air, and food. Low concentrations (1.58 μg/g- 12.5 μg/g) of OPFRs have been found in homes (He *et al.*, 2015), offices (Carignan *et al.*, 2013), and in the air of offices and aircrafts (Yang *et al.*, 2014). Furthermore, high levels were found in cereals (Poma *et al.*, 2017), pastries (Poma, *et al.*, 2017), sweets and beverages (Poma, *et al.*, 2017), and breast milk (Hoffman *et al.*, 2014; Kim *et al.*, 2014). *In vitro* studies have shown that OPFRs interact with a variety of nuclear receptors, like PPARγ and ERα (Belcher *et al.*, 2014; Liu, *et al.*, 2015). Specifically, triphenyl phosphate (TPP) and tricresyl phosphate (TCP) are ERα agonists (Liu, et al., 2012; Pillai, et al., 2014), while Tris (1,3-dichloro-2-propyl)phosphate (TDCPP) was found to be an ERα antagonist and upregulate ERα target genes (Liu *et al.*, 2013a; Liu, *et al.*, 2013c). It should be noted that transactivation assays of TPP and TCP did show an activation of ERα/β, but were not as potent as the endogenous ligand, 17β-estradiol (E2) (Liu, *et al.*, 2012; Pillai, *et al.*, 2014).

Though there are studies expanding our knowledge on OPFR exposure, the effects on neuroendocrine control are few and far between. Studies in zebrafish were found to impact steroidogenesis (Wang *et al.*, 2015a), liver functions (Wang, *et al.*, 2015a), lipogenesis (Dishaw *et al.*, 2014b), glucose homeostasis (Wang, *et al.*, 2015a), neural growth and

hypothalamic functions during early developmental stages (Liu, *et al.*, 2013a); these findings were also found in chicks. Furthermore, OPFR exposure in chicks was found to induce cholestatic liver and biliary fibrosis, disrupt lipid metabolism, reduce plasma cholesterol, and alter apolipoprotein E (ApoE), hepatocyte nuclear factor 4 alpha (HNF4α), and PPARα expression (Liu, *et al.*, 2013a; Liu, *et al.*, 2013c). Studies conducted by the Patisaul lab are one of few that assess developmental OPFR effects in rodent models and have found OPFR found in FM550 accumulate in the placenta in a sex-specific manner, alter exploratory behaviors in offspring and male cardiac physiology at levels lower than the purported NOAEL (Baldwin *et al.*, 2017; Patisaul *et al.*, 2013a). Together, these studies show that maternal or early developmental exposures can impact ER and PPAR signaling, potentially causing a change in neural growth, exploratory behavior, steroid- and lipogenesis, and hypothalamic function.

The hypothalamus is the master regulator of energy homeostasis in the brain and also includes the arcuate nucleus. This area encompasses many different types of neurons, like kisspeptin-neurokinin B (*Tac2*)-dynorphin (KNDy), proopiomelanocortin (POMC), neuropeptide Y (NPY), agouti-related peptide (AgRP), and growth hormone-releasing hormone (GHRH) neurons. These neurons are associated with local and peripheral hormones throughout the body, and consequently, their ability to send and receive information regarding energy homeostasis is due to their proximity to a "leaky" area of the blood-brain barrier (Morton *et al.*, 2006; Schwartz *et al.*, 2000).

ERα is highly expressed in the ARC, and thus there is the potential for OPFR exposure to disrupt ERα mediated pathways (Roepke *et al.*, 2007). However, most experiments only

noted changes in early developmental stages in non-rodent models, so it should be of high concern to understand the effects of developmental OPFR exposure in an adult rodent model.

A well known scientist updated the barker theory, stating that that diet can alter hypothalamic developmental programming (Barker *et al.*, 1990; Simmons, 2009). Due to rising incidences of obesity, type 2 diabetes, cardiovascular disease, and changes in lipogenesis, these symptoms and diseases can be characteristic of metabolic syndrome. Furthermore, symptoms of metabolic syndrome could have long lasting effects on the fetus of a pregnant mother because of its sensitivity to hormones and EDCs. Taken together, if few rodent models have shown the large phenotypic effect ERα potentially has, and diet alters hypothalamic programming, then more research is needed. This gap in knowledge led our hypothesis that maternal OPFR exposure followed by adult high-fat diet (HFD) or low-fat diet (LFD) challenges results in sexually dimorphic differences in behavior, activity and neuropeptides through interactions with steroid and nuclear receptors. The purpose of these studies is to understand the effects of maternal exposure on behavior, activity, and gene expression.

MATERIALS AND METHODS

Animal Care

All animal treatments and procedures were completed in accordance with institutional guidelines based on National Institutes of Health standards and were performed with Institutional animal Care and Use Committee approval at Rutgers University. Wild-type C57Bl/6J female and male mice were bred in-house, maintained under controlled

temperature (25°C) and 12/12h light/dark cycle with water and food *ad libitum*. All mice prior to experimentation had *ad libitum* access to a standard chow diet (LabDiet PicoLab Verified 5v75 IF, <75 ppm phytoestrogens) and water.

Maternal Exposures

Prior to mating, dams (8-12 weeks of age, n = 20 per treatment) were habituated to peanut butter 3-4 days. Gestation day (GD) 0 was determined with confirmation of a vaginal plug or evidence of intromission. Females were housed with males for one week. Dams were weighed every three days to ensure appropriate dosing. From GD7 to postnatal day (PND) 14, dams were dosed with sesame seed oil or a mixture of OPFR (tris(1,3-dichloro-2-propyl)phosphate (TDCPP, Sigma Aldrich, St. Louis, MO), triphenyl phosphate (TPP, Sigma Aldrich, St. Louis, MO), and tricresyl phosphate (TCP, AccuStandard, New Haven, CT), each at 1mg/kg), in a sesame seed vehicle. Both treatments were delivered orally using dried peanut butter. To prepare the OPFR stock, each compound was then dissolved into 1 ml of acetone for a stock solution. 100 ul of stock solution was dissolved into 10mL of sesame oil and allowed to vent for 24-48 h, as previously reported (Krumm *et al.*, 2018). Daily ingestion of the OPFR mixture or sesame oil occurred at 1000h. Dams were given a standard breeder diet (25% fat kCal, 3.83 kcal/g, Lab Diet 5015; Lab Diet, St. Louis, MO, USA) *ad libitum*. Only litters between 6 and 8 pups were used to reduce the effects of litter size on offspring energy homeostasis (Roepke *et al.*, 2017). Litters less than 6 were culled or paired with other litters.

Male and female pup body weights were measured on postnatal day (PND) 2, 7, and 14. Anogenital distance (AGD) was recorded on post-natal day 7 (PND7) to determine the

feminization or masculinization of male and female pups, respectively. At week 3, pups were weaned, housed by sex within litter (2-4 per cage), and fed a low-fat (LFD, 10% kCal fat, Cat D12450K, Research Diets, New Brunswick, NJ, USA) or high-fat (HFD, 45% kCal fat, Cat D12451, Research Diets) diet *ad libitum.* All mice were weighed weekly along with food intake per cage. Weekly food intake was calculated as grams per cage per mouse. Two consecutive experiments with 3-4 litters per treatment in the first experiment and 4-5 litters per treatment in the second. After the first batch of experimental litters, we gained access to an open field box, elevated plus maze, and a Biological Data Acquisition System (BioDAQ®) (Research Diets, Inc., New Brunswick, NJ, USA). Between week 19 and 24, mice were run through a battery of test including open field test (OFT), elevated plus maze (EPM), locomotor activity (Comprehensive Lab Animal Monitoring System (CLAMS), and Biological Data Acquisition System (BioDAQ).

Assessment of locomotor, exploratory, and feeding behaviors

Exploratory behavior assessments were completed in each mouse at week 19. Mice were first tested using an open field test (OFT) followed by elevated plus maze (EPM) 5 days later to eliminate an ordering effect. Mice were acclimated by moving the home cage to the behavior testing room 1 day prior to the start of the exploratory behavior tests.

At the start and end of testing for each mouse, the testing apparatus was sanitized. The main lights in the behavior room were turned off while a spotlight illuminated the testing area until all tests were completed for the day. Mice were returned to their home cage and placed back in their original housing area upon completion of OFT and EPM testing.

Minimization of stress and potential biases were done by avoiding any excessive movements and sounds.

For the OFT, testing started at 1000h and mice were placed into the middle of the chamber and allowed to explore the novel testing area for a full, uninterrupted 10 minute span. Video scoring was blind to three investigators. Analysis of OFT videos compared the velocity, number of crossings, time spent in inner and outer portions of the apparatus based on diet, treatment, sex, or a combination. For the EPM, testing started at 1000h and mice were placed into the middle of the maze and allowed to explore the novel testing area for a full, uninterrupted 5 minute span. Video scoring was blind to three investigators. Analysis of EPM videos compared the total number of closed and open arm entries and the average time in closed and open arms based on diet, treatment, sex, or a combination.

At week 20, mice were placed in a Comprehensive Lab Animal Monitoring System (CLAMS) (Columbus Instruments, Columbus, OH, USA) unit to measure activity in the X and Z plane for 48h. The last 24 h of recording was used to calculate general locomotor activity, x total, and x ambulatory activity. At week 21, assessment of feeding behaviors and meal patterns were measured individually using the for 7 days using the BioDAQ. The first 4 days of each trial were considered the acclimation period and the final 3 days of recording were used for data collection. Assessment of total ingested (g), daily food intake (g), average hourly food intake (g), meal frequency (meals/h), meal size (g), average meal duration (min), number of meals and meals per day were completed based on diet, treatment, sex, or a combination, during the day or night.

Arcuate RNA Extraction and quantitative real-time PCR

At the end of all physiological and behavioral assessments, each mouse was allowed to recover for 5-6 days. For collection of hypothalamic nuclei, mice were decapitated after an injection of ketamine at 1000h (100 µL of 100 mg/mL, IP) and the brain was extracted from the skull and rinsed in ice-cold Sorensen's buffer for 30 sec. Brain slices were done using a brain matrix (Ted Pella, Redding, CA, USA) that cut the brain into 1-mm thick coronal rostral and caudal blocks corresponding to Plates 42 to 47 and Plates 48 to 53, respectively, from *The Mouse Brain in Stereotaxic Coordinates* (Paxinos & Franklin 2008, 3rd Edition). Blocks of the basal hypothalamus (BH) were transferred to RNAlater (Life Technologies) and stored overnight at 4 °C. The rostral and caudal parts of the arcuate nucleus were dissected from slices using a dissecting microscope and stored at –80 °C until needed for extraction.

Pure RNA was extracted from the arcuate nucleus (ARC) using Ambion RNAqueous® Micro Kits (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol, followed by DNase-I treatment to remove contamination by genomic DNA (Life Technologies). Quantity of RNA was assessed by the NanoDrop™ ND-2000 spectrophotometer (ThermoFisher, Inc., Waltham, MA, USA), followed by the RNA 6000 Nano Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) to assess quality. Samples with an RNA integrity number (RIN) greater than 8 were used for quantitative real-time PCR (qpCR). Complementary DNA (cDNA) was synthesized from 250 ng of total ARC RNA using Superscript III reverse transcription (Life Technologies), 4 µL 5× buffer, 25 mM $MgCl_2$, 10 mM dNTP (Clontech Laboratories, Mountain View, CA), 100 ng random hexamer primers (Promega, Madison, WI), 40 U/µL Rnasin (Promega), and 100 mM dithiothreitol in diethylpyrocarbonate-treated water (Bioexpress, Kaysville, UT) in a total

volume of 20 µL. Reverse transcription was conducted using the following protocol: 5 minutes at 25°C, 60 minutes at 50°C, and 15 minutes at 70°C. cDNA was diluted to 1:20 with nuclease-free water (Gene Mate/Bioexpress) for a final cDNA concentration of 0.5 ng/µL and stored at –20°C. Untreated ARC tissue RNA was used for the calibrator and negative control (no reverse transcription) and processed simultaneously with the experimental samples. All values were normalized and are expressed as relative mRNA expression.

Efficiencies were calculated as a percent efficiency and are approximately equal (90%–110% or one doubling per cycle, See Table 1 for a list of primer sequences). Amplification protocol for genes was as follows initial denaturing at 95 °C for 3 (SsoAdvanced) or 10 minutes (PowerSYBR) followed by 40 cycles of amplification at 94°C for 10 seconds (denaturing), 60°C for 45 seconds (annealing), and completed with a dissociation step for melting point analysis with 60 cycles of 95 °C for 10 seconds, 65 °C to 95 °C (in increments of 0.5 °C) for 5 seconds and 95°C for 5 seconds. The reference genes used were *Gapdh, Hprt, and Actb.* Positive, negative and water blank controls were included in the qPCR plate design. The geomean of the Cq values from each reference gene was used to calculate relative gene expression (Yasrebi *et al.*, 2017).

Statistical Analysis

All data are expressed as mean ± SEM and were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) or Statistica (StatSoft, Tulsa, OK, USA). Body weight gain, AGD, average weekly food intake, BioDAQ, activity, EPM, and OFT

data were analyzed using a multifactorial (sex, diet, OPFR exposure) ANOVA with a *post hoc* Newman-Keuls test. All gene expression data were analyzed using 2-way ANOVA with a *post hoc* Newman-Keuls test within each genotype. In all experiments, effects were considered significant at $\alpha \leq 0.05$.

RESULTS

Anogenital Distance and Adult Body Weights

To determine if OPFR exposure has potential estrogenic or androgenic effects on the developing pups, we measured anogenital distance on PND7. OPFR-exposed male pups had a reduced AGD compared to control (Fig. 2a; sex = $F(1,32 = 441.0$, $P < 0.0001$; sex*EDC = $F(1,32) = 9.692$). OPFR-exposed males had an average AGD ($AGD/\sqrt[3]{}$ of Body Weight) of 1.68 ± 0.4, while oil-exposed males had an average AGD of 1.81 ± 0.2 ($P < 0.05$). At 20 weeks average bodyweight for all mice was measured and analyzed by litter. Male body weight was only altered by diet ($F(1, 27) = 110.5$, $P < 0.0001$, Fig. 2b). HFD-fed Males from oil- and OPFR-treated dams weighed 44.6 ± 1.3 g and 42.9 ± 0.8 g, respectively, which was higher than their LFD-fed counterparts (oil: 33.3 ± 1.1 g, $P < 0.0001$; OPFR: 32.9 ± 0.5 g, $P < 0.0001$). Females were also only affected by diet ($F(1, 28) = 19.21$, $P<.0001$, Fig. 2c) fed a HFD also weighed more (26.0 ± 0.9 g (oil) vs. 26.5 ± 1.2 g (OPFR)) than LFD-fed females (oil: 22.5 ± 0.5 g, $P < 0.01$; OPFR: 22.1 ± 0.5 g, $P < 0.01$), regardless of maternal treatment. Overall, these studies identified a feminization of male mice from OPFR exposure.

Food intake and meal patterns

Average weekly food intake was measured for all mice from weaning until tissue collection. Average weekly energy intake was altered by diet in males ($F(1, 27) = 18.65$, $P < 0.0001$, Fig. 2b) and females ($F(1, 27) = 10.33$, $P < 0.001$; Fig. 2d). In contrast, there was no effect of EDC or diet on average weekly food intake in males (Fig. 3a) or females (Fig. 3c). HFD-fed males consumed more energy than LFD in both the oil-exposed ($P < 0.01$) and OPFR-exposed ($P < 0.05$) groups. However, only OPFR-exposed HFD females consumed more energy ($P < 0.05$) compared to LFD.

At 21 weeks, we analyzed meal patterns using a BioDAQ system. In the last 72 h of run, oil-exposed, HFD-fed males consumed less than the oil-exposed, LFD-fed males ($P < 0.001$; Fig. 4a). However, in OPFR-exposed males there was no difference between diets in total food ingested. In fact, HFD-fed males exposed to OPFR consumed more HFD than oil-exposed males ($P < 0.01$, diet: $F(1, 12) = 7.03$, $P<.05$; interaction of diet and OPFR: $F(1, 12) = 5.98$, $P < 0.05$). When separated into daily food ingested, oil-exposed, LFD-fed males consumed more than HFD-fed males ($P < 0.001$), which consumed less food than OPFR-exposed, HFD-fed males ($P < 0.001$). While there was no difference in day 2, oil-exposed, HFD-fed males consumed less food than their LFD-fed counterparts ($P < 0.05$, adult diet: $F(1, 12) = 5.44$, $P<.05$; adult diet*OPFR: $F(1, 12) = 8.08$, $P < 0.05$). During the last 72 h, hourly consumption of food was altered by time and an interaction of time and OPFR (time: $F(23, 276) = 8.56$, $P < 0.0001$; time*EDC: $F(23, 276) = 1.76$, $P < 0.05$; Fig. 4c). LFD-fed, oil-exposed males consumed more food at 0800h, 1700h, and 2000h ($P < 0.05$), compared to both HFD-fed, oil-exposed and LFD-fed, OPFR-exposed. In females, there was no effect of maternal treatment or diet on total ingested (Fig. 4d) or daily food

intake (Fig. 4e). However, hourly food intake was affected by time and interactions of time and OPFR and time and diet (time: $F(23, 276) = 9.1$, $P < 0.0001$; time*OPFR: $F(23, 276) = 2.56$, $P < 0.0001$; time*adult diet: $F(23, 276) = 1.55$, $P < 0.05$; Fig. 4f). The effects were observed during the night, specifically, at 1900, 2200, and 2300h when oil-exposed LFD females consumed less than their HFD-fed counterparts ($P < 0.05$) and their OPFR-exposed counterparts ($P < 0.01$).

Meal parameters were determined from the last 72h of each run. There was no effect of diet or maternal treatment on meal frequency (Fig. 5a and Fig. 5d) or meal size (Fig. 5b and Fig. 5e) for male and female mice. However, meal duration was longer in oil-exposed, LFD-fed male mice than their HFD-fed counterparts (Fig 5c; $P < 0.05$, diet: $F(1, 12) = 5.09$, $P < 0.05$), but no effect of diet was observed in the OPFR-exposed males or in female mice (Fig. 5f). No effect of diet or maternal treatment on total number of meals or meals per day was observed in males or females (data not shown). Overall, these studies identified sexual dimorphism from diet and maternal OPFR exposure..

Locomotor Activity and Exploratory Behaviors

Locomotor activity was measured using a CLAMS and collected from the last 24 h of each run. Total x-plane, ambulatory x-plane, and z-plane activity was measured. In males, OPFR exposure reduced nighttime activity (total and ambulatory x) in LFD-fed males and reduced the effects of HFD on activity ($P < 0.05$, Fig. 6a & b). Collectively, total and ambulatory x-plane activity were affected by diet ($F(1, 54) = 8.33$; $P < 0.01$), time ($F(1, 54) = 34.42$; $P < 0.0001$), and maternal treatment ($F(1, 54) = 6.43$; $P < 0.05$). A similar pattern was found in the z plane activity although there were few pairwise differences (diet:

F (1, 54) = 6.50; P < 0.05, time: F (1, 54) = 16.18; P < 0.001, and maternal treatment: F (1, 54) = 6.79; P < 0.05; Fig. 6c). Female activity (total and ambulatory x and z) were not altered by diet or maternal treatment but were subjected to an influence of time (x: F (1, 54) = 23.32; P < 0.0001 and z: F (1, 56) = 19.16; P < 0.0001; Fig. 6d, 6e, & 6f).

Because EDC are known to alter exploratory behaviors (Baldwin, *et al.*, 2017; Jarema *et al.*, 2015; Patisaul, *et al.*, 2013a), we tested males and females from 4-5 litters on an open field test and an elevated plus maze. When fed a HFD, OPFR exposure reduced total number of closed entries (Fig. 7a; F (1,14) = 4.925; P < 0.05) and the total number of open entries for in males (Fig. 7c; F (1,14) = 7.021; P < 0.01). An effect of diet was found for both oil- and OPFR-exposed male mice spending more time in the closed arms (Fig. 7e; F (1,14) = 8.508; P < 0.05; P < 0.01) and less in the open arms (Fig. 7g; P < 0.05; P < 0.01) compared to LFD-fed male mice. No effect of diet or OPFR was observed in the number of closed or open arm entries in females (Fig., 7b & 7d). However, there was an overall effect of diet in average time spent in closed arms (Fig. 7f; F (1,14) = 4.613; P < 0.05) and in open arms (Fig 7h; F (1,14) = 4.981, P < 0.05) for females.

For the open field test, OPFR exposure reduced the time in the inner zone in females fed a HFD (Fig. 8b; F(1,14) = 4.936, P<0.05). However, the total number of crossings was higher in HFD-fed females only after OPFR exposure (Fig. 8c; F(1,14) = 8.661; P < 0.05). There was no effect on velocity or time spent in the outer zone. Average distance traveled was reduced by OPFR exposure in LFD-fed females (Fig. 8f; F(1,14) = 4.799; P<0.05), while OPFR exposure increased outer distance traveled (Fig 8g; F(1,14) = 4.832; P<0.05), and total distance traveled (Fig 8i; F(1,14) = 5.306; P < 0.05) in HFD-fed females. Finally, HFD increased the distance traveled in the inner zone only in OPFR-exposed females (Fig.

8h; F(1,14) = 8.414, P<0.05). For males, there were few effects of diet or OPFR on OFT parameters. OPFR exposure reduced time spent in the inner zone in HFD-fed males (Fig. 9b, F (1, 14) = 4.75, P < 0.05). Similarly, OPFR exposure reduced average distance in LFD-fed males (Fig. 9f; F (1, 14) = 4.79, P < 0.05). Overall these studies identified a sexual dimorphic effect from diet and maternal OPFR exposure.

Arcuate Gene Expression

To determine if maternal OPFR exposure altered hypothalamic expression of neuropeptides and receptors involved in energy homeostasis and feeding behaviors, we analyzed gene expression in the arcuate nucleus of the hypothalamus. In males (Table 2), there was no effect of diet or OPFR exposure on any of the ten genes analyzed. Conversely, in females (Table 3), adult diet altered the expression of *Pparg* (F (1, 26) = 6.96, P<.05), *Pomc* (F (1, 26) = 7.42, P<.05), *Cart* (F (1, 26) = 6.51, P<.05), and *Kiss1* (F (1, 26) = 4.96, P<.05). Exposure to OPFR altered the expression of *Agrp* (F (1, 26) = 12.27, P<.01) and *Esr1* (F (1, 26) = 6.70, P<.05). Overall, these studies identified a sexual dimorphic effect in gene expression from diet and maternal OPFR exposure.

DISCUSSION

Understanding the impact of maternal OPFR exposure on offspring behaviors, activity and neuropeptides is one of many factors in addressing signs and symptoms related to metabolic syndrome. While there are few studies on the effects of OPFR exposure during development, we hypothesized that that maternal OPFR exposure along with a high-fat diet (HFD) challenge results in sexually dimorphic differences in behavior, activity, and

neuropeptide expression through interactions with steroid and nuclear receptors and, in particular, ERα or PPARγ.

As previously mentioned, chemicals during pregnancy can have long lasting effects on physiological and endocrine processes controlled by the hypothalamus. An example of this is the anogenital distance (AGD), which is a biomarker of perinatal androgen exposure. In males, a shorter AGD is a sign of reduced androgen production, while in females an increased AGD is increased androgen production (Macleod *et al.*, 2010; Vandenbergh *et al.*, 1995). In our current study, OPFR exposure reduced AGD in male mice demonstrating a feminization in male mice, that, in our study, is from maternal OPFR exposure. This observation was also found in PBDE experiments on Long Evans rats (Kodavanti *et al.*, 2010; Lilienthal *et al.*, 2006). However, one of the experiments did not have statistical significance, most likely due to low dose exposure (Kodavanti, *et al.*, 2010). AGD in rodents exposed to bisphenol A (BPA) was found to be reduced in males for prenatal exposure, but increased in multigenerational studies that had prenatal and postnatal exposure (Gupta, 2000; Honma *et al.*, 2002; Talsness, 2000; Tyl *et al.*, 2008; Tyl *et al.*, 2002). These findings also translated to human studies as well (Mammadov *et al.*, 2018; Sun *et al.*, 2018).

It is also known that androgen-dependent behavioral and physiological observations, like increased body weight, during adulthood is correlated to the degree of androgenization (Vandenbergh, *et al.*, 1995). Although we did find an increase in body weight across both sexes and treatments due to HFD, the absence of an increase in body weight due to OPFR exposure on a LFD suggests that the mechanism is not an androgenization effect. Furthermore, our findings are supported by Daubie and colleagues, but contradicts the

well-known study by Rubin and Soto (Daubie *et al.*, 2011; Rubin *et al.*, 2009). Furthermore, we are the first to show food intake on a HFD in male mice is suppressed by OPFR in the meal pattern studies, providing more evidence that OPFR potentially produces sex-specific effects in feeding behaviors (Fig. 4a, b). No effects were seen in total ingested and daily intake for female mice, which is supported by a study that assessed crude food intake in mice (Taylor *et al.*, 2018). Both sexes consumed more during, or right before, lights out (1700-1800), but this is expected as they are most active during this time.

Our exploratory tests illustrated an interactive effect of HFD and OPFR exposure. Specifically, OPFR-exposed females spent less time in the inner section, but crossed more times compared to their counterparts with the same maternal exposure fed a LFD, while oil HFD spent more time in inner compared to oil LFD. These findings are supported by Chen and colleagues, but they did not assess sex differences when exposing mice to BPA (Chen *et al.*, 2015a). A recent OPFR study observed different results, stating there was no difference in OFT time in the center or center entries for either sex or exposure to OPFR in rats (Baldwin, *et al.*, 2017). However, the toxicokinetics between the two rodents models could explain the differences. An additional study also found no difference in OFT from female rats exposed to BPA, while another saw no difference in number of crossings but did notice males spent more time in the center compared to females (Kubo *et al.*, 2001; Xu *et al.*, 2015). Though we did not notice a difference in time spent in OPFR exposed females compared to males, we did notice, that average distance was reduced in OPFR LFD females, total distance traveled was increased in OPFR HFD, and regardless of diet, travel increased in the outer and inner areas for HFD females. In males, it appeared that EDC alone caused the reduction in time and average distance for OPFR HFD and OPFR LFD,

respectively. These results suggest there are sex differences in males and females from maternal exposure and diet.

Likewise, EPM results only showed changes in male activity. Specifically, males spent more time in the center of the EPM than anywhere else and regardless of exposure, HFD males spent more time in the closed arms compared to open when they did explore which is supported by one study (Aslani *et al.*, 2015). A similar study assessing perinatal exposure to BPA with a HFD challenge noted no difference in diet or EDC (Wise *et al.*, 2018). Possible explanation of our findings could be due to increased BW, causing less movement overall and reduced likelihood of males to explore compared to females. Though findings were also evident in numerous rats studies of varying EDCs at lower doses (15-1500 μg/kg), with a few contradicting other papers, it should be noted for most of these only one sex was used and no sex differences were found in controls (Funabashi *et al.*, 2004; Negishi *et al.*, 2004; Patisaul *et al.*, 2008). No effects were observed in females. Taken together, these results show that OPFR can have an anxiogenic effect. This finding is supported by numerous studies that exposed mice to bisphenol A (BPA) and assessed their anxiogenic effects with EPM or OFT. Since it is known that EDC can alter exploratory behaviors and our findings are supported by another report that tests a known EDC, this provides evidence that OPFRs can be a potential EDC (Baldwin, *et al.*, 2017; Jarema, *et al.*, 2015; Patisaul, *et al.*, 2013a).

Similar findings were observed for locomotor activity in CLAMS. That is, most effects were observed in males, while only x total activity changed in females. Specifically, both sexes, only HFD for females, had a higher x total count at night compared to day. Increased x total activity is expected to occur at night due to our animal model choice. However, a

reduction in x total activity in OIL-exposed HFD males compared to LFD at night was not. These exact effects were also observed in male x ambulatory activity. Z total activity, or rearing, was increased at night in oil-exposed LFD males compared to day but decreased at night in oil-exposed HFD males compared to LFD. Possible explanation for the data departing from expectations could be due to changes in the lateral hypothalamus, ARC, and ventromedial hypothalamus, and paraventricular nucleus all of which play a critical role in sleeping behavior and arousal, food intake, and feeding behavior. Other studies have contradicting results, even with differing exposure windows, EDC, and an absence of a diet challenge (Batista *et al.*, 2012; Chen *et al.*, 2013; Eriksson *et al.*, 2001; Eriksson *et al.*, 2002; Riu *et al.*, 2011; Zhao *et al.*, 2014). Overall, these changes in neurological pathways and reductions in exploratory behavior from maternal OPFR exposure during acrophase in a mouse can increase susceptibility of obesity and metabolic syndrome.

We also sought to understand if there were any effects in arcuate expression of neuropeptides and hormone receptors since any changes could explain any differences observed. Though there were no changes in males, there was a notable increase in *Pparg*, *Cart*, *Agrp*, and *Pomc, Kiss1*, and *Esr1* in female mice, all of which play critical roles in energy balance, appetite, or locomotor behavior (Table 3). A derivative of BPA, bisphenol S, was also found to increase *Agrp* with no change in *Pomc*, or *Cart*, but was only noted in male mice as they didn't include females (Rezg *et al.*, 2018). These modest changes in *Agrp* signify the mice are more orexigenic. In comparison, perinatal BPA exposed mice had a reduction in *Pomc* with no change in *Agrp*, while Salehi and colleagues saw an increase in *Pomc* relating it to PPARγ mediated mechanisms (MacKay *et al.*, 2017; Salehi *et al.*, 2019).This could potentially explain the increased in total ingested for OPFR-

exposed HFD males compared to control in the BioDAQ as well as the increase in both *Pomc* and *Pparg* gene expression.

In regards to epigenetic changes with nuclear receptors following postnatal exposure to BPA, two studies noticed an increase in *esr1* and *kiss1* in a mouse and zebrafish model (Johnson *et al.*, 2018; Qiu *et al.*, 2016). Though in the zebrafish model changes were only observed at higher concentrations (100 and 1000 μg/L) (Qiu, *et al.*, 2016). As these receptors play a crucial role in neurodevelopment, behavior, and maturation, this could explain the AGD differences in OPFR-exposed males.

To conclude, our findings along with other studies from known EDCs, appear to support the hypothesis that maternal OPFR exposure along with a high-fat diet (HFD) or low-fat diet (LFD) challenge results in sexually dimorphic differences in behavior, activity, and neuropeptides through interactions with steroid and nuclear receptors. Though these interactions are multiple and complex, we were the first to show food intake on a HFD in male mice is suppressed by OPFR in the meal pattern studies, providing more evidence that OPFR potentially produces sex-specific effects in feeding behaviors. Furthermore, there are a few that refute our findings, but these studies did not measure the exact parameters we tested such as crude versus individual food intake, different exposure windows, or only testing in one sex. However, in studies that have ablated ERα they have found that it blocks the influence of diet of energy homeostasis, hypothalamic and liver gene expression in comparison to PPARγ KO mice showing signs of enhanced emotional response from stress and anxiety (Domi *et al.*, 2016; Roepke, *et al.*, 2017). In addition, a study noted that a diet low in phytoestrogens, like our study, can lead to higher body fat

and serum leptin levels, which we observed in this study and an unpublished study by our lab (Ruhlen *et al.*, 2008).

Though our experiments have found many new, exciting results from OPFR exposure, more tests could be conducted to further understand the effect of maternal exposure and diet on behavior, activity, and gene expression. As mentioned before, ERα and PPARγ play a critical role in development. If we were to use a ERα knockout model (KO) mouse model, we could expand our knowledge on the role ERα plays in behavior, activity and gene expression. Additionally, ovariectomizing female mice to understand the potential effects amongst pair housed females, and potentially even males would be interesting to assess if the same results are observed. For a PPARγ mice, tissue specific KO models would be used (i.e. full PPARγ KO's are fatal) to understand food and energy intake and whether KO in one tissue has a greater effect on gene expression than another.

Therefore, it would be interesting to repeat these studies to determine if behavioral and gene expression patterns are also observed in a PPARγ or ERα.

Table 1. Primer Sequences.

Gene Name	Forward Primer	Reverse Primer	Accession #
*Actb**	GCCCTGAGGCTTTTTCCA	TAGTTTCATGGATGCCACAGGA	NM_007393.3
Agrp	CTCCACTGAAGGGCATCAGAA	ATCTAGCACCTCCGCCAAA	NM_007427.2
Cart	GCTCAAGAGTAAACGCATTCC	GTCCCTTCACAAGCACTTCAA	NM_013732
Esr1	GCGCAAGTGTTACGAAGTG	TTCGGCCTTCCAAGTCATC	NM_007956
*Gapdh**	TGACGTGCCGCCTGGAGAAA	AGTGTAGCCCAAGATGCCCTTCAG	NM_008084.2
Ghsr	CAGGGACCAGAACCACAAAC	AGCCAGGCTCGAAAGACT	NM_177330
*Hprt**	GCTTGCTGGTGAAAAGGACCTCTCGAAG	CCCTGAAGTACTCATTATAGTCAAGGG CAT	NM_013556
Insr	GTGTTCGGAACCTGATGAC	GTGATACCAGAGCATAGGAG	NM_010568
Kiss1	TGATCTCAATGGCTTCTTGGCAGC	CTCTCTGCATACCGCGATTCCTTT	NM_178260
Lepr	AGAATGACGCAGGGCTGTAT	TCCTTGTGCCCAGGAACAAT	NM_146146.2
Npy	ACTGACCCTCGCTCTATCTC	TCTCAGGGCTGGATCTCTTG	NM_023456
Pomc	GGAAGATGCCGAGATTCTGC	TCCGTTGCCAGGAAACAC	NM_008895
Pparg	CTGCTCAAGTATGGTGTCCATGAG	GAGGAACTCCCTGGTCATGAATC	NM_011146.3

Actb, beta-actin; *Agrp*, agouti-related peptide; *Cart*, cocaine- and amphetamine-regulated transcript; *Esr1*, estrogen receptor alpha; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Ghsr*, growth hormone secretagogue receptor; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase; *Insr*, insulin receptor; *Kiss1*, kisspeptin; *Lepr*, leptin receptor; *Npy*, neuropeptide Y; *Pomc*, proopiomelanocortin; *Pparg*, peroxisome proliferator-activated receptor γ. *denotes reference genes.

Table 2. Arcuate expression of neuropeptides and hormone receptors in males.

	Oil		OPFR	
Gene	**LFD**	**HFD**	**LFD**	**HFD**
Agrp	1.1 ± 0.1	0.7 ± 0.1	1.6 ± 0.5	0.8 ± 0.2
Cart	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Esr1	1.0 ± 0.1	1.3 ± 0.4	1.5 ± 0.3	0.9 ± 0.2
Ghsr	1.0 ± 0.1	0.8 ± 0.1	1.4 ± 0.3	1.1 ± 0.2
Insr	1.0 ± 0.1	1.3 ± 0.1	1.5 ± 0.3	1.2 ± 0.2
Kiss1	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.3	1.3 ± 0.2
Lepr	1.0 ± 0.1	1.4 ± 0.2	1.6 ± 0.4	0.8 ± 0.2
Npy	1.1 ± 0.2	0.5 ± 0.0	1.1 ± 0.2	0.9 ± 0.2
Pomc	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.3 ± 0.2
Pparg	1.1 ± 0.1	1.3 ± 0.2	1.6 ± 0.9	0.9 ± 0.1

All data were normalized to oil-treated within each sex, n = 7-9 litters.

Table 3. Arcuate expression of neuropeptides and hormone receptors in females.

	Oil		OPFR	
Gene	**LFD**	**HFD**	**LFD**	**HFD**
Agrp	1.1 ± 0.2	0.9 ± 0.1	2.2 ± 0.4**A**	2.0 ± 0.4**A**
Cart	1.0 ± 0.2	1.8 ± 0.5	1.3 ± 0.2	2.5 ± 0.5**a**
Esr1	1.0 ± 0.1	1.5 ± 0.3	1.6 ± 0.2	2.0 ± 0.2
Ghsr	1.1 ± 0.1	1.5 ± 0.4	1.8 ± 0.5	1.4 ± 0.3
Insr	1.2 ± 0.2	1.4 ± 0.4	0.9 ± 0.2	1.7 ± 0.6
Kiss1	1.1 ± 0.2	1.8 ± 0.5	1.6 ± 0.3	2.5 ± 0.4
Lepr	1.1 ± 0.2	1.4 ± 0.3	1.3 ± 0.4	1.7 ± 0.5
Npy	1.2 ± 0.2	0.9 ± 0.1	2.0 ± 0.6	1.3 ± 0.2
Pomc	1.0 ± 0.2	1.7 ± 0.5	1.4 ± 0.1	2.6 ± 0.4 **a**
Pparg	1.1 ± 0.2	2.2 ± 0.4	1.1 ± 0.2	1.8 ± 0.5

All data were normalized to oil-treated within each sex. Lowercase letters denote differences within maternal treatment between adult diets. Uppercase letters denote differences within adult diets between maternal treatments. **a** = $P < 0.05$; **b** = $P < 0.01$; **c** = $P < 0.001$; **d** = P <0 .0001. n = 7-9 litters.

Figure 1. Experimental design. Prior to mating dams were habituated to peanut butter for 3-4 days before mating. Pairing of the male and female mice was noted as gestation day (GD) 0. From GD7 until post-natal day (PND) 14, dams were dosed with the OPFR mixture (1 mg/kg/day of each OPFR) or sesame oil vehicle mixed with dried peanut butter. At week 3, pups were weaned, separated by sex and housed within litter. Offspring were fed either a low-fat diet (LFD) or a high-fat diet (HFD) for a total of 7-9 litters per treatment per diet. Between week 19 wand 24, mice were tested for differences in behavior (open field test (OFT), elevated plus maze (EPM), body composition (MRI), metabolic rate, substrate utilization, and locomotor activity (Comprehensive Lab Animal Monitoring System (CLAMS)), Biological Data Acquisition System (BioDAQ)), hemodynamics (CODA).

Figure 2. Anogenital distance at PND7 and adult body weights for mice fed a LFD or HFD at week 20. A: Anogenital Distance (AGD/body weight) for male and female pups at PND7. B: Body weights for adult male (B) and female (C) mice (age 20 weeks) on a low-fat diet (LFD) or a high-fat diet (HFD). Column number represents the sample size (number of litters) per treatment per sex. Capped lines with letters denote significance between adult diets within maternal treatments (a = $P < 0.05$; b = $P < 0.01$; d = $P < 0.0001$).

Figure 3. Average weekly food and energy intake in oil- and OPFR-exposed mice fed a LFD or HFD from week 4 to week 20. Average weekly food intake (g) for male (A) and female (C) mice. Average weekly energy intake (kCal) for male (B) and female (D) mice. Column number represents the samples size (number of litters) per treatment per sex. Capped lines with letters denote significance between adult diets within maternal treatments (a = $P < 0.05$; b = $P < 0.01$).

Figure 4. Total ingested over 72 h; daily food intake and hourly food intake from BioDAQ for oil- and OPFR-exposed mice fed a LFD or HFD. Total ingested (g) for male (A) and female (D) mice. Daily food intake (g) for male (B) and female (E) mice. Average hourly food intake (g) for male (C) and female (F) mice. Thick lines in C and F denote nighttime compared to daytime recording. Data was collected from the last 96 h of a 7-day run on the BioDAQ. Column number represent sample size (number of litters) per treatment. For A, B, D, and E, capped lines with letters denote significance between diets within maternal treatments. Letters denote significance between maternal treatments within adult diets. For C and F, lower case letters denote significance between adult diets within maternal treatment and uppercase letters denote significance between maternal treatments within adult diets (a, A = $P < 0.05$; b, B = $P < 0.01$, c = $P < 0.001$).

Figure 5. Meal frequency, meal size, and meal duration in BioDAQ for oil and OPFR-exposed mice fed a LFD or HFD. Meal frequency (meals/h) for male (A) and female (D) mice. Average meal size (g) for male (B) and female (E) mice. Average meal duration (min) for male (C) and female (F). Data was collected from the last 96 h of a 7-day run in the BioDAQ. Column number represent sample size (number of litters) per treatment. Capped lines with letters denote significance between adult diets within maternal treatments (a = $P < 0.05$).

Figure 6. Day and night locomotor activity in the CLAMS for oil- and OPFR-exposed mice fed a LFD or HFD at week 20. A: X total activity (counts). B: X ambulatory activity (counts). C: Z total activity (counts). D: X total activity (counts). E: X ambulatory activity (counts). F: Z total activity (counts). Capped lines with letters denote significance between adult diets within maternal treatments. Letters denote significance between maternal treatments within adult diets (a = $P < 0.05$; c = $P < 0.001$).

Figure 7. Exploratory behavior for oil- and OPFR-exposed mice fed a LFD or HFD in an elevated plus maze. Total number of closed entries for male (A) and female (B). Total number of open entries for oil- and OPFR-exposed male (C) and female (D) mice. Average time in closed arms for male (E) and female (F) mice. Average time in open arms for male (G) and female (H) mice. Column number represent sample size (number of litters) per treatment. Capped lines with letters denote significance between adult diets within maternal treatments. Letters denote significance between maternal treatments within adult diet (a = $P < 0.05$; b = $P < 0.01$).

Figure 8. Open field test for oil- and OPFR-exposed female mice fed a LFD or HFD. Time spent in outer (A) and inner (B) section of open field and the number of crossings (C); velocity in the outer (D) and inner (E) sections of open field; distance traveled: Average (F), outer (G), inner (H), and total (I). Column number represent sample size (number of litters) per treatment. Capped lines with letters denote significance between adult diets within maternal treatments. Letters denote significance between maternal treatments within adult diet (a = $P < 0.05$).

Figure 9. Open field test for oil- and OPFR-exposed male mice fed a LFD or HFD. Time spent in outer (A) and inner (B) section of open field and the number of crossings (C); velocity in the outer (D) and inner (E) sections of open field; distance traveled: Average (F), outer (G), inner (H), and total (I). Column number represent sample size (number of litters) per treatment. Capped lines with letters denote significance between adult diets within maternal treatments. Letters denote significance between maternal treatments within adult diet (a = $P < 0.05$).

Figure 1.

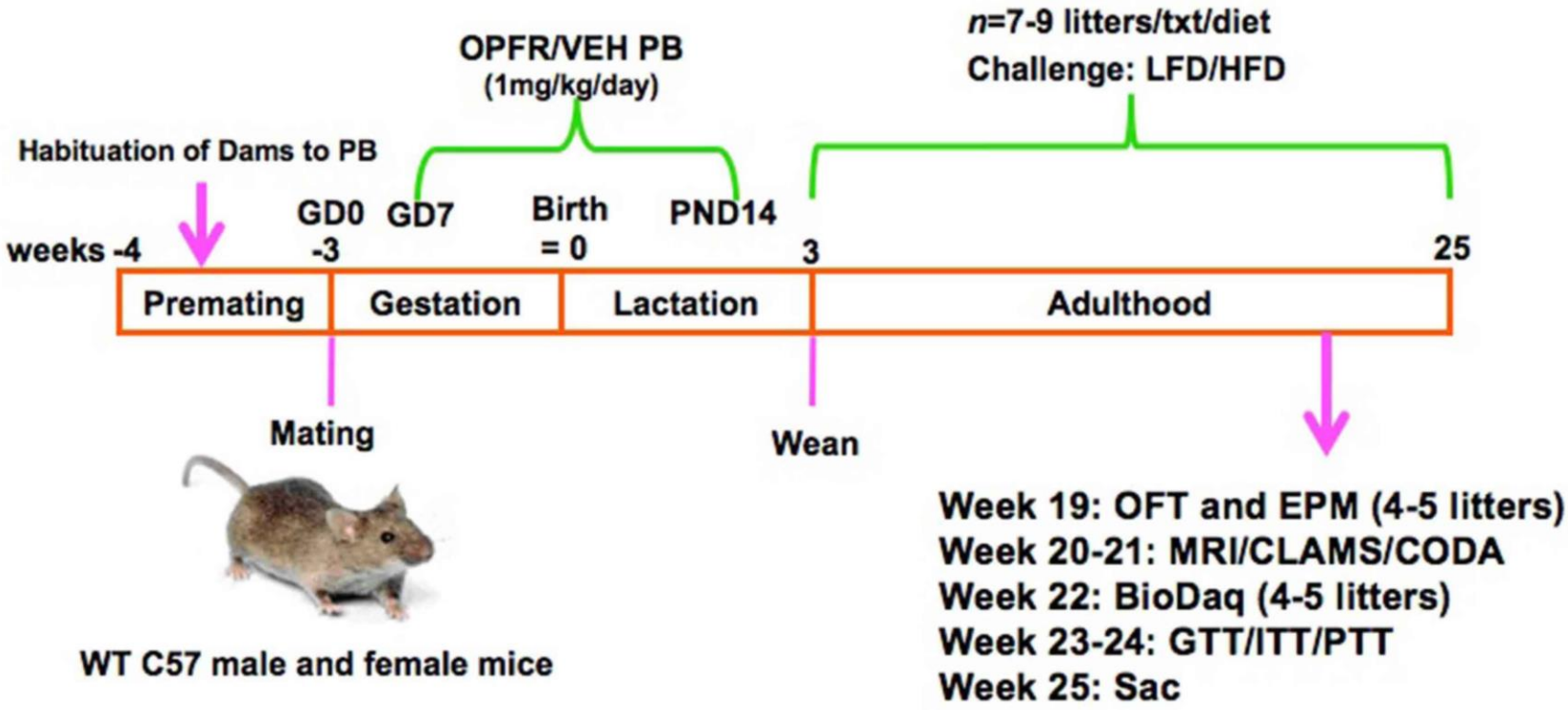

OPFR/VEH PB
(1mg/kg/day)
n=7-9 litters/txt/diet
Challenge: LFD/HFD
Habituation of Dams to PB
weeks -4
GD0
-3
GD7
Birth
= 0
PND14
3
25
Premating
Gestation
Lactation
Adulthood
Mating
Wean
WT C57 male and female mice
Week 19: OFT and EPM (4-5 litters)
Week 20-21: MRI/CLAMS/CODA
Week 22: BioDaq (4-5 litters)
Week 23-24: GTT/ITT/PTT
Week 25: Sac

Figure 2.

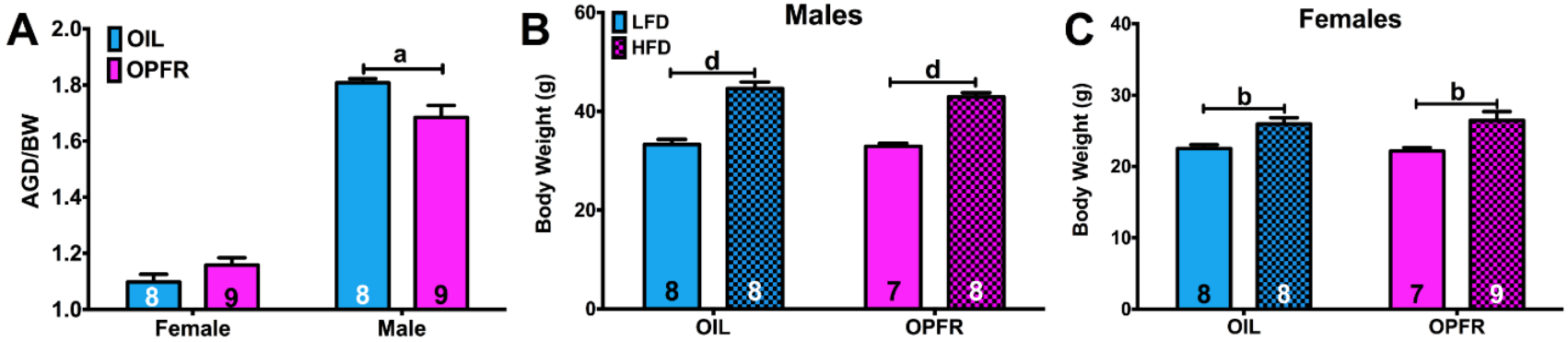

A
OIL
OPFR
AGD/BW
2.0
1.8
1.6
1.4
1.2
1.0
a
8
9
8
9
Female
Male
B
LFD
HFD
Males
Body Weight (g)
60
40
20
0
d
d
8
8
7
8
OIL
OPFR
C
Females
Body Weight (g)
40
30
20
10
0
b
b
8
8
7
9
OIL
OPFR

Figure 3.

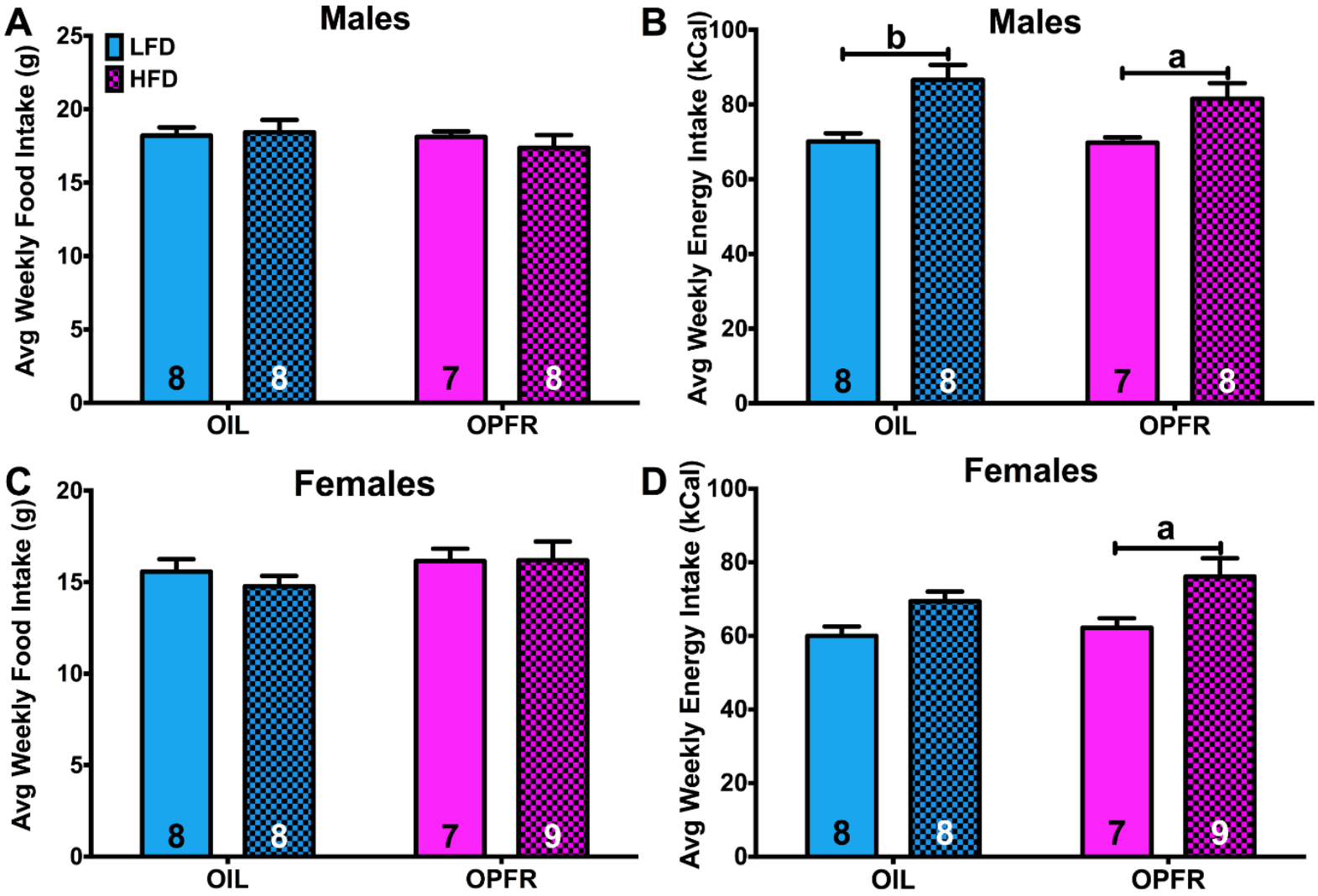

A
Males
LFD
HFD
Avg Weekly Food Intake (g)
25
20
15
10
5
0
8
8
7
8
OIL
OPFR
B
Males
Avg Weekly Energy Intake (kCal)
100
80
60
40
20
0
b
a
8
8
7
8
OIL
OPFR
C
Females
Avg Weekly Food Intake (g)
20
15
10
5
0
8
8
7
9
OIL
OPFR
D
Females
Avg Weekly Energy Intake (kCal)
100
80
60
40
20
0
a
8
8
7
9
OIL
OPFR

Figure 4.

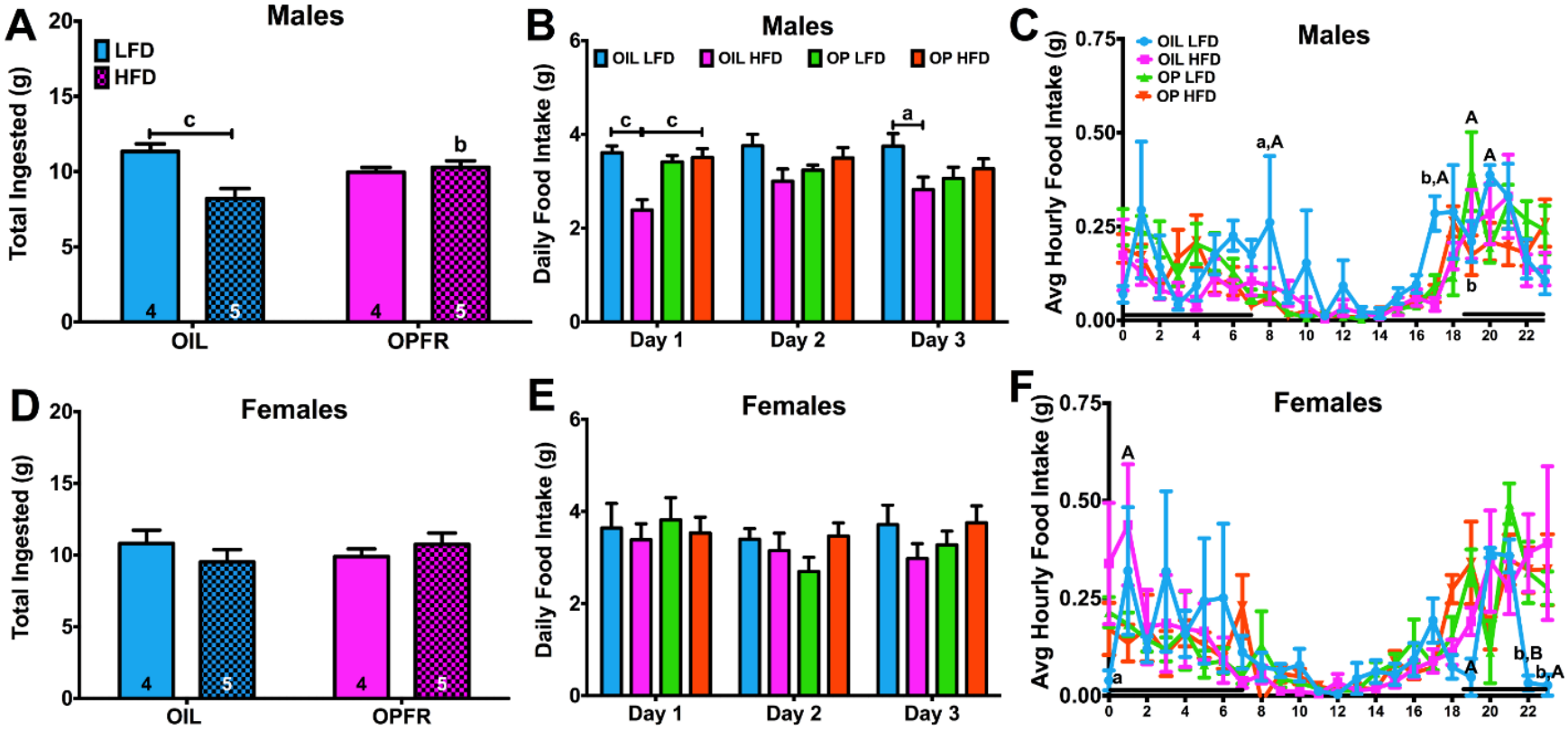

A
Males
LFD
HFD
Total Ingested (g)
c
b
4
5
OIL
OPFR
B
Males
OIL LFD
OIL HFD
OP LFD
OP HFD
Daily Food Intake (g)
c
c
a
Day 1
Day 2
Day 3
C
Males
OIL LFD
OIL HFD
OP LFD
OP HFD
Avg Hourly Food Intake (g)
a,A
b,A
A
A
b
D
Females
Total Ingested (g)
OIL
OPFR
E
Females
Daily Food Intake (g)
Day 1
Day 2
Day 3
F
Females
Avg Hourly Food Intake (g)
A
a
A
b,B
b,A

Figure 5.

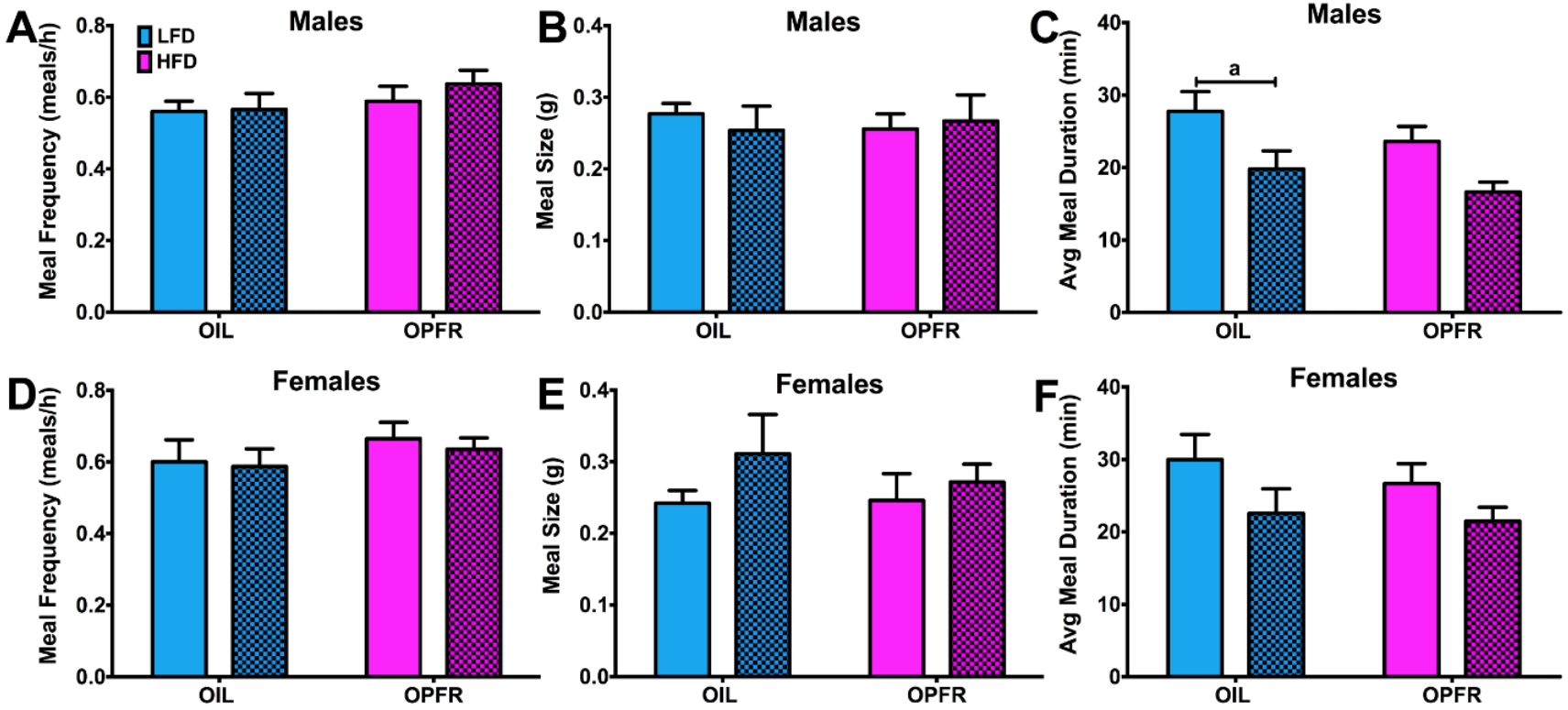

A
Males
LFD
HFD
Meal Frequency (meals/h)
0.0
0.2
0.4
0.6
0.8
OIL
OPFR
B
Males
Meal Size (g)
0.0
0.1
0.2
0.3
0.4
OIL
OPFR
C
Males
Avg Meal Duration (min)
a
0
10
20
30
40
OIL
OPFR
D
Females
Meal Frequency (meals/h)
0.0
0.2
0.4
0.6
0.8
OIL
OPFR
E
Females
Meal Size (g)
0.0
0.1
0.2
0.3
0.4
OIL
OPFR
F
Females
Avg Meal Duration (min)
0
10
20
30
40
OIL
OPFR

Figure 6.

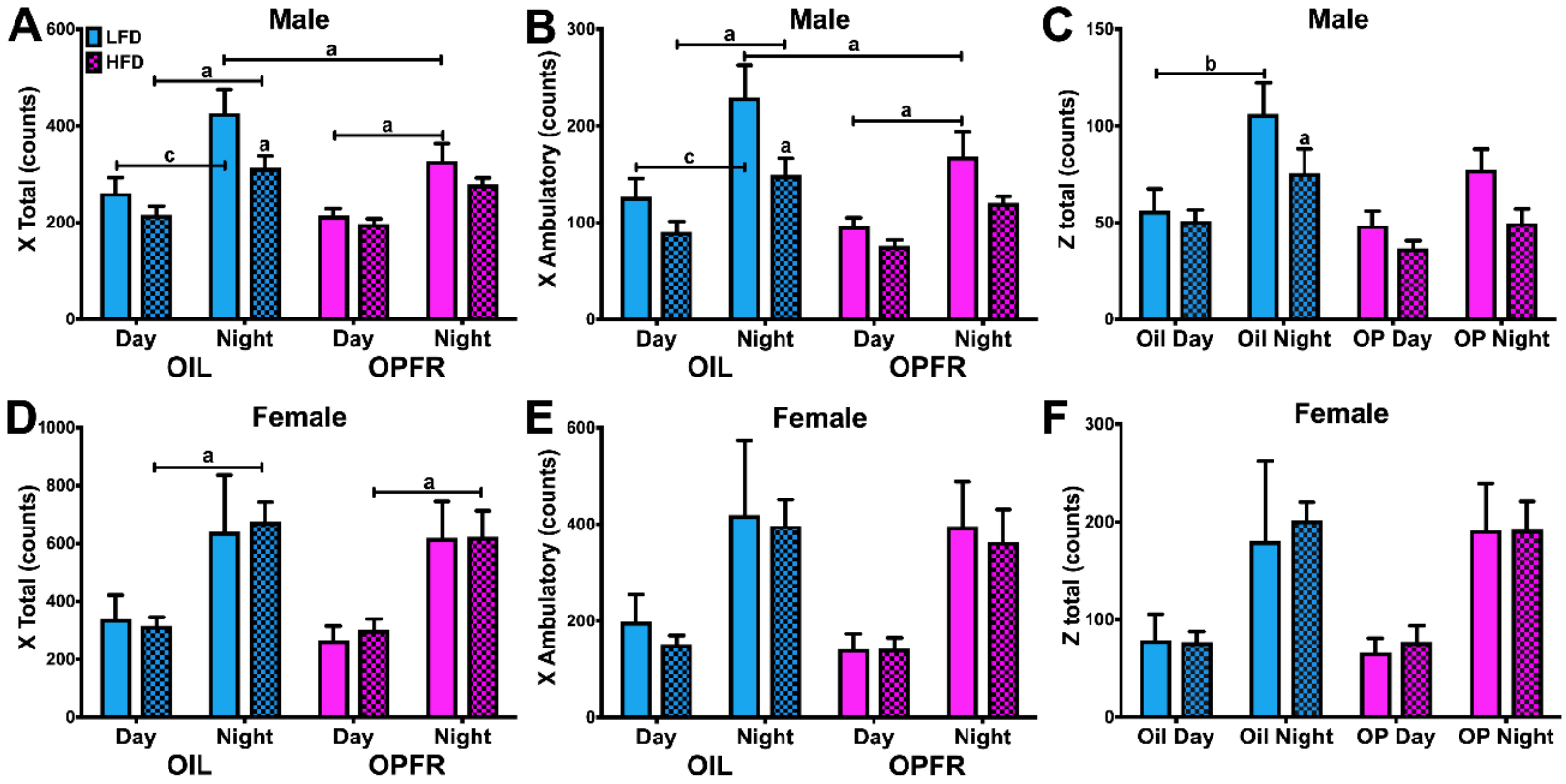

A
Male
LFD
HFD
X Total (counts)
600
400
200
0
a
a
a
c
a
Day
Night
Day
Night
OIL
OPFR
B
Male
X Ambulatory (counts)
300
200
100
0
a
a
c
a
a
Day
Night
Day
Night
OIL
OPFR
C
Male
Z total (counts)
150
100
50
0
b
a
Oil Day
Oil Night
OP Day
OP Night
D
Female
X Total (counts)
1000
800
600
400
200
0
a
a
Day
Night
Day
Night
OIL
OPFR
E
Female
X Ambulatory (counts)
600
400
200
0
Day
Night
Day
Night
OIL
OPFR
F
Female
Z total (counts)
300
200
100
0
Oil Day
Oil Night
OP Day
OP Night

Figure 7.

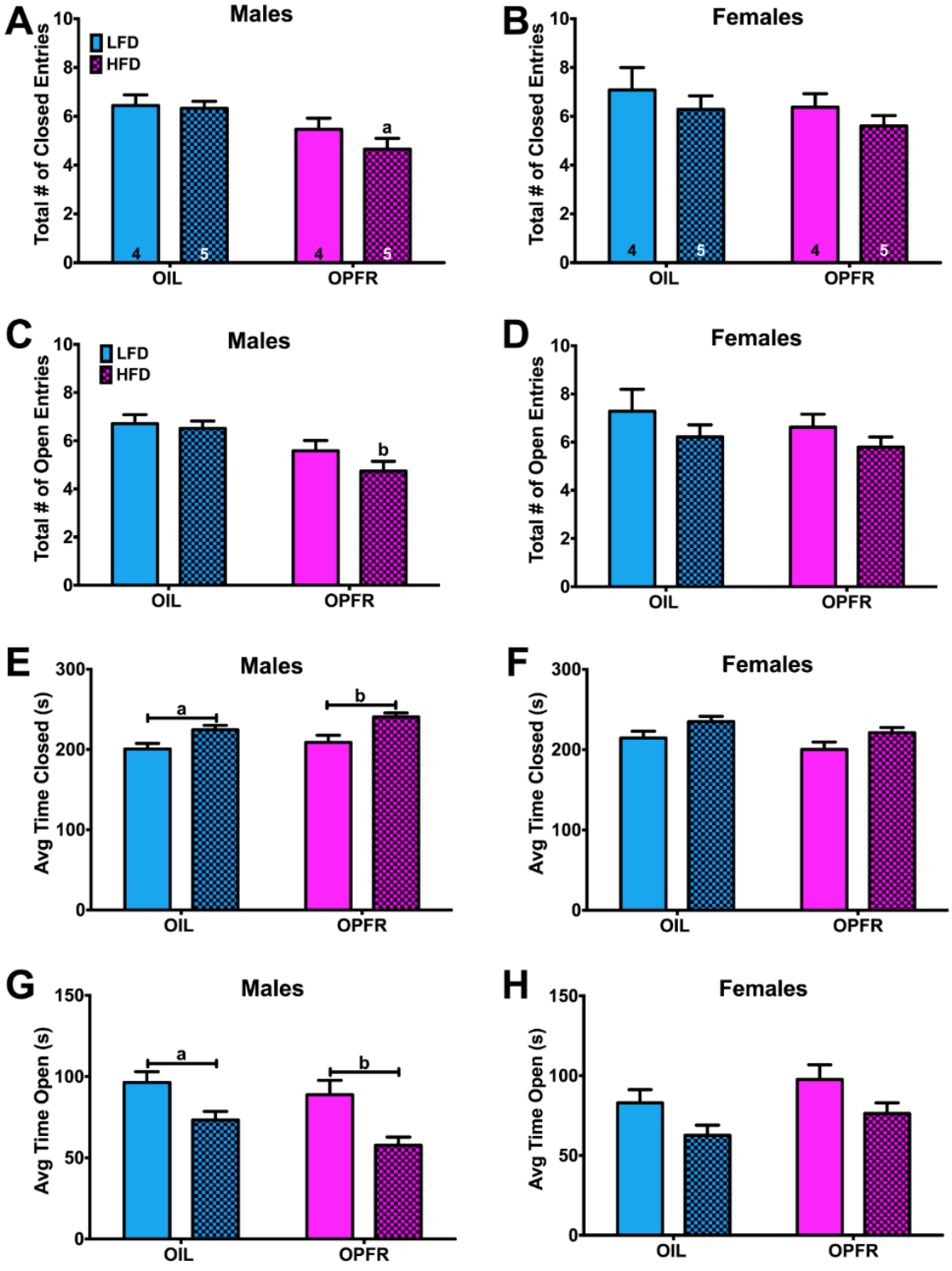

A
Males
LFD
HFD
Total # of Closed Entries
10
8
6
4
2
0
4
5
4
5
a
OIL
OPFR
B
Females
Total # of Closed Entries
4
5
4
5
OIL
OPFR
C
Males
LFD
HFD
Total # of Open Entries
b
OIL
OPFR
D
Females
Total # of Open Entries
OIL
OPFR
E
Males
Avg Time Closed (s)
300
200
100
0
a
b
OIL
OPFR
F
Females
Avg Time Closed (s)
OIL
OPFR
G
Males
Avg Time Open (s)
150
100
50
0
a
b
OIL
OPFR
H
Females
Avg Time Open (s)
OIL
OPFR

Figure 8.

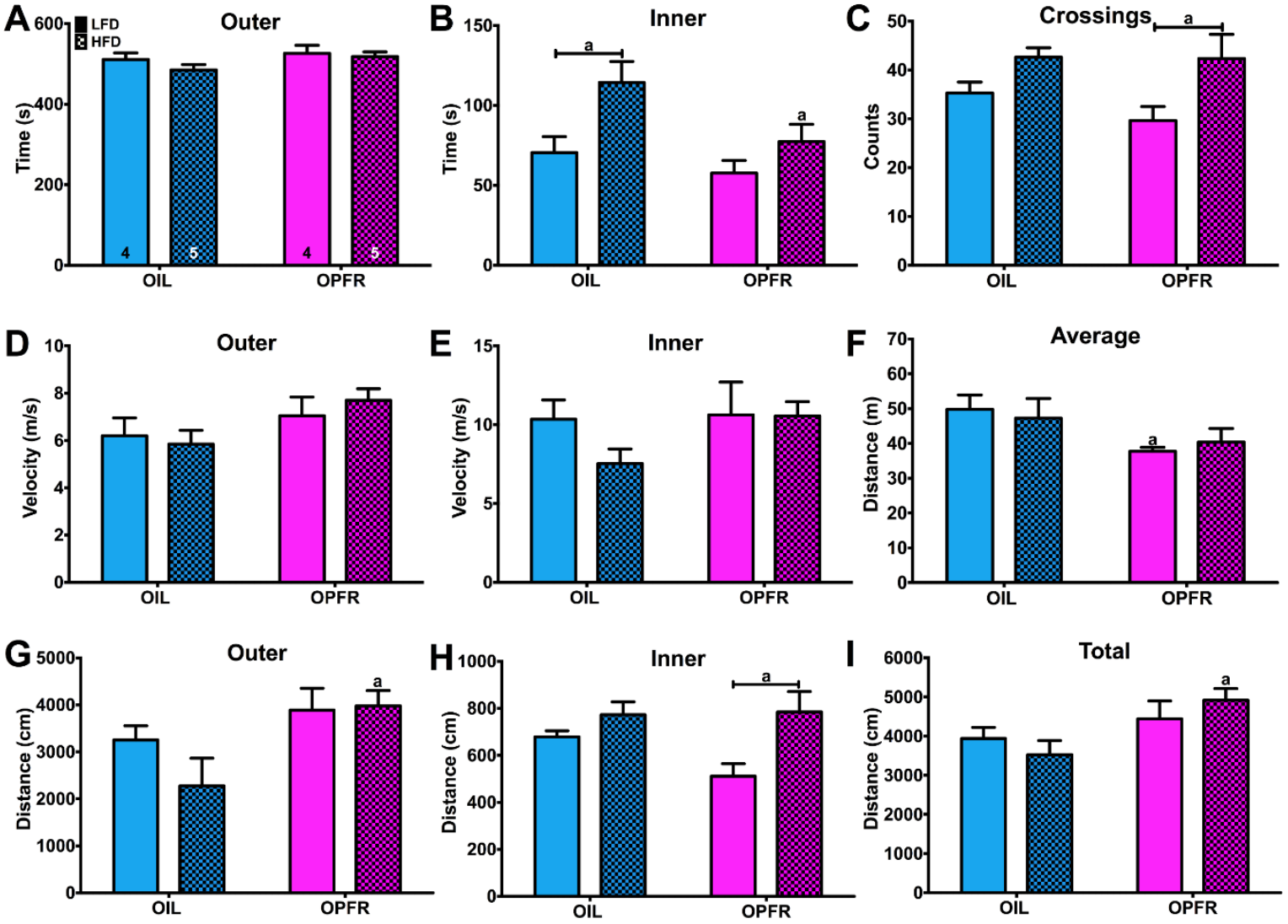
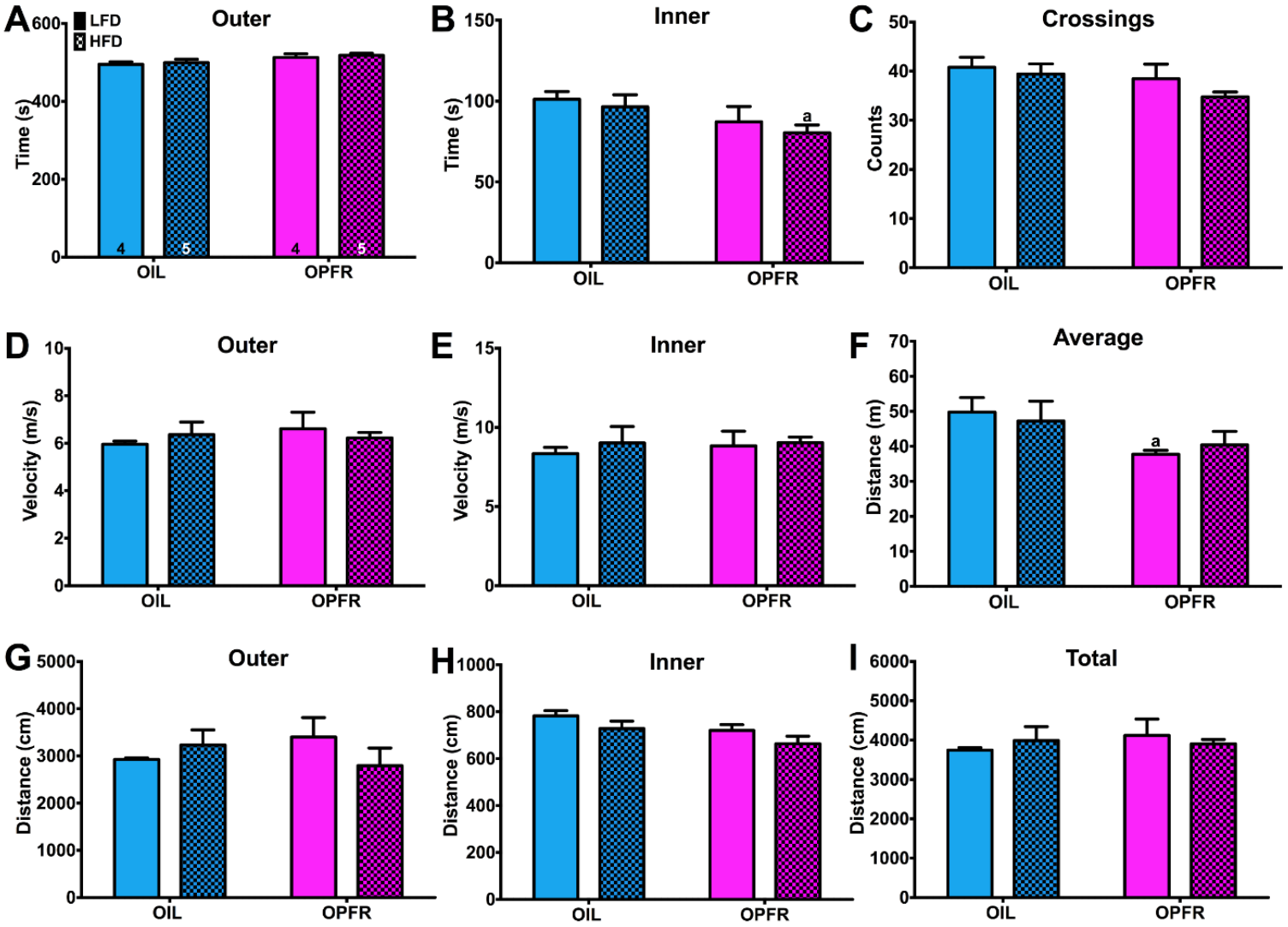

A
Outer
LFD
HFD
Time (s)
600
400
200
0
4
5
4
5
OIL
OPFR
B
Inner
Time (s)
150
100
50
0
a
a
OIL
OPFR
C
Crossings
Counts
50
40
30
20
10
0
a
OIL
OPFR
D
Outer
Velocity (m/s)
10
8
6
4
2
0
OIL
OPFR
E
Inner
Velocity (m/s)
15
10
5
0
OIL
OPFR
F
Average
Distance (m)
70
60
50
40
30
20
10
0
a
OIL
OPFR
G
Outer
Distance (cm)
5000
4000
3000
2000
1000
0
a
OIL
OPFR
H
Inner
Distance (cm)
1000
800
600
400
200
0
a
OIL
OPFR
I
Total
Distance (cm)
6000
5000
4000
3000
2000
1000
0
a
OIL
OPFR

Figure 9.


A
LFD
HFD
Outer
Time (s)
600
400
200
0
4
5
4
5
OIL
OPFR
B
Inner
Time (s)
150
100
50
0
a
OIL
OPFR
C
Crossings
Counts
50
40
30
20
10
0
OIL
OPFR
D
Outer
Velocity (m/s)
10
8
6
4
2
0
OIL
OPFR
E
Inner
Velocity (m/s)
15
10
5
0
OIL
OPFR
F
Average
Distance (m)
70
60
50
40
30
20
10
0
a
OIL
OPFR
G
Outer
Distance (cm)
5000
4000
3000
2000
1000
0
OIL
OPFR
H
Inner
Distance (cm)
1000
800
600
400
200
0
OIL
OPFR
I
Total
Distance (cm)
6000
5000
4000
3000
2000
1000
0
OIL
OPFR

CHAPTER 3:

INTERACTIONS OF MATERNAL OPFR EXPOSURE AND ADULT HIGH-FAT DIET ON ENERGY AND GLUCOSE METABOLISM, HEMODYNAMICS, AND LIVER GENE EXPRESSION IN A SEXUALLY DIMORPHIC MANNER

ABSTRACT

Persistent organic pollutant flame retardants (FRs) like polybrominated diphenyl ethers and organophosphate FRs (OPFR) can accumulate in the body and interact with nuclear receptors, like the estrogen (ER) and peroxisome proliferating activated receptor (PPAR). Still, there is little research on OPFR effects on mammalian neuroendocrine functions. Therefore, we investigated if maternal OPFR exposure with a high-fat diet (HFD) or low-fat diet (LFD) challenge results in sexually dimorphic changes in gene expression and higher susceptibility to symptoms of metabolic syndrome. WT C57Bl/6J dams were orally dosed with vehicle (oil) or an OPFR mixture {1mg/kg combination each of tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate} from gestation day 7 to postnatal day 14. After weaning, pups were challenged with a high-fat (HFD) or low-fat diet (LFD). To assess symptoms of metabolic syndrome, we not only analyzed bodyweight and body composition, but other metabolic activity parameters. OPFR altered substrate utilization in both sexes, altered carbon dioxide and oxygen consumption in females following CLAMS use. OPFR altered fasting glucose in females, and glucose and hepatic glucose homeostasis in males. Plasma leptin was reduced in males while liver enzymes and receptors were reduced in both sexes. The physiological implications are that OPFRs alter ARC and liver homeostatic gene expression and energy balance in a sex-dependent manner, and that these effects are mediated, in part, by ERα and PPARγ.

Glossary: 17-β estradiol (E2), anogenital distance (AGD), apolipoprotein E (ApoE), area under the curve (AUC), bisphenol A (BPA), body weight (BW), cardiovascular diseases (CVD), comprehensive lab animal monitoring system (CLAMS), diacylglycerol

transferase (*Dgat2*), endocrine-disrupting compound (EDC), estrogen receptor (ER), estrogen receptor alpha (*Esr1*), fatty acid synthase (*Fasn)*, flame retardants (FR), Forkhead box protein O1 (*Foxo1*), gestational day (GD), glucose-6-phosphatase catalytic subunit (*G6pc*), glucose tolerance test (GTT), hepatocyte nuclear factor 4 alpha (HNF4α), high-fat diet (HFD), Insulin receptor (*Insr*), insulin tolerance test (ITT), intraperitoneally (ip), knockout (KO), Leptin receptor (*Lepr*), low-fat diet (LFD), no observable adverse effect level (NOAEL), organophosphate FR (OPFR), peanut butter (PB), peroxisome proliferator activated receptor (PPAR), PPAR alpha (*Ppara*), PPAR gamma (*Pparg*), Phosphoenolpyruate carboxykinase (*Pepck*), polybrominated diphenyl ether (PBDE), postnatal day (PND), pyruvate tolerance test (PTT), respiratory exchange ratio (RER), tricresyl phosphate (TCP), triphenyl phosphate (TPP), tris (1,3-dichloro-2-propyl) phosphate (TDCPP)

INTRODUCTION

Increased production and use of organophosphate flame retardants (OPFR) are due to the phase out of polybrominated diphenyl ethers (PBDE). This is because numerous studies discovered PBDE bioaccumulation in the environment (Darnerud, et al., 2001). As a result, many human studies assessed PBDE affects and found that they can affect endocrine and neurological systems. Specifically, they have been found to target nuclear receptors involved with homeostasis, like peroxisome proliferator activated receptor (PPAR) and the estrogen receptor (ER) (Fang, *et al.*, 2015; Liu, *et al.*, 2015). Subsequently, PBDEs were labeled as an endocrine disrupting compound (EDC) and human concentrations have steadily declined since their phase out.

Consequently, another class of flame retardants (FR), organophosphate FR (OPFR) were produced and used as a replacer for PBDE. In addition to their similar structure to PBDEs, OPFR are not chemically bound to most products they are applied to and can be released into dust, air, and food. Although low levels of OPFRs have been found in homes (He, *et al.*, 2015), offices (Carignan, *et al.*, 2013), and in the air of offices and aircrafts (Yang, *et al.*, 2014), high levels were found in cereals, pastries, sweets and beverages (Poma, *et al.*, 2017), and breast milk (Hoffman, *et al.*, 2014; Kim, *et al.*, 2014). *In vitro* studies have shown that OPFRs interact with a variety of nuclear receptors, like PPAR and ER (Belcher, *et al.*, 2014; Liu, *et al.*, 2015).

PBDEs have been detected in similar products and in humans and land animals (Betts, 2002; Goralczyk *et al.*, 2002; Hale *et al.*, 2003; Herzke *et al.*, 2003; Hooper *et al.*, 2000; Hooper *et al.*, 2003; Linares *et al.*, 2015; Meironyte Guvenius *et al.*, 2001). Extensive presence of PBDEs is because they are not chemically bound to the commercial and

household goods they are applied to and are more prone to degradation and persistence from their weak carbon-bromine bonds (Burreau *et al.*, 2000; Hale, *et al.*, 2003). Therefore, PBDEs have been shown to activate ER and PPAR mediated pathways involving hepatic enzyme activity, behavior and fetal development like cardio- and hepatic physiology (Darnerud, *et al.*, 2001; Dellovade *et al.*, 1996; Fang, *et al.*, 2015; Meerts *et al.*, 2001a; Riu, *et al.*, 2011; Zhou *et al.*, 2001).

While studies on OPFR exposure is expanding, the consequences on the neuroendocrine system are few and far between. Studies in zebrafish were found to impact steroidogenesis (Wang, *et al.*, 2015a), liver functions (Wang, *et al.*, 2015a), lipogenesis (Dishaw, *et al.*, 2014b), glucose homeostasis (Wang, *et al.*, 2015a), neural growth and hypothalamic functions during early developmental stages (Liu, *et al.*, 2013a); these findings were also found in chicks. Furthermore, OPFR exposure in chicks was found to induce cholestatic liver and biliary fibrosis, disrupt lipid metabolism, reduce plasma cholesterol, and alter apolipoprotein E (ApoE), hepatocyte nuclear factor 4 alpha (HNF4α), and PPARγ expression (Liu, *et al.*, 2013a; Liu, *et al.*, 2013c). Studies conducted by the Patisaul lab are one of the few that assess developmental OPFR effects in rodent models and have found OPFR in FM550 accumulate in the placenta in a sex-specific manner, increased running wheel activity in females, and alter male cardiac physiology at levels lower than the purported no observable adverse effect level (NOAEL) (Baldwin, *et al.*, 2017; Patisaul, *et al.*, 2013a). Together, these studies show that maternal or early developmental exposures can impact ER and PPAR signaling, potentially causing a change in neural growth, exploratory behavior steroid- and lipogenesis, glucose homeostasis, and liver function. Specifically, triphenyl phosphate (TPP) and tricresyl phosphate (TCP) are ERα agonists

(Liu, *et al.*, 2012; Pillai, *et al.*, 2014), while Tris (1,3-dichloro-2-propyl)phosphate (TDCPP) was found to be an ERα antagonist and upregulate ERα target genes (Liu, *et al.*, 2013a; Liu, *et al.*, 2013c). It should be noted that transactivation assays of TPP and TCP did show an activation of ERα/β, but were not as potent as the endogenous ligand, 17b-estradiol (E2) (Liu, *et al.*, 2012; Pillai, *et al.*, 2014).

Presence of both ERα and PPARγ are not only present in the hypothalamus, but also the liver. Signals from this "leaky area" allows the brain to send and receive signals to other regions of the body (Morton, *et al.*, 2006; Schwartz, *et al.*, 2000). Ultimately, this has the potential to disrupt ERα/PPAR mediated pathways (Roepke, *et al.*, 2007; Tyagi *et al.*, 2011). Recent studies noted that ERα knockout (KO) female mice are phenotypically obese and resistant to E2's effects on energy balance and glucose clearance in adulthood, while PPARα/γ KO mice were found to be resistant to HFD induced obesity and insulin resistance (Guerre-Millo *et al.*, 2001; Jones *et al.*, 2005; Shi *et al.*, 2013; Yasrebi *et al.*, 2016). However, most experiments only noted changes in early developmental stages in non-rodent models, so it should be of high concern to understand the effects of OPFR exposure in a rodent model. EDC's like FR's and BPA, have the potential to disrupt metabolic function and can be classified as "metabolic disrupting chemicals" when implicated in the etiology of metabolic disease (Heindel *et al.*, 2017).

The rise of obesity, diabetes, dyslipidemia, cardiovascular diseases (CVD) and those related together are known as metabolic syndrome. As metabolic syndrome has steadily increased so has the economic burden and understanding its etiology. EDCs have recently been regarded as the answer for the steady increase observed worldwide. Due to the

many causative factors associated with metabolic disease there is much to understand. This includes, but is not limited to, the liver and pancreas which can lead to changes in glucose homeostasis, plasma peptide hormones, cellular apoptosis, hemodynamics, and other metabolic parameters. Specifically, it has been shown that exposure to EDCs during critical windows of development can alter glucose intolerance in mice (Asahi *et al.*, 2010; Bindhumol *et al.*, 2003; Moon *et al.*, 2012; Nakagawa *et al.*, 2000). Many proteins and plasma peptide hormones can affect glucose homeostasis, and thus, metabolic syndrome. Furthermore, they can up- and downregulate based on environmental conditions or exposure (Camargo *et al.*, 2010; Gallou-Kabani *et al.*, 2005; Grayson *et al.*, 2011). Taken together, rodent models have shown that ERα and PPARα/γ are potential targets of EDC altering hypothalamic programming. However, little is known about the role of these receptors in the effects of maternal OPFR exposure on energy balance. This gap in knowledge led our hypothesis that maternal OPFR exposure along with a high-fat diet (HFD) or low-fat diet (LFD) challenge results in sexually dimorphic changes in gene expression and higher susceptibility to symptoms of metabolic syndrome like metabolic parameters, glucose homeostasis, and hemodynamics through interactions with steroid and nuclear receptors. Therefore, the purpose of these studies was to understand the effects of diet and maternal exposure on symptoms of metabolic syndrome and gene expression.

MATERIALS AND METHODS

Animal Care

All animal treatments and procedures were completed in accordance with institutional guidelines based on National Institutes of Health standards and were performed with Institutional animal Care and Use Committee approval at Rutgers University. Wild-type C57Bl/6J female and male mice were bred in-house, maintained under controlled temperature (25°C) and 12/12h light/dark cycle with water and food *ad libitum*. Mice were fed *ad libitum* a chow diet (LabDiet PicoLab Verified 5v75 IF, <75 ppm phytoestrogens) and water.

Maternal Exposures

Prior to mating Dams were habituated to PB 3-4 weeks before mating. Pairing of the male and female mouse for mating was noted as gestation day (GD) 0. Females were housed with males for one week and acclimated to peanut butter at least three days prior to male removal. Dams were weighed every three days to ensure appropriate dosing and after confirmation of successful copulation (vaginal plug). From GD7, through birth, until post-natal day (PND) 14, Dams were dosed with OPFR (1 mg/kg/day) or vehicle peanut butter (PB) to maternally expose pups via breast milk.

The OPFR mixture was a 1mg/kg combination of tris(1,3-dichloro-2-propyl)phosphate (TDCPP, Sigma Aldrich, St. Louis, MO), triphenyl phosphate (TPP, Sigma Aldrich, St. Louis, MO), and tricresyl phosphate (TCP, AccuStandard, New Haven, CT) at 1mg/kg each. Each compound was then dissolved into acetone for a stock solution and 100 ul of stock was dissolved into 10mL of sesame oil and allowed to vent for 24h. Daily ingestion

of the OPFR mixture or sesame oil mixed with powdered peanut butter at 1000 was the route of exposure for Dams. Dams were given a standard breeder diet (25% fat kCal, 3.83 kcal/g, Lab Diet 5015; Lab Diet, St. Louis, MO, USA) *ad libitum*. Litters were between 6 and 8 pups to reduce the effects of litter size on offspring energy homeostasis (Roepke, et al., 2017).

During maternal exposure to OPFRs, male and female pup body weights were measured on postnatal day (PND) 2, 7, and 14. Anogenital distance (AGD) was recorded on post-natal day 7 (PND7) to determine the feminization or masculinization of male and female pups. At week 3, pups were weaned, pair housed by treatment, and challenged with a LFD or HFD diet *ad libitum* for a total of 7-9 litters per treatment per diet. All mice were weighed weekly along with weekly food intake per cage. Weekly food intake was calculated as grams per cage per mouse basis. We ran two successive maternal experiments with 3-4 litters per treatment in the first experiment and 4-5 litters per treatment in the second. Between week 19 and 24, all mice were tested for body composition, and metabolic rate, substrate utilization, hemodynamics, and glucose, insulin, and pyruvate tolerance test.

Assessment of metabolism

To determine oxygen consumption, substrate utilization, and energy expenditure, each mouse was placed in a Comprehensive Lab Animal Monitoring System (CLAMS) (Columbus Instruments, Columbus, OH, USA) unit for 48 h with the last 24 h of recording used for analysis. $V.O_2$ (ml/min/kg), $V.CO_2$ (ml/min/kg), respiratory exchange ratio (RER) ($V.CO_2/V.O_2$), and energy expenditure (kCal/hr/lean mass (g)) were assessed in after 17 weeks on a LFD or HFD.

Assessment of cardiovascular parameters

For the second round 4-5 litters, we were given access to a CODA® (Kent Scientific, Torrington, CT, USA). During week 23, mice were run through CODA® to determine if maternal OPFR exposure affected blood pressure and heart rate. Mice were acclimated to CODA for 20-30 min in a quiet room for 4 days followed by the experimental day (day 5). One run for each mouse was 25 cycles with the first 5 cycles as acclimation. One cycle was about 1 minute. More than 3 accepted recordings were required for each run. Results from this non-invasive tail-cuff blood pressure system include diastolic, systolic, average blood pressure, heart rate, and tail volume blood flow.

Assessment of glucose metabolism

Following the week of CODA data collection, each mouse was prepared for a glucose tolerance test (GTT) to determine glucose clearance. Mice were fasted 5 h in a new cage with access to water and all experiments starting between 1300 and 1400h. Thirty minutes after local anesthetizing of the tail with lidocaine, mice were placed in a plexiglass restrainer and tails were nicked to collect a baseline (time = 0) glucose reading using a glucometer (AlphaTRAK2). Immediately after baseline, mice were injected intraperitoneally (ip) with a bolus of glucose (2.0 g/kg body weight) and then individually put into clean cages with no food. Tail blood samples were collected at 15, 30, 60, 90, and 120 min post-injection. After sufficient recovery (~4 d), an insulin tolerance test (ITT) was performed after a 4 h fast in a similar manner as the GTT with an ip injection of insulin made the same day (0.75 units/kg in sterile saline). Blood samples were collected from the

tail in individual cages at 0, 15, 30, 60, 90, and 120 min post-injection. To assess hepatic glucose production, a pyruvate tolerance test (PTT) was conducted ~4 d post-ITT with an ip injection of pyruvate (2.0 g/kg body weight) following a 5 h fast. Post injection, tail blood samples were collected at 0, 15, 30, 60, 90, and 120 min. After each test, each mouse was returned to its home cage with its original cage mate with *ad libitum* access to water and food.

Tissue collection, RNA extraction, and quantitative real-time PCR

At week 25 after suitable recovery (4-5 d) prior to tissue and blood collection. On the day of tissue collection, all mice were fasted for 1 h and decapitated after sedation with ketamine at 1000. Trunk blood was immediately collected in a K^+ EDTA collection tube with the addition of a protease inhibitor, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (1 mg/ml, Sigma-Aldrich, St. Louis, MO, USA), for peptide hormone analysis. Samples were maintained on ice until centrifugation at 1, 100 rcf for 15 min at 4 °C and stored at –80 °C until analysis. Plasma insulin and leptin levels were determined by multiplex assay (MMHMAG-44 K, EMD Millipore, Billerica, Massachusetts).

The left lateral lobe of the liver was fixed in RNAlater (Life Technologies, Grand Island, NY, USA), and stored at –80 °C. Liver RNA was extracted using a standard TRIzol® extraction (Life Technologies) coupled with Macherey-Nagel NucleoSpin® RNA extraction and DNase-1 kit (Bethlehem, PA, USA). Total RNA was treated with DNase I using the extraction kit protocol at 37 °C for 30 min to minimize any genomic DNA contamination. RNA quantity and quality were determined using a NanoDrop ND-2000

spectrophotometer (ThermoFisher, Waltham, MA, USA) and an Agilent 2100 Bioanalyzer and RNA Nano Chips (Agilent Technologies, Santa Clara, CA, USA). Only samples with RNA Integrity Number (RIN) greater than 8 were used. Complementary DNA (cDNA) was synthesized from 500 ng of total liver RNA using Superscript III reverse transcription (Life Technologies), 4 μL 5× buffer, 25 mM $MgCl_2$, 10 mM dNTP (Clontech Laboratories, Mountain View, CA), 100 ng random hexamer primers (Promega, Madison, WI), 40 U/μL Rnasin (Promega), and 100 mM dithiothreitol in diethylpyrocarbonate-treated water (Bioexpress, Kaysville, UT) in a total volume of 20 μL. A 1:20 dilution of the cDNA was made with nuclease-free water (Gene Mate) for a final cDNA concentration of 0.5 ng/μL and stored at -20 °C until needed. Reverse transcription was conducted using a standard Superscript III reverse transcriptase protocol: 5 min at 25 °C, 60 min at 50 °C, and 15 min at 70 °C.

All primers were designed to span exon-exon junctions and synthesized by Life Technologies, using Clone Manager 5 software (Sci Ed Software, Cary, NC, USA, See Table 1 for a list of primer sequences). A standard curve was generated for each primer pair using serial dilutions of ARC and liver cDNA in triplicate. Efficiencies were calculated as a percent efficiency and are approximately equal (90%–110% or one doubling per cycle). Untreated liver tissue RNA was used for the calibrator and negative control (no reverse transcription) and processed simultaneously with the experimental samples. All values were normalized and are expressed as relative mRNA expression. Amplification protocol for genes was as follows: initial denaturing at 95 °C for 3 (SsoAdvanced) or 10 minutes (PowerSYBR) followed by 40 cycles of amplification at 94 °C for 10 seconds (denaturing), 60 °C for 45 seconds (annealing), and completed with a dissociation step for

melting point analysis with 60 cycles of 95 °C for 10 sec, 65 °C to 95 °C (in increments of 0.5 °C) for 5 seconds and 95 °C for 5 seconds. The reference genes used were *Gapdh, Hprt,* and *Actb.* The geomean of the Cq values from each reference gene was used to calculate relative gene expression. Positive, negative and water blank controls were included in the qPCR plate design.

Statistical Analysis

All data are expressed as mean ± SEM and were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) or Statistica (StatSoft, Tulsa, OK, USA). Data from cumulative body weight gain, GTT/ITT/PTT, body composition, CLAMS, EPM, OFT, and CODA were analyzed using a multifactorial (sex, diet, OPFR exposure) ANOVA with a *post hoc* Newman-Keuls test. All gene expression data were analyzed using 2-way ANOVA with a *post hoc* Newman-Keuls test. In all experiments, effects were considered significant at $\alpha \leq 0.05$.

RESULTS

Body weights and body composition

All mice were weighed weekly during the study. HFD-fed males weighed more than LFD-fed males by week 7 in OPFR-exposed males and by week 8 in oil-exposed males (Fig. 1a; diet: $F(1, 27) = 78.72$, $P < 0.0001$; time: $F(17, 459) = 950.2$, $P < 0.0001$ F; time*diet: $(17, 459) = 52.07$, $P < 0.0001$). HFD-fed females weighed more than LFD-fed females by week

10 in OPFR-exposed mice and by week 12 in oil-exposed mice (Fig. 1b; diet: $F(1, 28) = 20.39$, $P < 0.0001$; time: $F(17, 476) = 499.9$, $P < .0001$ F; time*diet: $(17, 476) = 13.45$, $P < 0.0001$). After 20 weeks, regardless of sex and chemical exposure, HFD increased fat mass normalized to body weight compared to LFD (Fig. 1c & 1d; male: $F(1, 27) = 52.08$, $P < 0.0001$; female: $F(1, 28) = 15.53$, $P < 0.001$). In males, lean mass normalized to body was lower in HFD-fed males, regardless of maternal treatment (Fig. 1e; $F(1, 27) = 25.33$, $P < 0.0001$). Lean mass in females was also affected by adult diet (Fig. 1f; $F(1, 28) = 7.58$, $P < 0.05$). Overall, these studies showed an effect of diet on body weight and composition.

Metabolic parameters

Metabolic parameters were measured in a 48 h run on CLAMS with the last 24 h of recorded data used for analysis. HFD-fed males consumed less oxygen than LFD-fed males exposed to OPFR day and night, showing an effect of adult diet (Fig. 2a; $F(1, 54) = 15.59$, $P < 0.001$), time (Fig. 2a; $F(1, 54) = 4.13$, $P<.05$), and an interaction of OPFR and diet (Fig. 2a; $F(1, 54) = 3.98$, $P < 0.05$). An effect of time was observed in females (Fig. 3a; ($F(1, 56) = 5.25$, $P < 0.05$). In LFD-fed males, carbon dioxide production was greater at night than during the day for control and was overall greater than HFD-fed males, showing an effect of adult diet (Fig. 2b; $F(1, 54) = 64.58$, $P < 0.0001$) and time: (Fig. 2b; $F(1, 54) = 5.29$, $P < 0.05$). HFD-fed females showed an effect of diet (Fig 3b; $F(1, 56) = 16.814$, $P < 0.0001$), and time (Fig 3b; $F(1, 56) = 4.41$, $P < 0.05$) by producing less carbon dioxide than LFD at night. RER, a measure of substrate utilization (fat vs. carbohydrates) was affected by OPFR (Fig. 2c; $F(1, 54) = 10.39$, $P < 0.01$) and adult diet (Fig. 2c; $F(1, 54) = 122.9$, $P < 0.0001$) in that OPFR exposed LFD-fed males had a higher RER compared to control

and all HFD-fed mice. The same effects, OPFR (Fig. 3c; $F(1, 56) = 17.20$, $P < 0.001$), adult diet (Fig. 3c; $F(1, 56) = 176.4$, $P < 0.0001$), as well as interaction of OPFR and diet (Fig. 3c; $F(1, 56) = 12.69$, $P < 0.001$) were observed in females. In HFD- fed males, energy expenditure was affected by OPFR (Fig. 2d; $F(1, 54) = 9.55$, $P < 0.01$), adult diet (Fig. 2d; $F(1, 54) = 32.3$, $P < 0.00001$), time (Fig. 2d; $F(1, 54) = 11.43$, $P < 0.01$), and an interaction of OPFR and adult diet (Fig. 2d; $F(1, 54) = 6.1$, $P < 0.05$). The same, OPFR (Fig. 3d; $F(1, 56) = 12.37$, $P < 0.001$), adult diet (Fig. 3d; $F(1, 56) = 23.12$, $P < 0.00001$), time (Fig. 3d; $F(1, 56) = 11.09$, $P < 0.01$), and an interaction of OPFR and adult diet (Fig. 3d; $F(1, 56) = 5.1$, $P < 0.05$). Overall, these studies showed an sexual dimorphic effect from diet, OPFR exposure, and an interaction of diet and OPFR exposure.

Hemodynamics

Using a CODA instrument, we measure blood pressure, heart rate, and blood flow and volume in mice from 4-5 litters from each treatment. In males, OPFR exposure increased blood pressure, systolic (Fig. 4a; $F (1, 14) = 16.06$, $P < 0.01$), diastolic (Fig 4b; $F (1, 14) = 11.19$, $P < 0.01$), and mean pressure (Fig. 4c; $F (1, 14) = 12.13$, $P < 0.01$) in male mice. There was no effect of adult diet or maternal treatment on heart rate (Fig. 4d) or blood flow (Fig. 4e). There was an effect of adult diet on tail blood volume (i.e. ml of blood in tail) (Fig. 4f, $F (1, 14) = 5.23$, $P < 0.05$), although there no pairwise differences. There was no effect of adult diet or maternal treatment on hemodynamic parameters in females (data not shown). Overall, these studies showed OPFR-exposed males were more susceptible to changes in cardiovascular parameters.

Glucose homeostasis

To determine the effects of maternal OPFR exposure on glucose homeostasis, we conducted glucose, insulin, and pyruvate tolerance tests on all mice. In males, HFD mice decreased glucose clearance in both oil- and OPFR-exposed mice (Fig. 5a; $F(1, 27)=32.728$, $P < 0.00001$). In oil-exposed, HFD-fed males, glucose levels were higher by 30 min post-injection compared to LFD-fed males ($P < 0.05$). While in OPFR-exposed, HFD-fed males, glucose levels were not higher until 60 min post-injection, both showing an effect of diet (Fig. 5a; $F(1, 27)=32.728$, $P < 0.00001$), time (Fig. 5a; $F(5, 135)=174.02$, $P < 0.0001$), and interaction of diet and time (Fig. 5a; $F(5, 135)=26.611$, $P < 0.0001$). Interestingly, OPFR-exposure increased glucose clearance in HFD-fed males by 120 min showing an interaction effect of time and EDC ($F(5, 135)=3.1189$, $P < 0.01$). AUC analysis was significantly greater for HFD mice compared to LFD, regardless of maternal exposure (Fig. 5b; $F(1, 27)=32.728$, $P < 0.00001$). Similarly, fasting glucose levels were elevated by adult diet ($F(1, 27)=32.728$, $P < 0.00001$) but with no effect of maternal treatment (Fig. 5c). In females, HFD also reduced glucose clearance in both maternal treatments showing an effect of diet (Fig. 5d; $F(1, 27)=30.561$, $P < 0.00001$), time (Fig. 5d; $F(5, 135)=172.61$, $P < 0.0001$), and interaction of diet and time (Fig. 5d; $F(5, 135)=8.1949$, $P < 0.00001$). At 60 min post-injections, there was a transient increase in glucose clearance in OPFR-exposed, HFD-fed females compared to oil-exposed, HFD-fed females and only in oil-exposed females did HFD reduce glucose clearance compared to LFD-fed females at 120 min ($P<0.001$). Female mice fed a HFD exhibited elevated AUC versus LFD-fed female mice (Fig. 5e; $F(1, 27)=30.561$, $P < 0.00001$). Fasting glucose levels were elevated by adult diet only in OPFR-exposed females (Fig. 5f; $F(1, 27)=30.561$, $P < 0.00001$). Overall, these

studies showed sexual dimorphic changes in glucose and hepatic glucose parameters following maternal OPFR exposure.

In the insulin tolerance test, HFD reduced insulin-induced glucose clearance in both oil- and OPFR-exposed male mice, although OPFR exposure reduced the effect of HFD on insulin tolerance in males (Fig. 6a, diet: $F(1, 27)=34.583$, $P < 0.00001$; maternal: $F(1, 27)=4.7833$, $P < 0.05$). In fact, this effect of OPFR exposure was apparent at 90 and 120 min post-injection ($P < 0.01$, $P < 0.05$, respectively). These effects of diet and maternal treatment were clearly illustrated by AUC analysis (Fig. 6b). Male mice fed a HFD exhibited elevated AUC (oil: $P < 0.05$; OPFR: $P < 0.0001$). However, when comparing HFD-fed groups, OPFR exposure reduced AUC compared to oil-exposed ($P < 0.05$). In females, only oil-exposed, HFD-fed female mice exhibited an increase in insulin intolerance at the last two time points (Fig. 6c, $F(1, 28) = 6.684$, $P < 0.05$), which is reflected in the AUC analysis (Fig 6d; $F (1, 28) = 9.964$, $P < 0.01$). This could be OPFR reducing the diet induced suppression of insulin tolerance in females. Overall, these studies show sexual dimorphism in glucose homeostasis tests following maternal exposure and diet challenge.

In the pyruvate tolerance test, OPFR exposure reduced the effects of pyruvate injection on glucose clearance in males by slightly decreasing glucose clearance in the LFD-fed males and increasing glucose clearance in HFD-fed males (Fig 6a; $F (1, 14) = 13.290$, $P < 0.01$). From 60 minutes onwards, glucose levels were higher in the oil-exposed, HFD-fed males compared to LFD-fed males ($P < 0.05$; $P < 0.05$, $P < 0.01$, respectively). This was reflected in the AUC analysis (Fig. 6b, diet: $F (1, 14) = 12.53$, $P < 0.01$). No effects of either diet or maternal treatment on pyruvate tolerance were observed in females (Fig. 6c & 6d). Overall,

these studies show sexual dimorphism in hepatic glucose homeostasis tests following maternal exposure and diet challenge.

Peptide hormones and liver gene expression

At the conclusion of the experiment liver gene expression leptin and insulin levels were analyzed. There was no effect of adult diet or maternal treatment on insulin levels in males (Fig. 9a) or females (Fig 9c). However, leptin levels were reduced by OPFR-exposure in both HFD-fed males, which is significant at biologically relevant levels (Fig. 9b; $F(1, 36) = 9.90$, $P < 0.01$) and females (Fig. 9d; $F(1, 31) = 16.03$, $P < 0.001$). Liver gene expression in males showed no effect of diet of OPFR exposure except *Esr1* (Fig. 8F $(1, 26) = 22.67$, $P < 0.0001$). Females had no effect of diet, but an OPFR effect on *Esr1* ($F(1, 29) = 6.836$, $P < 0.05$); *Lepr* ($F(1, 27) = 5.81$, $P < 0.05$), *Fasn* ($F(1, 27) = 4.953$, $P < 0.05$), and *Pepck* ($F(1, 27) = 11.05$, $P < 0.01$). Overall, these studies showed sexual dimorphic effects in peptide hormones and gene expression following maternal OPFR exposure.

DISCUSSION

The negative impact maternal exposure to EDCs, like BPA and PBDEs has been extensively studied. As these chemicals have been phased out and replaced by others, it is of great importance in understanding whether their replacements have the same effects. This gap in knowledge led us to investigate the effects of maternal OPFR exposure along with HFD or LFD challenge in offspring resulted in sexually dimorphic disturbances and changes in gene expression, metabolic parameters, glucose homeostasis, and

hemodynamics through interactions with steroid and nuclear receptors, particularly ERα or PPARγ.

Exposure to EDCs like BPA and PBDEs has been linked to an increased risk for obesity, diabetes, and metabolic syndrome in humans (citation). Our study did not observe an increase in body weight due to OPFR exposure, but only by diet as mice had an increased weight at week 7 and 8 for males and week 10 and 12 for females. While the results from our study did not find evidence of an increase or decrease in bodyweight following exposure (Alonso-Magdalena *et al.*, 2010; Branchi *et al.*, 2002; Howdeshell *et al.*, 1999; Moon *et al.*, 2015b; Rubin *et al.*, 2001; Suvorov *et al.*, 2009; Taylor, et al., 2018; Wei *et al.*, 2011), we did find alterations to multiple metabolic endpoints. This also includes a couple human studies that had conflicting results in regards to weight from exposure to PBDEs, BPA, and other EDCs (Lignell *et al.*, 2013b; Woods *et al.*, 2017). However, most, with the exception of Moon et al., do not assess diet with exposure or exposure window was different. The same result was also observed in mice upon analyzing fat mass normalized to bodyweight in our study. That is, regardless of exposure, we found mice fed a HFD had a higher fat mass to body weight ratio. This was also observed in males for lean mass normalized to body weight, with no effect seen in females. There is one study that supports our findings for a higher fat mass to body weight ratio, but chronic exposure was during gestation and diet challenge was during adulthood (Patel *et al.*, 2014). A separate study assessed lean mass in mice maternally exposed to BPA and found at the highest exposure of 50 ug/kg/day, females had a higher lean mass compared to low dose (10ug/kg/day) and control (Silva *et al.*, 2018). No effect was observed in males in their study.

In trying to understand the metabolic parameters of perinatal OPFR exposure along with a diet challenge, we are the first to show a sexual dimorphic effect of EDC on oxygen consumption. Specifically, OPFR males had decreased oxygen consumption regardless of the time of day compared to LFD. No effect was observed in females. HFD male mice regardless of exposure and time of day had a reduced carbon dioxide production compared to oil, while oil-treated LFD-fed males produced more carbon dioxide at night compared to day. In comparison, oil HFD females had a reduced carbon dioxide production compared to oil-treated LFD-fed. Although there are no other comparable studies currently available, these results are expected with HFD-fed mice, which explains the RER results for mice. However, there was one novel finding because we were the first to show OPFR-treated LFD-fed mice had a greater RER compared to oil-treated during the day and night. In addition, we were also the first to show OPFR-treated HFD-fed males and OPFR-treated, LFD-fed females had a reduced energy expenditure compared oil-treated HFD-fed and oil-treated LFD-fed, respectively. We also observed a higher energy expenditure for oil-treated HFD-fed males and OPFR-treated HFD-fed females compared to oil-treated LFD-fed and OPFR-treated LFD-fed, respectively, but this was an effect of diet. Though there are no studies as of now to compare these findings to, there is one recently published study that noted symptoms of metabolic disease induced by maternal exposure to TPP could be interfered by a HFD (Wang *et al.*, 2018).

Hemodynamics was analyzed to understand if maternal OPFR exposure following a HFD/LFD challenge had any differences. We are the first to show that OPFR males had a higher mean blood pressure and systolic and diastolic pressure compared to oil, regardless of diet. In addition, because no effect was seen in females, this also provides evidence that

OPFR has a sexually dimorphic effect on hemodynamics. This evidence, that blood pressure is increased from exposure to EDCs, is supported by multiple studies when assessing BPA and PBDEs in clinical studies, as well as animal models with similar exposure windows (Bae *et al.*, 2012; Domi, et al., 2016; Gump *et al.*, 2014; Wang *et al.*, 2015c). One study following perinatal exposure to PBDE showed differences in systolic blood pressure; they did not asses females (Shah *et al.*, 2011). This, along with alterations in energy expenditure can lead to cardiovascular problems like hypertension, a contributing factor for metabolic syndrome.

Glucose homeostasis was assessed with relevant tolerance tests. We found HFD males had higher blood glucose than LFD between 60 and 120 min for oil and 30-120 min for OPFR. Further, the same effect was observed in females but between 30 and 120 min for oil and 30 and 90 for OPFR. As expected, AUC was higher in HFD males and females compared to LFD. Similarly, fasting glucose levels were higher in HFD males regardless of exposure, but in females was only noted for OPFR-treated HFD-fed. Support for these findings was noted in a couple studies, one of which had a different exposure window (Moon *et al.*, 2015a; Wei, et al., 2011). OPFR effects were only noted at the last time point for HFD males and 60 minutes for HFD females. In addition, other studies that have a different exposure window or do not include a diet challenge, have mixed results (Liu *et al.*, 2013b; Patel, et al., 2014; Patisaul *et al.*, 2013b; Tung *et al.*, 2017).

Similar to GTT results, ITT showed male HFD blood glucose levels were higher compared to LFD for all time points and all but 30 min for oil-treated HFD-fed and OPFR-treated HFD-fed, respectively. However, this was only observed at the last two time points for female oil-treated HFD-fed compared to LFD. AUC results showed HFD was greater than

oil-treated in males, but only oil-treated for females. These findings are supported by a few similar studies, though most differ from our findings due to differences in exposure windows, diet challenge, different rodent strain, and use of only male or female mice (Liu, et al., 2013b; Patel, et al., 2014; Tung, et al., 2017; Wei, et al., 2011). However, our results are expected with mice fed a HFD. There was an OP effect in HFD-fed males showing a lower AUC compared to HFD-fed control mice as well as at the last two time points. This decreased sensitivty to insulin is supported in two studies that had a similar exposure window, with no diet challenge, one of which only studied in females (Kozlova *et al.*, 2017; Wang, et al., 2018).

PTT was also analyzed and showed a diet effect in males, with no changes observed in females. Namely, a higher hepatic glucose production in oil HFD versus LFD at the last three time points. This result is to be expected and is supported by other studies even after using a different mouse model, exposure window, or used one sex (Hanf *et al.*, 2014; Lin *et al.*, 2004; Stanya *et al.*, 2013). That is, as pyruvate increases blood glucose by promoting gluconeogenesis, HFD-fed mice are more prone to higher blood glucose levels. AUC results were only noted in males, showing a higher AUC in oil-exposed HFD-fed males compared to LFD-fed. One study appears to refute our findings but they did not assess diet and EDC exposure (Lai *et al.*, 2018). Though there aren't many studies that support our exact PTT, GTT, or ITT findings, it is evident in ours and previous studies that males are more susceptible and potentially at higher risk for symptoms related to metabolic disease.

Assessment of any potential effects of metabolic syndrome from maternal OPFR exposure and diet challenge were assessed via peptide hormones and liver enzyme and receptors. Leptin peptide hormone levels showed an OPFR effect in both sexes of HFD mice. That

is, leptin levels were significantly lower compared to oil HFD. Human and animal studies from other notable EDCs support our findings, showing a decrease in leptin levels (Leijs *et al.*, 2017; Ronn *et al.*, 2014; Zhao *et al.*, 2012). Liver expression showed increased levels of *esr1* and *pepck* for OPFR HFD males and OPFR females, regardless of diet, respectively, signifying an OPFR effect in both sexes. Though one study assessed PBDE exspoure at a different window of time and only assesed changes in males, their findings confirm ours in that *pepck* levels increased (Nash *et al.*, 2013). Furthermore, it should be noted that *pepck* plays a crucial role in hepatic gluconeogenesis and an overexpression in *pepck* can cause either increased gluconeogenesis or hyperglycemia (Rosella *et al.*, 1993; Valera *et al.*, 1994). In addition, there are recent papers that explicitly state whether a fasting or chronic state of hyperglycemia alters *pepck* expression (Samuel *et al.*, 2009; Shao *et al.*, 2005). A correlation between leptin and hepatic gluconeogenesis is still unknown as there are studies supporting a promotion and reduction in hepatic gluconeogenesis from changes in leptin (German *et al.*, 2011; Liu *et al.*, 1998; Rossetti *et al.*, 1997).

In conclusion, our findings along with other studies from known EDCs, appear to support the hypothesis that maternal OPFR exposure with a high-fat diet (HFD) or low-fat diet (LFD) challenge results in sexually dimorphic changes in gene expression and higher susceptibility to symptoms of metabolic syndrome. Though these interactions are multiple and complex, we were the first to show a sexual dimorphic effect of EDC on metabolic and cardiovascular parameters. These results not only show that OPFR can increase the likelihood of metabolic syndrome in mice, but also the importance of analyzing metabolic parameters compared to simple crude food intake and body weight. Furthermore, there are a few studies that refute our findings, but they did not measure the

exact parameters we tested such as analyzing sex differences, window of exposure, and animal model. However, studies that have ablated ERα, identified inhibition of the influence of diet of energy homeostasis, hypothalamic and liver gene expression. In comparison, PPARγ KO mice show signs of enhanced emotional response from stress and anxiety (Domi, et al., 2016; Roepke, et al., 2017). In addition, previously published studies coincide with our published and unpublished findings relating a diet low in phytoestrogens to higher body fat and serum leptin levels (Ruhlen, et al., 2008).

Future studies that would in in our understanding of maternal OPFR exposure effects, could include ERα and tissue specific PPARγ KO models. Both nuclear receptors play a crucial role in physical activity, energy expenditure, and glucose homeostasis. Therefore, utilizing a KO model can help paint a better picture of potential downstream effects. As previously mentioned, changes in symptoms of metabolic syndrome can be due to disruptions in the central nervous system or peripheral organs, like the liver. Therefore, it would be beneficial to understand if the changes that we observed in glucose homeostasis, hepatic glucose homeostasis, and hemodynamics are due to such changes.

Table 4. Primer sequences.

Gene Name	Forward Primer	Reverse Primer	Accession #
*Actb**	GCCCTGAGGCTCTTTTCCA	TAGTTTCATGGATGCCACAGGA	NM_007393.3
Dgat2	ACTCTGGAGGTTGGCACCAT	GGGTGTGGCTCAGGAGGAT	NM_026384.3
Esr1	GCGCAAGTGTTACGAAGTG	TTCGGCCTTCCAAGTCATC	NM_007956
Fasn	GGGTTCTAGCCAGCAGAGTC	TCAGCCACTTGAGTGTCCTC	NM_007988.3
Foxo1	CAATGGCTATGGTAGGATGG	TTTAAATGTAGCCTGCTCAC	NM_019739
G6pc	GCCTCCTGTCGGATACAGAA	TGCACCGCAAGAGCATT	NM_008061.4
*Gapdh**	TGACGTGCCGCCTGGAGAAA	AGTGTAGCCCAAGATGCCCTTCAG	NM_008084.2
*Hprt**	GCTTGCTGGTGAAAAGGACCTCTCG AAG	CCCTGAAGTACTCATTATAGTCAAGGG CAT	NM_013556
Insr	GTGTTCGGAACCTGATGAC	GTGATACCAGAGCATAGGAG	NM_010568
Lepr	AGAATGACGCAGGGCTGTAT	TCCTTGTGCCCAGGAACAAT	NM_146146.2
Pepck	AGCGGATATGGTGGGAAC	GGTCTCCACTCCTTGTTC	NM_011044.2
Ppara	CTAACCTTGGGCCACACCT	CGGGTAACCTCGAAGTCTGA	NM_001113418.1
Pparg	CTGCTCAAGTATGGTGTCCATGAG	GAGGAACTCCCTGGTCATGAATC	NM_011146.3

Actb, beta-actin; *Agrp*, agouti-related peptide; *Dgat2*, Diacylglycerol O-Acyltransferase 2 ; *Esr1*, estrogen receptor alpha; *Fasn*, Fatty acid synthase ; *Foxo1*, forkhead box O1; *G6pc*, glucose 6 phosphatase catalytic subunit; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase; *Insr*, insulin receptor; *Lepr*, Leptin receptor; *Pepck*, phosphoenolpyruvate carboxykinase; *Ppara*, peroxisome proliferator-activated receptor α; *Pparg*, peroxisome proliferator-activated receptor γ.* denotes reference genes.

Table 5. Liver expression of enzymes and receptors in males.

	Oil		OPFR	
Gene	**LFD**	**HFD**	**LFD**	**HFD**
Dgat2	1.1 ± 0.2	1.0 ± 0.2	1.9 ± 0.8	1.0 ± 0.5
Esr1	1.2 ± 0.1	1.6 ± 0.2	3.5 ± 0.8**a**	4.6 ± 0.7**b**
Fasn	1.3 ± 0.3	0.8 ± 0.2	2.5 ± 1.1	1.7 ± 0.6
Foxo1	1.2 ± 0.3	1.5 ± 0.1	1.5 ± 0.8	1.8 ± 0.7
G6pc	1.2 ± 0.2	0.7 ± 0.2	3.1 ± 1.6	1.2 ± 0.7
Insr	1.3 ± 0.3	0.7 ± 0.1	1.8 ± 0.8	1.1 ± 0.3
Lepr	1.5 ± 0.6	2.3 ± 1.1	1.9 ± 1.0	0.4 ± 0.2
Pepck	1.1 ± 0.2	0.7 ± 0.2	1.3 ± 0.5	1.4 ± 0.8
Ppara	1.2 ± 0.3	0.8 ± 0.1	1.3 ± 0.4	1.3 ± 0.4
Pparg	1.5 ± 0.4	2.7 ± 0.5	2.1 ± 1.0	3.3 ± 1.2

All data were normalized to oil-treated within each sex. Compared to oil within same diet: **a** = $P < 0.05$; **b** = $P < 0.01$. n = 7-9.

Table 6. Liver expression of enzymes and receptors in females.

	Oil		OPFR	
Gene	**LFD**	**HFD**	**LFD**	**HFD**
Dgat2	1.3 ± 0.9	2.7 ± 1.7	2.8 ± 0.5	5.6 ± 1.6
Esr1	1.1 ± 0.1	1.5 ± 0.5	2.9 ± 0.8	2.8 ± 0.8
Fasn	1.4 ± 0.4	1.4 ± 0.6	3.2 ± 1.2	3.4 ± 1.0
Foxo1	1.6 ± 0.5	3.6 ± 2.1	4.9 ± 2.0	7.0 ± 3.6
G6pc	1.3 ± 0.2	1.1 ± 0.2	2.1 ± 0.4	1.4 ± 04
Insr	1.4 ± 0.3	2.0 ± 0.9	2.4 ± 0.9	2.1 ± 0.8
Lepr	1.3 ± 0.3	1.5 ± 0.9	4.3 ± 1.2	2.7 ± 1.0
Pepck	1.6 ± 0.4	3.5 ± 1.7	6.7 ± 1.**a**	7.5 ± 1.5**a**
Ppara	1.4 ± 0.4	2.7 ± 1.4	2.5 ± 0.7	3.1 ± 0.9
Pparg	1.4 ± 0.5	2.4 ± 1.0	2.3 ± 0.6	2.5 ± 0.6

All data were normalized to oil-treated within each sex. Compared to oil within each diet: **a** = $P < 0.05$. n = 7-9.

Figure 10. Body weights and body composition for oil- and OPFR-exposed mice fed a LFD or HFD between week 3 and 20. Weekly body weight for male (A) and female (B) mice between week 3 and 20. Fat Mass normalized to body weight for male (C) and female (D) mice. Lean Mass normalized to body weight for male (E) and female F) mice. For A & B: Underlined letter denotes significance between adult diets within OPFR treatment. Regular letter denotes significance between adult diets within the oil treatment. For C-F: Capped lines with letters denote significance between adult diets within maternal treatments (a = $P < 0.05$; b= $P < 0.01$; d = $P < 0.0001$). Column number represents the sample size (number of litters) per treatment per sex.

Figure 11. Day and night metabolic parameters from the CLAMS in oil- and OPFR-exposed males fed LFD or HFD at week 20. A: Oxygen consumption ($V.O_2$, ml/min/kg). B: Carbon dioxide production ($V.CO_2$, ml/min/kg). C: Respiratory exchange ratio (RER, $V.CO_2/V.O_2$). D: Energy expenditure (kCal/hr). Capped lines with letters denote significance between maternal treatment within adult diet or between time periods but within a maternal treatment and adult diet. Letters denote significance between adult diets within maternal treatment and time (a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$; d = $P < 0.0001$).

Figure 12. Day and night metabolic parameters from the CLAMS in oil- and OPFR-exposed females fed LFD or HFD at week 20. A: Oxygen consumption (V.O2, ml/min/kg). B: Carbon dioxide production (V.CO2, ml/min/kg). C: Respiratory exchange ratio (RER, V.CO2/V.O2). D: Energy expenditure (kCal/hr). Capped lines with letters denote

significance between maternal treatment within adult diet or between time periods but within a maternal treatment and adult diet. Letters denote significance between adult diets within maternal treatment and time (a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$; d = $P < 0.0001$).

Figure 13. Hemodynamics using a Kent Scientific CODA instrument in males at week 21. A: Systolic pressure (mmHg) B: Diastolic pressure (mmHg) C: Mean pressure (mmHg) D: Heart rate (bpm). E: Tail blood flow (μl/min) F: Tail blood volume (ml) Column number represent sample size (number of litters) per treatment. Letters denote significance between maternal treatments within adult diet (a = $P < 0.05$; b = $P < 0.01$).

Figure 14. Glucose tolerance test (GTT) for oil- and OPFR-exposed male (A) and female (D) mice fed a LFD or HFD. Area under the curve (AUC) analysis for male (B) and female (E) GTT. Fasting (5 h) glucose levels in males (C) and females (F). Column number represent sample size (number of litters) per treatment. For A and C: letters denote significance between adult diet within maternal treatment and underlined letters denote significance between maternal treatments within adult diet. For B, C, E, and F: capped lines with letters denote significance between adult diets within maternal treatments (a = $P < 0.05$; b = $P < 0.01$, c = $P < 0.001$, d = $P < 0.0001$).

Figure 15. Insulin tolerance test (ITT) for oil- and OPFR-exposed exposed male (A) and female (C) mice fed a LFD or HFD. Area under the curve (AUC) analysis for male (B) and female (D) ITT. Column number represent sample size (number of litters) per treatment.

For A and C: letters denote significance between adult diet within maternal treatment and underlined letters denote significance between maternal treatments within adult diet. For B and D: capped lines with letters denote significance between adult diets within maternal treatments and letters denote significance between maternal treatments within adult diets ($a = P < 0.05$; $b = P < 0.01$, $c = P < 0.001$, $d = P < 0.0001$).

Figure 16. Pyruvate tolerance test (PTT) for oil- and OPFR-exposed male (A) and female (C) mice fed a LFD or HFD. Area under the curve (AUC) analysis for male (B) and female (D) PTT. Column number represent sample size (number of litters) per treatment. For A and C: letters denote significance between adult diet within maternal treatment and underlined letters denote significance between maternal treatments within adult diet. For B and D: capped lines with letters denote significance between adult diets within maternal treatments ($a = P < 0.05$; $b = P < 0.01$).

Figure 17. Peptide hormones for oil- and OPFR-exposed mice fed a LFD or HFD. Terminal plasma insulin levels (pg/ml) for males (A) and females (C). Terminal plasma leptin levels (pg/ml) for males (B) and females (D). Letters denote significance between maternal treatments within adult diet ($b = P < 0.01$; $c = P < 0.001$).

Figure 10.

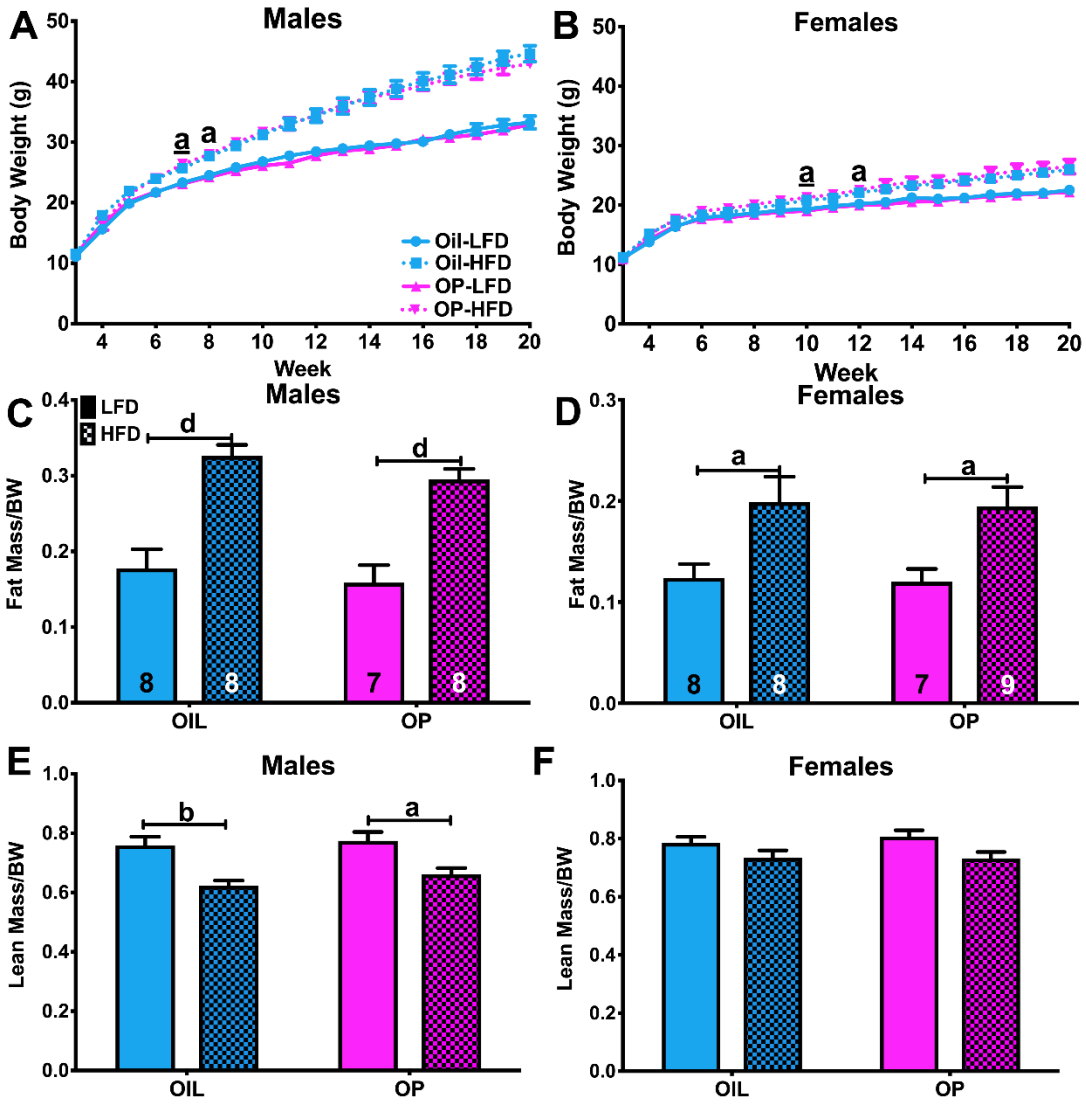

A
Males
Body Weight (g)
Week
a a
Oil-LFD
Oil-HFD
OP-LFD
OP-HFD
B
Females
Body Weight (g)
Week
a a
C
Males
LFD
HFD
Fat Mass/BW
d
d
8
8
7
8
OIL
OP
D
Females
Fat Mass/BW
a
a
8
8
7
9
OIL
OP
E
Males
Lean Mass/BW
b
a
OIL
OP
F
Females
Lean Mass/BW
OIL
OP

Figure 11.

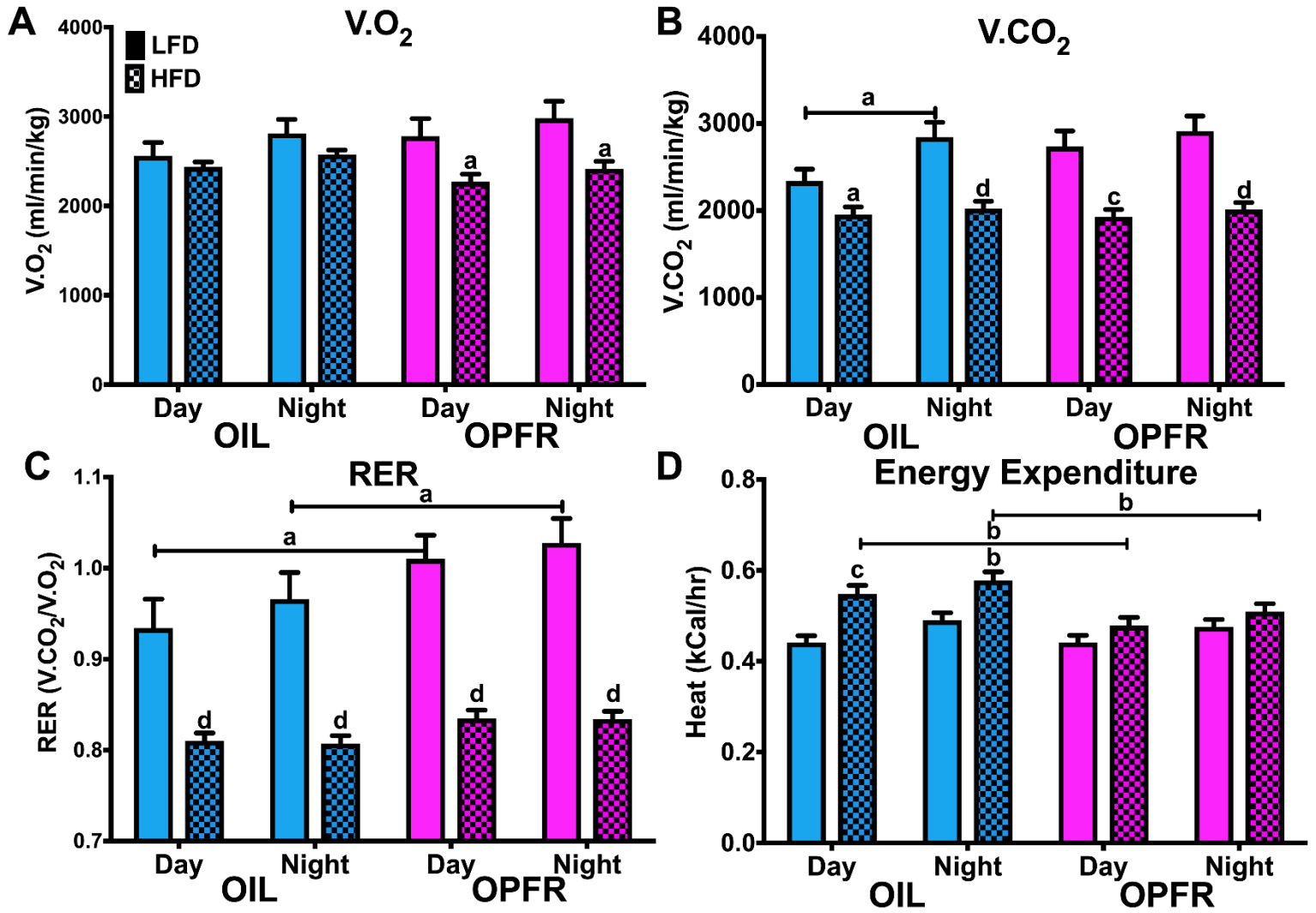

A
V.O2
LFD
HFD
V.O2 (ml/min/kg)
4000
3000
2000
1000
0
a
a
Day
Night
Day
Night
OIL
OPFR
B
V.CO2
V.CO2 (ml/min/kg)
a
a
d
c
d
C
RER a
RER (V.CO2/V.O2)
1.1
1.0
0.9
0.8
0.7
a
d
d
d
d
D
Energy Expenditure
Heat (kCal/hr)
0.8
0.6
0.4
0.2
0.0
b
b
b
c

Figure 12.

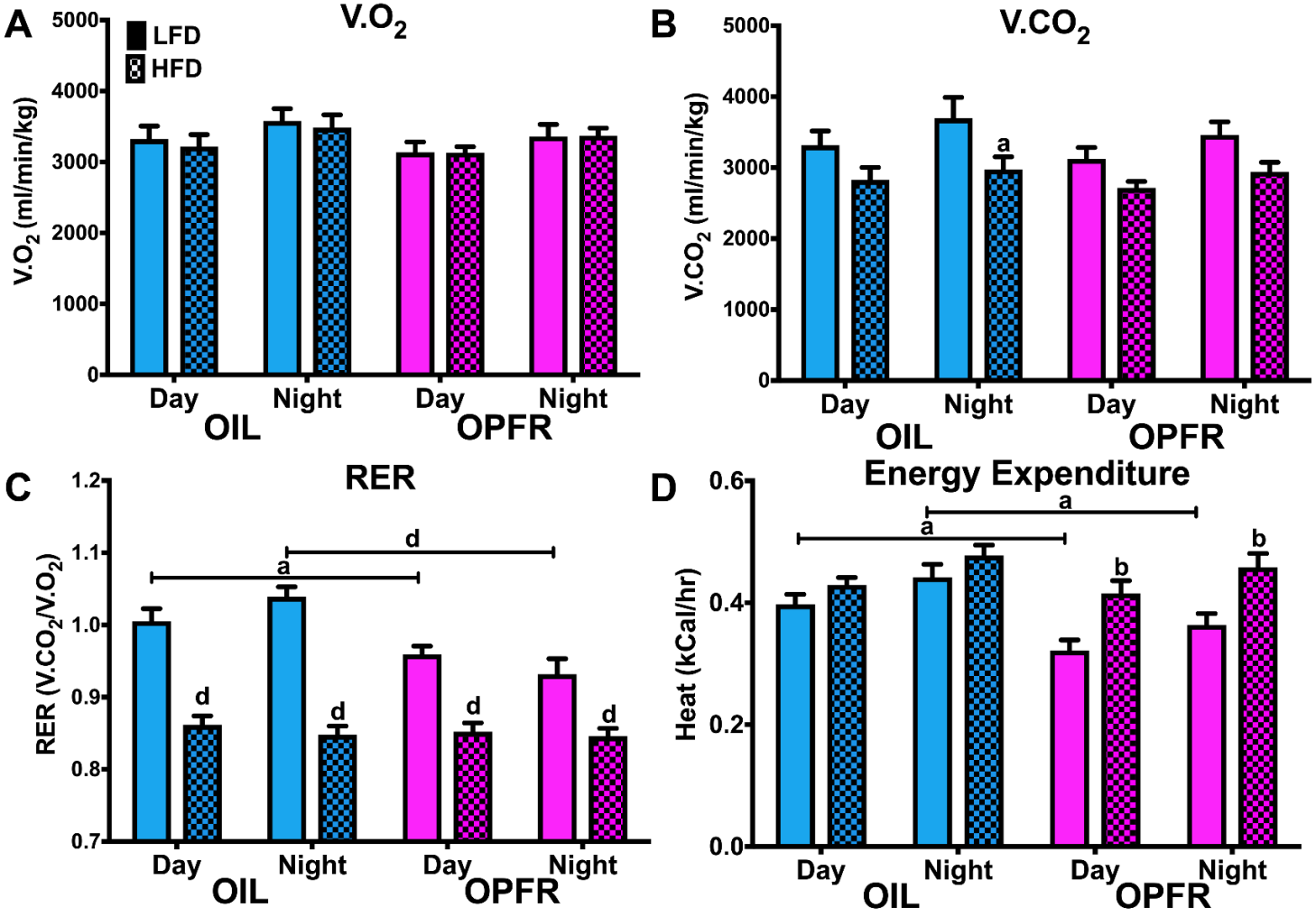

A
V.O2
LFD
HFD
V.O2 (ml/min/kg)
5000
4000
3000
2000
1000
0
Day
Night
Day
Night
OIL
OPFR
B
V.CO2
V.CO2 (ml/min/kg)
a
C
RER
RER (V.CO2/V.O2)
1.2
1.1
1.0
0.9
0.8
0.7
d
a
D
Energy Expenditure
Heat (kCal/hr)
0.6
0.4
0.2
0.0
b

Figure 13.

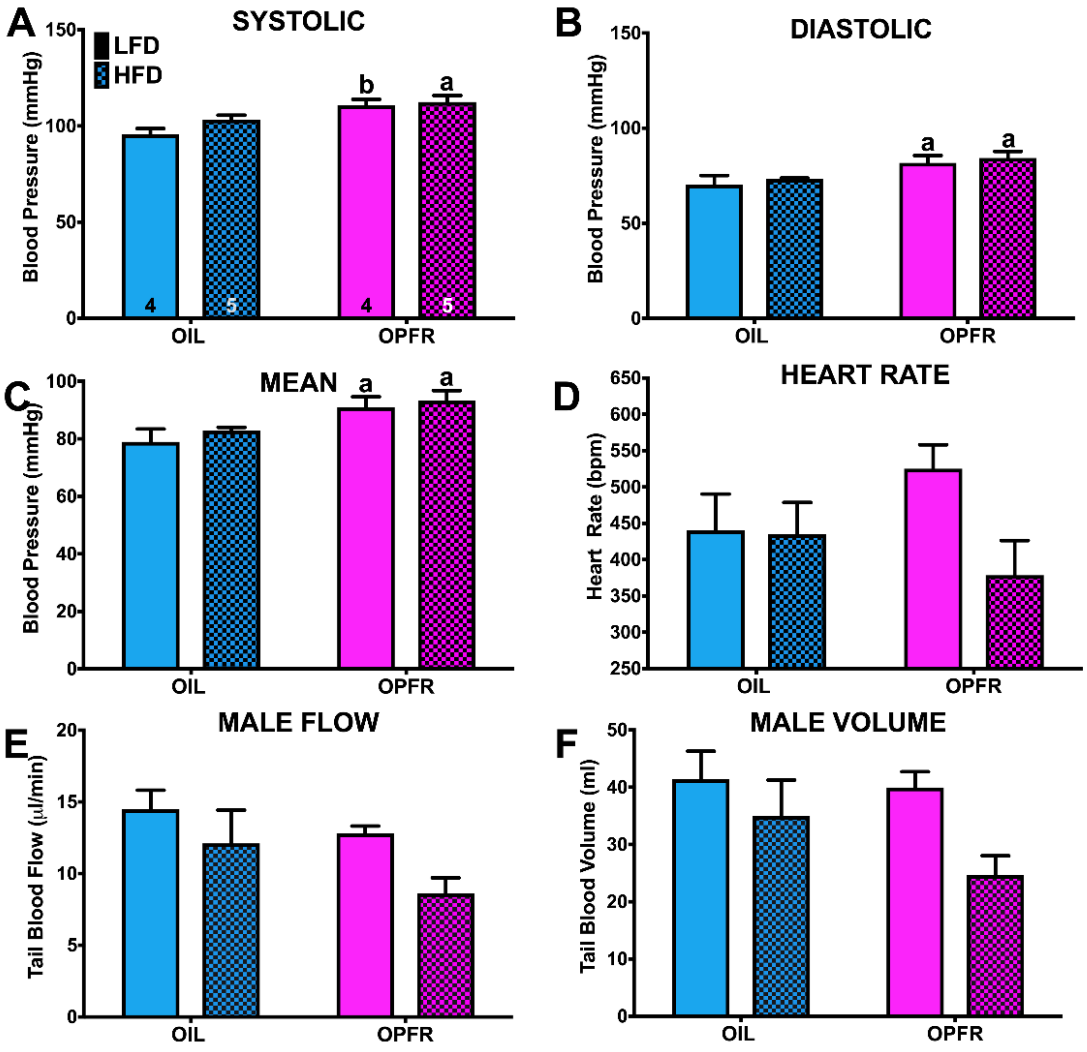

A
SYSTOLIC
LFD
HFD
Blood Pressure (mmHg)
150
100
50
0
b
a
4
5
4
5
OIL
OPFR
B
DIASTOLIC
Blood Pressure (mmHg)
150
100
50
0
a
a
OIL
OPFR
C
MEAN
Blood Pressure (mmHg)
100
80
60
40
20
0
a
a
OIL
OPFR
D
HEART RATE
Heart Rate (bpm)
650
600
550
500
450
400
350
300
250
OIL
OPFR
E
MALE FLOW
Tail Blood Flow (µl/min)
20
15
10
5
0
OIL
OPFR
F
MALE VOLUME
Tail Blood Volume (ml)
50
40
30
20
10
0
OIL
OPFR

Figure 14.

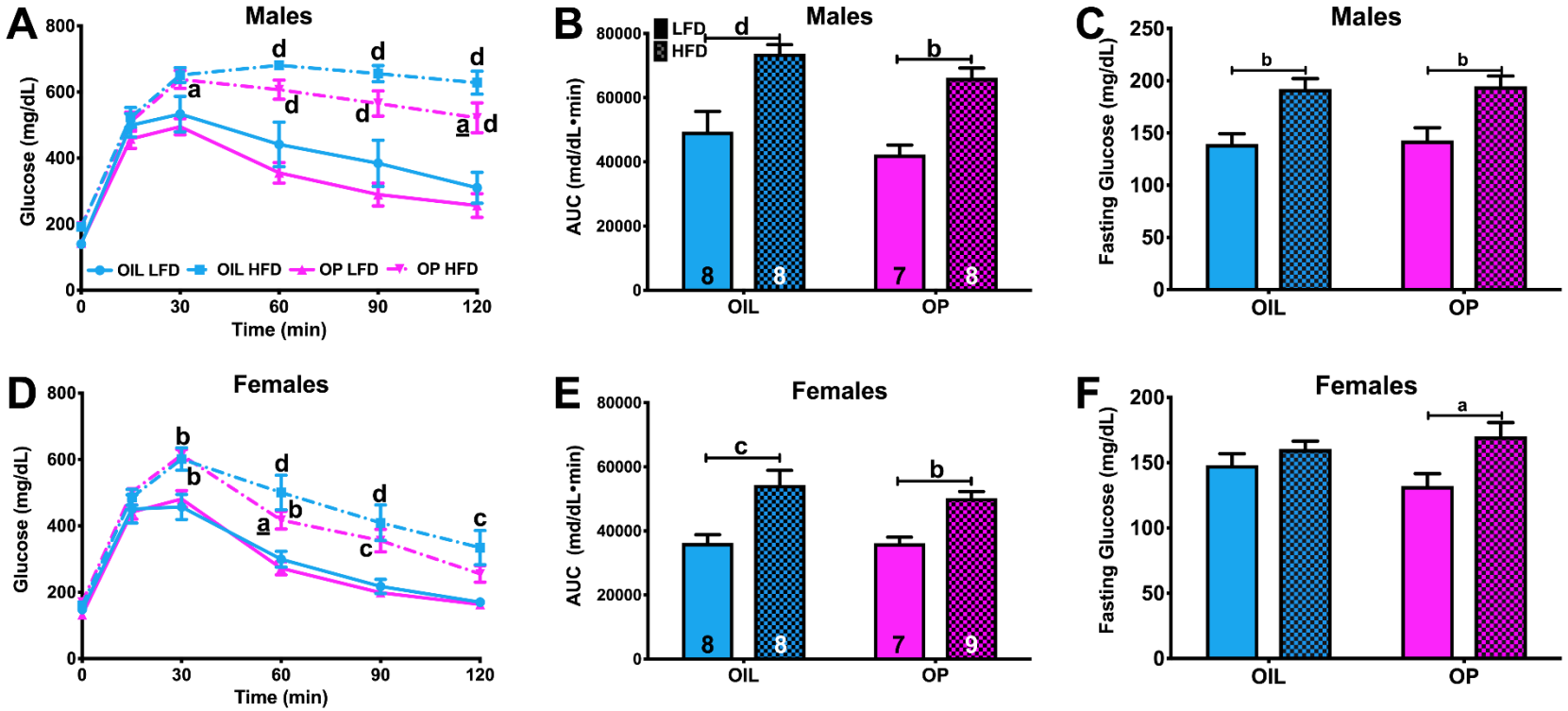

A
Males
Glucose (mg/dL)
Time (min)
OIL LFD
OIL HFD
OP LFD
OP HFD
B
Males
LFD
HFD
AUC (md/dL•min)
OIL
OP
C
Males
Fasting Glucose (mg/dL)
OIL
OP
D
Females
Glucose (mg/dL)
Time (min)
E
Females
AUC (md/dL•min)
OIL
OP
F
Females
Fasting Glucose (mg/dL)
OIL
OP

Figure 15.

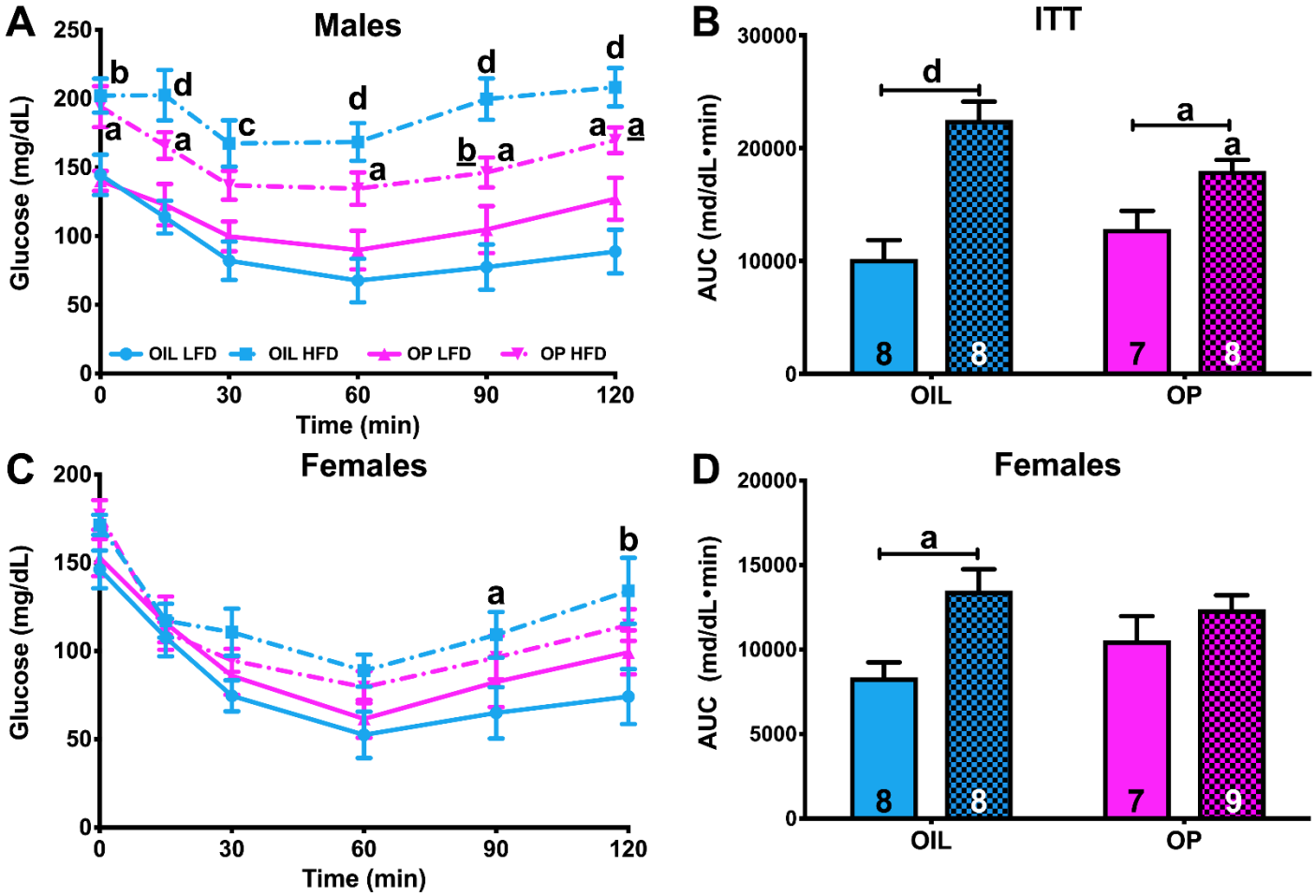

A
Males
Glucose (mg/dL)
Time (min)
OIL LFD
OIL HFD
OP LFD
OP HFD
B
ITT
AUC (md/dL•min)
OIL
OP
C
Females
Glucose (mg/dL)
Time (min)
D
Females
AUC (md/dL•min)
OIL
OP

Figure 16.

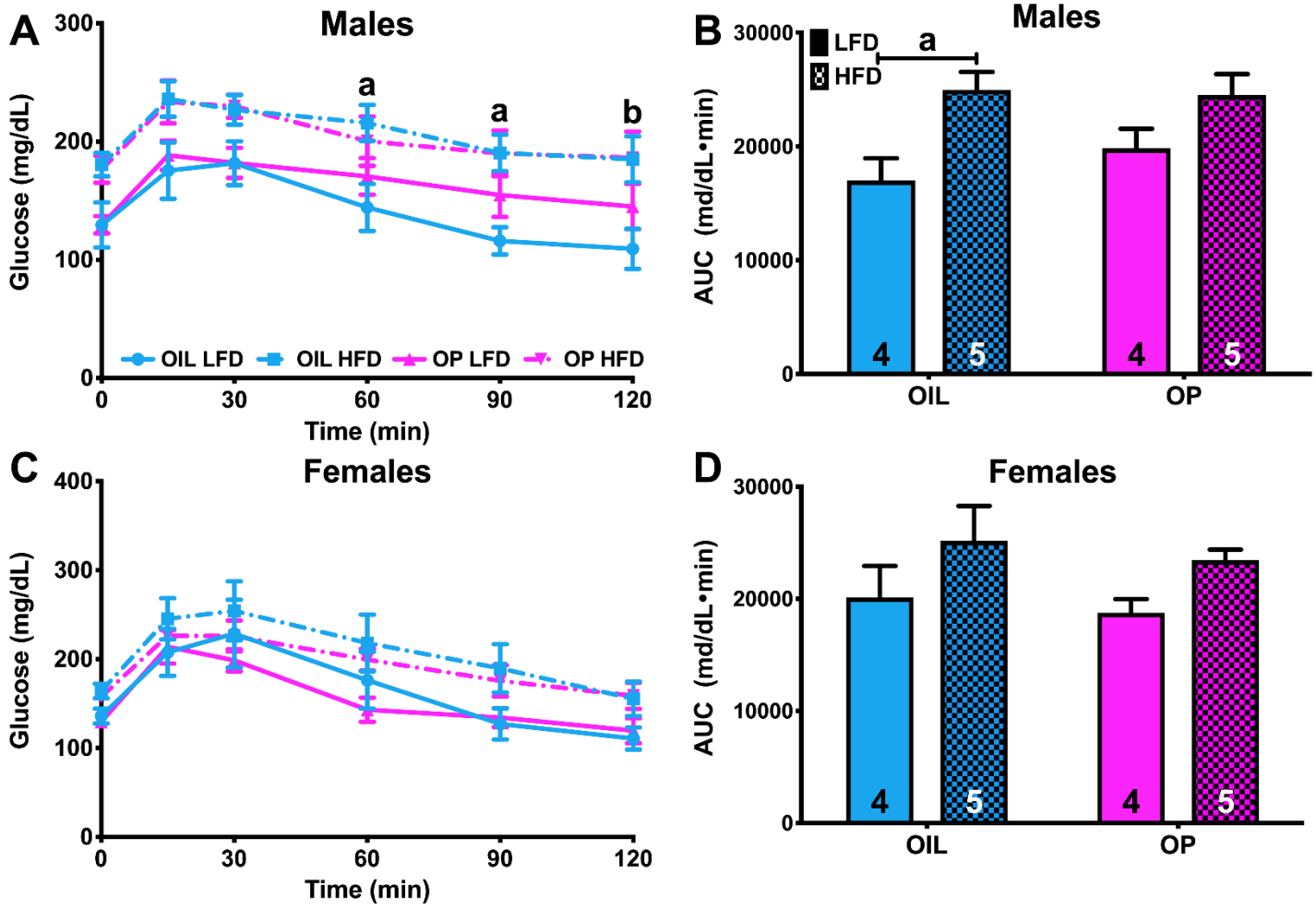
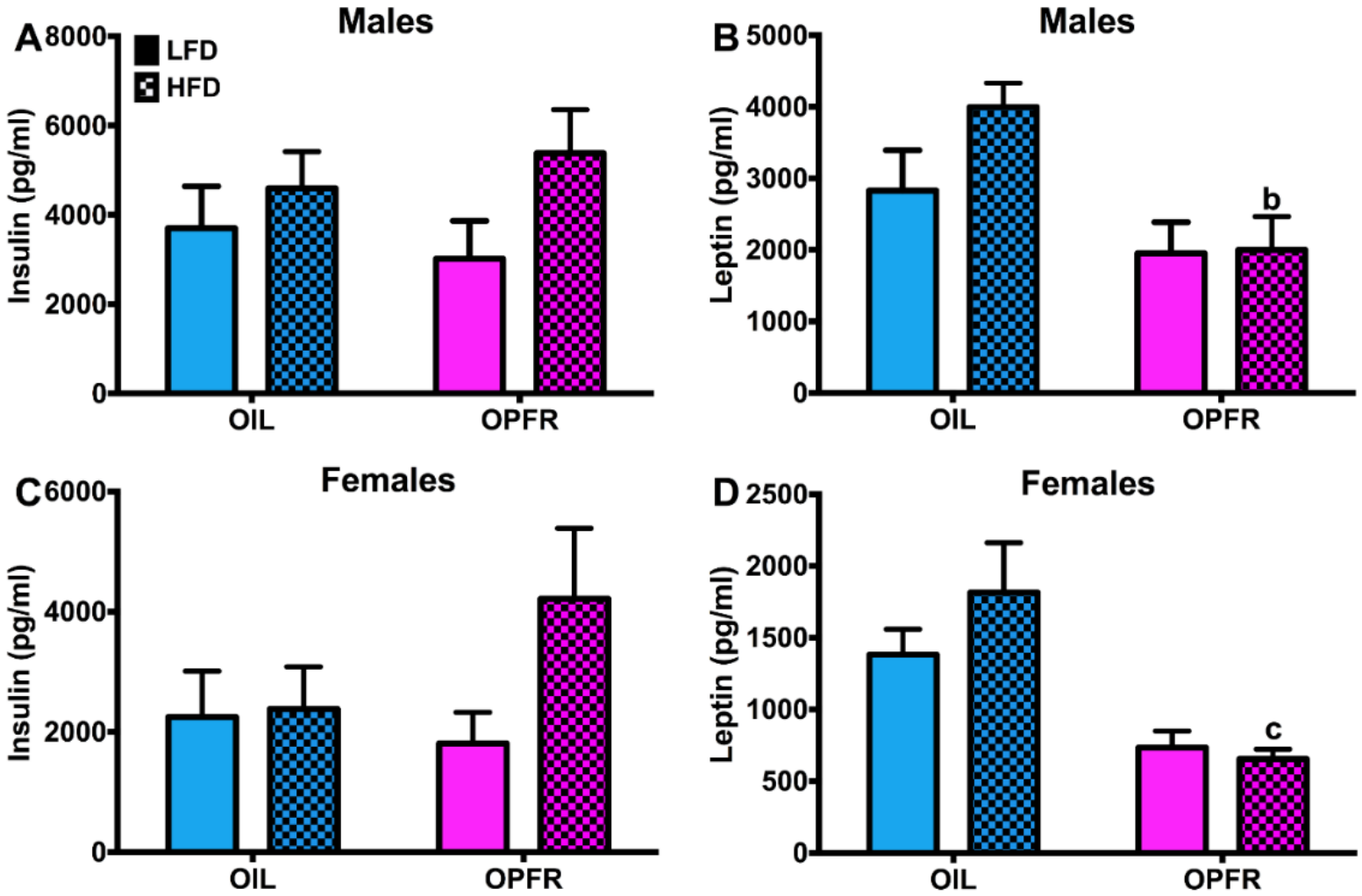

A
Males
Glucose (mg/dL)
300
200
100
0
a
a
b
OIL LFD
OIL HFD
OP LFD
OP HFD
0
30
60
90
120
Time (min)
B
Males
LFD
HFD
a
AUC (md/dL•min)
30000
20000
10000
0
4
5
4
5
OIL
OP
C
Females
Glucose (mg/dL)
400
300
200
100
0
0
30
60
90
120
Time (min)
D
Females
AUC (md/dL•min)
30000
20000
10000
0
4
5
4
5
OIL
OP

Figure 17.


A
Males
LFD
HFD
Insulin (pg/ml)
8000
6000
4000
2000
0
OIL
OPFR
B
Males
Leptin (pg/ml)
5000
4000
3000
2000
1000
0
b
OIL
OPFR
C
Females
Insulin (pg/ml)
6000
4000
2000
0
OIL
OPFR
D
Females
Leptin (pg/ml)
2500
2000
1500
1000
500
0
c
OIL
OPFR

CHAPTER 4:

SUMMARY

SUMMARY

Presence of organophosphate flame retardants (OPFRs) like tris(1,3-dichloro-2-propyl)phosphate (TDCPP), triphenyl phosphate (TPP), and tricresyl phosphate (TCP) are steadily increasing to alarming concentrations in our environment, with little known in regard to their effects on reproduction, energy homeostasis, and their modulation on hypothalamic functions (Walley *et al.*, 2018). In the present studies, we found developmental OPFR exposure altered anogenital distance, hypothalamic and liver homeostatic gene expression, glucose homeostasis, daily food intake, exploratory activity, oxygen consumption, energy expenditure, and blood pressure. These OPFR-induced effects in central and peripheral parameters of energy balance occurred more often in males. Though we observed negative outcomes of OPFR exposure on energy balance, it would be useful to examine the central and peripheral OPFR-induced mechanisms of action that elicit these effects.

It was expected that male mice would be more susceptible to OPFR-induced alteration energy homeostasis as our lab ran a similar set of experiments (Krumm *et al.*, 2018). However, it is still unclear how OPFRs are exerting these effects. It is known that OPFR interact with multiple nuclear receptors that play a critical role in metabolism centrally and peripherally *in vitro* such as the ER, androgen receptor (AR), and PPAR, thus explaining the difficulty in differentiating receptor-mediated mechanisms (Hu *et al.*, 2017; Kojima *et al.*, 2013; Kojima *et al.*, 2016a; Kojima *et al.*, 2016b; Pillai *et al.*, 2014; Suzuki *et al.*, 2013; Zhang *et al.*, 2014). In addition, their expression is dynamic and can vary with each other and other receptors, further adding to the difficulty in isolating these receptor mediated mechanisms (Chu *et al.*, 2014; Houston *et al.*, 2003; Keller *et al.*, 1995; Lemberger *et al.*,

1996; Min *et al.*, 2002; Naderi *et al.*, 2008; Olokpa *et al.*, 2017; Wang *et al.*, 2002). Therefore, acquiring global and tissue-specific knockout models of both sexes and specific tissues to understand possible receptor-mediated mechanisms that are related to sexual dimorphism would be the next step.

In addition, use of cellular harvesting of ARC neurons, RNA sequencing or quantitative real-time PCR (RT qPCR) on genes in tissues such as the liver, fat and hypothalamus can be done to further understand OPFR mechanisms in central and peripheral tissues throughout the body. In the present studies we have found that OPFR exposure alters gene expression in the ARC, which plays an integrative role in controlling energy homeostasis via hypothalamic melanocortin neurocircuitry (Cowley *et al.*, 2001; Elmquist *et al.*, 1999; Kalra *et al.*, 1999; Schwartz *et al.*, 2000). However, as previously mentioned discrete hypothalamic nuclei project numerous reciprocal neural connections between each other and to other brain regions such as the hindbrain all of which are involved in feeding behavior. Therefore, utilization of RT qPCR to examine if maternal OPFR exposure alters gene expression in the PVN, DMH, LH, and VMH would be a valuable study to conduct. In addition, understanding which components of the melanocortin neurocircuitry that are activated from maternal OPFR exposure such as POMC, AGRP, and CART can be used in immunohistochemistry experiments to visualize the density of innervated neuronal projections.

Moreover, neuronal cell type is another part in understanding the expression of genes and proteins that are ubiquitously expressed throughout the hypothalamus, including the ARC (Vail *et al.*, 2018). Following maternal OPFR treatment, POMC and NPY can be marked with a green fluorescent protein to visualize individual neurons in the ARC . Additionally,

qPCR analysis can provide insight if OPFR affects gene expression as noted in our study is also affected in individual neurons. Use of electrophysiology on neurons involved in gluconeogenesis, effects of maternal OPFR exposure on insulin, leptin, and ghrelin sensitivity and cation channel activity can be understood.

Additional experiments to create a more accurate dose-response curve for these compounds should be conducted due to numerous studies that have documented effects at or below the no-observed-adverse-effect-level as designated by the U.S. Environmental Protection Agency (Baldwin *et al.*, 2017; EPA, 2005; Patisaul *et al.*, 2013). This, along with maternal toxicity and care studies, could potentially help us understand the reason for difficulty in breeding successful pregnancies in dams exposed to OPFR. Other notable compounds like BPA, its replacement BPS, and other EDCs have shown to decrease maternal care in numerous animal models, but these behaviors have not been assessed in subjects exposed to OPFRs (Boudalia *et al.*, 2014; Catanese *et al.*, 2017; Della Seta *et al.*, 2005; Engell *et al.*, 2006; Johnson *et al.*, 2015; Kundakovic *et al.*, 2013; Palanza, 2017; Palanza *et al.*, 2002).

While our lab and current studies expanded on the knowledge pertaining to central and peripheral consequences of maternal OPFR exposure on energy homeostasis, there is much that still needs to be done. Recent studies are recording maternal OPFR exposure above the NOAEL and at environmentally relevant levels, which are causing these central and peripheral consequences in male mice (Dishaw *et al.*, 2014). Additionally, if these environmentally relevant levels are reaching the human population through our drinking water, food, or dermal exposure via commercial and household products, the call for further

investigation of OPFR exposure in the environment and how it impacts the general public should be a major focus.

REFERENCES

1. Abdallah MA, Covaci A (2014) Organophosphate flame retardants in indoor dust from Egypt: implications for human exposure. Environ Sci Technol 48(9):4782-9 doi:10.1021/es501078s.
2. Aigars, J., Suhareva, N., and Poikane, R. (2017). Distribution of Polybrominated Diphenyl Ethers in Sewage Sludge, Sediments, and Fish from Latvia. *Environments* **4**(1), 12.
3. Alonso-Magdalena P, Vieira E, Soriano S, et al. (2010) Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118(9):1243-50 doi:10.1289/ehp.1001993.
4. Amin S, Moore RW, Peterson RE, Schantz SL (2000) Gestational and lactational exposure to TCDD or coplanar PCBs alters adult expression of saccharin preference behavior in female rats. Neurotoxicol Teratol 22(5):675-82.
5. Anderson OS, Peterson KE, Sanchez BN, Zhang Z, Mancuso P, Dolinoy DC (2013) Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 27(4):1784-92 doi:10.1096/fj.12-223545.
6. Andrade NA, McConnell LL, Torrents A, Ramirez M (2010) Persistence of Polybrominated Diphenyl Ethers in Agricultural Soils after Biosolids Applications. Journal of Agricultural and Food Chemistry 58(5):3077-3084 doi:10.1021/jf9034496.
7. Andresen JA, Grundmann A, Bester K (2004) Organophosphorus flame retardants and plasticisers in surface waters. Science of The Total Environment 332(1):155-166 doi:https://doi.org/10.1016/j.scitotenv.2004.04.021.
8. Anim, A. K., Drage, D. S., Goonetilleke, A., Mueller, J. F., and Ayoko, G. A. (2017). Distribution of PBDEs, HBCDs and PCBs in the Brisbane River estuary sediment. *Marine Pollution Bulletin* **120**(1), 165-173.
9. Aoyama H, Hojo H, Takahashi KL, et al. (2012) Two-generation reproduction toxicity study in rats with methoxychlor. Congenital anomalies 52(1):28-41 doi:10.1111/j.1741-4520.2011.00344.x.
10. Arora S, Anubhuti (2006) Role of neuropeptides in appetite regulation and obesity--a review. Neuropeptides 40(6):375-401 doi:10.1016/j.npep.2006.07.001.
11. Asahi J, Kamo H, Baba R, et al. (2010) Bisphenol A induces endoplasmic reticulum stress-associated apoptosis in mouse non-parenchymal hepatocytes. Life Sciences 87:431-438.
12. Asakawa A, Toyoshima M, Fujimiya M, et al. (2007) Perfluorooctane sulfonate influences feeding behavior and gut motility via the hypothalamus. International journal of molecular medicine 19(5):733-9.
13. Asakawa A, Toyoshima M, Harada KH, Fujimiya M, Inoue K, Koizumi A (2008) The ubiquitous environmental pollutant perfluorooctanoicacid inhibits feeding behavior via peroxisome proliferator-activated receptor-alpha. International journal of molecular medicine 21(4):439-45.
14. Asarian L, Geary N (2007) Estradiol enhances cholecystokinin-dependent lipid-induced satiation and activates estrogen receptor-alpha-expressing cells in the

nucleus tractus solitarius of ovariectomized rats. Endocrinology 148(12):5656-66 doi:10.1210/en.2007-0341.
15. Aslani S, Vieira N, Marques F, Costa PS, Sousa N, Palha JA (2015) The effect of high-fat diet on rat's mood, feeding behavior and response to stress. Translational Psychiatry 5:e684 doi:10.1038/tp.2015.178.
16. Bacaloni A, Cucci F, Guarino C, Nazzari M, Samperi R, Laganà A (2008) Occurrence of Organophosphorus Flame Retardant and Plasticizers in Three Volcanic Lakes of Central Italy. Environmental Science & Technology 42(6):1898-1903 doi:10.1021/es702549g.
17. Bae S, Kim JH, Lim YH, Park HY, Hong YC (2012) Associations of bisphenol A exposure with heart rate variability and blood pressure. Hypertension (Dallas, Tex : 1979) 60(3):786-93 doi:10.1161/hypertensionaha.112.197715.
18. Baldwin KR, Phillips AL, Horman B, et al. (2017) Sex Specific Placental Accumulation and Behavioral Effects of Developmental Firemaster 550 Exposure in Wistar Rats. Sci Rep 7(1):7118 doi:10.1038/s41598-017-07216-6.
19. Barker DJ, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. BMJ 301(6746):259-62.
20. Barros RP, Gustafsson JA (2011) Estrogen receptors and the metabolic network. Cell metabolism 14(3):289-99 doi:10.1016/j.cmet.2011.08.005.
21. Batista TM, Alonso-Magdalena P, Vieira E, et al. (2012) Short-Term Treatment with Bisphenol-A Leads to Metabolic Abnormalities in Adult Male Mice. PLOS ONE 7(3):e33814 doi:10.1371/journal.pone.0033814.
22. Batterman S, Godwin C, Chernyak S, Jia C, Charles S (2010) Brominated flame retardants in offices in Michigan, USA. Environ Int 36(6):548-56 doi:10.1016/j.envint.2010.04.008.
23. Behl M, Rice JR, Smith MV, et al. (2016) Editor's Highlight: Comparative Toxicity of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers to Caenorhabditis elegans. Toxicol Sci 154(2):241-252 doi:10.1093/toxsci/kfw162.
24. Belcher SM, Cookman CJ, Patisaul HB, Stapleton HM (2014) In vitro assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components. Toxicol Lett 228(2):93-102 doi:10.1016/j.toxlet.2014.04.017.
25. Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA (2009) Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. Environmental Science & Technology 43(3):597-603 doi:10.1021/es801845a.
26. Berthoud HR (2002) Multiple neural systems controlling food intake and body weight. Neuroscience and biobehavioral reviews 26(4):393-428.
27. Betts KS (2002) Rapidly rising PBDE levels in North America. Environ Sci Technol 36(3):50A-52A.
28. Bindhumol V, Chitra K, Mathur P (2003) Bisphenol A induces reactive oxygen species generation in the liver of male rats. Toxicology 188:117-124.
29. Boucher O, Muckle G, Bastien CH (2009) Prenatal exposure to polychlorinated biphenyls: a neuropsychologic analysis. Environmental health perspectives 117(1):7-16 doi:10.1289/ehp.11294.

30. Boudalia S, Berges R, Chabanet C, et al. (2014) A multi-generational study on low-dose BPA exposure in Wistar rats: effects on maternal behavior, flavor intake and development. Neurotoxicol Teratol 41:16-26 doi:10.1016/j.ntt.2013.11.002.
31. Bradley M, Rutkiewicz J, Mittal K, Fernie K, Basu N (2015) In ovo exposure to organophosphorous flame retardants: survival, development, neurochemical, and behavioral changes in white leghorn chickens. Neurotoxicol Teratol 52(Pt B):228-35 doi:10.1016/j.ntt.2015.08.003.
32. Branchi I, Alleva E, Costa LG (2002) Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. Neurotoxicology 23(3):375-84.
33. Brandsma SH, Leonards PEG, Leslie HA, de Boer J (2015) Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Science of The Total Environment 505:22-31 doi:https://doi.org/10.1016/j.scitotenv.2014.08.072.
34. Burreau S, Broman D, Orn U (2000) Tissue distribution of 2,2',4,4'-tetrabromo[14C]diphenyl ether ([14C]-PBDE 47) in pike (Esox lucius) after dietary exposure--a time series study using whole body autoradiography. Chemosphere 40(9-11):977-85.
35. Butt CM, Congleton J, Hoffman K, Fang M, Stapleton HM (2014) Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. Environ Sci Technol 48(17):10432-8 doi:10.1021/es5025299.
36. Butt CM, Stapleton HM (2013) Inhibition of thyroid hormone sulfotransferase activity by brominated flame retardants and halogenated phenolics. Chemical research in toxicology 26(11):1692-702 doi:10.1021/tx400342k.
37. Camargo, A., Ruano, J., Fernandez, J. M., Parnell, L. D., Jimenez, A., Santos-Gonzalez, M., Marin, C., Perez-Martinez, P., Uceda, M., Lopez-Miranda, J., *et al.* (2010). Gene expression changes in mononuclear cells in patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil. *BMC Genomics* **11**(1), 253 (journal article).
38. Cao J, Echelberger R, Liu M, et al. (2015) Soy but not bisphenol A (BPA) or the phytoestrogen genistin alters developmental weight gain and food intake in pregnant rats and their offspring. Reproductive toxicology (Elmsford, NY) 58:282-94 doi:10.1016/j.reprotox.2015.07.077.
39. Carignan CC, McClean MD, Cooper EM, et al. (2013) Predictors of tris(1,3-dichloro-2-propyl) phosphate metabolite in the urine of office workers. Environ Int 55:56-61 doi:10.1016/j.envint.2013.02.004.
40. Carté B, Faulkner DJ (1981) Polybrominated diphenyl ethers from Dysidea herbacea, Dysidea chlorea and Phyllospongia foliascens. Tetrahedron 37(13):2335-2339 doi:https://doi.org/10.1016/S0040-4020(01)88886-4.
41. Catanese, M. C., and Vandenberg, L. N. (2017). Bisphenol S (BPS) Alters Maternal Behavior and Brain in Mice Exposed During Pregnancy/Lactation and Their Daughters. *Endocrinology* **158**(3), 516-530.
42. Cederroth CR, Vinciguerra M, Kühne F, et al. (2007) A phytoestrogen-rich diet increases energy expenditure and decreases adiposity in mice. Environmental health perspectives 115(10):1467-1473 doi:10.1289/ehp.10413.

43. Chen F, Zhou L, Bai Y, Zhou R, Chen L (2015) Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety- and depression-like behaviors in rats perinatally exposed to bisphenol A. J Biomed Res 29(3):250-8 doi:10.7555/JBR.29.20140058.
44. Chen G, Bunce NJ (2003) Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. Toxicol Sci 76(2):310-20 doi:10.1093/toxsci/kfg236.
45. Chen L, Wang C, Cui C, et al. (2015) Prenatal exposure to polybrominated diphenyl ethers and birth outcomes. Environ Pollut 206:32-7 doi:10.1016/j.envpol.2015.06.019.
46. Chen L, Huang Y, Huang C, Hu B, Hu C, Zhou B (2013) Acute exposure to DE-71 causes alterations in visual behavior in zebrafish larvae. Environ Toxicol Chem 32(6):1370-5 doi:10.1002/etc.2168.
47. Chen L, Yu K, Huang C, et al. (2012) Prenatal transfer of polybrominated diphenyl ethers (PBDEs) results in developmental neurotoxicity in zebrafish larvae. Environ Sci Technol 46(17):9727-34 doi:10.1021/es302119g.
48. Cheng, J.-O., and Ko, F.-C. (2018). Occurrence of PBDEs in surface sediments of metropolitan rivers: Sources, distribution pattern, and risk assessment. *Science of The Total Environment* **637-638**, 1578-1585.
49. Choi, G. , Wang, Y. B., Sundaram, R., Chen, Z., Barr, D. B., Buck Louis, G. M., and Smarr, M. M. (2019). Polybrominated diphenyl ethers and incident pregnancy loss: The LIFE Study. *Environ Res* **168**, 375-381.
50. Choo, G., Cho, H.-S., Park, K., Lee, J.-W., Kim, P., and Oh, J.-E. (2018). Tissue-specific distribution and bioaccumulation potential of organophosphate flame retardants in crucian carp. *Environmental Pollution* **239**, 161-168.
51. Chopra IJ, Wu SY, Teco GN, Santini F (1992) A radioimmunoassay for measurement of 3,5,3'-Hazlet, NJ triiodothyronine sulfate: studies in thyroidal and nonthyroidal diseases, pregnancy, and neonatal life. The Journal of clinical endocrinology and metabolism 75(1):189-94 doi:10.1210/jcem.75.1.1619009
52. Chu, R., van Hasselt, A., Vlantis, A. C., Ng, E. K., Liu, S. Y., Fan, M. D., Ng, S. K., Chan, A. B., Liu, Z., Li, X. Y., *et al.* (2014). The cross-talk between estrogen receptor and peroxisome proliferator-activated receptor gamma in thyroid cancer. *Cancer* **120**(1), 142-53.
53. Chung, H. W., and Ding, W. H. (2009). Determination of organophosphate flame retardants in sediments by microwave-assisted extraction and gas chromatography-mass spectrometry with electron impact and chemical ionization. *Analytical and bioanalytical chemistry* **395**(7), 2325-34.
54. Cincinelli, A., Martellini, T., Misuri, L., Lanciotti, E., Sweetman, A., Laschi, S., and Palchetti, I. (2012). PBDEs in Italian sewage sludge and environmental risk of using sewage sludge for land application. *Environmental Pollution* **161**, 229-234.
55. Cooke GM, Tryphonas H, Pulido O, Caldwell D, Bondy GS, Forsyth D (2004) Oral (gavage), in utero and postnatal exposure of Sprague-Dawley rats to low doses of tributyltin chloride. Part 1: Toxicology, histopathology and clinical chemistry. Food Chem Toxicol 42(2):211-20.

56. Costa LG, Giordano G (2007) Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. Neurotoxicology 28(6):1047-67 doi:10.1016/j.neuro.2007.08.007.
57. Cowley MA, Smart JL, Rubinstein M, et al. (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411(6836):480-4 doi:10.1038/35078085.
58. Crimmins BS, Pagano JJ, Xia X, Hopke PK, Milligan MS, Holsen TM (2012) Polybrominated diphenyl ethers (PBDEs): turning the corner in Great Lakes trout 1980-2009. Environ Sci Technol 46(18):9890-7 doi:10.1021/es302415z.
59. Crump D, Chiu S, Kennedy SW (2012) Effects of tris(1,3-dichloro-2-propyl) phosphate and tris(1-chloropropyl) phosphate on cytotoxicity and mRNA expression in primary cultures of avian hepatocytes and neuronal cells. Toxicol Sci 126(1):140-8 doi:10.1093/toxsci/kfs015.
60. Da, C., Wu, K., Ye, J., Wang, R., Liu, R., and Sun, R. (2019). Temporal trends of polybrominated diphenyl ethers in the sediment cores from different areas in China. *Ecotoxicology and environmental safety* **171**, 222-230.
61. Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M (2001) Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. Environ Health Perspect 109 Suppl 1:49-68 doi:10.1289/ehp.01109s149.
62. Daubie S, Bisson JF, Lalonde R, Schroeder H, Rychen G (2011) Neurobehavioral and physiological effects of low doses of polybrominated diphenyl ether (PBDE)-99 in male adult rats. Toxicol Lett 204(1):57-63 doi:10.1016/j.toxlet.2011.04.009.
63. Daso, A. P., Fatoki, O. S., Odendaal, J. P., and Olujimi, O. O. (2013). Polybrominated diphenyl ethers (PBDEs) and 2, 2′, 4, 4′, 5, 5′-hexabromobiphenyl (BB-153) in landfill leachate in Cape Town, South Africa. *Environmental monitoring and assessment* **185**(1), 431-439.
64. de Boer J, Wester PG, van der Horst A, Leonards PE (2003) Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands. Environ Pollut 122(1):63-74.
65. de Cock M, van de Bor M (2014) Obesogenic effects of endocrine disruptors, what do we know from animal and human studies? Environ Int 70:15-24 doi:10.1016/j.envint.2014.04.022.
66. Della Seta, D., Minder, I., Dessi-Fulgheri, F., and Farabollini, F. (2005). Bisphenol-A exposure during pregnancy and lactation affects maternal behavior in rats. *Brain research bulletin* **65**(3), 255-60.
67. Dellovade TL, Zhu YS, Krey L, Pfaff DW (1996) Thyroid hormone and estrogen interact to regulate behavior. Proc Natl Acad Sci U S A 93(22):12581-6.
68. Diaz Weinstein S, Villafane JJ, Juliano N, Bowman RE (2013) Adolescent exposure to Bisphenol-A increases anxiety and sucrose preference but impairs spatial memory in rats independent of sex. Brain research 1529:56-65 doi:10.1016/j.brainres.2013.07.018.
69. Dimicco JA, Zaretsky DV (2007) The dorsomedial hypothalamus: a new player in thermoregulation. American journal of physiology Regulatory, integrative and comparative physiology 292(1):R47-63 doi:10.1152/ajpregu.00498.2006.

70. Ding G, Yu J, Cui C, et al. (2015) Association between prenatal exposure to polybrominated diphenyl ethers and young children's neurodevelopment in China. Environ Res 142:104-11 doi:10.1016/j.envres.2015.06.008.
71. Dirbaba, N. B., Li, S., Wu, H., Yan, X., and Wang, J. (2018). Organochlorine pesticides, polybrominated diphenyl ethers and polychlorinated biphenyls in surficial sediments of the Awash River Basin, Ethiopia. *PLoS One* **13**(10), e0205026.
72. Dirbaba, N. B., Li, S., Wu, H., Yan, X., and Wang, J. (2018). Organochlorine pesticides, polybrominated diphenyl ethers and polychlorinated biphenyls in surficial sediments of the Awash River Basin, Ethiopia. *PLoS One* **13**(10), e0205026.
73. EPA (2014). AN ALTERNATIVES ASSESSMENT FOR THE FLAME RETARDANT DECABROMODIPHENYL ETHER (DecaBDE).
74. Dishaw LV, Hunter DL, Padnos B, Padilla S, Stapleton HM (2014) Developmental exposure to organophosphate flame retardants elicits overt toxicity and alters behavior in early life stage zebrafish (Danio rerio). Toxicol Sci 142(2):445-54 doi:10.1093/toxsci/kfu194.
75. Dishaw LV, Macaulay LJ, Roberts SC, Stapleton HM (2014) Exposures, mechanisms, and impacts of endocrine-active flame retardants. Curr Opin Pharmacol 19:125-33 doi:10.1016/j.coph.2014.09.018.
76. Dishaw LV, Powers CM, Ryde IT, et al. (2011) Is the PentaBDE replacement, tris (1,3-dichloro-2-propyl) phosphate (TDCPP), a developmental neurotoxicant? Studies in PC12 cells. Toxicology and applied pharmacology 256(3):281-9 doi:10.1016/j.taap.2011.01.005.
77. Dodd GT, Worth AA, Nunn N, et al. (2014) The thermogenic effect of leptin is dependent on a distinct population of prolactin-releasing peptide neurons in the dorsomedial hypothalamus. Cell metabolism 20(4):639-49 doi:10.1016/j.cmet.2014.07.022.
78. Domi E, Uhrig S, Soverchia L, et al. (2016) Genetic Deletion of Neuronal PPARgamma Enhances the Emotional Response to Acute Stress and Exacerbates Anxiety: An Effect Reversed by Rescue of Amygdala PPARgamma Function. J Neurosci 36(50):12611-12623 doi:10.1523/JNEUROSCI.4127-15.2016.
79. Domingo JL, Nadal M (2017) Per- and Polyfluoroalkyl Substances (PFASs) in Food and Human Dietary Intake: A Review of the Recent Scientific Literature. J Agric Food Chem 65(3):533-543 doi:10.1021/acs.jafc.6b04683.
80. Eaton DL, Daroff RB, Autrup H, et al. (2008) Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical reviews in toxicology 38 Suppl 2:1-125 doi:10.1080/10408440802272158.
81. Eggen T, Heimstad ES, Stuanes AO, Norli HR (2013) Uptake and translocation of organophosphates and other emerging contaminants in food and forage crops. Environmental science and pollution research international 20(7):4520-31 doi:10.1007/s11356-012-1363-5.
82. Elias CF, Lee CE, Kelly JF, et al. (2001) Characterization of CART neurons in the rat and human hypothalamus. The Journal of comparative neurology 432(1):1-19.

83. Elmquist JK, Elias CF, Saper CB (1999) From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 22(2):221-32.
84. Engell, M. D., Godwin, J., Young, L. J., and Vandenbergh, J. G. (2006). Perinatal exposure to endocrine disrupting compounds alters behavior and brain in the female pine vole. *Neurotoxicol Teratol* **28**(1), 103-10.
85. EPA (2005). U. S. Furniture flame retardancy partnership: environmental profiles of chemical flame retardant alternatives doi: EPA 742-R-05-002A.
86. Eriksson P, Jakobsson E, Fredriksson A (2001) Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environ Health Perspect 109(9):903-8 doi:10.1289/ehp.01109903.
87. Eriksson P, Viberg H, Jakobsson E, Orn U, Fredriksson A (2002) A brominated flame retardant, 2,2',4,4',5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development. Toxicol Sci 67(1):98-103.
88. Eskenazi B, Chevrier J, Rauch SA, et al. (2013) In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. Environ Health Perspect 121(2):257-62 doi:10.1289/ehp.1205597.
89. EU (2001) Risk assessment report: diphenyl ether, pentabromo derivative (pentabromodiphenyl ether). European Chemicals Bureau.
90. Eulaers I, Jaspers VL, Halley DJ, et al. (2014) Brominated and phosphorus flame retardants in White-tailed Eagle Haliaeetus albicilla nestlings: bioaccumulation and associations with dietary proxies (delta(1)(3)C, delta(1)(5)N and delta(3)(4)S). The Science of the total environment 478:48-57 doi:10.1016/j.scitotenv.2014.01.051.
91. Evenset A, Christensen GN, Skotvold T, et al. (2004) A comparison of organic contaminants in two high Arctic lake ecosystems, Bjornoya (Bear Island), Norway. The Science of the total environment 318(1-3):125-41 doi:10.1016/s0048-9697(03)00365-6.
92. Fang M, Webster TF, Ferguson PL, Stapleton HM (2015) Characterizing the peroxisome proliferator-activated receptor (PPARgamma) ligand binding potential of several major flame retardants, their metabolites, and chemical mixtures in house dust. Environ Health Perspect 123(2):166-72 doi:10.1289/ehp.1408522.
93. Farhat A, Crump D, Chiu S, et al. (2013) In Ovo effects of two organophosphate flame retardants--TCPP and TDCPP--on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. Toxicol Sci 134(1):92-102 doi:10.1093/toxsci/kft100.
94. Frederiksen M, Thomsen M, Vorkamp K, Knudsen LE (2009) Patterns and concentration levels of polybrominated diphenyl ethers (PBDEs) in placental tissue of women in Denmark. Chemosphere 76(11):1464-9 doi:10.1016/j.chemosphere.2009.07.017.
95. Fries E, Puttmann W (2001) Occurrence of organophosphate esters in surface water and ground water in Germany. Journal of environmental monitoring : JEM 3(6):621-6.

96. Fu X, Schmitz FJ, Govindan M, et al. (1995) Enzyme inhibitors: new and known polybrominated phenols and diphenyl ethers from four Indo-Pacific Dysidea sponges. Journal of natural products 58(9):1384-91.
97. Funabashi T, Kawaguchi M, Furuta M, Fukushima A, Kimura F (2004) Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. Psychoneuroendocrinology 29(4):475-85.
98. Gallou-Kabani, C., and Junien, C. (2005). Nutritional Epigenomics of Metabolic Syndrome. *New Perspective Against the Epidemic* **54**(7), 1899-1906.
99. Gandhi N, Gewurtz SB, Drouillard KG, et al. (2017) Polybrominated diphenyl ethers (PBDEs) in Great Lakes fish: Levels, patterns, trends and implications for human exposure. The Science of the total environment 576:907-916 doi:10.1016/j.scitotenv.2016.10.043.
100. German JP, Thaler JP, Wisse BE, et al. (2011) Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia. Endocrinology 152(2):394-404 doi:10.1210/en.2010-0890.
101. Giulivo, M., Capri, E., Kalogianni, E., Milacic, R., Majone, B., Ferrari, F., Eljarrat, E., and Barcelo, D. (2017). Occurrence of halogenated and organophosphate flame retardants in sediment and fish samples from three European river basins. *The Science of the total environment* **586**, 782-791.
102. Golden RJ, Noller KL, Titus-Ernstoff L, et al. (1998) Environmental endocrine modulators and human health: an assessment of the biological evidence. Critical reviews in toxicology 28(2):109-227 doi:10.1080/10408449891344191.
103. Gomara B, Herrero L, Ramos JJ, et al. (2007) Distribution of polybrominated diphenyl ethers in human umbilical cord serum, paternal serum, maternal serum, placentas, and breast milk from Madrid population, Spain. Environ Sci Technol 41(20):6961-8.
104. Goralczyk K, Strucinski P, Czaja K, Hernik A, Ludwicki JK (2002) [Flame retardants--use and hazards for human]. Rocz Panstw Zakl Hig 53(3):293-305.
105. Grayson, B. L., Wang, L., and Aune, T. M. (2011). Peripheral blood gene expression profiles in metabolic syndrome, coronary artery disease and type 2 diabetes. *Genes and immunity* **12**(5), 341-351.
106. Grill HJ, Hayes MR (2012) Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. Cell metabolism 16(3):296-309 doi:10.1016/j.cmet.2012.06.015.
107. Guerre-Millo M, Rouault C, Poulain P, et al. (2001) PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes 50(12):2809-14.
108. Gump BB, Yun S, Kannan K (2014) Polybrominated diphenyl ether (PBDE) exposure in children: possible associations with cardiovascular and psychological functions. Environmental research 132:244-250 doi:10.1016/j.envres.2014.04.009.
109. Gupta C (2000) Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. Proc Soc Exp Biol Med 224(2):61-8.
110. Hagenmaier H, She J, Benz T, Dawidowsky N, Düsterhöft L, Lindig C (1992) Analysis of sewage sludge for polyhalogenated dibenzo-p-dioxins, dibenzofurans,

and diphenylethers. Chemosphere 25(7):1457-1462 doi:https://doi.org/10.1016/0045-6535(92)90169-R.
111. Hale RC, La Guardia MJ, Harvey EP, Mainor TM, Duff WH, Gaylor MO (2001) Polybrominated diphenyl ether flame retardants in Virginia freshwater fishes (USA). Environ Sci Technol 35(23):4585-91.
112. Hale RC, Alaee M, Manchester-Neesvig JB, Stapleton HM, Ikonomou MG (2003) Polybrominated diphenyl ether flame retardants in the North American environment. Environ Int 29(6):771-9 doi:10.1016/S0160-4120(03)00113-2.
113. Hallgren S, Darnerud PO (2002) Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats-testing interactions and mechanisms for thyroid hormone effects. Toxicology 177(2-3):227-43.
114. Hany J, Lilienthal H, Sarasin A, et al. (1999) Developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicology and applied pharmacology 158(3):231-43 doi:10.1006/taap.1999.8710.
115. Hayes MR, Covasa M (2006) Gastric distension enhances CCK-induced Fos-like immunoreactivity in the dorsal hindbrain by activating 5-HT3 receptors. Brain research 1088(1):120-30 doi:10.1016/j.brainres.2006.03.018.
116. He CT, Zheng J, Qiao L, et al. (2015) Occurrence of organophosphorus flame retardants in indoor dust in multiple microenvironments of southern China and implications for human exposure. Chemosphere 133:47-52 doi:10.1016/j.chemosphere.2015.03.043.
117. Heindel JJ, Blumberg B, Cave M, et al. (2017) Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol 68:3-33 doi:10.1016/j.reprotox.2016.10.001.
118. Hendriks HS, Meijer M, Muilwijk M, van den Berg M, Westerink RH (2014) A comparison of the in vitro cyto- and neurotoxicity of brominated and halogen-free flame retardants: prioritization in search for safe(r) alternatives. Arch Toxicol 88(4):857-69 doi:10.1007/s00204-013-1187-1.
119. Herbstman JB, Mall JK (2014) Developmental Exposure to Polybrominated Diphenyl Ethers and Neurodevelopment. Curr Environ Health Rep 1(2):101-112 doi:10.1007/s40572-014-0010-3.
120. Herzke D, Gabrielsen GW, Evenset A, Burkow IC (2003) Polychlorinated camphenes (toxaphenes), polybrominated diphenylethers and other halogenated organic pollutants in glaucous gull (Larus hyperboreus) from Svalbard and Bjornoya (Bear Island). Environ Pollut 121(2):293-300.
121. Hill RN, Crisp TM, Hurley PM, Rosenthal SL, Singh DV (1998) Risk assessment of thyroid follicular cell tumors. Environ Health Perspect 106(8):447-57 doi:10.1289/ehp.98106447.
122. Hoffman K, Butt CM, Chen A, Limkakeng AT, Jr., Stapleton HM (2015) High Exposure to Organophosphate Flame Retardants in Infants: Associations with Baby Products. Environ Sci Technol 49(24):14554-9 doi:10.1021/acs.est.5b03577.

123. Hoffman K, Daniels JL, Stapleton HM (2014) Urinary metabolites of organophosphate flame retardants and their variability in pregnant women. Environ Int 63:169-72 doi:10.1016/j.envint.2013.11.013.
124. Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T (2002) Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod Toxicol 16(2):117-22.
125. Hooper K, McDonald TA (2000) The PBDEs: an emerging environmental challenge and another reason for breast-milk monitoring programs. Environ Health Perspect 108(5):387-92 doi:10.1289/ehp.00108387.
126. Hooper K, She J (2003) Lessons from the polybrominated diphenyl ethers (PBDEs): precautionary principle, primary prevention, and the value of community-based body-burden monitoring using breast milk. Environ Health Perspect 111(1):109-14 doi:10.1289/ehp.5438.
127. Houston, K. D., Copland, J. A., Broaddus, R. R., Gottardis, M. M., Fischer, S. M., and Walker, C. L. (2003). Inhibition of proliferation and estrogen receptor signaling by peroxisome proliferator-activated receptor gamma ligands in uterine leiomyoma. *Cancer research* **63**(6), 1221-7.
128. Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS (1999) Exposure to bisphenol A advances puberty. Nature 401:763 doi:10.1038/44517.
129. Horvath TL (2006) Synaptic plasticity in energy balance regulation. Obesity (Silver Spring, Md) 14 Suppl 5:228s-233s doi:10.1038/oby.2006.314.
130. I Bd (1981) A Two-year Oral Toxicity/Carcinogenicity Study on Fyrol FR-2 in Rats (Final Report).
131. Hu, W., Gao, F., Zhang, H., Hiromori, Y., Arakawa, S., Nagase, H., Nakanishi, T., and Hu, J. (2017). Activation of Peroxisome Proliferator-Activated Receptor Gamma and Disruption of Progesterone Synthesis of 2-Ethylhexyl Diphenyl Phosphate in Human Placental Choriocarcinoma Cells: Comparison with Triphenyl Phosphate. *Environmental Science & Technology* **51**(7), 4061-4068.
132. Ikonomou MG, Rayne S, Fischer M, Fernandez MP, Cretney W (2002) Occurrence and congener profiles of polybrominated diphenyl ethers (PBDEs) in environmental samples from coastal British Columbia, Canada. Chemosphere 46(5):649-63.
133. Ivry Del Moral L, Le Corre L, Poirier H, et al. (2016) Obesogen effects after perinatal exposure of 4,4'-sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. Toxicology 357-358:11-20 doi:10.1016/j.tox.2016.05.023.
134. J.H. Christensen, B. G., J. Vikelsoe, K. Vorkamp (2004). Polybrominated Diphenyl Ethers (PBDEs) in Sewage Sludge and Wastewater: Method Development and Validation. *NERI Technical Report* **481**.
135. Jarema KA, Hunter DL, Shaffer RM, Behl M, Padilla S (2015) Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicol Teratol 52(Pt B):194-209 doi:10.1016/j.ntt.2015.08.010.
136. Jeong JH, Lee DK, Jo YH (2017) Cholinergic neurons in the dorsomedial hypothalamus regulate food intake. Molecular metabolism 6(3):306-312 doi:10.1016/j.molmet.2017.01.001.

137. Jin, M. Q., Zhang, D., Zhang, Y., Zhou, S. S., Lu, X. T., and Zhao, H. T. (2018). Neurological responses of embryo-larval zebrafish to short-term sediment exposure to decabromodiphenylethane. *Journal of Zhejiang University. Science. B* **19**(5), 400-408.
138. Johnson SA, Ellersieck MR, Rosenfeld CS (2018) Hypothalamic gene expression changes in F1 California mice (Peromyscus californicus) parents developmentally exposed to bisphenol A or ethinyl estradiol. Heliyon 4(6):e00672 doi:10.1016/j.heliyon.2018.e00672.
139. Johnson, S. A., Javurek, A. B., Painter, M. S., Peritore, M. P., Ellersieck, M. R., Roberts, R. M., and Rosenfeld, C. S. (2015). Disruption of parenting behaviors in california mice, a monogamous rodent species, by endocrine disrupting chemicals. *PLoS One* **10**(6), e0126284.
140. Johnson SA, Painter MS, Javurek AB, et al. (2015) Sex-dependent effects of developmental exposure to bisphenol A and ethinyl estradiol on metabolic parameters and voluntary physical activity. Journal of developmental origins of health and disease 6(6):539-52 doi:10.1017/s2040174415001488.
141. Jolous-Jamshidi B, Cromwell HC, McFarland AM, Meserve LA (2010) Perinatal exposure to polychlorinated biphenyls alters social behaviors in rats. Toxicol Lett 199(2):136-43 doi:10.1016/j.toxlet.2010.08.015.
142. Jones JR, Barrick C, Kim KA, et al. (2005) Deletion of PPARgamma in adipose tissues of mice protects against high fat diet-induced obesity and insulin resistance. Proc Natl Acad Sci U S A 102(17):6207-12 doi:10.1073/pnas.0306743102.
143. Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS (1999) Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocrine reviews 20(1):68-100 doi:10.1210/edrv.20.1.0357.
144. Kaya H, Hany J, Fastabend A, Roth-Harer A, Winneke G, Lilienthal H (2002) Effects of maternal exposure to a reconstituted mixture of polychlorinated biphenyls on sex-dependent behaviors and steroid hormone concentrations in rats: dose-response relationship. Toxicology and applied pharmacology 178(2):71-81 doi:10.1006/taap.2001.9318.
145. Keller, H., Givel, F., Perroud, M., and Wahli, W. (1995). Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. *Molecular endocrinology (Baltimore, Md.)* **9**(7), 794-804.
146. Kim B, Colon E, Chawla S, Vandenberg LN, Suvorov A (2015) Endocrine disruptors alter social behaviors and indirectly influence social hierarchies via changes in body weight. Environmental health : a global access science source 14:64 doi:10.1186/s12940-015-0051-6.
147. Kim JW, Isobe T, Muto M, et al. (2014) Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. Chemosphere 116:91-7 doi:10.1016/j.chemosphere.2014.02.033.
148. Kim JW, Isobe T, Chang KH, et al. (2011) Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ Pollut 159(12):3653-9 doi:10.1016/j.envpol.2011.07.020.

149. Kim JY, Yi BR, Go RE, Hwang KA, Nam KH, Choi KC (2014) Methoxychlor and triclosan stimulates ovarian cancer growth by regulating cell cycle- and apoptosis-related genes via an estrogen receptor-dependent pathway. Environmental toxicology and pharmacology 37(3):1264-74 doi:10.1016/j.etap.2014.04.013.
150. Kim, M., Guerra, P., Theocharides, M., Barclay, K., Smyth, S. A., and Alaee, M. (2013). Polybrominated diphenyl ethers in sewage sludge and treated biosolids: effect factors and mass balance. *Water research* **47**(17), 6496-505.
151. Kinyua AW, Yang DJ, Chang I, Kim KW (2016) Steroidogenic Factor 1 in the Ventromedial Nucleus of the Hypothalamus Regulates Age-Dependent Obesity. PLOS ONE 11(9):e0162352 doi:10.1371/journal.pone.0162352.
152. Kirchner S, Kieu T, Chow C, Casey S, Blumberg B (2010) Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. Molecular endocrinology (Baltimore, Md) 24(3):526-39 doi:10.1210/me.2009-0261.
153. Kodavanti PR, Coburn CG, Moser VC, et al. (2010) Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. Toxicological sciences : an official journal of the Society of Toxicology 116(1):297-312 doi:10.1093/toxsci/kfq105.
154. Kojima H, Takeuchi S, Itoh T, Iida M, Kobayashi S, Yoshida T (2013) In vitro endocrine disruption potential of organophosphate flame retardants via human nuclear receptors. Toxicology 314(1):76-83 doi:10.1016/j.tox.2013.09.004.
155. Kojima, H., Takeuchi, S., Van den Eede, N., and Covaci, A. (2016a). Effects of primary metabolites of organophosphate flame retardants on transcriptional activity via human nuclear receptors. *Toxicology Letters* **245**, 31-39.
156. Kojima, H., Takeuchi, S., Van den Eede, N., and Covaci, A. (2016b). Effects of primary metabolites of organophosphate flame retardants on transcriptional activity via human nuclear receptors. *Toxicol Lett* **245**, 31-9.
157. Kovner, K. (2009). Persistent organic pollutants: a global issue, a global response. *US Environmental Protection Agency (EPA), December*.
158. Krumm EA, Patel VJ, Tillery TS, et al. (2018) Organophosphate Flame-Retardants Alter Adult Mouse Homeostasis and Gene Expression in a Sex-Dependent Manner Potentially Through Interactions With ERalpha. Toxicol Sci 162(1):212-224 doi:10.1093/toxsci/kfx238.
159. Kubo K, Arai O, Ogata R, Omura M, Hori T, Aou S (2001) Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat. Neurosci Lett 304(1-2):73-6.
160. Kuehl DW, Haebler R (1995) Organochlorine, organobromine, metal, and selenium residues in bottlenose dolphins (Tursiops truncatus) collected during an unusual mortality event in the Gulf of Mexico, 1990. Arch Environ Contam Toxicol 28(4):494-9.
161. Kundakovic, M., Gudsnuk, K., Franks, B., Madrid, J., Miller, R. L., Perera, F. P., and Champagne, F. A. (2013). Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. *Proc Natl Acad Sci U S A* **110**(24), 9956-61.

162. Kuriyama SN, Wanner A, Fidalgo-Neto AA, Talsness CE, Koerner W, Chahoud I (2007) Developmental exposure to low-dose PBDE-99: tissue distribution and thyroid hormone levels. Toxicology 242(1-3):80-90 doi:10.1016/j.tox.2007.09.011.
163. La Merrill M, Karey E, Moshier E, et al. (2014) Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. PLoS One 9(7):e103337 doi:10.1371/journal.pone.0103337.
164. La Merrill M, Kuruvilla BS, Pomp D, Birnbaum LS, Threadgill DW (2009) Dietary fat alters body composition, mammary development, and cytochrome p450 induction after maternal TCDD exposure in DBA/2J mice with low-responsive aryl hydrocarbon receptors. Environ Health Perspect 117(9):1414-9 doi:10.1289/ehp.0800530.
165. Lai KP, Ng AH-M, Wan HT, et al. (2018) Dietary Exposure to the Environmental Chemical, PFOS on the Diversity of Gut Microbiota, Associated With the Development of Metabolic Syndrome. Frontiers in microbiology 9:2552-2552 doi:10.3389/fmicb.2018.02552.
166. Lai S, Xie Z, Song T, et al. (2015) Occurrence and dry deposition of organophosphate esters in atmospheric particles over the northern South China Sea. Chemosphere 127:195-200 doi:10.1016/j.chemosphere.2015.02.015.
167. Lassiter TL, Brimijoin S (2008) Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. Neurotoxicol Teratol 30(2):125-30 doi:10.1016/j.ntt.2007.10.004.
168. Lassiter TL, Ryde IT, Levin ED, Seidler FJ, Slotkin TA (2010) Neonatal exposure to parathion alters lipid metabolism in adulthood: Interactions with dietary fat intake and implications for neurodevelopmental deficits. Brain research bulletin 81(1):85-91 doi:10.1016/j.brainresbull.2009.07.002.
169. Lassiter TL, Ryde IT, Mackillop EA, et al. (2008) Exposure of neonatal rats to parathion elicits sex-selective reprogramming of metabolism and alters the response to a high-fat diet in adulthood. Environ Health Perspect 116(11):1456-62 doi:10.1289/ehp.11673.
170. Lechan RM, Fekete C (2006) The TRH neuron: a hypothalamic integrator of energy metabolism. Progress in brain research 153:209-35 doi:10.1016/s0079-6123(06)53012-2.
171. Lee, H. J., Jeong, H. J., Jang, Y. L., and Kim, G. B. (2018a). Distribution, accumulation, and potential risk of polybrominated diphenyl ethers in the marine environment receiving effluents from a sewage treatment plant. *Marine Pollution Bulletin* **129**(1), 364-369.
172. Lee, S., Cho, H.-J., Choi, W., and Moon, H.-B. (2018b). Organophosphate flame retardants (OPFRs) in water and sediment: Occurrence, distribution, and hotspots of contamination of Lake Shihwa, Korea. *Marine Pollution Bulletin* **130**, 105-112.
173. Legler J, Brouwer A (2003) Are brominated flame retardants endocrine disruptors? Environ Int 29(6):879-85 doi:10.1016/s0160-4120(03)00104-1.
174. Leijs MM, Koppe JG, Vulsma T, et al. (2017) Alterations in the programming of energy metabolism in adolescents with background exposure to dioxins, dl-PCBs and PBDEs. PLOS ONE 12(9):e0184006 doi:10.1371/journal.pone.0184006.

175. Lema SC, Dickey JT, Schultz IR, Swanson P (2008) Dietary exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) alters thyroid status and thyroid hormone-regulated gene transcription in the pituitary and brain. Environ Health Perspect 116(12):1694-9 doi:10.1289/ehp.11570.
176. Lemberger, T., Braissant, O., Juge-Aubry, C., Keller, H., Saladin, R., Staels, B., Auwerx, J., Burger, A. G., Meier, C. A., and Wahli, W. (1996). PPAR tissue distribution and interactions with other hormone-signaling pathways. *Annals of the New York Academy of Sciences* **804**, 231-51.
177. Leonetti C, Butt CM, Hoffman K, Miranda ML, Stapleton HM (2016) Concentrations of polybrominated diphenyl ethers (PBDEs) and 2,4,6-tribromophenol in human placental tissues. Environ Int 88:23-29 doi:10.1016/j.envint.2015.12.002.
178. Li J, Yu N, Zhang B, et al. (2014) Occurrence of organophosphate flame retardants in drinking water from China. Water research 54:53-61 doi:10.1016/j.watres.2014.01.031.
179. Li, Y., Chen, L., Wen, Z. H., Duan, Y. P., Lu, Z. B., Meng, X. Z., and Zhang, W. (2015). Characterizing distribution, sources, and potential health risk of polybrominated diphenyl ethers (PBDEs) in office environment. *Environ Pollut* **198**, 25-31.
180. Li Z, Jennings A (2017) Worldwide Regulations of Standard Values of Pesticides for Human Health Risk Control: A Review. International journal of environmental research and public health 14(7) doi:10.3390/ijerph14070826.
181. Liao C, Kannan K (2013) Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J Agric Food Chem 61(19):4655-62 doi:10.1021/jf400445n.
182. Lignell S, Aune M, Darnerud PO, Hanberg A, Larsson SC, Glynn A (2013) Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: a cross-sectional study. Environmental health : a global access science source 12:44 doi:10.1186/1476-069x-12-44.
183. Lilienthal H, Hack A, Roth-Harer A, Grande SW, Talsness CE (2006) Effects of developmental exposure to 2,2 ,4,4 ,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. Environ Health Perspect 114(2):194-201 doi:10.1289/ehp.8391.
184. Lin J, Wu PH, Tarr PT, et al. (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 119(1):121-35 doi:10.1016/j.cell.2004.09.013.
185. Linares V, Belles M, Domingo JL (2015) Human exposure to PBDE and critical evaluation of health hazards. Arch Toxicol 89(3):335-56 doi:10.1007/s00204-015-1457-1.
186. Liu C, Wang Q, Liang K, et al. (2013a) Effects of tris(1,3-dichloro-2-propyl) phosphate and triphenyl phosphate on receptor-associated mRNA expression in zebrafish embryos/larvae. Aquat Toxicol 128-129:147-57 doi:10.1016/j.aquatox.2012.12.010.
187. Liu H, Tang S, Zheng X, et al. (2015) Bioaccumulation, biotransformation, and toxicity of BDE-47, 6-OH-BDE-47, and 6-MeO-BDE-47 in early life-stages of

zebrafish (Danio rerio). Environ Sci Technol 49(3):1823-33 doi:10.1021/es503833q.

188. Liu J, Yu P, Qian W, et al. (2013b) Perinatal bisphenol A exposure and adult glucose homeostasis: identifying critical windows of exposure. PLoS ONE 8(5):e64143-e64143 doi:10.1371/journal.pone.0064143.
189. Liu, J., Lu, G., Zhang, F., Nkoom, M., Yan, Z., and Wu, D. (2018). Polybrominated Diphenyl Ethers (PBDEs) in a Large, Highly Polluted Freshwater Lake, China: Occurrence, Fate, and Risk Assessment. *International journal of environmental research and public health* **15**(7).
190. Liu L, Karkanias GB, Morales JC, et al. (1998) Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes. The Journal of biological chemistry 273(47):31160-7.
191. Liu X, Ji K, Choi K (2012) Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquat Toxicol 114-115:173-81 doi:10.1016/j.aquatox.2012.02.019.
192. Liu X, Ji K, Jo A, Moon HB, Choi K (2013) Effects of TDCPP or TPP on gene transcriptions and hormones of HPG axis, and their consequences on reproduction in adult zebrafish (Danio rerio). Aquat Toxicol 134-135:104-11 doi:10.1016/j.aquatox.2013.03.013.
193. Liu Y, Liggio J, Harner T, Jantunen L, Shoeib M, Li SM (2014) Heterogeneous OH initiated oxidation: a possible explanation for the persistence of organophosphate flame retardants in air. Environ Sci Technol 48(2):1041-8 doi:10.1021/es404515k.
194. Long L, Toda C, Jeong JK, Horvath TL, Diano S (2014) PPARgamma ablation sensitizes proopiomelanocortin neurons to leptin during high-fat feeding. The Journal of clinical investigation 124(9):4017-27 doi:10.1172/jci76220.
195. Lopez-Espinosa MJ, Costa O, Vizcaino E, et al. (2015) Prenatal Exposure to Polybrominated Flame Retardants and Fetal Growth in the INMA Cohort (Spain). Environ Sci Technol 49(16):10108-16 doi:10.1021/acs.est.5b01793.
196. Lu Q, Cai Z, Fu J, et al. (2014) Molecular docking and molecular dynamics studies on the interactions of hydroxylated polybrominated diphenyl ethers to estrogen receptor alpha. Ecotoxicology and environmental safety 101:83-9 doi:10.1016/j.ecoenv.2013.12.018.
197. MacKay H, Patterson ZR, Abizaid A (2017) Perinatal Exposure to Low-Dose Bisphenol-A Disrupts the Structural and Functional Development of the Hypothalamic Feeding Circuitry. Endocrinology 158(4):768-777 doi:10.1210/en.2016-1718.
198. Mackay H, Patterson ZR, Khazall R, Patel S, Tsirlin D, Abizaid A (2013) Organizational effects of perinatal exposure to bisphenol-A and diethylstilbestrol on arcuate nucleus circuitry controlling food intake and energy expenditure in male and female CD-1 mice. Endocrinology 154(4):1465-75 doi:10.1210/en.2012-2044.
199. Macleod DJ, Sharpe RM, Welsh M, et al. (2010) Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl 33(2):279-87 doi:10.1111/j.1365-2605.2009.01005.x

200. Mai, ChenChen, Luo, et al. (2005) Distribution of Polybrominated Diphenyl Ethers in Sediments of the Pearl River Delta and Adjacent South China Sea. Environmental Science & Technology 39(10):3521-3527 doi:10.1021/es048083x.
201. Malarvannan G, Belpaire C, Geeraerts C, Eulaers I, Neels H, Covaci A (2015) Organophosphorus flame retardants in the European eel in Flanders, Belgium: Occurrence, fate and human health risk. Environ Res 140:604-10 doi:10.1016/j.envres.2015.05.021.
202. Mammadov E, Uncu M, Dalkan C (2018) High Prenatal Exposure to Bisphenol A Reduces Anogenital Distance in Healthy Male Newborns. J Clin Res Pediatr Endocrinol 10(1):25-29 doi:10.4274/jcrpe.4817.
203. Marsh G B, A., Bladh, L.-G., Gillner, M., Jakobsson, E. (1998) Synthesis of p-hydroxybromodiphenyl ethers and binding to the thyroid receptor. Organohalogen Compd.
204. Mastorakos G, Zapanti E (2004) The hypothalamic-pituitary-adrenal axis in the neuroendocrine regulation of food intake and obesity: the role of corticotropin releasing hormone. Nutritional neuroscience 7(5-6):271-80 doi:10.1080/10284150400020516.
205. Mazda T, Yamamoto H, Fujimura M, Fujimiya M (2004) Gastric distension-induced release of 5-HT stimulates c-fos expression in specific brain nuclei via 5-HT3 receptors in conscious rats. American journal of physiology Gastrointestinal and liver physiology 287(1):G228-35 doi:10.1152/ajpgi.00373.2003.
206. Meeker JD, Cooper EM, Stapleton HM, Hauser R (2013) Urinary metabolites of organophosphate flame retardants: temporal variability and correlations with house dust concentrations. Environ Health Perspect 121(5):580-5 doi:10.1289/ehp.1205907.
207. Meerts IA, Letcher RJ, Hoving S, et al. (2001) In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PDBEs, and polybrominated bisphenol A compounds. Environ Health Perspect 109(4):399-407 doi:10.1289/ehp.01109399.
208. Meironyte Guvenius D, Bergman A, Noren K (2001) Polybrominated diphenyl ethers in Swedish human liver and adipose tissue. Arch Environ Contam Toxicol 40(4):564-70.
209. Min, G., Kim, H., Bae, Y., Petz, L., and Kemper, J. K. (2002). Inhibitory Cross-talk between Estrogen Receptor (ER) and Constitutively Activated Androstane Receptor (CAR): CAR INHIBITS ER-MEDIATED SIGNALING PATHWAY BY SQUELCHING p160 COACTIVATORS. *Journal of Biological Chemistry* **277**(37), 34626-34633.
210. Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H (2007) Perinatal and postnatal exposure to bisphenol a increases adipose tissue mass and serum cholesterol level in mice. Journal of atherosclerosis and thrombosis 14(5):245-52.
211. Moon BH, Hong CG, Kim SY, et al. (2008) A single administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin that produces reduced food and water intake induces long-lasting expression of corticotropin-releasing factor, arginine vasopressin, and proopiomelanocortin in rat brain. Toxicology and applied pharmacology 233(2):314-22 doi:10.1016/j.taap.2008.09.001.

212. Moon M, Kim M, Jung I, et al. (2012) Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. Journal of Korean Medical Science 27:644-652.
213. Moon MK, Jeong I-K, Oh TJ, et al. (2015a) Long-term oral exposure to bisphenol A induces glucose intolerance and insulin resistance. 226(1):35 doi:10.1530/joe-14-0714.
214. Moon MK, Jeong IK, Jung Oh T, et al. (2015b) Long-term oral exposure to bisphenol A induces glucose intolerance and insulin resistance. J Endocrinol 226(1):35-42 doi:10.1530/JOE-14-0714.
215. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. Nature 443(7109):289-95 doi:10.1038/nature05026.
216. Muenhor, D., and Harrad, S. (2018). Polybrominated diphenyl ethers (PBDEs) in car and house dust from Thailand: Implication for human exposure. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering* **53**(7), 629-642.
217. Mystkowski P, Seeley RJ, Hahn TM, et al. (2000) Hypothalamic melanin-concentrating hormone and estrogen-induced weight loss. J Neurosci 20(22):8637-42.
218. Naderi, A., and Hughes-Davies, L. (2008). A functionally significant cross-talk between androgen receptor and ErbB2 pathways in estrogen receptor negative breast cancer. *Neoplasia (New York, N.Y.)* **10**(6), 542-8.
219. Nahon JL (2006) The melanocortins and melanin-concentrating hormone in the central regulation of feeding behavior and energy homeostasis. Comptes rendus biologies 329(8):623-38; discussion 653-5 doi:10.1016/j.crvi.2006.03.021.
220. Nakagawa Y, Tayama S (2000) Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Archives of toxicology 74:99-105.
221. Nash JT, Szabo DT, Carey GB (2013) Polybrominated diphenyl ethers alter hepatic phosphoenolpyruvate carboxykinase enzyme kinetics in male Wistar rats: implications for lipid and glucose metabolism. Journal of toxicology and environmental health Part A 76(2):142-56 doi:10.1080/15287394.2012.738457.
222. Negishi T, Kawasaki K, Suzaki S, et al. (2004) Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. Environ Health Perspect 112(11):1159-64 doi:10.1289/ehp.6961.
223. Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN (2007) Perinatal exposure to environmental estrogens and the development of obesity. Molecular nutrition & food research 51(7):912-7 doi:10.1002/mnfr.200600259.
224. Ngo HT, Hetland RB, Sabaredzovic A, Haug LS, Steffensen IL (2014) In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice. Environ Res 132:251-63 doi:10.1016/j.envres.2014.03.033.
225. Norris, J. M., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., Jewett, G. L., Gehring, P. J., and Mailhes, J. B. (1975). Toxicology of octabromobiphenyl and decabromodiphenyl oxide. *Environmental health perspectives* **11**, 153-161.

226. NTP (1994). NTP Toxicology and Carcinogenesis Studies of Tricresyl Phosphate (CAS No. 1330-78-5) in F344/N Rats and B6C3F1 Mice (Gavage and Feed Studies). *National Toxicology Program technical report series* **433**, 1-321.
227. Odusanya, D. O., Okonkwo, J. O., and Botha, B. (2009). Polybrominated diphenyl ethers (PBDEs) in leachates from selected landfill sites in South Africa. *Waste management* **29**(1), 96-102.
228. Olokpa, E., Moss, P. E., and Stewart, L. V. (2017). Crosstalk between the Androgen Receptor and PPAR Gamma Signaling Pathways in the Prostate. *PPAR Research* **2017**, 13.
229. Olsson A, Valters K, Burreau S (2000) Concentrations of Organochlorine Substances in Relation to Fish Size and Trophic Position: A Study on Perch (Perca fluviatilis L.). Environmental Science & Technology 34(23):4878-4886 doi:10.1021/es991400t.
230. Ontiveros-Cuadras, J. F., Ruiz-Fernández, A. C., Sanchez-Cabeza, J.-A., Sericano, J., Pérez-Bernal, L. H., Páez-Osuna, F., Dunbar, R. B., and Mucciarone, D. A. (2019). Recent history of persistent organic pollutants (PAHs, PCBs, PBDEs) in sediments from a large tropical lake. *Journal of hazardous materials* **368**, 264-273.
231. Padmanabhan V, Sarma HN, Savabieasfahani M, Steckler TL, Veiga-Lopez A (2010) Developmental reprogramming of reproductive and metabolic dysfunction in sheep: native steroids vs. environmental steroid receptor modulators. Int J Androl 33(2):394-404 doi:10.1111/j.1365-2605.2009.01024.x.
232. Palanza, P. (2017). The "Plastic" Mother. *Endocrinology* **158**(3), 461-463.
233. Palanza, P. L., Howdeshell, K. L., Parmigiani, S., and vom Saal, F. S. (2002). Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environ Health Perspect* **110 Suppl 3**, 415-22.
234. Parolini M, Guazzoni N, Binelli A, Tremolada P (2012) Polybrominated diphenyl ether contamination in soil, vegetation, and cow milk from a high-mountain pasture in the Italian Alps. Arch Environ Contam Toxicol 63(1):29-44 doi:10.1007/s00244-012-9753-8.
235. Patel BB, Di Iorio M, Chalifour LE (2014) Metabolic response to chronic bisphenol A exposure in C57bl/6n mice. Toxicol Rep 1:522-532 doi:10.1016/j.toxrep.2014.07.012.
236. Patisaul HB, Roberts SC, Mabrey N, et al. (2013a) Accumulation and endocrine disrupting effects of the flame retardant mixture Firemaster(R) 550 in rats: an exploratory assessment. J Biochem Mol Toxicol 27(2):124-36 doi:10.1002/jbt.21439.
237. Patisaul HB, Roberts SC, Mabrey N, et al. (2013b) Accumulation and endocrine disrupting effects of the flame retardant mixture Firemaster® 550 in rats: an exploratory assessment. Journal of biochemical and molecular toxicology 27(2):124-136 doi:10.1002/jbt.21439.
238. Patisaul HB, Bateman HL (2008) Neonatal exposure to endocrine active compounds or an ERbeta agonist increases adult anxiety and aggression in gonadally intact male rats. Horm Behav 53(4):580-8 doi:10.1016/j.yhbeh.2008.01.008.

239. Perez-Leighton CE, Boland K, Billington CJ, Kotz CM (2013) High and low activity rats: elevated intrinsic physical activity drives resistance to diet-induced obesity in non-bred rats. Obesity (Silver Spring, Md) 21(2):353-60 doi:10.1002/oby.20045.
240. Perez-Leighton CE, Boland K, Teske JA, Billington C, Kotz CM (2012) Behavioral responses to orexin, orexin receptor gene expression, and spontaneous physical activity contribute to individual sensitivity to obesity. American journal of physiology Endocrinology and metabolism 303(7):E865-74 doi:10.1152/ajpendo.00119.2012.
241. Pillai HK, Fang M, Beglov D, et al. (2014) Ligand binding and activation of PPARgamma by Firemaster(R) 550: effects on adipogenesis and osteogenesis in vitro. Environ Health Perspect 122(11):1225-32 doi:10.1289/ehp.1408111.
242. Pohjanvirta R, Tuomisto J (1990) 2,3,7,8-Tetrachlorodibenzo-p-dioxin enhances responsiveness to post-ingestive satiety signals. Toxicology 63(3):285-99.
243. Pohjanvirta R, Wong JM, Li W, Harper PA, Tuomisto J, Okey AB (1998) Point mutation in intron sequence causes altered carboxyl-terminal structure in the aryl hydrocarbon receptor of the most 2,3,7,8-tetrachlorodibenzo-p-dioxin-resistant rat strain. Molecular pharmacology 54(1):86-93.
244. Poma G, Glynn A, Malarvannan G, Covaci A, Darnerud PO (2017) Dietary intake of phosphorus flame retardants (PFRs) using Swedish food market basket estimations. Food Chem Toxicol 100:1-7 doi:10.1016/j.fct.2016.12.011.
245. Rashid CS, Carter LG, Hennig B, Pearson KJ (2013) Perinatal Polychlorinated Biphenyl 126 Exposure Alters Offspring Body Composition. Journal of pediatric biochemistry 3(1):47-53 doi:10.3233/jpb-120072.
246. Rebolledo-Solleiro D, Roldan-Roldan G, Diaz D, et al. (2017) Increased anxiety-like behavior is associated with the metabolic syndrome in non-stressed rats. PLoS One 12(5):e0176554 doi:10.1371/journal.pone.0176554.
247. Regnery J, Püttmann W (2009) Organophosphorus Flame Retardants and Plasticizers in Rain and Snow from Middle Germany. CLEAN – Soil, Air, Water 37(4-5):334-342 doi:doi:10.1002/clen.200900050.
248. Rezg R, Abot A, Mornagui B, Aydi S, Knauf C (2018) Effects of Bisphenol S on hypothalamic neuropeptides regulating feeding behavior and apelin/APJ system in mice. Ecotoxicol Environ Saf 161:459-466 doi:10.1016/j.ecoenv.2018.06.001.
249. Riediger T (2012) The receptive function of hypothalamic and brainstem centres to hormonal and nutrient signals affecting energy balance. The Proceedings of the Nutrition Society 71(4):463-77 doi:10.1017/s0029665112000778.
250. Riu A, Grimaldi M, le Maire A, et al. (2011) Peroxisome proliferator-activated receptor gamma is a target for halogenated analogs of bisphenol A. Environ Health Perspect 119(9):1227-32 doi:10.1289/ehp.1003328.
251. Roepke TA, Malyala A, Bosch MA, Kelly MJ, Ronnekleiv OK (2007) Estrogen regulation of genes important for K+ channel signaling in the arcuate nucleus. Endocrinology 148(10):4937-51 doi:10.1210/en.2007-0605.
252. Roepke TA, Yasrebi A, Villalobos A, Krumm EA, Yang JA, Mamounis KJ (2017) Loss of ERalpha partially reverses the effects of maternal high-fat diet on energy homeostasis in female mice. Sci Rep 7(1):6381 doi:10.1038/s41598-017-06560-x.

253. Ronn M, Lind L, Orberg J, et al. (2014) Bisphenol A is related to circulating levels of adiponectin, leptin and ghrelin, but not to fat mass or fat distribution in humans. Chemosphere 112:42-8 doi:10.1016/j.chemosphere.2014.03.042.
254. Rosella G, Zajac JD, Kaczmarczyk SJ, Andrikopoulos S, Proietto J (1993) Impaired suppression of gluconeogenesis induced by overexpression of a noninsulin-responsive phosphoenolpyruvate carboxykinase gene. Molecular endocrinology (Baltimore, Md) 7(11):1456-62 doi:10.1210/mend.7.11.8114759.
255. Rossetti L, Massillon D, Barzilai N, et al. (1997) Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. The Journal of biological chemistry 272(44):27758-63.
256. Rubin BS, Soto AM (2009) Bisphenol A: Perinatal exposure and body weight. Mol Cell Endocrinol 304(1-2):55-62 doi:10.1016/j.mce.2009.02.023.
257. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM (2001) Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 109(7):675-80 doi:10.1289/ehp.01109675.
258. Ruhlen RL, Howdeshell KL, Mao J, et al. (2008) Low phytoestrogen levels in feed increase fetal serum estradiol resulting in the "fetal estrogenization syndrome" and obesity in CD-1 mice. Environ Health Perspect 116(3):322-8 doi:10.1289/ehp.10448.
259. Sagiv SK, Kogut K, Gaspar FW, et al. (2015) Prenatal and childhood polybrominated diphenyl ether (PBDE) exposure and attention and executive function at 9-12 years of age. Neurotoxicol Teratol 52(Pt B):151-61 doi:10.1016/j.ntt.2015.08.001.
260. Salehi A, Loganathan N, Belsham DD (2019) Bisphenol A induces Pomc gene expression through neuroinflammatory and PPARgamma nuclear receptor-mediated mechanisms in POMC-expressing hypothalamic neuronal models. Mol Cell Endocrinol 479:12-19 doi:10.1016/j.mce.2018.08.009.
261. Saper CB, Chou TC, Elmquist JK (2002) The need to feed: homeostatic and hedonic control of eating. Neuron 36(2):199-211.
262. Schlingemann J, Hess J, Wrobel G, et al. (2003) Profile of gene expression induced by the tumour promotor TPA in murine epithelial cells. International journal of cancer 104(6):699-708 doi:10.1002/ijc.11008.
263. Schneider JE, Brozek JM, Keen-Rhinehart E (2014) Our stolen figures: the interface of sexual differentiation, endocrine disruptors, maternal programming, and energy balance. Hormones and behavior 66(1):104-19 doi:10.1016/j.yhbeh.2014.03.011.
264. Schreder ED, La Guardia MJ (2014) Flame retardant transfers from U.S. households (dust and laundry wastewater) to the aquatic environment. Environ Sci Technol 48(19):11575-83 doi:10.1021/es502227h.
265. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404(6778):661-71 doi:10.1038/35007534.
266. Schwartz GJ, Moran TH (1996) Sub-diaphragmatic vagal afferent integration of meal-related gastrointestinal signals. Neuroscience and biobehavioral reviews 20(1):47-56.

267. Seefeld MD, Corbett SW, Keesey RE, Peterson RE (1984) Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology and applied pharmacology 73(2):311-22.
268. Shah A, Coburn CG, Watson-Siriboe A, et al. (2011) Altered cardiovascular reactivity and osmoregulation during hyperosmotic stress in adult rats developmentally exposed to polybrominated diphenyl ethers (PBDEs). Toxicology and applied pharmacology 256(2):103-13 doi:10.1016/j.taap.2011.07.014.
269. Shao J, Qiao L, Janssen RC, Pagliassotti M, Friedman JE (2005) Chronic hyperglycemia enhances PEPCK gene expression and hepatocellular glucose production via elevated liver activating protein/liver inhibitory protein ratio. Diabetes 54(4):976-84 .
270. She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D (2002) PBDEs in the San Francisco Bay Area: measurements in harbor seal blubber and human breast adipose tissue. Chemosphere 46(5):697-707.
271. Shi L, Bian X, Qu Z, et al. (2013) Peptide hormone ghrelin enhances neuronal excitability by inhibition of Kv7/KCNQ channels. Nat Commun 4:1435 doi:10.1038/ncomms2439.
272. Sibiya, I., Olukunle, O., and Okonkwo, O. (2017). Seasonal variations and the influence of geomembrane liners on the levels of PBDEs in landfill leachates, sediment and groundwater in Gauteng Province, South Africa. *Emerging Contaminants* **3**(2), 76-84.
273. Siddiqi, M. A., Laessig, R. H., and Reed, K. D. (2003). Polybrominated diphenyl ethers (PBDEs): new pollutants-old diseases. *Clinical medicine & research* **1**(4), 281-290.
274. Silva BS, Bertasso IM, Pietrobon CB, et al. (2018) Effects of maternal bisphenol A on behavior, sex steroid and thyroid hormones levels in the adult rat offspring. Life Sci doi:10.1016/j.lfs.2018.12.039.
275. Simmons, R. A. (2009). Developmental origins of adult disease. *Pediatric clinics of North America* **56**(3), 449-Contents.
276. Smyth PP, Hetherton AM, Smith DF, Radcliff M, O'Herlihy C (1997) Maternal iodine status and thyroid volume during pregnancy: correlation with neonatal iodine intake. The Journal of clinical endocrinology and metabolism 82(9):2840-3 doi:10.1210/jcem.82.9.4203.
277. Somm E, Schwitzgebel VM, Toulotte A, et al. (2009) Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 117(10):1549-55 doi:10.1289/ehp.11342.
278. Sprague GL, Castles TR, Bickford AA (1984) Assessment of the delayed neurotoxic potential of isopropyl triphenylphosphate using a nontraditional testing strategy. Journal of toxicology and environmental health 14(5-6):773-88 doi:10.1080/15287398409530626.
279. Sprague GL, Sandvik LL, Brookins-Hendricks MJ, Bickford AA (1981) Neurotoxicity of two organophosphorus ester flame retardants in hens. Journal of toxicology and environmental health 8(3):507-18 doi:10.1080/15287398109530087.

280. Stanya KJ, Jacobi D, Liu S, et al. (2013) Direct control of hepatic glucose production by interleukin-13 in mice. The Journal of clinical investigation 123(1):261-271 doi:10.1172/JCI64941.
281. Stern GA, Ikonomou M (2000) Temporal trends of polybrominated diphenyl ethers in SE Baffin beluga: Increasing evidence of long range atmospheric transport. Organohalogen Compd 47:81–84.
282. Strakovsky RS, Lezmi S, Flaws JA, Schantz SL, Pan YX, Helferich WG (2014) Genistein exposure during the early postnatal period favors the development of obesity in female, but not male rats. Toxicol Sci 138(1):161-74 doi:10.1093/toxsci/kft331.
283. Sugai E, Yoshioka W, Kakeyama M, Ohsako S, Tohyama C (2014) In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin modulates dysregulation of the lipid metabolism in mouse offspring fed a high-calorie diet. Journal of applied toxicology : JAT 34(3):296-306 doi:10.1002/jat.2881.
284. Sun L, Tan H, Peng T, et al. (2016) Developmental neurotoxicity of organophosphate flame retardants in early life stages of Japanese medaka (Oryzias latipes). Environ Toxicol Chem 35(12):2931-2940 doi:10.1002/etc.3477.
285. Sun X, Li D, Liang H, et al. (2018) Maternal exposure to bisphenol A and anogenital distance throughout infancy: A longitudinal study from Shanghai, China. Environ Int 121(Pt 1):269-275 doi:10.1016/j.envint.2018.08.055.
286. Sundkvist AM, Olofsson U, Haglund P (2010) Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. Journal of environmental monitoring : JEM 12(4):943-51 doi:10.1039/b921910b.
287. Suvorov A, Battista MC, Takser L (2009) Perinatal exposure to low-dose 2,2',4,4'-tetrabromodiphenyl ether affects growth in rat offspring: what is the role of IGF-1? Toxicology 260(1-3):126-31 doi:10.1016/j.tox.2009.03.018.
288. Suzuki, G., Tue, N. M., Malarvannan, G., Sudaryanto, A., Takahashi, S., Tanabe, S., Sakai, S.-i., Brouwer, A., Uramaru, N., Kitamura, S., *et al.* (2013). Similarities in the Endocrine-Disrupting Potencies of Indoor Dust and Flame Retardants by Using Human Osteosarcoma (U2OS) Cell-Based Reporter Gene Assays. *Environmental Science & Technology* **47**(6), 2898-2908.
289. Ta N, Li C, Fang Y, et al. (2014) Toxicity of TDCPP and TCEP on PC12 cell: changes in CAMKII, GAP43, tubulin and NF-H gene and protein levels. Toxicol Lett 227(3):164-71 doi:10.1016/j.toxlet.2014.03.023.
290. Talsness C, Fialkowski, O., Gericke, C., Merker, H.J., Chahoud, I. (2000) The effects of low and high doses of bisphenol A on the reproductive system of female and male rat offspring. Cogenital Anomalies 40:s94-s107.
291. Taylor JA, Sommerfeld-Sager JM, Meng CX, Nagel SC, Shioda T, Vom Saal FS (2018) Reduced body weight at weaning followed by increased post-weaning growth rate interacts with part-per-trillion fetal serum concentrations of bisphenol A (BPA) to impair glucose tolerance in male mice. PLoS ONE 13(12):e0208846 doi:10.1371/journal.pone.0208846.
292. Tiwari, M., Sahu, S. K., Bhangare, R. C., Ajmal, P. Y., and Pandit, G. G. (2018). Polybrominated diphenyl ethers (PBDEs) in core sediments from creek ecosystem: occurrence, geochronology, and source contribution. *Environmental geochemistry and health* **40**(6), 2587-2601.

293. Toda C, Santoro A, Kim JD, Diano S (2017) POMC Neurons: From Birth to Death. Annual review of physiology 79:209-236 doi:10.1146/annurev-physiol-022516-034110.
294. Tung EWY, Kawata A, Rigden M, et al. (2017) Gestational and Lactational Exposure to an Environmentally-Relevant Mixture of Brominated Flame Retardants: Effects on Neurodevelopment and Metabolism. Birth Defects Res 109(7):497-512 doi:10.1002/bdr2.1021.
295. Tuomisto JT, Viluksela M, Pohjanvirta R, Tuomisto J (2000) Changes in food intake and food selection in rats after 2,3,7, 8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. Pharmacology, biochemistry, and behavior 65(3):381-7.
296. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S (2011) The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. J Adv Pharm Technol Res 2(4):236-40 doi:10.4103/2231-4040.90879.
297. Tyl RW, Myers CB, Marr MC, et al. (2008) Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicological sciences : an official journal of the Society of Toxicology 104(2):362-84 doi:10.1093/toxsci/kfn084.
298. Tyl RW, Myers CB, Marr MC, et al. (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicological sciences : an official journal of the Society of Toxicology 68(1):121-46.
299. Vail, G., and Roepke, T. A. (2018). Membrane-initiated estrogen signaling via Gq-coupled GPCR in the central nervous system. *Steroids* doi: https://doi.org/10.1016/j.steroids.2018.01.010.
300. Valera A, Pujol A, Pelegrin M, Bosch F (1994) Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. Proceedings of the National Academy of Sciences 91(19):9151 doi:10.1073/pnas.91.19.9151.
301. Vandenbergh JG, Huggett CL (1995) The anogenital distance index, a predictor of the intrauterine position effects on reproduction in female house mice. Lab Anim Sci 45(5):567-73.
302. van Esterik JC, Verharen HW, Hodemaekers HM, et al. (2015) Compound- and sex-specific effects on programming of energy and immune homeostasis in adult C57BL/6JxFVB mice after perinatal TCDD and PCB 153. Toxicology and applied pharmacology 289(2):262-75 doi:10.1016/j.taap.2015.09.017.
303. vom Saal FS, Myers JP (2008) Bisphenol A and risk of metabolic disorders. Jama 300(11):1353-5 doi:10.1001/jama.300.11.1353.
304. Walley, S. N., and Roepke, T. A. (2018). Perinatal exposure to endocrine disrupting compounds and the control of feeding behavior-An overview. *Hormones and behavior* **101**, 22-28.
305. Wang D, Zhu W, Wang Y, et al. (2017) Metabolomics Approach to Investigate Estrogen Receptor-Dependent and Independent Effects of o,p'-DDT in the Uterus and Brain of Immature Mice. J Agric Food Chem 65(18):3609-3616 doi:10.1021/acs.jafc.7b00292.
306. Wang Q, Lam JC, Han J, et al. (2015) Developmental exposure to the organophosphorus flame retardant tris(1,3-dichloro-2-propyl) phosphate:

estrogenic activity, endocrine disruption and reproductive effects on zebrafish. Aquat Toxicol 160:163-71 doi:10.1016/j.aquatox.2015.01.014.

307. Wang Q, Lam JC, Man YC, et al. (2015) Bioconcentration, metabolism and neurotoxicity of the organophorous flame retardant 1,3-dichloro 2-propyl phosphate (TDCPP) to zebrafish. Aquat Toxicol 158:108-15 doi:10.1016/j.aquatox.2014.11.001.
308. Wang T, Xu M, Xu Y, et al. (2015b) Association of Bisphenol A Exposure With Hypertension and Early Macrovascular Diseases in Chinese Adults: A Cross-Sectional Study. Medicine 94(43):e1814-e1814 doi:10.1097/MD.0000000000001814.
309. Wang, X., and Kilgore, M. W. (2002). Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. *Mol Cell Endocrinol* **194**(1-2), 123-33.
310. Watanabe I MK, Y. Wang, Y. Chen, and R. Tatsukawa (1992) Polybrominated benzo-p-dioxins (PBDDs) and -dibenzofurnas (PBDFs) in atmospheric air in Taiwan and Japan. Organohalogen Compd.
311. Watkins DJ, McClean MD, Fraser AJ, et al. (2011) Exposure to PBDEs in the office environment: evaluating the relationships between dust, handwipes, and serum. Environ Health Perspect 119(9):1247-52 doi:10.1289/ehp.1003271
312. Wei J, Lin Y, Li Y, et al. (2011) Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology 152(8):3049-61 doi:10.1210/en.2011-0045.
313. Williams DL, Schwartz MW (2011) Neuroanatomy of body weight control: lessons learned from leptin. The Journal of clinical investigation 121(6):2152-5 doi:10.1172/jci58027.
314. Williams G, Bing C, Cai XJ, Harrold JA, King PJ, Liu XH (2001) The hypothalamus and the control of energy homeostasis: different circuits, different purposes. Physiology & behavior 74(4-5):683-701.
315. Williams SA, Jasarevic E, Vandas GM, et al. (2013) Effects of developmental bisphenol A exposure on reproductive-related behaviors in California mice (Peromyscus californicus): a monogamous animal model. PLoS One 8(2):e55698 doi:10.1371/journal.pone.0055698.
316. Wise LM, Hernandez-Saavedra D, Boas SM, Pan YX, Juraska JM (2018) Perinatal High-Fat Diet and Bisphenol A: Effects on Behavior and Gene Expression in the Medial Prefrontal Cortex. Dev Neurosci:1-16 doi:10.1159/000494879.
317. Woods MM, Lanphear BP, Braun JM, McCandless LC (2017) Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: a Bayesian analysis of the HOME Study. Environmental health : a global access science source 16(1):115-115 doi:10.1186/s12940-017-0332-3.
318. Xi W, Lee CK, Yeung WS, et al. (2011) Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus-pituitary-gonadal axis of CD-1 mice. Reproductive toxicology (Elmsford, NY) 31(4):409-17 doi:10.1016/j.reprotox.2010.12.002.

319. Xu X, Dong F, Yang Y, Wang Y, Wang R, Shen X (2015) Sex-specific effects of long-term exposure to bisphenol-A on anxiety- and depression-like behaviors in adult mice. Chemosphere 120:258-66 doi:10.1016/j.chemosphere.2014.07.021.
320. Xu X, Tan L, Himi T, et al. (2011) Changed preference for sweet taste in adulthood induced by perinatal exposure to bisphenol A-A probable link to overweight and obesity. Neurotoxicol Teratol 33(4):458-63 doi:10.1016/j.ntt.2011.06.002.
321. Yang L, Scott KA, Hyun J, et al. (2009) Role of dorsomedial hypothalamic neuropeptide Y in modulating food intake and energy balance. J Neurosci 29(1):179-90 doi:10.1523/jneurosci.4379-08.2009.
322. Yang F, Ding J, Huang W, Xie W, Liu W (2014) Particle size-specific distributions and preliminary exposure assessments of organophosphate flame retardants in office air particulate matter. Environ Sci Technol 48(1):63-70 doi:10.1021/es403186z.
323. Yasrebi A, Rivera JA, Krumm EA, Yang JA, Roepke TA (2017) Activation of Estrogen Response Element-Independent ERalpha Signaling Protects Female Mice From Diet-Induced Obesity. Endocrinology 158(2):319-334 doi:10.1210/en.2016-1535.
324. Yasrebi A, Hsieh A, Mamounis KJ, et al. (2016) Differential gene regulation of GHSR signaling pathway in the arcuate nucleus and NPY neurons by fasting, diet-induced obesity, and 17beta-estradiol. Mol Cell Endocrinol 422:42-56 doi:10.1016/j.mce.2015.11.007.
325. Zhang, Q., Lu, M., Dong, X., Wang, C., Zhang, C., Liu, W., and Zhao, M. (2014). Potential Estrogenic Effects of Phosphorus-Containing Flame Retardants. *Environmental Science & Technology* **48**(12), 6995-7001.
326. Zhang X, Zou W, Mu L, et al. (2016) Rice ingestion is a major pathway for human exposure to organophosphate flame retardants (OPFRs) in China. Journal of hazardous materials 318:686-693 doi:10.1016/j.jhazmat.2016.07.055.
327. Zhao J, Xu T, Yin DQ (2014) Locomotor activity changes on zebrafish larvae with different 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) embryonic exposure modes. Chemosphere 94:53-61 doi:10.1016/j.chemosphere.2013.09.010.
328. Zhao HY, Bi YF, Ma LY, et al. (2012) The effects of bisphenol A (BPA) exposure on fat mass and serum leptin concentrations have no impact on bone mineral densities in non-obese premenopausal women. Clinical biochemistry 45(18):1602-6 doi:10.1016/j.clinbiochem.2012.08.024.

329. Zhen, X., Tang, J., Xie, Z., Wang, R., Huang, G., Zheng, Q., Zhang, K., Sun, Y., Tian, C., Pan, X., *et al.* (2016). Polybrominated diphenyl ethers (PBDEs) and alternative brominated flame retardants (aBFRs) in sediments from four bays of the Yellow Sea, North China. *Environ Pollut* **213**, 386-394.
330. Zhou T, Ross DG, DeVito MJ, Crofton KM (2001) Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. Toxicol Sci 61(1):76-82.
331. Zhu S, Eclarinal J, Baker MS, Li G, Waterland RA (2016) Developmental programming of energy balance regulation: is physical activity more 'programmable' than food intake? The Proceedings of the Nutrition Society 75(1):73-77 doi:10.1017/s0029665115004127.
332. Zota AR, Linderholm L, Park JS, et al. (2013) Temporal comparison of PBDEs, OH-PBDEs, PCBs, and OH-PCBs in the serum of second trimester pregnant women recruited from San Francisco General Hospital, California. Environ Sci Technol 47(20):11776-84 doi:10.1021/es402204y.