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AN INVESTIGATION OF PERINATAL POLYCHLORINATED BIPHENYL EXPOSURE ON BODY COMPOSITION AND GLUCOSE HOMEOSTASIS

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AN INVESTIGATION OF PERINATAL POLYCHLORINATED BIPHENYL EXPOSURE ON BODY COMPOSITION AND GLUCOSE HOMEOSTASIS

DISSERTATION

A dissertation has been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Medicine Graduate Center for Nutritional Sciences at the University of Kentucky

By
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ABSTRACT OF DISSERTATION

AN INVESTIGATION OF PERINATAL POLYCHLORINATED BIPHENYL EXPOSURE ON BODY COMPOSITION AND GLUCOSE HOMEOSTASIS

Recent advancements have uncovered environmental contributions to obesity and diabetes etiology. In fact, perinatal malnutrition resulting in low birth weight (LBW) has been shown to correlate with later life obesity and impaired glucose tolerance in aged offspring. LBW can result from a myriad of developmental perturbations including macronutrient restriction, hypoxia, maternal stress and toxin exposure.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants that bioaccumulate in the food chain resulting in dietary exposure in humans. Maternal and cord blood PCB levels are inversely associated with birth weight, and recent studies indicate that perinatal exposures to PCBs contribute to gender-specific obesity development in children. PCBs have also been shown to enter breast milk resulting in direct exposure in early postnatal life. Therefore, we hypothesized that perinatal PCB exposure cause developmental blight resulting in decreased birth weight and increased adiposity and glucose intolerance with aging. We found that mice perinatally exposed to PCBs did not differ in birth weight, but exhibited sex-specific effects on adiposity. Females perinatally exposed to PCBs were significantly more obese at 7 weeks of age while male offspring exhibited no difference in fat mass but had decreased lean mass compared to controls. With aging, the differences in females dissipated while the male offspring decreased lean mass persisted. Male offspring perinatally exposed to PCBs displayed impaired glucose tolerance at 7 weeks of age but normalized over time, while the females were glucose intolerant only after 6 months of age. This impairment of glucose tolerance was not attributed to insulin resistance. These data illustrate time-dependent and sex-specific perturbations of maternal PCB exposure on offspring body composition and glucose homeostasis.

As the liver is a major facilitator in glucose homeostasis and xenobiotic detoxification, we investigated PCB-induced alterations in hepatic gene expression and

found attenuated expression of glycolytic genes and increased expression of detoxifying and antioxidant genes in both PCB-exposed maternal and offspring livers. Taken together, these data demonstrate a role for perinatal pollutant exposure in the etiology of glucose intolerance. Further studies are required to elucidate the mechanisms causing sex-specific modulation of body composition and glucose intolerance.

Keywords: polychlorinated biphenyls, obesity, diabetes, developmental programming, environmental pollutants

Cetewayo Saif Rashid
Student's Signature

November 18, 2013
Date

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CHAPTER 1

INTRODUCTION

1.1 Diabetes

1.1.1 Diabetes in America and the Clinic Diabetes is a chronic noncommunicable disease characterized by hyperglycemia beyond a normal range. A hormone, insulin, is secreted by the pancreas in response to a meal and signals for glucose transporters to be translocated to cell surface of skeletal muscles and adipose tissue to facilitate transport into the cells from the periphery. By doing so, blood glucose levels decrease. The liver, too, takes up glucose thereby decreasing blood glucose levels. Diabetes is an inability to modulate blood glucose by either lack of insulin secretion, an inefficient response to insulin by peripheral tissues, but most commonly, a combination of the two. Diabetes is clinically characterized as having fasted blood glucose levels over 126 mg/dL or blood glucose of over 200 mg/dL following a 2 hour oral glucose challenge while normal levels are <100 and <140 mg/dL, respectively [1, 2]. Blood glucose levels above normal but below diabetes criteria are called prediabetes, and patients are characterized as having either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Prediabetics have an increased risk of developing overt diabetes if lifestyle interventions are not adopted [2].

Diabetes affects over 340 million people worldwide and 25 million of those are in the United States. In The United States, the prevalence of diabetes has increased over the past 20 years [1, 3]. Over 8% of Americans have diabetes and it is estimated that 1 in 3 Americans born after the year 2000 will develop diabetes in their lifetime [1, 4]. This

disease has cost the United States an estimated $245 billion dollars in 2012, up 39% from 2007, and $174 billion dollars are from direct medical costs [5]. Moreover, diabetes is associated with various serious comorbidities such as hypertension, stroke, heart disease and renal failure. In addition, it is the leading cause of non-traumatic limb amputations, and a major cause of blindness [1].

There are two types of diabetes, Type 1 diabetes, or insulin-dependent diabetes, which occurs primarily in children and is an autoimmune disease causing destruction of insulin-secreting pancreatic β-cells. Treatment is administration of insulin via subcutaneous injection [2]. The second and more prevalent type is type 2 diabetes mellitus (T2DM), or insulin-independent diabetes. T2DM is different from Type 1 in that it is not caused by immunological β-cell destruction, but rather by an inability of insulin to solicit its normal glucoregulatory response [6]. This inability of insulin to influence glucose homeostasis is referred to as insulin resistance. To compensate, the pancreatic β-cells hypertrophy in an attempt to secrete enough insulin to elicit an effective response, and in doing so, eventually leads to β-cell destruction [7]. Ninety-five to 99% of all diabetes cases in the United States are T2DM, and there are behaviors that can increase and, fortunately, others that can decrease the risk of developing this type of diabetes [1].

One risk factor for T2DM is excessive weight gain. Particularly individuals who are overweight and obese have increased rates of diabetes [8, 9]. Physical inactivity is also a risk factor for developing diabetes [10-12]. This could be a confounding factor since physical inactivity is also a cause for overweight and obesity. Interestingly however, exercise and healthy diet, even in overweight individuals can decrease the risk

of developing diabetes, and this is independent of weight loss [13]. Poor diet can also lead to the development of diabetes, particularly diets high in fat and simple sugars [6]. Western diets, those high in saturated fats, refined carbohydrates and low in micronutrients, are particularly keen to the development of diabetes. This points to the fact that the specific macronutrients are important as to whether glucose intolerance will result, as certain fats are protective while others are not [14, 15]. Finally, tobacco smoking is a major risk factor for diabetes [16, 17]. Luckily, these risk factors are mostly modifiable, such that if one adopts healthy lifestyle changes including a healthy diet and consistent exercise and cessation of tobacco use, one can greatly reduce his/her risk of developing this chronic disease.

There are additional risk factors that are not modifiable, those being male gender, certain ethnicities and age [18, 19]. Further, there is a rather large and unknown genetic component in T2DM etiology as concordance in identical twins is 90% although the genetic contribution is determined to be only 10% [20, 21]. Also, Americans from African and Hispanic descent develop the disease at higher rates than those of European and Asian descent [18]. Finally, there is evidence that diabetes risk can be affected by the intrauterine environment as determined by the maternal and developmental influences [22]. This idea that that the intrauterine environment can "program" the developing fetus and have long-term health consequences on offspring is called the Developmental Origins of Health and Disease (DOHaD) Hypothesis. This hypothesis is fundamental to my dissertation and will be discussed fully in the upcoming sections.

1.1.2 Glucose homeostasis: Overview Upon ingestion of a meal, carbohydrates are broken down to their simplest sugars, monosacchrides. This digestion begins with

salivary and pancreatic amylase [23]. Villus enterocytes of the duodenum and jejunum produce hydrolases on their brush-border membrane which further digest carbohydrates into their monomeric sugars [23]. Absorption into enterocytes is carried out by sodium-monosaccharide cotransporter, $SGLT_1$. Sugars exit the cells at the basal lateral membrane via sodium-independent transporter, glucose transporter 2 (GLUT2) and enter blood stream via the portal vein [23]. Under normal conditions, the increased blood sugar then signals the pancreas to secrete insulin, an endocrine hormone whose function is to initiate glucose uptake from the blood stream into peripheral tissues to meet energy requirements. Glucose uptake is mediated by glucose transporters (GLUTs), the main ones being GLUT1, GLUT2, GLUT3 and GLUT4 [6]. GLUT1 is ubiquitously expressed and is a moderate affinity, low capacity transporter of glucose, while GLUT2 is a low affinity, high capacity transporter expressed primarily in the intestine, kidneys, pancreas, and liver [6]. GLUT4 is an insulin-dependent glucose transporter whose expression facilitates glucose uptake in highly metabolic tissues of skeletal muscle and adipose [6]. When insulin binds its receptor, signal transduction results in the translocation of cytoplasmic GLUT4 to the cellular membrane where it transports glucose from the bloodstream into its respective tissue. Type 2 diabetes is often caused by a defect in this insulin-dependent glucose uptake mechanism, particularly the insulin signal transduction. In addition to insulin's role in peripheral glucose uptake, attenuated insulin signal transduction in the liver can lead to augmented hepatic glucose production and impaired adipose free fatty acid (FFA) uptake further elevating blood glucose levels and exacerbating glucose intolerance.

1.1.3 Insulin secretion and function Insulin is the primary mediator of glucose tolerance. The β-cells of the Islets of Langerhans take up glucose from the blood by way of GLUT2. Once inside the β-cell, glucose undergoes the normal process of glycolysis, providing substrates for mitochondrial respiration. The increased intracellular ATP:AMP ratio begins a signaling cascade that results in depolarization of the cellular membrane, increased cytosolic calcium concentration and degranulation of stored insulin into the blood for systemic circulation [24].

There are insulin secretagogues that modulate insulin secretion, some being sympathetic and parasympathetic innervations, but of particular interest in normal glucose homeostasis, are incretin gut hormones, gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1) [25]. GIP and GLP-1 are secreted prandially by K and L cells of the intestine, respectively [26, 27]. These incretins potentiate glucose-stimulated insulin secretion, but they are rapidly degraded by dipeptidyl peptidase 4 (DPP4). Fifty percent of GLP-1 is degraded even before it reaches the portal vein, and an additional 40% is degraded in the liver [28-32]. In fact, the half-life of GLP-1 is only 60-90 seconds. GLP-1 also inhibits β-cell apoptosis and promotes β-cell survival and proliferation [33, 34]. In T2DM patients, GIP insulinotropic activity has been shown to be diminished by 54% and GLP-1 prandial release is attenuated [25, 35-38]. These incretins are being investigated for their potential use as anti-diabetic agents because their effects on augmenting insulin secretion are absent when blood sugar levels are low, thereby decreasing the risk of life-threatening hypoglycemia [39, 40]. Once insulin is secreted from the β-cell into the systemic circulation, its actions in glucose regulation are mediated by insulin receptors on liver, skeletal muscle and adipose tissue.

The insulin receptor is a receptor tyrosine kinase. Upon ligand binding, autophosphorylation of the β subunit of the insulin receptor occurs [41-43]. Insulin receptor substrate (IRS) is recruited to the activated receptor and is itself phosphorylated [44]. Phosphorylated IRS then interacts with p85, the regulatory subunit of phosphotidylinositol 3 kinase (PI3K), targeting the enzyme to the plasma membrane [44, 45]. The product of this enzyme's activity is phosphotidylinositol 3,4,5,-triphosphate (PIP_3). This molecule then recruits and activates other kinases including protein kinase C (PKC) ζ and protein kinase B (Akt) [46, 47]. Insulin signaling results in several events including translocation of GLUT4-containing intracellular vesicles to the plasma membrane in skeletal muscle and adipose tissue to facilitate glucose uptake [42]. In liver, insulin signaling deactivates glycogen synthase kinase 3 (GSK3) leading to the disinhibition of glycogen synthase, thereby increasing glycogen storage and quelling hepatic glucose production [48, 49]. Insulin resistance occurs when circulating insulin no longer elicits glucose normalizing responses. As previously mentioned, as insulin insensitivity progresses, the pancreatic β cells hypertrophy and eventually cause β cell dysfunction and apoptosis and insulin deficiency [50].

1.1.4 Role of adipose, liver and muscle in glucose intolerance Insulin resistance can result from increased free fatty acid metabolism and ceramide production or also from inflammation [50-57]. Studies have shown that cytokines TNF-α, IL-1β and IL-6 disrupt insulin signaling and lead to insulin resistance [58-63]. These cytokines in the context of diabetes are secreted primarily by adipose tissue, either the adipocyte itself or the infiltrating immune cells that accompany the tissue's hypoxia [64]. Also, adipokines such as leptin and adiponectin modulate energy homeostasis and insulin activity, and

their levels are also disrupted in obesity [65-67]. This may explain the association often observed between obesity and diabetes. Adipose tissue then is a major player in diabetes as it not only takes up free fatty acids and glucose, but also contributes to diabetes by modulation of its endocrine function [64, 68, 69]. Adipose tissue is responsible for less than 10% of whole body glucose uptake, yet genetic knockout of adipose tissue GLUT4 causes hyperinsulinemia and hyperglycemia characterized by liver and skeletal muscle insulin resistance, exemplifying its role in diabetes etiology despite its minor contribution to peripheral glucose uptake [70].

Similar to the pancreas, the liver expresses the insulin-independent glucose transporter GLUT2 [6]. Once inside the hepatocytes, glucose is phosphorylated by glucokinase (GCK), preventing its retrotransport back into the systemic circulation [71, 72]. Hepatic insulin signaling activates transcription of glucokinase [72, 73]. The phosphorylated glucose molecule then undergoes several fates such as glycolysis, stored as glycogen for later use, or can be used in the pentose phosphate pathway [6]. Moreover, in the fasted state when insulin levels are low, the liver undergoes glycogenolysis and gluconeogenesis to supply glucose to the periphery [74, 75]. Insulin signaling terminates these hepatic glucose production processes. Consequently, hepatic insulin resistance causes decreased hepatic glucose flux due to decreased rate of intracellular glucose phosphorylation, thereby permitting GLUT2-mediated retrotransport of glucose back into the periphery [75]. The liver is also responsible for approximately 70% of insulin degradation [76-78]. In diabetic subjects, hepatic insulin clearance is decreased anywhere from 20-65% [75, 79-81]. This presumably is protective against hyperglycemia by mediating increased circulating insulin to be used by skeletal muscle

and adipose tissue to facilitate glucose uptake. However, this decreased hepatic insulin clearance may precipitate peripheral insulin resistance and in turn produce a positive feedback loop of hyperglycemia and insulin resistance. Further, it was observed that visceral fat is more detrimental to insulin sensitivity than subcutaneous fat [82-84]. This is interesting because glucoregulatory adipokines, cytokines and FFAs from visceral fat drain to the portal vein directly to the liver mediating impaired hepatic glucose homeostasis and insulin resistance [85]. Hepatic insulin resistance permits hepatic glucose production in the presence of elevated insulin which further increases blood sugar [86]. Together, the liver contributes to hyperglycemia both by decreasing glucose uptake and storage, but also by dysregulated glucose excursion.

Skeletal muscle is a major site of glucose disposal, accounting for upwards of 90% of insulin-dependent peripheral blood glucose uptake, however approximately one third of postprandial glucose is taken up by the liver [7, 87-90]. Skeletal muscle intracellular transport is dose-dependently mediated by insulin. Hyperinsulinemic euglycemic clamp studies in both lean and obese type 2 diabetics revealed a greater than 50% decrease in whole body glucose disposal [87]. This reduction in insulin-stimulated whole body glucose uptake was mirrored by a 50% increase in skeletal muscle EC50 for insulin-mediated glucose uptake [87]. Additionally, not only were the skeletal muscles of diabetic subjects resistant to insulin activity, a delay in the onset of insulin activity was also observed [87, 90]. Intramyocellular FFAs and impaired mitochondrial β-oxidation are strongly associated with muscle insulin resistance, but it is difficult to establish a causal relationship between the two [90-92]. Nonetheless, the result is impaired insulin

signal transduction, impaired skeletal muscle metabolic flexibility, and consequently, attenuated glucose uptake.

1.2 Developmental Origins of Health and Disease

1.2.1 Thrifty Phenotype. Barker and Hales conducted epidemiological studies in the United Kingdom and observed that areas with the highest mortality rates were the same areas with the highest infant mortality rates several decades earlier [93-95]. Upon further investigation, it was discovered that not only did these geographical areas have higher rates of mortality but also higher rates of metabolic disease as well [22, 96-98]. They then put forth the Thrifty Phenotype Hypothesis which explains that developmental perturbations result in low birth weight offspring who have an increased risk of developing cardiovascular and metabolic disease later in life. Low birth weight is a phenotype resulting from intrauterine growth restriction which can have several causes including maternal undernutrition and uteroplacental insufficiency. Initial studies investigated the role of maternal undernutrition on offspring body weight and aging-associated sequelae. In addition to low birth weight babies having increased risk of developing metabolic disease, large babies do as well. Figure 1.2.1 diagrams the different intrauterine environments that precipitate metabolic disease that will be discussed in more detail.

1.2.2 Maternal undernutrition. This hypothesis was evaluated in humans by investigating outcomes of offspring who were in utero during times of famine. One of the first and most famous studies is that of the Dutch famine. During the winter of 1944, the Germans established a blockade of food to the Netherlands causing severe famine.

Investigations of offspring who were in utero during this period revealed that children were born with decreased placental and body weights although length and head circumference were relatively less affected [99]. It is thought that reduced fetal nutrient availability results in a brain-sparing effect [100, 101]. This describes vascular development and subsequent nutrient redistribution to more important organs such as the brain, heart and adrenal gland and the sequestration of nutrients away from visceral organs of the liver, and kidneys [102-104]. As a result, growth-restricted infants often have normal sized brains but underdeveloped visceral organs. Often the kidneys of small infants have decreased nephron size and number, and moreover, the livers are smaller and hypoperfused [105-107].

Ravelli et al. showed upon 2 hr glucose tolerance test that offspring who were in utero during famine had increased glucose and insulin levels indicating impaired glucose homeostasis and insulin resistance compared to those conceived after or born before the famine [108]. It also appeared that those who were affected during the last trimester exhibited the most marked derangements. Studies of Chinese Famine of 1959-1961 revealed elevated risk of impaired glucose homeostasis of those adults who were in utero during famine [109, 110]. Those in utero during the most severe famine showed marked impairments over those whose nutrient restriction was less severe. Additionally, the researchers found those adults who were exposed to nutrient restriction during famine but whose diets where rich later in life had the highest prevalence of glucose intolerance in adulthood [109, 110]. Developmentally malnourished offspring upon later life nutrient abundance demonstrated exacerbated glucose homeostatic maladaptation, illustrating continued nutrient response postpartum.

1.2.3 Catch-up growth There is now a theory that low birth weight offspring, when exposed to nutrient rich postnatal environment, display "catch-up" growth. Catch-up growth is a phenomenon by which underweight infants experience rapid postnatal growth eventually equaling and even surpassing the size of those born with normal birth weight. Low birth weight offspring that would later develop type 2 diabetes were taller, heavier, and had increased BMI by 7 years of age and during adolescents compared to normal weight offspring [111-113]. A study of low birth weight South African children revealed that those infants that underwent rapid weight gain during childhood exhibited the most profound impairments in glucose tolerance [114, 115]. This same association between catch-up growth and glucose intolerance was confirmed in Finnish children. Those infants who were small for gestational age and experienced the most rapid increase in BMI from 2 to 11 years of age were most likely to develop type 2 diabetes in adulthood [114, 116]. Ong et al conducted a literature review and found that rapid growth in infancy conferred a two to three fold increased risk of obesity with aging [117]. Not only is reduced birth weight and subsequent catch up growth associated with glucose intolerance, but reduced birth weight is also associated with obesity in older individuals. As mentioned in previous sections, the association between obesity and diabetes is well established; therefore it is possible that the increased risk of diabetes in small babies may be concomitant or secondary to the development of obesity. However the association with impaired glucose disposal is generally greater than with obesity, indicating that lifestyle probably plays more of a role in development of obesity than diabetes in the context of developmental programming.

1.2.4 Animal models: general A wide variety of experimental techniques have been employed to corroborate the human data linking low birth weight to metabolic dysfunction in adults and also to elucidate the mechanisms involved. Intrauterine growth restriction results in infants that are of decreased birth weight, or more precisely, small for their gestational age. It is important to distinguish between the two as an infant may be small because of premature birth and the ramifications may be different depending upon prematurity or decreased fetal growth. Experimental techniques use maternal low protein or low calorie diets, and uterine artery ligation to facilitate growth restriction. Each of the experimental animal models of growth restriction exhibit decreased birth weights compared to controls [118-122]. Although maternal undernutrition and uteroplacental insufficiency have reduced birth weight offspring, the responses may be quite different and consequently have varying mechanisms to disease progression [123].

1.2.5 Animal models of undernutrition Low protein (8% vs. 20%) isocaloric diets utilized later in gestation result in growth restricted offspring, demonstrated by decreased birth weights in rats despite comparable placental weights [121]. The brain sparing effect is also observed in this model. Desai et al. showed offspring born to dams on low protein diet had the smallest changes in absolute brain and lung weights, while reductions in kidney, heart and thymus were proportional to the reduction in body weight, and the pancreas, spleen, liver and skeletal muscle had reduced weights even when taken as a proportion of body weight [124]. Regarding glucose homeostasis, both male and female offspring born to protein restricted dams display glucose intolerance and insulin resistance upon aging [125, 126]. Furthermore, pancreatic disturbances were observed including decreased β-cell mass, diminished islet proliferation and vascularization,

decreased islet insulin levels, and attenuated glucose-stimulated insulin secretion [119-122, 127-130]. The fetal pancreas also showed increased islet apoptosis [131]. Caloric restriction to at least 50% of ad libitum control animals also caused decreased birth weight and age related impairments in glucose tolerance [120]. This model also exhibits disturbances in the endocrine pancreas including decreased β-cell mass and differentiation [120, 128]. Abrogated insulin signaling mediated by decreased PKC ζ and IRS-1 expression and increased serine phosphorylation of IRS-1 in skeletal muscle indicates additional disruption in extrapancreatic tissues [132, 133].

As mentioned earlier, postnatal nutrition can further program the offspring toward age-associated metabolic disease. Cross fostering studies in which offspring from malnourished dams were nursed to control dams, and vice-versa, support early postnatal nutrition as important factors in later life disease susceptibility. Growth-restricted offspring nursed to low protein diet dams have growth rates that are comparable to controls that persist as the offspring age, but the growth-restricted offspring weigh less than controls at every time point [124]. The greatest pre-weaning growth was observed in growth restricted pups that were fostered to control dams [124]. This intervention group actually weighed the same as controls by weaning in a different study [134, 135]

Tissue specific effects of maternal undernutrition include adaptations of the liver lobules which are larger in size yet fewer in number in growth-restricted animals [105]. Glucagon-stimulated glucose production was reduced, but insulin and glucagon together caused an initial increase in glucose output and delayed glucose output suppression [105, 136]. Skeletal muscle glucose uptake diminishes with age, owing at least in part to decreased expression of insulin signaling mediator PKC ζ [133, 137]. The adipose tissue

from growth restricted animals resulted in age-dependent impairments in insulin-stimulated glucose uptake and impaired inhibition of lipolysis resulting from attenuated activation of insulin-stimulated PI3K activation [138]. Taken together, the endocrine pancreas is disrupted with maternal undernutrition, but organs involved in glucose uptake are also disrupted and exhibit insulin resistance upon aging.

1.2.6 Uteroplacental insufficiency Uteroplacental insufficiency refers to incomplete attachment of the placenta to the uterus leading to diminished placenta vascularization and subsequent reduction of placental and fetal blood flow. Uteroplacental insufficiency thus results in placentas and fetuses that are hypoxic, undernourished, and consequently growth restricted. It is the most common cause of intrauterine growth restriction in humans, especially in developed countries [139]. Uteroplacental insufficiency may have several causes including hypertension and maternal tobacco smoking [140].

Between 13 and 20% of American woman smoke cigarettes during pregnancy, delivering one million infants exposed in utero to tobacco smoke [141]. Cigarette smoke contains approximately 4800 compounds including aryl hydrocarbon receptor (AhR) agonists in the class of polycyclic aryl hydrocarbons such as benzo(a)pyrene and chlorinated dioxins and furans [141-144]. Maternal cigarette smoking is associated with a myriad of developmental defects including uteroplacental insufficiency resulting in reduced birth weight [145, 146]. It is also associated with increased risk of offspring obesity development [147-150]. An investigation of American Indian children up to 3 years of age revealed the children whose mothers smoked at the initial prenatal visit were twice as likely to be in the 85th percentile of weight despite having reduced birth weight.

These 3 year old children born to mothers who smoked additionally had increased weight-to-length ratio z-scores due to increased adiposity as compared to those toddlers whose mothers were nonsmokers [147]. Data analysis from the 1958 British birth cohort showed smoking during pregnancy resulted in reduced birth weights compared to nonsmokers and that the odds ratio for obesity increased with age, and by 33 years of age both men and women born to smokers had statistically significant odds ratio for diabetes (1.56 and 1.41, respectively) [149]. Scientific data regarding maternal cigarette smoking and subsequent diabetes risk in the context of developmental programming is lacking. However, one study by Montgomery and Ekbom showed that maternal cigarette smoking not only increased the risk of obesity with aging, but showed a strong association between maternal smoking and later life diabetes prevalence by age 33 [150]. Moreover, offspring born to mothers who smoked at least 10 cigarettes per day during pregnancy were 4 times as likely to develop diabetes by age 33 than adults whose mothers did not smoke during pregnancy [150].

1.2.7 Animal models of uteroplacental insufficiency Sequelae of uteroplacental insufficiency is studied experimentally by employing uterine artery ligation. Uterine artery ligation growth restricts the developing fetus by reducing blood flow to the placenta [139, 151]. Consistent with uteroplacental insufficiency, the model exhibits reduced β-cell mass, impaired glucose homeostasis and decreased IGF-1 levels in growth restricted offspring born to uterine artery ligated dams [130, 152]. Rat pups born to ligated dams show global adiposity and insulin resistance as early as 10 weeks of age which is in stark contrast to the experimentally undernourished model whose offspring exhibit increased insulin sensitivity in early life and only exhibit insulin resistance and

diabetes upon significant aging [120, 123, 125, 126, 130, 153]. Oxidative phosphorylation is impaired in the liver and skeletal muscle in offspring from ligated dams, and insulin-mediated GLUT4-dependent glucose transport in muscle is attenuated contributing to hyperglycemia [154, 155]. Pancreatic derangements may be mediated in part by aberrant pancreatic and duodenal homeobox 1 (Pdx1) expression via epigenetic modulations [118, 128, 156]. The Pdx1 gene plays pivotal roles in pancreatic development [118, 119]. Three month old offspring from uterine artery ligated dams displayed reductions in Pdx1 gene expression which was concomitant with an 80% reduction in β-cell mass. Interestingly, GLP-1 receptor agonist, Exendin-4, administered at birth restored Pdx1 mRNA levels and ultimately β-cell function [157, 158]. Exendin-4 further reduced hepatic oxidative stress and insulin resistance [157, 159].

1.2.8 Developmental Programming associated with high birth weight

Developmental programming of adult disease is not limited to those growth restricted offspring; high birth weight offspring, too, have increased risk of later life metabolic diseases [160-163]. There is a loss of maternal insulin sensitivity in late gestation in normal pregnancies so as to increase insulin and nutrient availability for the developing fetus, but this can lead to gestational diabetes when this insulin resistance becomes pathologic. The Pima Indian population have increased incidence of diabetes [164-166]. Studies on this unique population revealed that both low birth weight and high birth weight babies have increased risk of developing type 2 diabetes suggesting a U-shaped relationship [167]. Pima Indian females whose mothers had gestational diabetes were also more likely to develop diabetes [165]. Not only does diabetes lead to high birth weight and metabolically perturbed offspring, but maternal obesity does as well. When a

high fat obesogenic diet was given before mating, during gestation, and lactation, the offspring were hyperphagic and had increased adiposity and insulin resistance [168]. Also, offspring of obese mothers have developmentally programmed nonalcoholic fatty liver disease which can have additional metabolic sequelae including insulin resistance [169]. Mestan et al showed correlations of maternal BMI and diabetes status (Type 2 or gestational) were both positively correlated with large-for-gestational age offspring phenotype, OR 2.64, 95% CI 1.31-6.20 and OR 5.58, 95% CI 2.06-15.13, respectively [170]. Also the adipose tissue secreted cytokine RANTES in cord blood was found to be correlated with large-for-gestational age and ponderal index in offspring [170]. Harder et al conducted a meta-analysis of 14 studies investigating the association between birth weight and adult Type 2 diabetes incidence and confirmed the U-shaped association [171].

1.2.9 Conclusion Perturbations in the plastic periods of development during gestation and infancy can have long lasting physiological effects which predispose one to increased metabolic disease susceptibility later in life. There are varying causes of reduced birth weight and similarly different yet converging paths to disease progression. Fortunately, excellent experimental models of growth restriction and developmental programming are available to help elucidate the mechanisms behind disease progression in the two most prevalent causes on reduced birth weight. Uteroplacental insufficiency can result from exposure to environmental toxins such as those found in cigarette smoke and predispose offspring from maternal smokers to metabolic disorders later in life. Similar to chemicals in cigarette smoke, polychlorinated biphenyls (PCBs) are also

environmental toxins that activate the AhR, suggesting that they may also diminish intrauterine growth and precipitate postnatal metabolic derangements.

1.3 Polychlorinated biphenyls

1.3.1 History of polychlorinated biphenyls PCBs are a class of persistent organochlorine pollutants that partition with lipids and biomagnify in the food chain resulting in dietary exposure [172-174]. PCBs are dielectric molecules whose heat resistant properties afforded many industrial uses including in electrical insulating fluids and heat exchange fluids. They were used in electrical equipment such as transformers and capacitors, fluorescent light ballasts, plastics, adhesives, paints, varnishes, and paper [174, 175]. PCBs were manufactured in the United States under the trade name Aroclor. In the 1970's two accidental exposures in Southeast Asia revealed some potential health hazards associated with dietary PCB exposure. These two incidents are the Yusho and Yu-Cheng incidents of Japan and Taiwan, respectively, in which populations were exposed to PCB via contaminated rice bran cooking oil [176-179]. Exposed subjects presented with chloracne, hyperpigmentation, ocular lesions, neurological symptoms including numbness, nausea and headaches, and digestive indications including vomiting and diarrhea [180]. Consequently, the US Environmental Protection Agency (EPA) banned production of PCBs in 1976 although use of the compounds was still permitted. Investigations of PCB-associated health hazards in Western and European countries confirm those of the Yusho and Yu-Cheng incidents, and additional health hazards associated with PCB exposure have been observed including cancer, hypertension, immune and thyroid dysfunction, asthma, obesity, diabetes and low birth weight [181-183]. Over one billion pounds of PCBs were produced in the United States in the 50

years of their production, and PCBs, particularly those with more chlorine substitutions, resist biodegradation and therefore persist in the environment [173]. Although their production has long ceased, currently over 450 million pounds have contaminated our environment and PCBs are found in over 29% of hazardous waste sites in America and have thus been categorized as ubiquitous environmental pollutants [173, 175, 184].

1.3.2 PCB Exposure PCBs enter the environment by improper disposal of PCB waste and leakage of PCB containing fluids from electrical equipment. They then partition to the nonpolar components of the environment, particularly soil and sediment, due to their hydrophobicity. When PCBs are volatilized they become airborne leading to their detection in the atmosphere [185], and these characteristics give rise to their ability to bioaccumulate in food chain [181]. Human exposure can be occupational for those whose work involves PCB-containing materials, but for the majority of Americans, PCB exposure is dietary coming primarily from animal based foods such as milk, beef and fish [174]. Greatest dietary exposure comes from beef consumption although fatty fish such as farm raised salmon have higher PCB concentrations on average [172, 174]. This disparity is owed the large proportion of beef in the Western diet compared to fish. Because fish have far greater levels of PCBs, some studies use sport fish consumption as an estimate of exposure levels above background [186, 187].

PCBs have been detected in lipid rich tissues including adipose tissue, central nervous system, liver, serum, placenta, cord blood and breast milk [186, 188, 189]. The discovery of PCBs in placenta, cord blood and breast milk indicate exposure to these pollutants as early as the time of embryonic implantation and throughout gestation, lactation and the entire life course after weaning [188]. Owing to cessation of production

and successful remediation procedures, environmental and body burdens of PCBs are declining [190-192]. Fetal development is quite plastic and responsive to subtle changes in its intrauterine and the early postnatal environment, as discussed regarding fetal programming of metabolic disease [193, 194]. Also, several of the PCB-associated health hazards were shown to have inverted U-shaped relationships suggesting that low level exposures associated with declining environmental levels and body burdens may actually be more detrimental than expected [195-198].

PCB exposure in the general population has been shown to be associated with low birth weight in several epidemiological studies [182, 199, 200]. It then stands that in the context of DOHaD, perinatal PCB exposure resulting in low birth weight may lead to programming of metabolic diseases such as obesity and diabetes. Studies investigating the association between PCBs and metabolic disease are done based upon postnatal PCB exposure [183, 201-205]. Few studies have looked at perinatal exposure and subsequent development of obesity with most prospective studies ending prior to adolescents and having inconsistent results [206, 207]. Epidemiological studies investigating dietary exposure and body burdens in adulthood show clear associations although causal relationships are difficult to ascertain [208-210]. Regarding diabetes, even fewer studies have investigated the association between early life pollutant exposure and diabetes risk later in life. It does seem clear however that there is a correlation between diabetes risk and PCB exposure as indicated by studies showing lack of association between obesity and diabetes in subjects with lowest levels of PCBs and diabetes incidence increasing with increasing plasma PCB concentrations [198, 204, 211]. Also, associations between PCB exposure and diabetes are found predominantly in females, demonstrating sex-

specific effects of PCBs [212]. The contribution of PCBs to diabetes etiology is under-investigated. Data regarding mechanisms of hyperglycemia in PCB-associated diabetes are inconclusive. Some studies suggest the effect of PCBs on diabetes is not mediated through insulin resistance but rather attenuated insulin secretion as evidenced by a negative association between PCBs and homeostatic model assessment (HOMA)-β (an estimate of β-cell function), but no association with HOMA-IR (measure of insulin resistance based upon fasting glucose and insulin levels) [211-213]. Some studies however, show positive associations with PCBs and insulin resistance [204]. Animal and in vitro studies demonstrate PCB-induced insulin resistance mediated predominately by adipose tissue [214, 215]. Although the association between PCB exposure and impaired glucose homeostasis is rather evident, the mechanism remains unknown, as do the effects of perinatal PCB exposure on glucose tolerance.

1.3.3 PCBs and AhR PCBs are composed chemically of two covalently bonded benzene rings with at least one chlorine molecule substitution. Given that there are 10 different locations for chlorines to bind, there are 209 different combinations of chlorine number and position; each different combination is called a congener [216]. There are some congeners that are non-ortho or mono-ortho substituted that have coplanar conformations while those having multiple ortho substituted chlorines display steric hindrance and are referred to as non-coplanar. Coplanar PCBs bind AhR whose canonical ligand is 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, also known as TCDD or simply dioxin [216]. For this reason, coplanar PCBs are often referred to as dioxin-like PCBs. The AhR is a ligand-activated DNA-binding transcription factor [217]. The receptor resides in the cytosol interacting with heat shock protein (HSP) 90, prostaglandin E

synthase 3 (p23), AhR interacting protein (AIP) [143, 218-221]. Upon ligand binding, a conformational change uncovers the receptor's nuclear localization signal causing its nuclear translocation [222]. Once in the nucleus, the receptor dimerizes with the aryl hydrocarbon nuclear receptor (ARNT) at which time it interacts with xenobiotic response elements and regulates gene transcription [223, 224]. AhR facilitates transcription of detoxifying enzymes such as cytochrome P450s (Cyp) 1a1, Cyp1b1 and phase II enzymes, glucuronosyltransferases (UGTs) and glutathione-s-transferases (GSTs) [143]. Moreover, the receptor can interact with other transcription factors including nuclear factor (erythroid-derived 2)-like 2 (Nrf2), estrogen receptor α (ERα) and RelA subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κb), thereby modulating their activities [225-230]. The detoxification process produces free radicals which can cause oxidative stress and disruption or activation of redox responsive signaling pathways [231]. These cellular AhR-dependent redox responses can contribute to a myriad of pathological phenotypes leading to the aforementioned PCB-associated diseases [232]. In fact, epidemiological studies find more positive correlations between coplanar PCB exposure than with non-coplanar PCBs [212, 233-237].

1.4 Scope of Dissertation

1.4.1 Aims of dissertation The main purpose of this dissertation was to determine whether PCB exposure during the critical windows of development, during gestation and lactation, has an adverse effect on offspring body composition and glucose homeostasis. Finally, I investigated changes in maternal hepatic gene expression during pregnancy and in fetal and offspring livers perinatally exposed to PCB. These studies elucidate a role of

early life environmental contaminant exposure in the subsequent development of obesity and diabetes.

1.4.2 Rational Epidemiological studies support an association between PCB exposure metabolic disease. In support of the human studies, Baker et al, have recently shown that direct exposure to coplanar PCBs cause adipose tissue inflammation and insulin resistance leading to impaired glucose tolerance in male mice [214]. It is clear however, that PCB exposure begins in utero and exposure this early in development can have detrimental effects such as increased rates of malformations. This perturbation of fetal development is evidenced also by decreased birth weights of offspring born to mothers with increased exposure to PCBs. In the context of the DOHaD then, it is reasonable to assume that these offspring will present with metabolic maladies of obesity and diabetes. Studies investigating prenatal PCB exposure on offspring body composition reveal sex specific increases in adiposity and reduced lean mass in female children 6.5 years of age, with boys of the same age being symmetrically smaller [238, 239]. At puberty, prenatal PCB exposure was associated with increased BMI in Caucasian adolescents [238]. Further, the observed increase in body weight with prenatal exposure holds true even when adjusted for postnatal exposure [239]. In vitro studies support a role of PCB exposure in this phenotype as these pollutants inhibit skeletal muscle differentiation and augment adipocyte differentiation [240, 241]. However, one study investigated the effects of estimated prenatal PCB exposure on adult body weight in females only, and it found no association between body weight and prenatal PCB exposure [242].

Epidemiological evidence shows positive associations between PCB body burden and perturbed glucose homeostasis, but the contribution of perinatal PCB exposure to this disease phenotype has not been investigated. Langer et al, in their investigation environmental pollution with impaired fasting glucose and thyroid dysfunction, concluded that there is likely transgenerational transmission of these phenotypes engendered by perinatal PCB and other pollutant exposure of the parent generation [187]. Despite the lack of evidence for a causal relationship between PCB exposure and diabetes, the DOHaD provides rationale for PCB-induced disruption of glucose tolerance in adults as sequelae to their disrupted fetal development. Nonetheless, further investigation into the role of perinatal pollutant exposure on offspring glucose tolerance is clearly warranted. Finally, the livers primary function is macronutrient homeostasis and xenobiotic detoxification. The effects of xenobiotic detoxification on hepatic gene expression has been studied, but not during pregnancy and fetal development, and not using PCBs. Moreover, a role of AhR in liver organogenesis has been identified, indicating temporal and spatial opportunity for AhR agonists to exert effects during hepatic development and maturation that may have long-term consequences.

1.4.3 Hypothesis and specific aims **Hypothesis:** Maternal coplanar PCB exposure during pregnancy and nursing will result in reduced birth weight offspring that will develop obesity and glucose intolerance with aging. Figure 1.4.1 diagrams the overall hypothesis and experimental approach to the dissertation. **Aim 1:** To test the hypothesis that perinatal PCB exposure will cause reduced birth weight and disease-associated modulation of body composition in aged offspring. **Aim 2:** To test the hypothesis that perinatal PCB exposure will impair glucose tolerance. **Aim 3:** To investigate alterations

in liver gene expression pattern upon PCB exposure during pregnancy and fetal development.

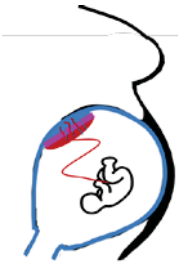
Causes	Maternal Undernutrition •Famine •Eating disorders	Placental Insufficiency •Hypertension •Cigarette smoking	Fetal Overnurtition •Western diet •Maternal diabetes
Animal Model	Maternal low protein or low calorie diets	Uterine artery ligation	Maternal high fat or high calorie diets
			
In Utero	•Normal placental function •IUGR due to lack of building blocks	•Small and dysfunctional placenta •IUGR due to decreased blood flow	•Large functional placenta •Fetal overgrowth due to increased building blocks and insulin
Outcome	Increased Risk of Obesity and Diabetes with Ageing		

Figure 1.2.1 Overview of developmental origins of metabolic disease. Maternal undernutrition and placental insufficiency both cause intrauterine growth restriction (IUGR) although by different mechanism. Fetal overnutrition can result in unusually large fetuses. Interestingly, however, these various fetal milieus precipitate a convergent phenotype of increased obesity and diabetes risk. Fortunately, experimental animal models have been developed to elucidate the mechanisms involved.

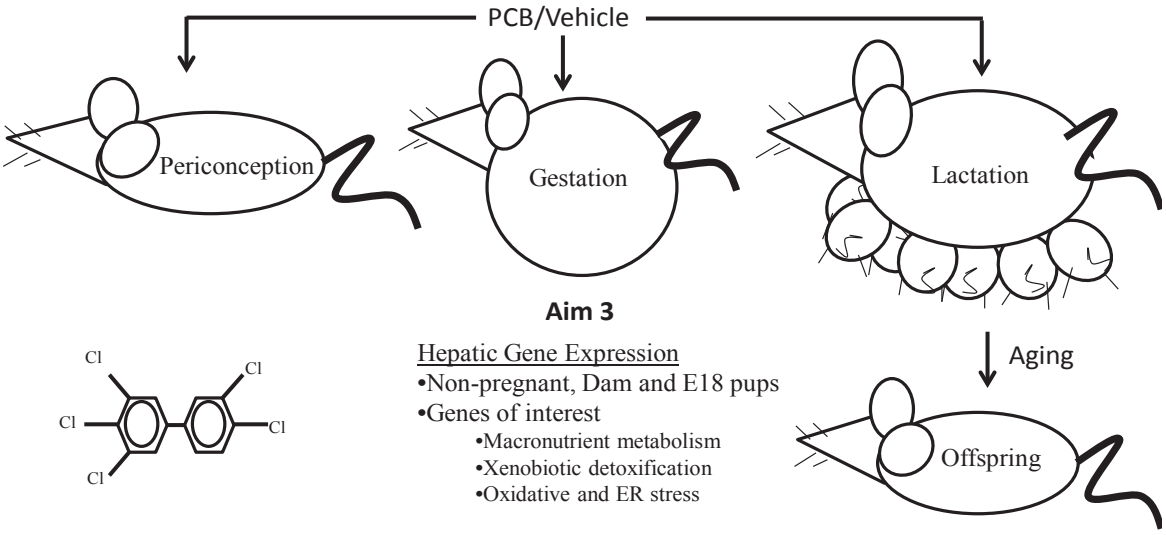


Figure 1.4.1 Specific Aims Diagram. Female CD-1 mice were exposed biweekly to safflower oil vehicle or PCB 126 via oral gavage such that mice exposure occurred near the time of conception, and once also during gestation and lactation. One cohort of mice was allowed to deliver their litters and nurse. These offspring were evaluated for body composition and glucose tolerance in Aims 1 and 2, respectively. A separate cohort was sacrificed at embryonic day 18 (E18) and pups and placentas were weighed to determine in utero developmental blight. Absolute messenger RNA quantitation of livers from fetuses, dams, and non-impregnated females were performed using NanoString nCounter Technology in Aim 3.

CHAPTER 2

PERINATAL POLYCHLORINATED BIPHENYL 126 EXPOSURE ALTERS OFFSPRING BODY COMPOSITION

2.1 Abstract

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants whose exposure levels are associated with various health hazards. We hypothesized that *in utero* and lactational exposure to PCBs can cause changes in body composition and obesity in a mouse model. Pregnant mice were exposed biweekly to increasing concentrations of PCB 126 via oral gavage. Maternal PCB exposure did not result in heavier offspring, however, dose-dependent and sex specific changes in body composition were observed. Female offspring displayed the most susceptibility to PCB-induced alterations in body composition, having less percent lean body mass and increased adiposity compared to females born to control dams, and these effects were largely dose-dependent. In contrast to females, and independent of the exposure level of PCB 126, male offspring had reduced lean body mass but no change in fat mass compared to males born to control dams. In conclusion, perinatal PCB 126 exposure did not affect body weight, but rather modulated body composition in a dose-dependent and gender-specific manner.

2.2 Introduction

Polychlorinated biphenyls (PCBs) were produced in the United States (US) until their ban in 1976 upon realization of their many hazards to human health [174]. Although production of PCBs has long ceased, environmental contamination remains, and PCBs are found in over 29% of hazardous waste sites in the US [174]. Primary sources of PCB exposure for the general population include contact with ground water or contaminated soil due to inappropriate disposal of materials containing PCBs, food contamination from food storage in silos with PCB-coated interiors, and consumption of fish from contaminated waterways [174]. Additionally, PCBs resist biodegradation, are lipophilic, and consequently bioaccumulate in the environment and in the food chain, thus exposing millions of Americans to these toxic compounds [173].

PCBs are ubiquitous and persistent environmental pollutants whose presence *in utero* perturbs fetal development. The developmental origins of health and disease hypothesis, derived from the thrifty phenotype hypothesis put forth by Barker and Hales in 1992, postulates that low birth weight offspring can result from insufficient nutrition in the intrauterine environment, leading to metabolic and other health derangements later in life [22, 243-247]. Animal models of low birth weight employing uterine artery ligation and protein or calorie restriction all show catch up growth and subsequent obesity with aging. In fact, maternal serum PCB levels in humans negatively correlate with infant birth weight and weight for gestational age [182, 199, 200, 248-251]. A meta-analysis comparing prenatal PCB exposure and birth weight effects across 12 European birth cohorts found a significant negative correlation between cord blood PCB levels and birth weight [182]. Other studies confirmed that low-level PCB exposure during the perinatal

period can significantly affect fetal growth and birth weight [199, 248-253]. In contrast, other studies found minimal or no correlation between perinatal PCBs and birth weight [254, 255].

Prospective studies investigating the effects of PCB exposure on body weight are incongruent, to say the least. Tang-Peronard et al. review of the literature in 2011 cited eight prospective studies looking at the effects of prenatal PCB exposure on body weight; 3 found positive associations with body weight, while another 3 found negative associations and the remaining 2 found no significant relationship between the two variables [200, 206, 208, 238, 242, 250, 256-258]. Further, non-monotonic dose-dependent relationships were observed [208]. The 2 prospective studies found positive associations with low PCB exposure (<1.0 ng/mg serum lipid) and body weight while high exposure (>4 ng/mg serum lipid) was correlated with decreased body weight [202, [200, 256, 257, 259]. Maternal serum levels between these amounts were divergent with some showing positive, negative and nonsignificant associations [206, 238, 242, 250, 258]. These effects were divergent regarding gender such that prenatal PCB exposure predominantly affected females in the nonmonotonic manner, while the male offspring were unaffected [208, 239]. These prospective studies, however, are limited in that only Karmaus et al investigated maternal PCB exposure on adult offspring which were of the female sex, and they found no association with maternal PCB exposure and BMI [242].

This is in contrast to cross sectional and case control studies that find significant associations with obesity and serum concentrations of PCBs in males [121, 208, 209, 260-263]. The lack of effect of prenatal PCB exposure in young males, but not females, but with effects observed in cross sectional studies is intriguing. Cross sectional studies

cannot determine causality, therefore the possibility of PCB induced developmental programming of obesity in males cannot be ruled out. Nonetheless, these studies indicate sex specific effects of PCB exposure on obesity.

Experimental studies utilizing animal models and in vitro techniques further demonstrate a role of PCB in adipose tissue development. Specifically, Arsenescu et al showed that dioxin-like PCB 77 or TCDD itself at low doses increased adipocyte differentiation in 3T3L1 preadipocytes whereas higher concentrations inhibited adipocyte differentiation. Additionally, in wildtype but not aryl hydrocarbon deficient mice, direct exposure of PCB 77 increased weight body weight mice [241]. These data provide a mechanism by which developmental exposure to dioxin-like pollutants can affect adipocyte differentiation in nonmonotonic dose-dependent manners observed in human studies.

Moreover, the decreased body weight observed primarily in females upon higher exposure levels can be concomitant with hypotonia and diminished physical activity in preschool aged children, but these effects are short-lived as they are not observed into puberty [258, 264]. These effects were thought to be due to lactational exposure rather than exposure in utero suggesting that heightened perinatal exposure, due in part to breast milk contamination, decreases body weight [258, 264]. The effect of perinatal PCB exposure on offspring lean mass is not well established, therefore using only BMI and body weight as marker of obesity is unavailing because they do not take into account the possible effects of PCB exposure on lean mass. Coletti et al found that Aroclor mixtures of PCBs dose-dependently diminish skeletal muscle differentiation in primary fetal myoblasts and in a myogenic cell line without affecting viability or proliferation,

supporting a role of PCB exposure on the disruption of skeletal muscle development in utero [240].

Evidence suggests that exposure to environmental pollutants can contribute to the pathology of obesity, and recent human studies support our hypothesis that perinatal exposure to PCBs is associated with overweight offspring [182, 239]. Thus, the purpose of this study was to investigate gender-specific differences in body composition of both young and aged offspring born to PCB-exposed dams.

2.3 Materials and Methods

2.3.1 Animals and diets: At 2 months of age, female ICR mice were bred and subsequently produced one litter at Taconic prior to their purchase and shipment to the University of Kentucky at roughly 4 months of age. The following studies were carried out at the University of Kentucky according to an approved Institutional Animal Care and Use Committee protocol. Females were housed 4 mice per cage for a 2 week acclimation period prior to mating. Female mice were fed a semi-purified low fat diet containing 10% kcal from fat (Research Diets D12450B) *ad libitum* for the duration of the study. Male ICR sires (Taconic) were fed standard chow (Teklad Global 18% Protein Rodent Diet #2018) during a 7 day acclimation period then mated to dams, 1 sire per 2 dams. In order to equilibrate nutrient availability, litters were culled to 6 pups on postnatal day 3. Pups from litters with more than 6 pups that were born on the same day were cross-fostered to dams nursing less than 6 pups. Body weight and maternal food intake were monitored weekly throughout weaning. Pups were weaned on postnatal day (PND) 21

onto standard chow, and male and female offspring were housed 3 and 5 mice per cage, respectively.

2.3.2 PCB Exposure: 3,3',4,4',5-pentachlorobiphenyl (PCB 126) was purchased from AccuStandard (C-126N New Haven, CT). PCB 126 was dissolved in tocopherol stripped safflower oil (Dyets # 403952 Bethlehem, PA). Female mice were orally gavaged with 0, 0.5 or 1.0 µmole of PCB 126 per kg body weight at a frequency of once per 14 days. The frequency was such that the dams were exposed to PCB 48 hours prior to mating, once during gestation, and once during lactation (Figure 1).

2.3.3 Body composition: At 7 weeks, 6 months and 9 months of age, nuclear magnetic resonance (EchoMRI; EchoMedical Systems; Houston, TX) was employed to quantify fat mass and lean body mass in both female and male offspring born to vehicle or PCB exposed dams. Although many tissues contribute to the lean mass output, there are undetectable components such as bone mineral content, hair, and claws which contribute to void mass. Body composition measurements in Tables 2 - 7 include data from only one female and one male offspring per litter, respectively. Body weight was measured just prior to body composition analysis.

2.3.4 Statistics: Overall differences in maternal body weight were determined by repeated measures ANOVA using IBM SPSS statistics 20 software. One-way ANOVA was used to make comparisons between groups for the remaining comparisons in Tables 1-3. Fisher LSD Method was used for post hoc comparisons. Upon the event of a failed Shapiro-Wilk normality test, data were transformed using natural log. Kruskal-Wallis ANOVA on Ranks was used when the data failed the equal variance test or failed

normality following transformation. These analyses were completed using SigmaPlot 12.0 software.

2.4 Results

2.4.1 Maternal effects of biweekly PCB 126 exposure and pup body weight: Figure 1A illustrates the perinatal exposure model used in the current study. Exposure at concentrations of 0.5 and 1 μmole PCB per kg body weight did not affect pregnancy rate (Table 2.1) or maternal body weight (Figure 2.1B) when compared to vehicle controls. In order to promote successful rearing, litters were not disturbed until postnatal day 3, at which time pups per litter were enumerated. The 1 μmole/kg dose led to significantly decreased litter size compared to controls. There was no significant difference in pup body weight on PND 3 although there was a trend toward decreased body weights in male offspring maternally exposed to PCB 126 compared to those from control dams at postnatal day 21 ($p=0.083$) (Table 2.1).

2.4.2 Body composition analysis: At 7 weeks, 6 months and 9 months of age, body composition analyses were conducted using EchoMRI, and the data are presented in Tables 2.2 – 2.7. Perinatal PCB exposure resulted in gender specific differences in body composition. Females born to PCB exposed dams had similar body weights, yet displayed significantly increased adiposity compared to females born to vehicle treated dams. Females perinatally exposed to 0.5 μmole/kg and 1 μmole/kg PCB 126 showed a dose-dependent 8.2% and 11.5% increase in percent body fat compared to females born to control dams ($p=0.044$ and $p<0.001$, respectively). The largest effect was seen in the high exposure group as these offspring additionally displayed significantly elevated fat

mass and a nearly 4% reduction in percent lean body mass (p=0.004 and p=0.005, respectively). Despite a lack of difference in body weight upon perinatal PCB 126 exposure, there were significant alterations in body composition at this early time point. While prospective epidemiological studies show an increase in body weight as representative of increased fatness in female offspring, we too demonstrated an increase in fatness. The decrease in lean mass contribute to comparable body weights despite increased grams of fat mass and increased % fat mass in these females. With aging, these PCB-induced effects are attenuated such that body composition is comparable in females perinatally exposed to PCB and control animals at 6 and 9 months of age, Tables 2.4 and 2.6, respectively.

For male offspring at 7 weeks of age, there was no significant effect of perinatal PCB exposure on body weight (p=0.368). In contrast to that which was observed in females, male offspring born to PCB exposed dams showed no difference in adiposity taken as either weight of fat mass or when expressed as a percentage of body weight (p=0.876 and p=0.536, respectively). There was, however, a significant reduction in grams of lean mass between the groups; specifically, males perinatally exposed to 0.5 μmole/kg and 1 μmole/kg PCB 126 showed significant reductions in lean mass (8.3% and 9.9%, respectively). However, there was no significant difference in percent lean mass (p=0.268). Perinatally exposed males at 6 months of age had a dose-dependent decrease in body weight compared to controls, most of which is attributed to reductions in grams of lean mass of PCB-exposed mice (Table 2.5). Specifically, these males born to dams administered 0.5 μmole/kg PCB 126 displayed 10.5 and 10.4 percent reductions in body weight and lean mass, respectively, while males born to dams given 1 μmole/kg PCB 126

exhibited 13.1 and 15.1 percent reductions in body weight and lean mass, respectively. This diminished lean mass accounted for 63 and 74% of the reduced lean mass in the 0.5 and 1 μmole/kg exposed males respectively. These trends persisted with further aging such that at 9 months of age there was again dose-dependent reductions in lean mass in PCB exposed males although the body weight difference was no longer statistically significant ($p=0.058$) (Table 2.7). The reduction in lean mass is dose-dependently, 11.2% and 17% for the two PCB exposed groups, respectively. Interestingly, the 0.5 μmole/kg decrease in lean mass is actually greater than the reduction in body weight for that group, an effect made possible by a slight increase in fat mass.

2.5 Discussion

The current study focused on the effects of perinatal PCB exposure on gender-specific body composition changes in the offspring. Because many studied causes of low birth weight are due to intrauterine growth restriction resulting from maternal undernutrition, it is important to note that maternal body weight was not affected by PCB exposure [22, 112, 265, 266]. It is uncertain whether or not the congener dosing and frequency used in this experiment caused low birth weight because initial pup weights were not measured until PND 3 instead of PND 0 so as to promote successful rearing. Nonetheless, there was no difference in body weight between pups born to control or PCB exposed dams at the first measurement of offspring body weight at PND 3. In fact, pup body weights were comparable throughout weaning. When mice were sexed at postnatal day 14, there was also no difference in body weight between treatment groups within each gender. In the event that low birth weight had occurred, it is unlikely that catch up growth would have occurred 3 days post-partum [267, 268]. Further, in Aim 3

we measured fetal weights at day 18 of gestation in vehicle and PCB exposed dams and no significant differences in fetal weights were noted.

Possible toxicity of our dosing regimen was evidenced by the reduced litter size in the 1 μmole/kg PCB 126 dose compared to control. The observed reduction in litter size is a likely consequence of poor rearing because unpublished data from our laboratory revealed no differences in fetal number at embryonic day 18 when dams were administered 1 μmole/kg PCB 126 as in the current exposure model (see Aim 3). Studies in humans have shown conflicting effects of maternal PCB exposure on fecundity, and specifically time to pregnancy [269-274].

In the present study, at 7 weeks of age, there was still no effect of perinatal PCB exposure on offspring body weight in these mice. Hertz-Picciotto et al. showed that in humans, *in* utero PCB exposure of males was associated with low birth weight and body weight for gestational age in boys, while in girls, in utero exposure was associated with shorter gestation [200]. In the same study, at five years of age, prenatal PCB exposure was associated with increased growth in girls but not boys [200]. Additionally, a recent prospective study conducted in humans revealed a significant positive correlation between cord blood concentrations of PCBs and overweight in females 6.5 years of age [239]. The males in that study did not exhibit such an association, although there was a trend toward a negative correlation between moderate prenatal PCB exposure and overweight. Our current experiment shows similar trends (Tables 2.2 and 2.3), such that perhaps with an increased sample size, we too would have observed statistically significant dose and gender-specific effects of maternal PCB exposure on body weight. Interestingly, when Valvi et al. adjusted for birth weight, the association between

overweight and PCBs strengthened, and the findings still held true even when only normal birth weight offspring were included, suggesting that prenatal PCB exposure in the absence of low birth weight may still contribute to the overweight phenotype [239]. Although it was assumed that increased weight at 6.5 years of age was caused by increased adiposity, confirmation was not provided [239].

In the current experiment, body composition analyses showed female offspring born to PCB exposed dams had increased adiposity as well as reduced lean body mass compared to offspring from vehicle treated dams despite comparable body weights. The observed adiposity is consistent with published data in mice which demonstrated coplanar PCB 77 augmented body weight gain, adiposity, and adipocyte differentiation [241]. There are previous reports in humans of PCB levels correlating with decreased muscle tone in females and from these studies, it appears evident hypotonia occurs preferentially in females [275-277]. Also, there is evidence that while *in utero* exposure best correlates with later life neurocognitive impairments, it is lactational exposure that best associates with hypotonia [258, 275, 276]. In the present study, male offspring displayed no such increased adiposity. In contrast to females, male offspring born to dams exposed to 1 μmole PCB 126 /kg body weight had decreased lean body mass compared to control males (Table 2.3).

It is interesting that with aging, the effects of PCB exposure on female offspring are attenuated. This could be indicative of a direct exposure effect in the females because without additional dietary exposure to PCBs the body burden would decrease mirroring the decreased difference in body composition observed between PCB exposed females and controls. The body composition effects in the male offspring persisted. Intriguingly,

there was no alteration in adiposity demonstrated at any time during the course of the study which is in agreement with prospective studies [200, 206, 208, 238, 239, 250, 258]. The majority of cross sectional studies found an increase in body weight in males [121, 260, 262]. The divergence between these cross-sectional studies and the present one may be attributed to a myriad of factors including timing and duration of exposure, nutritional status and of course species.

Timing of maternal exposure may be important in PCB-induced abnormal development, and consequently future studies will include cross-fostering of pups born to PCB-treated dams with those of vehicle-treated dams to further clarify compositional changes induced by exposure to PCB. Cross-fostering will allow us to establish whether PCB exposure *in utero* or during lactation precipitates these aberrant changes in body composition. If lactational exposure is deemed most detrimental, then perhaps steps could be taken to minimize lactational exposure such as formula feeding.

Results from the presented experiment are similar to findings in humans, thus supporting current and future use of this exposure model to assess effects of maternal PCB exposure on altered body composition in the offspring. In addition, this model can be utilized to determine mechanisms causing or regulating long-term outcomes in body composition. Future studies will investigate impacts these maladaptations of body composition will have on metabolic disease risks, specifically glucose tolerance. Because skeletal muscle and adipose tissue are key components of glucose disposal, one would suspect differences in body composition of female mice exposed to PCBs may predispose their offspring to impaired glucose tolerance at a young age and perhaps the male offspring too will display impairments in glucose tolerance as a result of their

decreased lean mass. My data clearly demonstrate that perinatal PCB exposure impacts body composition in young females and males of all ages, increasing adiposity and reducing lean body mass, respectively. Later life implications of such early PCB exposure and the development of obesity-associated pathologies remain uncertain.

Table 2.1 | Litter number, size and mean pup bodyweight

Dose of PCB 126 (µmole/kg)	# Deliveries	Mean # Pups per litter on PND 3	Mean Pup Weight (g) (SEM)					
			PND 3	PND 7	PND 14		PND 21	
					Male	Female	Male	Female
0	13/15	10.462 (0.781)	2.446 (0.0967)	5.473 (0.164)	11.429 (0.364)	11.299 (0.263)	17.082 (0.490)	16.242 (0.392)
0.5	15/15	9.133 (0.646)	2.527 (0.0839)	5.344 (0.145)	11.012 (0.308)	11.031 (0.257)	15.866 (0.396)	15.510 (15.510)
1	13/15	7.923* (0.560)	2.595 (0.132)	5.463 (0.259)	10.901 (0.473)	11.902 (0.370)	15.276 (0.724)	16.229 (0.713)
P	-	0.043	0.617	0.865	0.615	0.123	0.083	0.433

*, ** significantly different from control, $p<0.05$ and $p<0.01$, respectively

Abbreviations: PCB : polychlorinated biphenyl, PND : Postnatal day, SEM : standard error of the mean

Table 2.2 | Female Body Composition at 7 Weeks of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=12)**	**0.5 (n=15)**	**1 (n=10)**	***P***
Body Weight (g)	29.33 (0.80)	30.43 (0.71)	31.56 (1.41)	0.298
Fat Mass (g)	5.76 (0.52)	6.91 (0.45)	8.57** (0.94)	0.015
Fat (%)	19.37 (1.21)	22.45* (0.92)	26.54** (1.93)	0.003
Lean Mass (g)	20.52 (0.39)	20.69 (0.31)	20.33 (0.59)	0.830
Lean (%)	70.23 (1.09)	68.23 (0.81)	65.00** (1.70)	0.017

*,** Significantly different from 0 µmole/kg control, $p<0.05$ and $p<0.01$, respectively.

Table 2.3 | Male Body Composition at 7 Weeks of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=10)**	**0.5 (n=11)**	**1 (n=13)**	***P***
Body Weight (g)	39.58 (1.64)	37.69 (0.91)	36.82 (1.45)	0.368
Fat Mass (g)	6.72 (1.05)	7.20 (0.73)	6.76 (0.42)	0.876
Fat (%)	16.35 (1.91)	18.80 (1.61)	18.28 (0.77)	0.476
Lean Mass (g)	28.51 (0.64)	26.15* (0.39)	25.68** (0.91)	0.024
Lean (%)	72.59 (1.83)	69.66 (1.49)	69.96 (0.82)	0.286

*, ** significantly different from control, $p<0.05$ and $p<0.01$, respectively.

Table 2.4 | Female Body Composition at 6 months of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=12)**	**0.5 (n=15)**	**1 (n=11)**	***P***
Body Weight (g)	45.83 (2.26)	46.39 (2.14)	47.01 (2.25)	0.938
Fat Mass (g)	16.33 (1.80)	17.51 (1.74)	18.82 (1.53)	0.629
Fat (%)	34.49 (2.42)	36.33 (2.42)	39.46 (1.54)	0.365
Lean Mass (g)	25.23 (0.59)	24.55 (0.51)	24.18 (0.83)	0.529
Lean (%)	55.97 (1.91)	54.05 (1.96)	51.87 (1.19)	0.313

Table 2.5 | Male Body Composition at 6 months of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=10)**	**0.5 (n=13)**	**1 (n=12)**	***P***
Body Weight (g)	54.55 (1.69)	48.80* (1.67)	47.39* (2.34)	0.043
Fat Mass (g)	13.49 (0.89)	11.72 (1.57)	11.97 (1.34)	0.724
Fat (%)	24.18 (1.26)	23.16 (2.56)	24.87 (2.42)	0.896
Lean Mass (g)	35.03 (0.92)	31.39* (0.73)	29.73** (1.45)	0.007
Lean (%)	64.74 (1.30)	65.01 (2.17)	63.11 (2.00)	0.835

*, ** significantly different from control, $p<0.05$ and $p<0.01$, respectively.

Table 2.6 | Female Body Composition at 9 months of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=12)**	**0.5 (n=15)**	**1 (n=9)**	***P***
Body Weight (g)	52.43 (1.84)	51.91 (2.64)	52.93 (2.74)	0.960
Fat Mass (g)	22.69 (1.50)	23.03 (2.17)	24.35 (1.69)	0.723
Fat (%)	42.78 (1.43)	42.48 (2.83)	45.74 (1.19)	0.334
Lean Mass (g)	26.24 (0.41)	25.55 (0.59)	25.27 (1.13)	0.944
Lean (%)	50.48 (1.27)	50.73 (2.29)	47.92 (0.84)	0.414

Table 2.7 | Male Body Composition at 9 months of Age

	Mean (SEM)			
Dose of PCB 126 (µmole/kg)	**0 (n=10)**	**0.5 (n=12)**	**1 (n=10)**	***P***
Body Weight (g)	55.50 (2.02)	51.78 (1.72)	47.96 (2.47)	0.058
Fat Mass (g)	13.44 (1.43)	14.22 (1.39)	13.03 (1.74)	0.848
Fat (%)	23.67 (2.06)	26.85 (2.10)	26.83 (3.28)	0.601
Lean Mass (g)	36.24 (0.83)	32.18* (0.62)	30.08** (1.85)	0.004
Lean (%)	65.74 (1.66)	62.68 (1.73)	62.91 (2.56)	0.459

*, ** significantly different from control, $p<0.05$ and $p<0.01$, respectively.

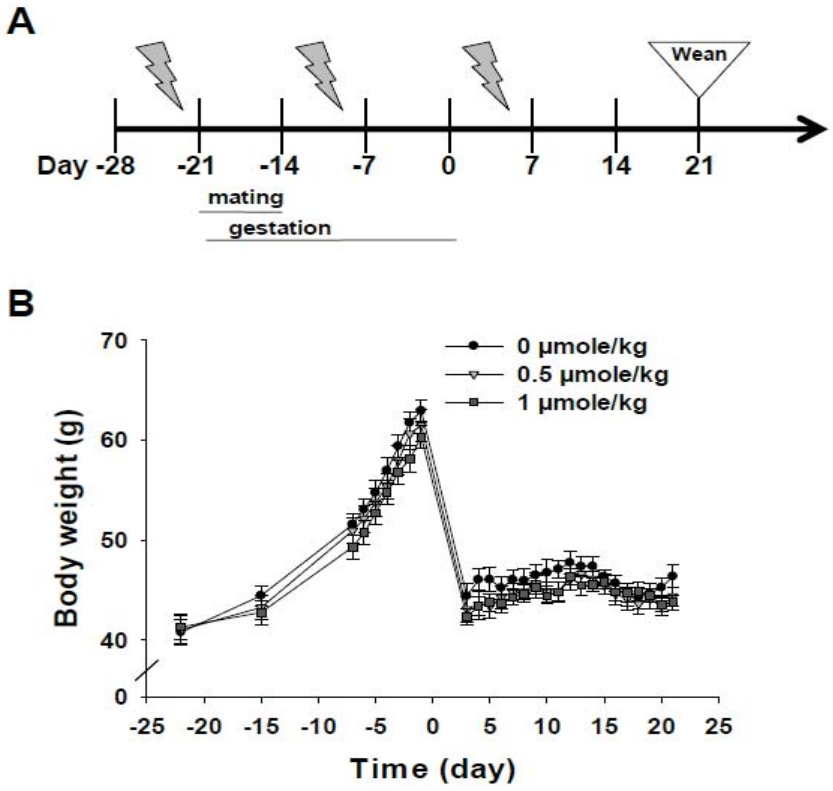


Figure 2.1 Maternal PCB 126 Exposure and Body Weight. A) Schematic diagram of exposure model. Dams were administered 0, 0.5 or 1.0 µmole of PCB 126 per kg body weight via oral gavage every 14 days beginning 48 hours prior to mating until approximately postnatal day 7, for a total of 3 exposures. Females were allowed to mate for 7 days; therefore, the exact developmental day of exposure was unknown. Day 0 signifies date of birth. Litters were delivered over a span of 7 days. Mice were weaned on postnatal day 21. B) Maternal body weight. Dam body weights were taken weekly prior to mating. During mating and gestation, dam body weights were taken daily until birth and again beginning on postnatal day 3 until weaning. There were no differences in maternal body weight between treatment groups (p=0.459).

CHAPTER 3

SEX-SPECIFIC IMPAIRMENTS OF GLUCOSE TOLERANCE AS A CONSEQUENCE OF PERINATAL POLYCHLORINATED BIPHENYL EXPOSURE

3.1 Abstract

Diabetes rates are increasing alarmingly. There is experimental evidence that environmental pollutants may be involved in diabetes etiology and there are plastic developmental periods in which xenobiotic exposure can have long term consequences on health. The present study investigated the effects of maternal polychlorinated biphenyl (PCB) exposure on offspring glucose homeostasis. Female CD-1 mice were orally gavaged biweekly with vehicle (safflower oil) or 1 μmole/kg coplanar PCB 126 beginning 48 hours prior to mating until weaning. Oral glucose tolerance tests were performed at 7 weeks, 6 and 9 months of age. Male offspring displayed impaired glucose tolerance at only 7 weeks of age while female offspring exhibited impaired glucose tolerance at 6 and 9 months of age. Interestingly, glucose intolerance in female offspring were dependent upon fasting time and route of glucose administration as prolonged fasting and intraperitoneal glucose administration resulted in glucose disposal rates comparable to control females. Insulin tolerance test was performed at 8 months of age and neither males nor females perinatally exposed to PCB 126 had attenuated insulin sensitivity. These data indicate sex-specific roles of perinatal PCB exposure on glucose homeostasis impairment. Further studies are needed to elucidate mechanisms involved in maternal PCB exposure-induced hyperglycemia and should investigate splanchnic disruptions that may be involved.

3.2 Introduction

Polychlorinated biphenyls (PCBs) are a class of persistent organochlorine pollutants that partition with lipids and biomagnify in the food chain resulting in dietary exposure [173, 174, 278]. Since production of PCBs was banned in 1976 and remediation has been established, their levels in the environment are decreasing, and consequently, dietary exposure and body burdens are also decreasing. Paradoxically, the incidence of diabetes has increased alarmingly over the past several decades and while advancements in understanding the mechanisms of hyperglycemia have been made, diabetes etiology remains elusive [181, 232]. Associations of diabetes and environmental pollutants in the form of organochlorines have recently been uncovered, but the findings across studies have been inconsistent.

Cross-Sectional studies of National Health and Nutrition Examination Survey (NHANES) data show clear associations between Type 2 diabetes mellitus (T2DM) and serum PCB levels [260, 279-281]. Everette et al found that PCB 126 was associated with diagnosed or undiagnosed diabetes, and also associated with prediabetes with HbA1c 5.9% - 6.4% [260, 279]. Turyk et al evaluated this association in Great Lakes fish consumers and found that dioxin-like PCBs but not total PCBs was associated with diabetes, but this finding did not hold true when adjusted for another organochlorine p,p'-diphenyl dichloroethene (DDE) [93]. Transnational studies confirm this positive association between PCBs, particularly dioxin-like PCBs, and diabetes [282-286]. These observed associations may be explained by an attenuation of xenobiotic clearance in diabetics, therefore one cannot conclude that PCB exposure is involved in diabetes etiology. Prospective studies are of better use in elucidating causality. Prospective

studies reveal increased risk of diabetes upon PCB exposure primarily in women [202, 287, 288]. However, two prospective case control studies failed to find an association between diabetes incidence and PCB levels; one was conducted in middle-aged Swedish women using only PCB 153 as an indicator of PCB exposure and the other was conducted from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study [259, 289]. Taken together, epidemiological studies are suggestive of a role of PCB exposure in the etiology of diabetes, particularly in women.

Transplacental transport of PCBs has been reported, and PCBs have been found in cord blood and breast milk indicating early life exposure beginning in utero [182] [188]. In an attempt to explain the paradox of decreasing exposure and increasing diabetes incidence, Lee at al hypothesized that low level exposure in utero may have pronounced epigenetic effects mediating diabetes susceptibility [261]. In fact, PCB exposure has been shown to be associated with low birth weight in some studies, and it is now well established that low birth weight offspring have an increased risk of developing diabetes later in life [182, 184, 200, 230, 248, 249, 290]. Despite the growing evidence of the importance of PCB exposure on glucose homeostasis, there are no reported investigations of perinatally exposed offspring and later life glucose tolerance.

In the present study, we employed a rodent model of perinatal PCB exposure which mimics body composition effects in young humans perinatally exposed to PCBs [291]. Previously, I have shown that mice born to dams exposed to a biweekly regimen of 1 μmole/kg PCB 126 displayed increased adiposity and decreased lean mass in female and male offspring, respectively. These alterations in body composition could have an impact of glucose tolerance, as increased adiposity may cause insulin resistance and

subsequent hyperglycemia. Additionally, a reduction in lean mass could also confer hyperglycemia as skeletal muscle is primarily responsible for glucose excursion. In the present study, I found that PCB exposure during the critical periods of periconception, gestation, and lactation engenders sex-specific and age-dependent perturbations of glucose homeostasis.

3.3 Materials and Methods

3.3.1 Animals and Diets The experiments were performed in accordance with an approved Institutional Animal Care and Use Committee protocol at the University of Kentucky. Experimental dams and offspring are the same as those in a published manuscript [291]. In summary, female ICR mice which had previously delivered one litter were purchased from Taconic at approximately 4 months of age. Dams were maintained on a low fat 10% Kcal diet (D12450B, Research Diets, New Brunswick, NJ) ad libitum for duration of study. One ICR sire was mated to two dams for one week. Pups were culled to 6 on postnatal day 3 and cross-fostering was employed whose litters had less than 6 pups to equilibrate nutrient availability. Mice were weaned onto standard chow (#2018, Teklad Global 18% Protein Rodent Diet) at 3 weeks of age and males were housed 3 per cage, and females were housed 5 per cage.

3.3.2 PCB exposure PCB 126 (3, 3', 4, 4', 5-pentachlorobiphenyl) was purchased from AccuStandard (C-126N, New Haven, CT). Dams were treated with either tocopherol-stripped safflower oil (vehicle) or 1 μmole/kg PCB 126 biweekly beginning 48 hours prior to mating [291].

3.3.3 Glucose tolerance test For all experimental procedures performed on offspring, only one male and one female per litter were used. At 7 weeks, 6 months, and 9 months of age glucose tolerance tests were performed on the offspring. Mice were fasted prior to glucose tolerance tests beginning at lights on. During glucose tolerance tests, mice were either orally gavaged (OGTT) or injected intraperitoneally (IPGTT) with 2 g/kg Dextrose (Bimeda, Le Sueur MN). Blood glucose was measured by tail vein venipuncture using an Ascensia Breeze 2 meter (Bayer, Mishawaka, IN) just prior to dextrose administration for baseline measurement. Blood was sampled again 15, 30, 60 and 120 minutes after glucose administration. For glucagon like peptide-1 (GLP-1) receptor antagonist studies, Exendin Fragment 9-39 (Ex-9) was purchased from Sigma (E7269) and administered at a dose of 5 μg in 200 μl sterile PBS 30 minutes prior to oral glucose tolerance test.

3.3.4 Insulin tolerance test Recombinant human insulin (Novolin) was used for insulin tolerance tests. Males and females were given 1.25 and 0.75 IU/kg insulin diluted in sterile phosphate buffered saline (PBS) intraperitoneally (IP), respectively. Mice were fasted at lights on for 3 hours prior to insulin administration. Blood glucose was measured via glucometer just prior to insulin administration and again 15, 30, 60, 120 and 180 minutes after insulin injection.

3.3.5 Pyruvate tolerance test Pyruvate tolerance tests were performed to indicate rate of gluconeogenesis. Pyruvate dissolved in sterile saline was injected IP at a concentration of 2 g/kg after 3 hours of fasting into female offspring. Blood glucose was measured in tail vein every 30 minutes for 3 hours. Again, the baseline measurement was taken just prior to pyruvate administration.

3.3.6 Statistics IBM SPSS statistics 20 software was used for repeated measures ANOVA for determining significance in experiments in which repeated samples were taken from a single mouse, as in the case of glucose, insulin and pyruvate tolerance tests. In the case that repeated measures identified a significant difference between groups, then OneWay ANOVA was performed for comparing the treatment groups at each time point. Areas under the curve were determined by SigmaPlot 12.0 software. Grubbs' Test was performed using GraphPad software to identify significant outliers which were excluded from further statistical analyses. Those animals whose area under the curve (AUC) was a significant outlier were removed for subsequent statistical analyses. Students T-test was used to determine significance between the areas under the curve of treatment groups.

3.4 Results

3.4.1 PCB-induced disruption of glucose homeostasis in gender specific and time-dependent At 7 weeks of age a glucose tolerance test was performed on both male and female offspring following a 3 hour fast. Male offspring displayed differences in glucose homeostasis (Figure 3.1A). Upon an oral glucose challenge, maternal PCB exposure resulted in impaired glucose tolerance in male offspring compared to controls. The AUC between the males born to dams exposed to 1 μmole/kg PCB 126 was increased 31% compared to controls, $p=0.041$ (Figure 3.1B) signifying attenuation of glucose disposal more than 4 weeks after the last perinatal PCB exposure. The PCB-exposed female offspring showed a trend toward impaired glucose disposal upon the experimental oral glucose challenge (Figure 3.1C) ($p=0.069$ and 0.162 for repeated measures ANOVA and AUC T-test, respectively). The female offspring born to PCB-exposed dams also had significantly elevated fasting blood glucose values.

At six months of age, both male and female offspring were fasted for 3 hours prior to administering oral glucose tolerance tests. Male offspring revealed no difference in glucose tolerance upon an oral glucose challenge (Figure 3.2A and 3.2B). The females born to dams perinatally exposed to 1 μmole/kg PCB 126 displayed impaired glucose tolerance, p=0.038 and p=0.055 for repeated measures ANOVA and AUC T-test, respectively (Figure 3.1D). Perhaps with a larger sample size, the AUC would have demonstrated statistically significant impairment in glucose homeostasis as well.

The male offspring control animals after 6 months of age displayed an overabundance of variability rendering it impossible to discern any statistically significant difference between them and PCB-exposed offspring regarding glucose tolerance. For this reason, male glucose tolerance data were not analyzed after 6 months of age and subsequent glucose tolerance tests were performed only on females born to vehicle and 1 μmole/kg PCB 126 exposed dams (Figures 3.4 and 3.5).

3.4.2 Insulin tolerance is comparable between treatment groups Insulin tolerance tests were employed to investigate whether impairments in glucose disposal were the result of insulin resistance. Blood glucose was monitored in both male and female offspring at 8 months of age following IP injection with insulin. Both sexes experienced declines in blood glucose at similar rates between vehicle and PCB exposed groups (Figure 3.3). Male offspring born to vehicle treated dams appeared to have higher levels of blood glucose beginning at baseline and continuing throughout the tolerance test, but the amount of variability in the controls made any difference statistically insignificant. The results from the insulin tolerance tests eliminate insulin resistance as an underlying mechanism of perinatal PCB-induced hyperglycemia.

3.4.3 Fasting time and route of glucose administration affect glucose tolerance in female offspring In aged females, glucose intolerance persisted in PCB exposed mice and was shown to reach statistical significance at 9 months of age (Figure 3.4). Interestingly, disruption of glucose homeostasis was only observed when the glucose was administered orally after a 3 hour fast (Figure 3.4A and 3.4B) because when glucose was injected IP, there were comparable glucose disposal rates between vehicle and PCB exposed offspring (Figure 3.4C). Additionally, when aged female offspring were administered glucose orally following an overnight 16 hour fast, there were again comparable rates of glucose disposal (Figure 3.4D). Taken together, these data illustrate disruption of an early event in glucose ingestion, one that occurs most likely prior to glucose appearance in the blood stream. This is further evidenced by the observation that the earliest time point after glucose challenge is the most severely impaired.

3.4.4 GLP-1 receptor antagonism does not extinguish PCB-induced glucose intolerance in female offspring Exendin Fragment 9-39 (Ex 9) is a competitive antagonist of the GLP-1 receptor. GLP-1 is secreted by L cells of the gut after a meal and signals in an endocrine fashion to slow gastric emptying and augment glucose-stimulated insulin secretion. The impairment in glucose tolerance is at the earliest time point, suggesting the bolus of glucose was absorbed into the bloodstream more quickly and completely in the female offspring born the PCB exposed dams than in the controls. Because GLP-1 slows gastric emptying, if impaired GLP-1 signaling was contributing to glucose intolerance, antagonism of the GLP-1 receptor would diminish glucose intolerance and result in comparable glucose tolerance curves, particularly at the 15 minute time point. The GLP-

1 receptor was inhibited by Ex-9 30 minutes prior to commencement of an oral glucose tolerance test. All mice were given Ex-9 due to the small sample size and inherent variability of the experiment. The effect of the Ex-9 was seen by the nearly identical 43% and 45% increase in blood glucose in the 30 minutes between Ex-9 injection and glucose administration. GLP-1 receptor antagonism mitigated much of the disturbance in glucose tolerance following an oral glucose challenge, p=0.225 and 0.468 for repeated measures and AUC, respectively. Antagonism of the GLP-1 receptor appeared to extenuate glucose intolerance yet the large impairment at 15 minutes post gavage remained, p=0.016 at that time point (Figure 3.5A).

3.4.5 Rate of gluconeogenesis is not different after 3 hour fast Hepatic glucose production (HGP) consists of glycogenolysis and gluconeogenesis, the latter utilizing pyruvate for de novo synthesis of glucose to be taken up by the periphery. The pyruvate tolerance test functions as an indirect measure of gluconeogenesis. Because glucose intolerance was observed after 3 hours of fasting and not 16 hours, this approach was used to investigate early initiation of HGP as a contributor to the glucose intolerant phenotype of perinatally PCB exposed female offspring. Figure 3.5B illustrates comparable blood glucose levels after pyruvate administration following a 3 hour fast, p=0.465 and 0.449, for repeated measures and AUC, respectively.

3.5 Discussion

The impairment in glucose tolerance observed at 7 weeks of age in perinatally exposed male offspring is likely due to direct exposure of PCBs through the breast milk. The partitioning of PCB 126 in goat kids upon maternal PCB exposure during gestation

and lactation revealed undetectable serum levels at birth but modest amounts at weaning [188]. Additionally, at 40 weeks of age, goat kids showed sequestration of PCB 126 in the liver as compared to adipose tissue and they determined its half-life to be 4 months [188]. Baker et al showed direct exposure of dioxin-like PCBs 77 and 126 elicit glucose intolerance and insulin resistance in male C57BL/6 mice [186]. Because insulin tolerance tests were not performed on the male offspring at the time of glucose intolerance, we cannot ascertain the role of insulin in PCB-induced hyperglycemia.

Female offspring born to PCB-exposed dams exhibited glucose intolerance at older ages, suggesting PCB-induced fetal programming of glucose intolerance rather than direct effects of PCB on glucose homeostasis. Impairment in glucose tolerance in female offspring upon an oral glucose challenge but not an IP glucose challenge, as well as its sensitivity to changes in fasting time, point to splanchnic system dysfunction rather than insulin resistance [192]. The insulin tolerance test results are evidence that perinatal PCB exposure does not impair insulin sensitivity. This is corroborated by Lee et al. who showed in a 2007 analysis of 1999-2002 NHANES data, that dioxin-like PCBs are not associated with HOMA-IR, an index of insulin resistance, in non-diabetic Americans [121]. Additionally, a case control study found no association between reference PCB 153 and insulin-dependent diabetes [292]. In further support of this is the fact that glucose reaches the pancreas in both routes of glucose administration, such that if there were impairment in glucose-stimulated insulin secretion, the disruption should be observed in both oral and IP glucose tolerance tests.

The pyruvate tolerance test reveals that gluconeogenesis is not affecting glucose tolerance, deducing hepatic glucose uptake as possible underlying mechanism of the

observed hyperglycemia. Studies have shown an involvement of the AhR in the development of the hepatic vasculature, specifically "failure of ductus venosus closure" resulting in hypoperfusion of the liver [293, 294]. If this effect of the AhR on liver development follows a U-shape, characteristic of endocrine disruptors, then it is possible that over activation of the receptor, too, could result in hepatic hypoperfusion and attenuated HGU. This phenomenon is also observed in hypoxic intrauterine growth restricted fetuses in order to spare nutrients for the proper development of more important organs like the brain [194, 226].

In conclusion, humans are exposed to persistent organic pollutants such as PCBs in our first environment, the womb of our mothers. Although this exposure continues for most likely the duration of our lives, the ramifications of early life pollutant exposure may not manifest until later in life. The present study demonstrated perturbations of PCB exposure on later life glucose homeostasis in both male and female offspring born and nursed to PCB exposed dams. More studies are needed to assess the contribution of pre-weaning xenobiotic exposure on the etiology of diabetes.

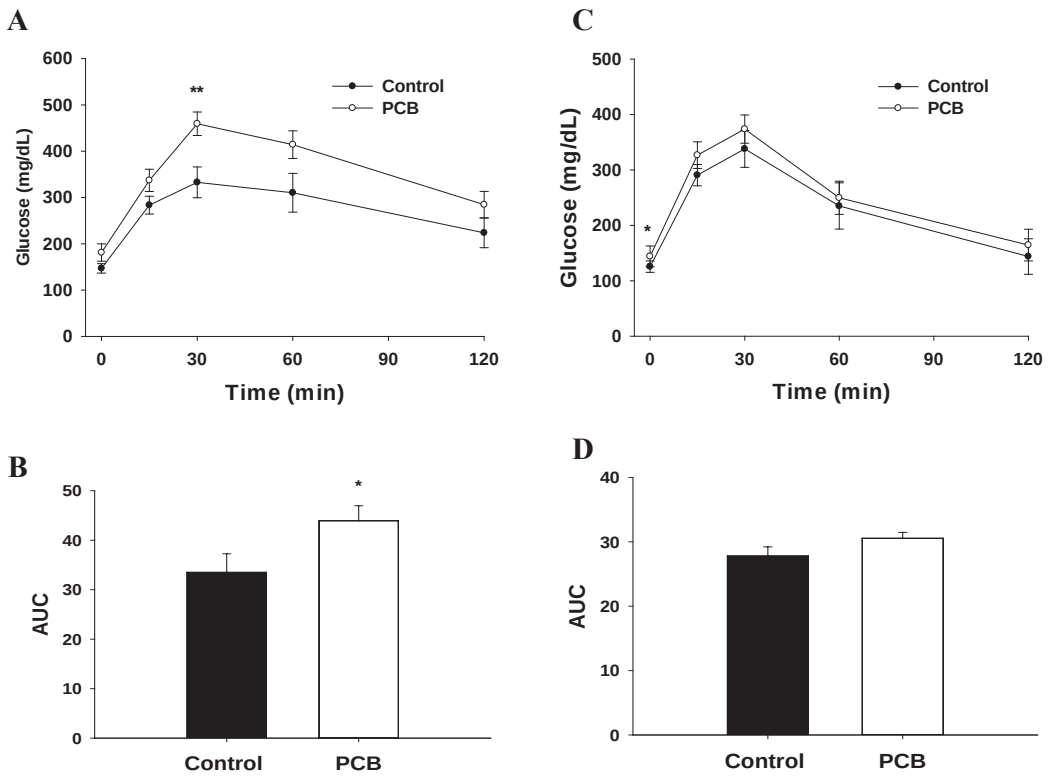


Figure 3.1. OGTT in offspring at 7 weeks of age. Mice were orally gavaged with 2 g/kg glucose after 3 hour fast. A) OGTT in male offspring; n=11 and n=12 for Control and PCB groups, respectively. Repeated measures p=0.038 B) Area under curve for OGTT of male offspring, $p<0.05$. C) OGTT in female offspring; n=11 and n=10 for Control and PCB groups, respectively. Repeated measures p=0.069. D) Area under curve for OGTT of female offspring. * indicates $p<0.05$ and ** indicates $p<0.01$.

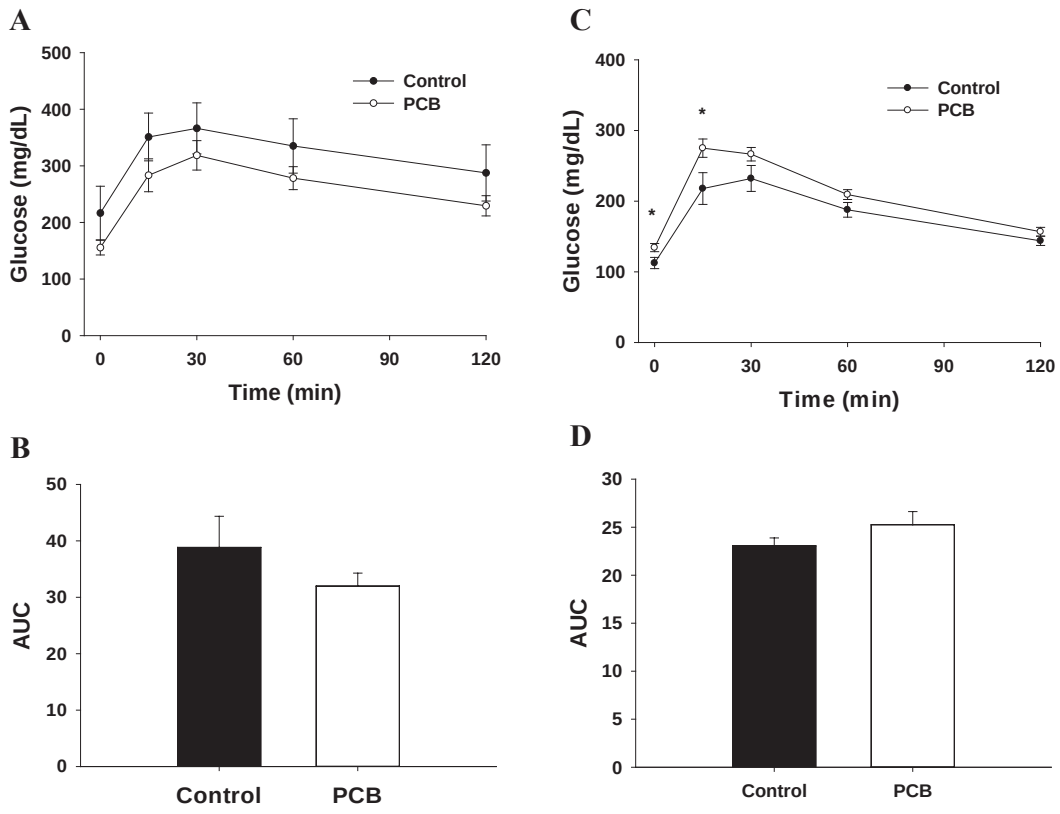


Figure 3.2. OGTT in offspring at 6 months of age. Mice were orally gavaged with 2 g/kg glucose after 3 hour fast. A) OGTT in male offspring; n=10 and n=11 for Control and PCB groups, respectively. Repeated measures p=0.494 B) Area under curve for OGTT of male offspring. C) OGTT in female offspring; n=11 and n=11 for Control and PCB groups, respectively. Repeated measures p=0.038. D) Area under curve for OGTT of female offspring, p=0.055. * indicates $p<0.05$.

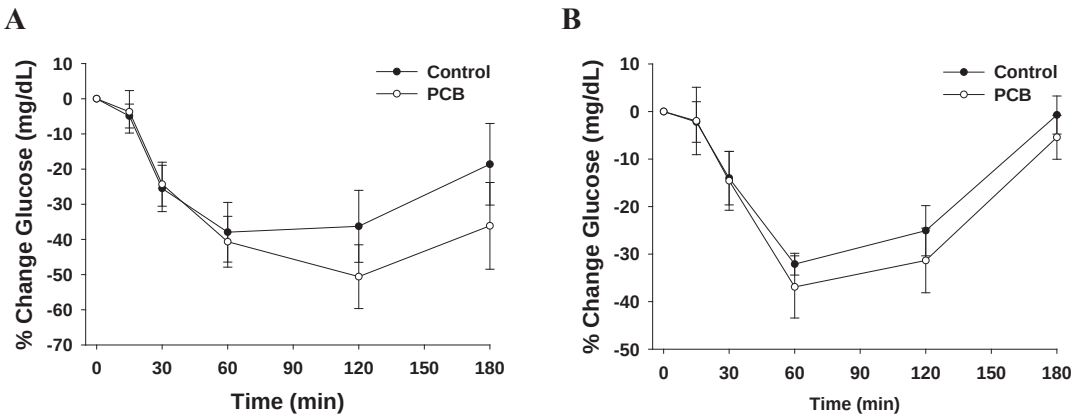


Figure 3.3. ITT in offspring at 8 months of age. Mice were fasted for 3 hours prior to insulin administration. A) Male offspring were IP injected with 1.25 IU/kg insulin; n=9 and n=10 for Control and PCB groups, respectively. B) Female offspring were IP injected with 0.75 IU/kg insulin; n=11 and n=10 for Control and PCB groups, respectively.

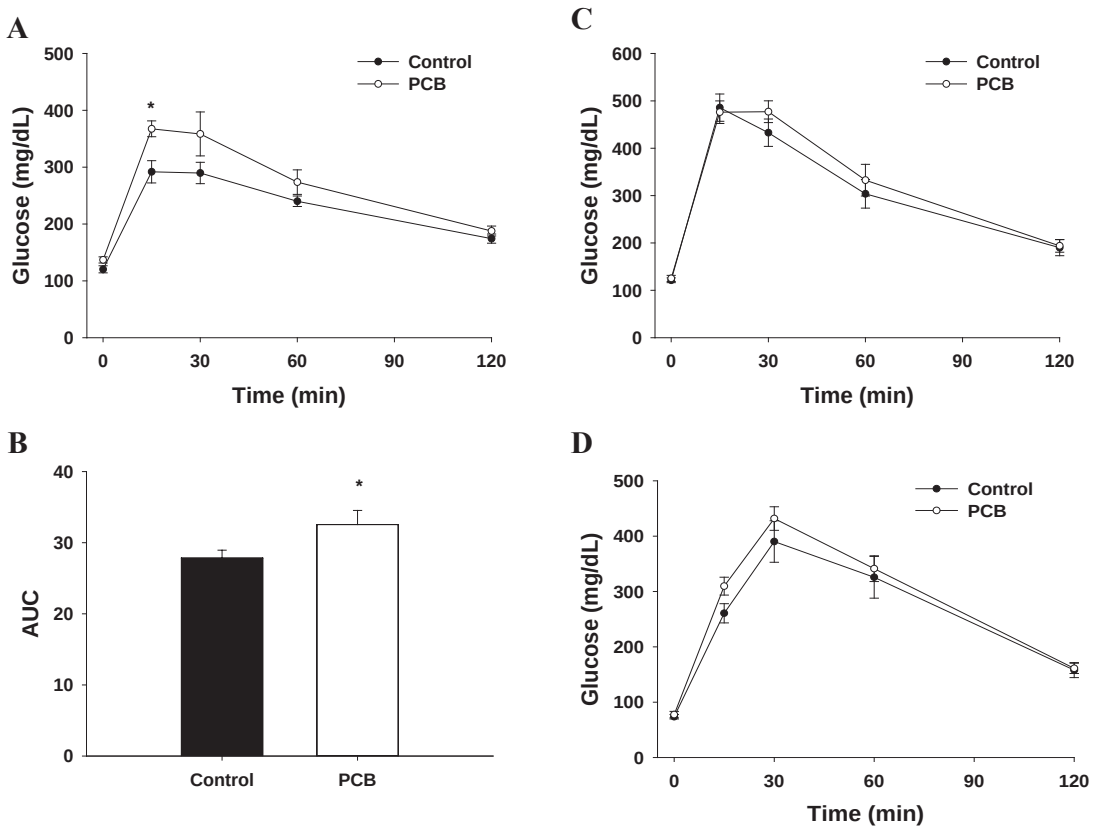


Figure 3.4. GTT in female offspring at 9 months of age. A) OGTT in female offspring; n=11 and n=9 for Control and PCB groups, respectively. Repeated measures p=0.019 B) Area under curve for OGTT of female offspring, $p<0.05$. C) IPGTT in female offspring; n=12 and n=9 for Control and PCB groups, respectively. D) OGTT of female offspring following an overnight (16 hour) fast; n=12 and n=9 for Control and PCB groups, respectively. * indicates $p<0.05$.

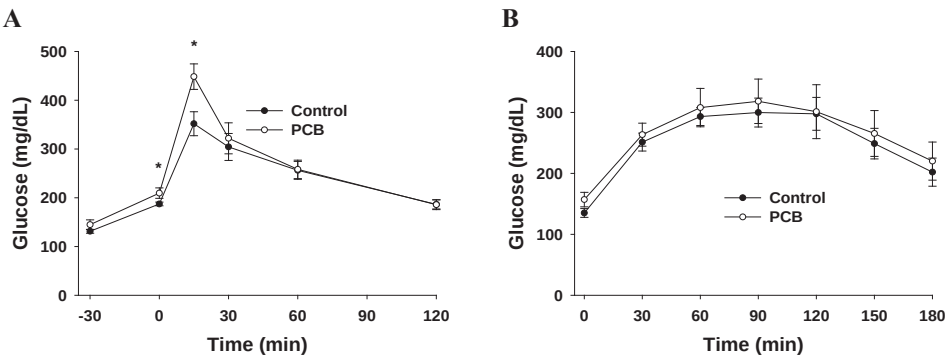


Figure 3.5. Splanchnic investigation. A) Female mice 1 year of age were administered GLP-1 receptor antagonist Ex-9 30 minutes prior to OGTT; n=12 and n=9 for Control and PCB groups, respectively. Repeated measures p=0.225. B) Pyruvate tolerance test was conducted on female offspring 1 year of age. Mice were injected IP with 2 g/kg sodium pyruvate and blood glucose measured every half hour for 3 hours; n=12 and n=9 for Control and PCB groups, respectively. * indicates $p<0.05$

CHAPTER 4

MATERNAL PCB EXPOSURE AFFECTS HEPATIC GENE EXPRESSION OF MOUSE DAM AND OFFSPRING BOTH IN UTERO AND AT 4 MONTHS OF AGE

4.1 Abstract

Polychlorinated biphenyls (PCBs) are environmental pollutants whose bioaccumulation and transplacental transfer results in exposure during critical windows of development which may cause reduced birth weight. The liver functions in macronutrient energy homeostasis and xenobiotic detoxification, and previous studies found changes in body composition and glucose tolerance upon perinatal PCB exposure. I therefore investigated effects of gestational PCB exposure on intrauterine growth and alterations in hepatic gene expression. Female ICR/CD1 mice were gavaged biweekly with 1 μmole/kg PCB 126 or safflower oil vehicle. In one cohort, females were euthanized at embryonic day 18 and pup and placental weights recorded. Also, livers were harvested from both non-pregnant and pregnant dams and cesarean pups. In a second cohort, dams were allowed to deliver and nurse their litters, and offspring livers were collected at 4 months of age (from breeding study in Aims 1-2). There were no differences in fetal body or placental weights. Absolute mRNA quantification revealed upregulation of dipeptidyl peptidase 4 along with canonically aryl hydrocarbon receptor (AhR) regulated genes cytochrome P450 (Cyp) 1a1, Cyp1b1, NAD(P)H quinone oxidoreductase 1 (NQO1) and nuclear factor erythroid-derived 2-like 2 (Nrf2) in livers of non-pregnant females, dams, and cesarean pups. PCB gavaged mice also displayed attenuated insulin-like growth factor binding protein 2 (IGFBP2) and alterations in genes involved in oxidative stress and macronutrient metabolism. Four month old offspring

exhibited decreased insulin-like growth factor (IGF) 1 expression and increased expression of Cyp1a1 and stress related genes, autophagy related protein 7 (Atg7) and DNA damage-inducible transcript 3 (CHOP) without any detectable PCB in their livers. Key findings in the study are significant changes in hepatic gene expression in adult offspring perinatally exposed to PCB even in the absence of any detectable parent compound or metabolite; and PCB-induced regulation of genes linked to metabolic disease states, DPP4 and IGFBP2.

4.2 Introduction

Polychlorinated biphenyls (PCBs) are a class of environmental pollutants whose lipophilicity and resistance to biodegradation enables biomagnifications in the food chain and resulting in lifetime of dietary exposure [181, 190]. PCB exposure begins in utero rather than after birth. These toxicants have been found in the placenta, cord blood and breast milk, signifying a continuum of exposure during perinatal development [278, 295-299]. Many animal studies employing PCBs often begin exposure after birth, which is not entirely exemplary of the human experience in that it dismisses a critical period when relatively small exposures are aggrandized and have potentially incessant repercussions. In fact, several studies in humans have correlated prenatal PCB exposure with low birth weight in offspring indicating PCB-induced developmental perturbations [182, 199, 200, 248-251].

Moreover, dioxin and dioxin like PCBs activate the AhR and studies have demonstrated a role of this xenobiotic receptor in hepatic organogenesis with AhR deficient animals exhibiting aberrant hepatic histology and attenuated perfusion [300-304]. Because the liver is the site of xenobiotic detoxification and central to energy homeostasis, I sought to investigate alterations of hepatic gene expression in murine offspring perinatally exposed to 3,3',4,4',5 pentachlorobiphenyl (PCB 126).

The classical and most studied ligand of the AhR is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or more commonly referred to as simply dioxin. Several studies have examined dioxin-induced alterations of hepatic gene expression [305-316]. Both in vitro and in vivo experiments confirm dioxin mediated induction of classical AhR responsive

genes cytochrome P450, 1a1 (Cyp1a1), 1b1 (Cyp1b1), and NAD(P)H dehydrogenase quinine 1 (NQO1) but genes involved in energy homeostasis and oxidant metabolism may be species and cell source specific [309, 311, 313-316]. AhR binding and nuclear translocation necessitates dioxin-induced hepatotoxicity and consequently studies have focused on dioxin-induced gene expression mediated by ligand binding [317-319]. However, Tijet et al in their investigation of TCDD-induced gene batteries demonstrated that some genes are regulated by AhR independent of TCDD, while others are regulated by TCDD when AhR is lacking, illustrating transcriptional modulation in the absence of canonical ligand:receptor activation [310].

Ovando et al. compared hepatic toxicogenomic effects of TCDD, PCB 126, and non-dioxin-like PCB 153 using a chronic exposure model in female Sprague Dawley rats. The rats were exposed to 100 ng/kg/day TCDD, 1000 ng/kg/day PCB 126, or 1000 μg/kg/day PCB 153 5 days per week for 13 or 52 weeks and of the 75, 70, and 9 genes, respectively, that were significantly regulated upon toxicant exposure at both time points, only 42 were shared between TCDD and dioxin-like PCB 126 [320]. These data accentuate the flaw in extrapolating dioxin-induced gene expression to dioxin-like PCB-mediated gene expression. Additionally, these studies fail to elucidate alterations in hepatic gene expression during fetal and postnatal development which contains critical periods of anatomical and epigenetic progression. As a result, PCB exposure during these windows of plasticity will likely exhibit changes in gene expression secondary and perhaps tertiary to AhR activation resulting from perturbed development. Previous studies employing this low dosing PCB exposure model have revealed sex-specific alterations in offspring body composition, and unpublished glucose and insulin tolerance

data alludes hepatic dysfunction. In the current experiment, I showed biweekly PCB 126 exposure at 1 μmole/kg alters hepatic gene expression in dam, fetal and offspring livers.

4.3 Methods:

4.3.1 PCB exposure during pregnancy These experiments were performed with the approval of the Institutional Animal Care and Use Committee at the University of Kentucky. Female CD1 mice were acquired from Taconic at approximately 4 months of age after having previously delivered one litter. Mice were fed a semi-purified low fat diet, 10% Kcal from fat (Research Diets D12450B) *ad libitum* beginning 2 weeks prior to mating. Male CD-1 breeders were also purchased from Taconic, and were fed low fat diet prior to mating. 48 hours prior to mating, female dams were exposed to male bedding in order to induce estrous. Female mice were then placed in the cage of a male breeder. Mating was permitted to commence overnight, at which time male mice were removed. On embryonic day zero dams were gavaged with 1 μmole/kg PCB 126 (AccuStandard, C-126N New Haven, CT) dissolved in tocopherol-stripped safflower oil (Dyets # 403952 Bethlehem, PA). A second dose was administered 14 days after the first oral exposure. This exposure was then on embryonic day 14. On embryonic day 18.5, mice were sacrificed, and there was no dam that delivered her litter prior to takedown. Livers were taken from both pregnant and non-pregnant dams. Also, the number of fetuses per dam was counted. Fetuses were weighed as well as their respective placentas. Livers were excised from the 2 most distal fetuses on each uterine horn as the position of the fetus within the uterus affects nutrient availability and perhaps subsequent hepatic gene expression. Tissues were snap frozen in liquid nitrogen until further processing.

4.3.2 PCB exposure of 4 month old offspring *In* a separate cohort of mice (same breeding paradigm as Aim 1 and 2), female ICR mice which had previously delivered one litter were purchased from Taconic at approximately 4 months of age. Dams were maintained on a low fat 10% Kcal diet (D12450B, Research Diets, New Brunswick, NJ) ad libitum for duration of study. One ICR sire was mated to two dams for one week. Dams were exposed bi-weekly to tocopherol-stripped safflower oil (vehicle) or 1 μmole/kg PCB 126 from 48 hours prior to mating until weaning. Pups were culled to 6 on postnatal day 3 and cross-fostering was employed whose litters had less than 6 pups to equilibrate nutrient availability. Mice were weaned onto standard chow (#2018, Teklad Global 18% Protein Rodent Diet) at 3 weeks of age and males were housed 3 per cage and females were housed 5 per cage. In this separate cohort, mice were mated for 1 week rather than overnight, therefore the day of gestation which dams were gavaged cannot be determined. At 4 months of age, a group of these mice were sacrificed; livers were collected and snap frozen until further processing.

4.3.3 PCB quantification Approximately 45 mg of liver was homogenized in 200 μl deionized water using 3 stainless steel 5/32" grinding balls and Geno/Grinder (SPEX Sample Prep) at 1250 rpm for 45 seconds. Eight hundred microliters of cold acetonitrile and 50 μl of 10μM ^{13}C labeled PCB 126 were added to homogenate with the latter serving as an internal standard. This solution was vortexed for 5 minutes, sonicated for 30 seconds in water bathe sonicator, then centrifuged for 5 minutes at 15,000 rpm. Superntant was transferred to a glass vial. This disruption was repeated on the pellet twice with 1 ml cold acetonitrile and 1 ml 50% cold acetonitrile, respectively.

Supernatants were pooled, dried under nitrogen and reconstituted in 100 µl of 99/1 methanol/water + 0.5% formic acid + 0.1% ammonium formate solution.

Liquid chromatography-tandem mass spectrometry of the reconstituted samples from vehicle and PCB exposed animals was performed by Manjula Sankara under the supervision of Dr. Andrew Morris. The following is their protocol for this procedure.

> *PCB 126 and hydroxy PCB 126 were measured using a Shimadzu UFLC coupled with an AB Sciex 4000-Qtrap hybrid linear ion trap triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode. 13C12 PCB 126 was used as internal standard for PCB 126 and hydroxy PCB 126 measurements. The mass spectrometer was operated in the positive APCI mode for PCB 126 measurements and in negative ESI mode for hydroxy PCB 126 measurements with optimal ion source settings determined by synthetic standards. PCB 126 was analyzed using Kinetex 2.6 u C18, 100 A, 100 X 2.10 mm (Phenomenex) column. The mobile phase consisted of water as solvent A and acetonitrile as solvent B. For the analysis of PCBs the separation was achieved using a gradient of 20 % B to 60 % B for 1 min, 60% B to 100% B in the next 7 min, and maintained at 100% B for the last 2 min with a flow rate of 0.25 ml/min. MRM transitions monitored were as follows: 325.9/256.1; 325.9/254.1 and 325.9/184 for PCB 126; and 338/268.1; 338/196.1 and 338/265.7 for 13C12 PCB 126. In the MRM ion transition the precursor ion represents the $M^{+\cdot}$ and the product ion represents either $[M-Cl]^{+}$ or $[M-2Cl]^{+}$.*
>
> *Hydroxy PCB 126 was analyzed using Luna 3 u C18 (2), 100 A, 250 X 2.00 mm (Phenomenex) column with a flow rate of 0.25 mL/min. The mobile phase consisted of 75/25 of methanol/ water with formic acid (0.5%) and 5 mM ammonium formate (0.1%) as solvent A and 99/1of methanol/ water with formic acid (0.5%) and 5 mM ammonium formate (0.1%) as solvent B. Hydroxy PCB 126 was eluted using a gradient of 10 % B to 100 % B in 4 min and maintained at 100% B for the next 11 min. MRM transitions monitored were as follows: 340.8/340.9 for hydroxy PCB 126 and 386.8/340.9 for dihydroxy PCB 126. Precursor ion of the ion transition is a formic acid adduct: [M+FA-H]- and product ion is [M-H]-.*

4.3.4 RNA isolation Total RNA was extracted from liver samples using either Qiagen RNeasy mini kits or Qiagen RNeasy lipid tissue mini kit. Approximately 30mg of tissue, three stainless steel 5/32" grinding balls and appropriate volume lysis buffer as indicated in the instructions was added to 2 ml tubes. Samples were disrupted and homogenized using Geno/Grinder (SPEX Sample Prep) at 1250 rpm for 30 seconds. Total RNA was silica-membrane purified from lysed homogenate according to manufacturer's protocol. Tissue RNA quality was determined using Bioanalyzer and only RNA with an RNA Integrity Number above 7 was used for Nanostring gene expression analysis.

4.3.5 Nanostring gene expression Nanostring technology uses capture and reporter probes specific to an mRNA sequence in a codeset to quantify the number of message RNA in a given sample. This technology can quantify over 500 genes in a given sample simultaneously in a given codeset. We picked over a hundred genes to investigate changes in hepatic gene expression across the stages of development. We used 2 different codesets and 2 different quantities of RNA. For a complete list of genes in each codeset, reference Tables 4.8 and 4.9. One hundred nanograms of RNA were run on the first codeset in which RNA from pregnant dam and fetal livers were used. On the second codeset, 750 nanograms of RNA from non-pregnant dam livers and 4 month old offspring livers were used. All of the target genes in the first codeset that showed significant changes in gene expression were included in the second codeset, along with several others.

4.3.6 Quantification of gene expression Manipulation of the raw counts from Nanostring was required to take into account variability in the amount of RNA used, and also the relative expression to housekeeping genes. To do this, the sums of the positive controls

for each sample were averaged. The sums of the samples' positive controls were then divided by this mean to obtain the first scaling-factor. A samples' count for a particular gene was then subtracted by its maximum negative control value and this difference was then multiplied by the first scaling factor. Housekeeping genes whose p value between treatment groups was greater than 0.5 and whose coefficient of variation across treatment groups was determined to be less than 0.5 were selected as reference genes. For each sample, the geometric mean of the reference genes were calculated and averaged across samples. This mean was then divided by the sample's respective geometric mean to obtain the second scaling factor. The adjusted counts were then multiplied by this second scaling factor, and it is these final values that were used in statistical analyses of gene expression.

4.3.7 Quantitative Real-time PCR Quantitative Real-Time PCR (qPCR) was performed to validate NanoString nCounter results. Four hundred nanograms of RNA were reverse transcribed using qScript cDNA SuperMix (Quanta Biosciences 95048). Primers were purchased from Eurofins MWG Operon (Table 4.8). Real-Time PCR was performed using PerfeCta SYBR Green FastMix Low Rox (Quanta Biosciences 101414) master mix and Stratagene Mx3000P real-time instrument. The $2^{\Delta\Delta CT}$ method was used to calculate the relative quantity (RQ) with the mean of control samples as the calibrator.

4.3.8 Statistics Fetal and placental weights were averaged such that each dam had an average fetal weight and an average placental weight, and these means were used in statistical analyses of pregnant dam parameters (Table 4.1). Students T-test was used to make comparisons between PCB treated and control animals. Interactions between

gender and PCB treatment were determined using 2way-ANOVA in SigmaPlot 12.0 software.

4.4 Results

4.4.1 PCB treatment does not affect fetal or placental weights Biweekly PCB 126 exposure did significantly affect dam, fetus, or placenta weights (Table 4.1). There was however a trend toward increased placenta weights upon PCB exposure ($p=0.07$). Placental weights often correspond with birth weight, such that heavy babies have heavier placentas and small babies have lighter placentas. This same trend is evident in this exposure model; PCB exposed fetuses weigh more than control fetuses, although this is difference is not statistically significant ($p=0.226$). The decreased number of fetuses per dam may account for this slight increase in mean fetus and placenta weight, as parity affects fetal weight. My hypothesis that PCB exposure would cause lower birth weight did not come to fruition in this mouse model and PCB dosing paradigm, if one is to extrapolate the data from late gestation.

4.4.2 Quantification of PCB 126 and hydroxyl metabolite Figure 4.1 displays amount of parent compound detected in the livers of the samples tested. Female mice gavaged with PCB that did not become impregnated had the highest average liver burden of parent compound at 20.96 pmol/mg wet weight, followed by pregnant dams at 6.16 pmol/mg with 1 of 9, and 6 of 9 samples having PCB levels below the limit of detection, respectively. Only 1 of 9 fetal livers had detectable PCB 126 and its concentration was 53.52 pmole/mg. PCB levels in control animals were all below the limit of detection. Moreover, there was no hydroxy-PCB metabolite detected in any of the samples.

4.4.3 Differential gene expression in dams administered PCB 126 PCB exposure during pregnancy resulted in 25 of 120 genes being differentially expressed. The first codeset primarily included genes involved in detoxification, stress response, epigenetics and lipid and carbohydrate metabolism. Table 4.2 shows the genes that were changed upon PCB 126 exposure during pregnancy. The genes are arranged in ascending order regarding the PCB/control ratio, beginning with the genes most downregulated and ending with the genes most upregulated. This is the arrangement for all of the gene expression tables. Maternal PCB 126 increases expression of detoxifying enzymes Cyp1a1 and Cyp1b1 as is expected upon AhR activation. Furthermore, Nuclear factor erythroid-derived 2-like 2 (Nrf2) and NAD(P)H quinone reductase 1 (NQO1) are increased, signifying interplay between the Nrf2 and AhR transcription factors [225, 321]. In addition to xenobiotic detoxifying cytochrome P450s being increased upon PCB exposure, antioxidative gene expression was increased (glutathione-s-transferase a1 (Gsta1), glutathione-s-transferase mu 4 (Gstm4), glutathione peroxidase (Gpx1), and catalase (Cat)). The majority of genes whose expression was decreased were involved in lipid metabolism e.g. perilipin 2 (Plin2), carnitine palmitoyltransferase 1a (CPT-1A), acetyl-CoA carboxylase 1 (ACC1), and peroxisome proliferator-activated receptor alpha (Pparα). However, other genes such as insulin-like growth factor (IGFBP2) and lactase dehydrogenase A (LDHA) were also decreased.

4.4.4 Non-pregnant mice display modulation of macronutrient metabolism PCB exposed female mice that did not get pregnant revealed interesting gene expression patterns as well. Of the 129 gene included on the 2nd codeset, 26 were changed upon PCB exposure. Again, cytochrome P450 enzymes involved in xenobiotic detoxification, Cyp1a1 and

Cyp1b1, as well as Nrf2 and NQO1 were significantly increased compared to controls. Genes involved in lipid homeostasis were attenuated, including fatty acid synthase (Fasn) and ACC1 and glucose-6-phosphate dehydogenase (G6PD). There appears to be an increase in glucose uptake in the PCB exposed tissues as glucose transporter 1 (GLUT1) is significantly increased. GLUT2 is increased by 13% compared to controls also, although this increase is not statistically significant, p=0.060. Increases in glycolytic genes hexokinase 3 (HK3) and phosphoglycerate kinase 1 (Pgk1) indicate possible increased glycolysis, but the decreased expression of glycogen synthase 2 (Gys2) and glycogen phosphorylase (Pygl) suggest neither glycogen synthesis nor glycogenolysis is increased, respectively. Glycogen synthesis however is not particularly transcriptionally regulated, as phosphorylation events related to insulin and glucagon signalling are responsible for regulation of glycogenesis [6, 23]. The decreased expression of G6PD also suggests less divergence of phosphorylated glucose to the pentose phosphate pathway. Figure 4.2 is a Venn diagram of the genes that are differentially expressed in pregnant and non-pregnant mice. The genes expressed in both pregnant and non-pregnant animals administered PCB are those involved in detoxification, either xenobiotic or oxidative.

4.4.5 Fetal livers respond to PCB exposure in utero Any changes in fetal liver gene expression upon maternal PCB administration is either through direct transplacental exposure to the toxicants or a response to a disrupted intrauterine environment as a consequence of a maternal response to the dioxin-like PCB. Table 4.4 displays genes that are significantly changed between those conceived by dams exposed to vehicle versus those exposed to 1 μmole/kg PCB 126. Xenobiotic and oxidative detoxification genes

were increased in fetal livers maternally exposed to PCB including cytochrome P450s, Gstm4, NQO1, peroxiredoxin-1 (Prdx1), and Cu-Zn superoxide dismutase (SOD1), but genes involved in glucose homeostasis exhibited attenuated expression (GLUT1 and lactate dehydrogenase type A (LDHA)). Figure 4.1 compares and contrasts gene expression among livers from non-pregnant and pregnant PCB exposed dams and the livers of fetuses. Interestingly, excluding the 4 marker genes (Cyp1a1, Cyp1b1, Nrf2 and NQO1) only dipeptidyl-peptidase 4 (Dpp4) gene expression was increased. Dpp4 plays a major role in glucose homeostasis by rapidly degrading the gut hormone, or incretin, glucagon-like peptide 1 (GLP-1) [32, 40, 322]. GLP-1 is secreted upon feeding and slows gastric emptying and potentiates glucose-stimulated insulin secretion [322, 323]. Increased Dpp4 expression and translation could augment glucose excursion and contribute to hyperglycemia. Together, the upregulation of Cyp1a1, Cyp1b1 and NQO1 point to direct activation of the fetal AhR and transactivation of these genes afforded by transplacental transport of PCB 126.

4.4.6 Maternal PCB exposure alters hepatic gene expression in 4 month old offspring

Recall that these offspring were born to dams that were exposed to PCB 126 forty-eight hours prior to mating and biweekly thereafter until weaning. These offspring were thus exposed during lactation in addition to transplacental exposure in utero. Alterations in hepatic gene expression persist in these animals. Across gender, perinatal PCB exposure decreased expression of hypoxia-inducible factor 1a (Hif1a) and insulin-like growth factor 1 (IGF1) and increased expression of autophagy related protein 7 (Atg7), C/EBP homologous protein (CHOP), insulin-like growth factor 1 receptor (IGF1R), and Cyp1a1 (Table 4.5). The divergent expression of IGF1 and its receptor was likely compensatory.

CHOP and Atg7 are involved in endoplasmic reticulum stress and autophagy, respectively [324, 325]. Cyp1a1 expression suggests direct exposure to PCB 126 or constitutive activation of the AhR, perhaps mediated by epigenetic mechanisms. Both male and female offspring exhibit a general decrease in gene expression, Tables 4.6 and 4.7, respectively. Only four genes are different in male offspring born to vehicle or PCB exposed dams, and interestingly one is DNA methyltransferase 1 (Dnmt1). If there were decreased DNA methylation in these livers, one might expect increased gene expression, but this is not the case. Female offspring have 8 genes differentially expressed and only 2 are upregulated, Nrf2 and Peroxisome proliferator-activated receptor gamma (Pparg). Igf1 and the insulin receptor (Insr) are both decreased demonstrating a possible hypoproliferative or hypotrophic state in these livers. Two-way ANOVA revealed significant interactions between gender and 5 genes, Insr, beta-2-microgobulin (B2m), CPT1A, Prdx1, and the mineral corticoid receptor (MCR). Only MCR was differentially expressed in males while the others were specific to females.

4.4.7 NanoString nCounter results were validated with Real-Time PCR Quantitative real-time PCR was performed to validate gene expression. Cyp1a1 and NQO1 were selected as verifier genes because it was not feasible to perform RT-PCR for every gene that was changed and these 2 genes are expressed during canonical AhR activation. Real-time PCR shows changes in gene expression comparable to those observed using absolute mRNA quantification via NanoString nCounter (Table 4.9).

4.5 Discussion

The object of the present study was to elucidate changes in hepatic gene expression upon perinatal PCB exposure as may be secondary to PCB-induced intrauterine growth restriction and subsequent abnormal liver physiology. The effects of PCB 126 on gene expression in livers was compared in female CD-1 mice, pregnant CD-1 dams, gestational day 18.5 fetuses, and 4 month old male and female offspring. Over one hundred genes were selected on 2 Nanostring codesets representing various biological processes, including but not limited to, xenobiotic and oxidant detoxification, inflammation, epigenetics, and metabolism of carbohydrates and lipids. Also, to evaluate the contribution of ligand activated AhR-dependent gene transcription, PCB 126 was measured in these tissues.

Intrauterine growth restricted animals are often small in respect to their gestational age and also small infants are usually accompanied by small placentas [326]. Because studies have shown that PCB exposure is inversely associated with birth weight, we hypothesized that gestational PCB exposure would result in fetuses and placentas that were smaller at gestation day 18.5. In the present study, however, I did not observe statistically significant differences in fetal weight or placenta weight. In fact, there were trends toward increased fetal and placental weights which are likely due a decrease in the number of fetuses per dam in the PCB exposed animals, although this too is not statistically significant. One of the drawbacks to this animal model is that PCB levels in the dams are distributed across many fetuses, while in singleton offspring this not the case. As a result, the toxicant transfer to the developing murine fetus is likely less than expected in singleton pregnancies.

Direct exposure to 1 μmole/kg PCB 126 via oral gavage resulted in changes in gene expression similar to those in previous studies using TCDD for AhR activation. Ligand-activated AhR has consistently been shown to activate gene transcription of detoxifying enzymes Cyp1a1, Cyp1b1 and NQO1 as was confirmed in the current experiment [299, 308, 310, 320, 327-329]. Lipid metabolism was shown to be decreased with CPT1a, Plin2, ACC1 and Fasn. This is inconsistent with previous studies using TCDD which showed increased lipid accumulation in mouse livers [308, 309, 316, 320, 330, 331]. However, Ovando et al demonstrated that fatty acid binding protein 5 (Fabp5) which is involved in fatty acid uptake, transport and metabolism, while it is upregulated in response to dioxin, it is not upregulated in response to PCB 126 [320]. The increase in GLUT1, HK3, and Pgk1 and concomitant attenuation of GLUT4, glucose-6-phosphate dehydogenase (G6pd), phosphorylase (pygl), LDHA, and glycogen synthase (GYS2) gene expression signifies modulation of carbohydrate metabolism. These data suggest an increase in basal glucose uptake and phosphorylation. G6pd is involved in shunting of glucose metabolism away from glycolysis and toward lipid synthesis or the pentose phosphate pathway [6, 23]. Its decreased gene expression in PCB-exposed animals suggests utilization of glucose for glycolysis while the decrease in LDHA and glycogen synthase point to increased utilization of glucose metabolites for mitochondrial respiration and not lactate production. However, genes involved in monocarboxylate mitochondrial transport and metabolism were not included in the codesets. Dere et al showed that TCDD-induced AhR activation increases AhR DNA binding at intragenic regions associated with lipid and carbohydrate metabolism at 2 and 24 hours after exposure [312]. Nonetheless, alterations in energy homeostasis in response to dioxin is

time-dependent, and there are no studies that evaluated changes in hepatic gene expression after 96 hour exposure in response to PCB 126 as in the current experiment.

Dioxin induced hepatic immune cell infiltration was reported by Boverhof et al. 168 hours post exposure, corroborating the current observation of increased adhesion molecule expression [316]. Insulin-like growth factor binding protein 2 (Igfpb2) is decreased in both pregnant and non-pregnant mice directly exposed to PCB. This is of importance because Igfbp2 is thought to mediate some of the glucose normalizing effects of leptin [332]. Additionally, Igfbp2 overexpression protects against high-fat-diet-induced obesity, glucose intolerance, and insulin resistance. [333]. Hepatic Igfbp2 mRNA levels have been shown to correlate with circulating Igfbp2 levels and low serum Igfbp2 are associated with obesity and insulin insensitivity [334-338]. The attenuated expression of Igfbp2 therefore serves as a mechanism of obesogenic activity of PCBs. Obesity-associated insulin resistance is also associated with inflammation and it is noteworthy that PCB exposure increased intercellular adhesion molecule (Icam1) expression independent of pregnancy.

Fetal liver gene expression in response to in utero PCB exposure is as expected with regard to the classical increase in Cyp1a1, Cyp1b1, and NQO1. Elevated expression of NQO1 in response to AhR activation has been shown to be dependent upon its binding to Nrf2. Therefore, it is no surprise that Nrf2 is increased in response to PCB 126 exposure as other Nrf2 responsive genes are also increased. This experiment is the first to show increased hepatic Dpp4 expression upon PCB 126 exposure. This may be an event particular to PCBs as many microarray studies using TCDD have not reported alterations in hepatic Dpp4 gene expression. As Dpp4 degrades incretins such as GLP-1,

increased Dpp4 expression could result in impaired oral glucose tolerance. Incretins have a role in potentiating insulin secretion and increasing insulin sensitivity, so it would not be surprising to observe impairments in oral glucose tolerance as a result of perinatal PCB exposure.

The 4 month old offspring perinatally exposed to PCB still exhibited augmented Cyp1a1 gene expression without detectable PCB in their livers although Cyp1b1 and NQO1 levels were comparable between treatment groups. This phenomenon alludes to either an epigenetic modification at this particular locus permitting gene transcription in the absence of exogenous ligand or PCB-induced upregulation of the receptors unknown endogenous ligand. Augmented CHOP and Atg7 gene expression is indicative of physiological stress, particularly stress of the endoplasmic reticulum. Autophagy related proteins have been shown to be modulated by TCDD exposure in previous studies, but this is the first report of increased expression of transcriptionally regulated unfolded protein response protein, CHOP [339]. It could be the case that developmental exposure to PCB resulted in sustained oxidant production via xenobiotic detoxifying enzymes which caused eventual disrupted normal hepatic physiology. Endoplasmic reticulum stress has recently been shown to mediate some of the pathology associated with obesity and insulin resistance [340-347].

Also, in these aged offspring, again not taking into account gender, there is a significant decrease in Igf-1 expression. Tijet et al showed decreased Igf-1 gene expression in mouse livers upon TCDD exposure and interestingly, this decrease was also demonstrated in wildtype mice compared to AhR null mice in the absence of ligand [310]. So it is not entirely surprising that the current exposure model resulted in

attenuated Igf-1 gene expression in the absence of detectable ligand. Decreased Igf-1 expression in utero can result in intrauterine growth restriction and poor growth during early life [348, 349]. It is intriguing that decreased Igf-1 expression was not observed in fetal livers, but only postnatally. Postnatal growth is mediated not only by growth hormone, but also by Igf-1. These male offspring, upon body composition analysis did exhibit decreased growth, specifically regarding lean mass, as the males weighed less and displayed decreased grams of lean mass while relative lean mass was unaltered (Tables 2.3 and 2.5 in Chapter 2).

In conclusion, perinatal PCB exposure alters offspring gene expression in fetuses and 4 month old offspring. These results illustrate the need to consider the ramifications of developmental xenobiotic exposure on liver physiology and gene expression and accentuate the pitfall of inferring effects of TCDD with those of all dioxin-like compounds. Finally, further studies are needed to evaluate epigenetic and AhR coregulatory alterations that contribute to PCB-mediated effects on gene expression in the absence of detectable ligand within the context of developmental PCB exposure.

Table 4.1 | Dam Parameters

Parameter	Control (n=9)	PCB (n=9)	*p*
Dam Body Weight (g)	57.22 (1.14)	57.58 (0.96)	0.810
Number of Pups per litter (g)	11.78 (0.32)	11.00 (0.47)	0.193
Fetal Body Weight (g)	1.210 (0.026)	1.258 (0.029)	0.226
Placental Weight (g)	0.095 (0.004)	0.107 (0.005)	0.066

Table 4.2 | Pregnant Dam

Gene	Control	PCB	PCB/control ratio	P
IGFBP2	256.07	133.96	0.52	0.006
Plin2	790.91	528.69	0.67	0.044
LDHA	3103.82	2092.78	0.67	0.001
CPT-1A	781.70	549.31	0.70	<0.001
Acc1	96.26	70.96	0.74	0.048
SNAT2	376.16	292.17	0.78	0.004
Pparα	426.90	333.57	0.78	0.002
eEF2	1597.35	1826.19	1.14	0.032
Eif3f	237.87	283.87	1.19	0.010
Cyp3a11	5755.76	7439.70	1.29	0.012
Rplp0	1459.02	1907.39	1.31	0.005
Cat	3288.02	4329.15	1.32	0.006
Pgk1	180.87	241.95	1.34	0.012
Dpp4	50.39	71.75	1.42	0.024
Hat1	14.84	22.35	1.51	0.030
Gpx1	1642.59	2526.97	1.54	<0.001
Icam1	22.06	35.07	1.59	<0.001
Gstm4	19.51	39.30	2.01	0.003
Nrf2	116.90	277.02	2.37	<0.001
Cyb5r1	3.91	10.55	2.70	0.031
Cyp1b1	13.83	40.30	2.91	<0.001
NQO1	8.43	28.19	3.35	0.001
MCP1	2.08	11.84	5.69	0.026
Gsta1	2.01	24.36	12.15	0.002
Cyp1a1	1.35	6265.34	4648.60	<0.001

Table 4.3 | Non-Pregnant

Gene	Control	PCB	PCB/Control Ratio	p
GLUT4	76.98	14.47	0.19	0.010
G6pd2	135.04	36.61	0.27	0.015
fasn	10968.28	3253.73	0.30	<0.001
Acc1	2952.94	888.85	0.30	<0.001
G6pdx	581.57	186.37	0.32	0.022
IGFBP2	622.54	292.11	0.47	<0.001
GYS2	1290.93	848.91	0.66	0.011
Mecp2	48.37	36.05	0.75	0.049
PGC1a	83.92	63.73	0.76	0.008
Pygl	5736.99	4387.09	0.76	0.002
Eif3f	1565.58	1903.12	1.22	<0.001
Pgk1	910.35	1110.83	1.22	0.007
eEF2	8784.30	10807.17	1.23	0.002
Hprt1	95.25	118.61	1.25	0.012
Dpp4	898.49	1142.08	1.27	0.001
Tubb5	124.13	160.80	1.30	0.012
Gpx1	11491.81	14940.43	1.30	0.032
GLUT1	33.40	60.83	1.82	0.005
Hk3	48.64	97.40	2.00	0.005
Icam1	110.09	245.95	2.23	0.008
Nrf2	632.41	1450.06	2.29	<0.001
NQO1	97.96	250.12	2.55	<0.001
Cyp1b1	79.53	311.07	3.91	0.013
Cyp1a1	12.07	36241.14	3002.50	<0.001

Table 4.4 | Fetal Liver

Gene	Control	PCB	PCB ratio	p
LDHA	6524.58	4670.05	0.72	<0.001
GLUT1	420.59	330.55	0.79	0.045
Gapdh	3285.93	2699.88	0.82	0.004
GCR	197.48	165.98	0.84	0.031
B2m	2345.53	2689.96	1.15	0.018
Rxrα	242.99	280.12	1.15	0.044
SOD1	2652.56	3099.92	1.17	0.025
Hif1α	126.23	153.90	1.22	0.010
Prdx1	132.05	161.75	1.22	0.004
Dpp4	58.97	77.87	1.32	0.026
GSTM4	19.26	30.18	1.57	0.015
Nrf2	151.59	244.04	1.61	<0.001
NQO1	24.12	65.86	2.73	<0.001
Cyp1b1	11.36	44.50	3.92	0.002
Cyp1a1	1.30	3177.66	2444.82	<0.001

Table 4.5 | Offspring Liver

Gene	Control	PCB	PCB/control ratio	p
Igf1	2129.19	1612.56	0.76	0.002
Hif1a	754.07	684.29	0.91	0.044
Atg7	462.78	515.69	1.11	0.028
CHOP	134.94	165.03	1.22	0.028
IGF1R	32.60	52.80	1.62	0.031
Cyp1a1	0.99	874.24	884.85	0.022

Table 4.6 | Male Offspring

Gene	Control	PCB	PCB/control ratio	p
Dnmt1	65.03	43.33	0.67	0.011
MCR	148.25	103.58	0.70	0.029
Igf1	2271.27	1785.08	0.79	0.040
FATP4	57.53	46.86	0.81	0.030

Table 4.7 | Female Offspring

Gene	Control	PCB	PCB/control ratio	p
Hnf6	614.12	272.85	0.44	0.043
Igf1	2018.30	1474.18	0.73	0.027
Insr	1055.10	931.09	0.88	0.023
LRP1	6446.90	5668.68	0.88	0.048
Prdx1	1078.49	935.22	0.87	0.004
Pygl	9739.07	8686.65	0.89	0.044
Nrf2	1068.59	1304.09	1.22	0.017
Pparg	70.46	114.08	1.62	0.019

Table 4.8 | Primer Sequences for Real-Time PCR

Gene	Primer Sequence
Cyp1a1 Fwd	GAC TTC CAG CCT TCG TGT CA
Cyp1a1 Rev	GGG TTC TTC CCC ACA GTC AG
NQO1 Fwd	ACC CCA CTC TAT TTT GCT CC
NQO1 Rev	ACT TAC TCC TTT TCC CAT CCT C
Cltc Fwd	CAG TAA CAA CAA CCC AGA GAG
Cltc Rev	TCA TAA GCA ACA CAA GCC AG

Table 4.9 │Real-Time PCR Validation of Hepatic Gene Expression

	Relative Quantity[a] Mean (SEM)				
Gene	Pregnant	Non-pregnant	Fetal	Male Offspring	Female Offspring
Cyp1a1	1940.10** (470.65)	3761.74** (1010.46)	3056.80*** (448.24)	177.64 (91.18)	72.30 (49.56)
NQO1	3.84*** (0.33)	2.02** (0.28)	2.71*** (0.32)	2.94 (1.22)	0.71 (0.22)

a: Gene expression was calculated using $2^{-\Delta\Delta Ct}$ method with the mean of the control group serving as the calibrator

** indicates $p<0.01$

*** indicates $p<0.001$

Table 4.10 | Genes on 1st Codeset. Underline indicates a putative housekeeping gene and Bold indicates gene is not included in the other codeset.

Common Name	Gene Symbol	Gene Name
Acc1	Acaca	Fatty acid synthase
Adipoq	Adipoq	Adiponectin
Adipor1	Adipor1	Adiponectin receptor 1
Adrenomedullin	**Adm**	**Adrenomedullin**
Ahr	Ahr	Aryl hydrocarbon receptor
Hif1b	Arnt	Aryl hydrocarbon receptor nuclear translocator
Bmal1	Arntl	Brain and muscle ARNT-like protein 1 Aryl hydrocarbon receptor nuclear translocator-like protein
ATF6	Atf6	Activating transcription factor 6
Atg7	Atg7	Autophagy related protein 7
B2m	B2m	beta-2 microglobulin
Bax	**Bax**	**Bcl-2-associated X protein**
Bcl2	**Bcl2**	**B-cell lymphoma 2**
Cat	Cat	Catalase
MCP1	Ccl2	Monocyte chemotractant protein-1
p16(INK4a)	**Cdkn2a**	**Cyclin-dependent kinase inhibitor 2A**
Cltc	Cltc	Clathrin heavy chain 1
CPT-1A	Cpt1a	Carnitine palmitoyltransferase 1A (liver)
Creb1	Creb1	CAMP responsive element binding protein 1
Cyb5r1	Cyb5r1	NADH-cytochrome b5 reductase 1
Aromatase	**Cyp19a1**	**Aromatase**
Cyp1a1	Cyp1a1	Cytochrome P450, family 1, subfamily A, polypeptide 1
Cyp1b1	Cyp1b1	Cytochrome P450, family 1, subfamily B, polypeptide 1
Cyp3a11	Cyp3a11	Alpha-methyl acyl-CoA racemase
CHOP	Ddit3	C/EBP homologous protein (CHOP) DNA damage-inducible transcript 3
Dnmt1	Dnmt1	DNA methyl transferase 1
Dnmt3a	Dnmt3a	DNA methyl transferase 3a
Dnmt3b	Dnmt3b	DNA methyl transferase 3b
Dpp4/CD26	Dpp4	Dipeptidyl peptidase 4
eEF2	Eef2	Eukaryotic elongation factor 2
Eif3f	Eif3f	Eukaryotic translation initiation factor-3
Epx	**Epx**	**Eosinophil peroxidase**
Esr1	Esr1	Estrogen receptor alpha

Table 4.10 (continued)

ERRa	**Esrra**	**Estrogen-related receptor alpha**
fasn	Fasn	Fatty acid synthase
Foxa2	Foxa2	forkhead box protein A2
Foxo1	Foxo1	forkhead box protein O1
G6pc	G6pc	Glucose-6-phosphatase, catalytic
Gapdh	Gapdh	Glyceraldehyde 3-phosphate dehydrogenase
Gck	Gck	Glucokinase
Gpx1	Gpx2	Glutathione peroxidase 1
Gsk3b	**Gsk3b**	**Glycogen synthase kinase 3 beta**
Gsta1	**Gsta1**	**Glutathione S-transferase A1**
Gstm4	Gstm4	Glutathione S-transferase mu 4
Gusb	Gusb	Beta-glucuronidase
GYS1	Gys1	Glycogen synthase 1
GYS2	Gys2	Glycogen synthase 2
Hat1	Hat1	Histone acetyltransferase 1
Hdac1	Hdac1	Histone deacetylase 1
Hdac3	Hdac3	Histone deacetylase 3
Hdac4	Hdac4	Histone deacetylase 4
HGF	Hgf	Hepatocyte growth factor
Hif1a	Hif1a	Hypoxia-inducible factor 1-alpha
Hprt1	Hprt	Hypoxanthine-guanine phosphoribosyltransferase
BiP/Grp78	**Hspa5**	**Binding immunoglobulin protein heat shock 70 kDa protein 5**
Icam1	Icam1	Intercellular adhesion molecule 1
IDE	Ide	Insulin-degrading enzyme
Ifng	Ifng	Interferon-gamma
Igf1	Igf1	Insulin-like growth factor 1
IGF1R	Igf1r	Insulin-like growth factor 1 receptor
Igfbp1	Igfbp1	Insulin-like growth factor-binding protein 1
IGFBP2	Igfbp2	Insulin-like growth factor-binding protein 2
Ikbkb	**Ikbkb**	**Inhibitor of nuclear factor kappa-B kinase subunit beta**
Il10	Il10	Interleukin-10
Il1a	Il1a	Interleukin-1a
Il1b	Il1b	Interleukin-1b
Il2	**Il2**	**Interleukin-2**
Il6	Il6	Interleukin-6
Insr	Insr	Insulin receptor
Irs1	Irs1	Insulin receptor substrate 1

Table 4.10 (continued)

Irs2	Irs2	Insulin receptor substrate 2
KLF14	**Klf14**	**Krüppel-like factor 14**
LDHA	Ldha	Lactate dehydrogenase A
LDH B	Ldhb	Lactate dehydrogenase A
Lep	Lep	Leptin
Lipe	Lipe	Hormone-sensitive lipase
LRP1	Lrp1	Low density lipoprotein receptor-related protein 1
MafA / RIPE3b1	**Mafa**	**V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A**
c-MET/HGFR	Met	Met protooncogene
mtor	Mtor	Mammalian target of rapamycin
Nampt	**Nampt**	**Nicotinamide phosphoribosyltransferase**
Nfe2l2	Nfe2l2	Nuclear factor (erythroid-derived 2)-like 2
NQO1	Nqo1	NAD(P)H dehydrogenase [quinone] 1
PXR1	Nr1i2	Pregnane X receptor 1
GCR	Nr3c1	Glucocorticoid receptor
Oit1/FAM3D	Oit1	Family with sequence similarity 3, member D
PEPCK	Pck1	Phosphoenolpyruvate carboxykinase
Pgk1	Pgk1	Phosphoglycerate kinase 1
cPLA2	**Pla2g4a**	**Cytosolic phospholipases A2**
ZAC/PLAGL1	**Plagl1**	**Zac tumor suppresor gene**
Plin2	Plin2	Perilipin 2
Ppara	Ppara	Peroxisome proliferator-activated receptor alpha
Ppard	**Ppard**	**Peroxisome proliferator-activated receptor delta**
Pparg	Pparg	Peroxisome proliferator-activated receptor gamma
Ppargc1a	Ppargc1a	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Prdx1	Prdx1	Peroxiredoxin-1
Prdx6	**Prdx6**	**Peroxiredoxin-6**
Ptgs2	**Ptgs2**	**Prostaglandin-endoperoxide synthase 2**
RCAN1	Rcan1	Regulator of calcineurin 1
Rela	**Rela**	**V-rel reticuloendotheliosis viral oncogene homolog A**
Rplp0/36B4	**Rplp0**	**Ribosomal phosphoprotein, large, P0**
Rxra	Rxra	Retinoid X receptor alpha
SHBG	Shbg	Sex hormone-binding globulin
SIRT1	Sirt1	Sirtuin 1
FATP1	Slc27a1	Fatty acid transport protein 1
FATP4	Slc27a4	Fatty acid transport protein 4

Table 4.10 (continued)

GLUT1	Slc2a1	Glucose transporter 1
GLUT2	Slc2a2	Glucose transporter 2
GLUT4	Slc2a4	Glucose transporter 4
SNAT1	Slc38a1	Sodium-coupled neutral amino acid transporter 1
SNAT2	Slc38a2	Sodium-coupled neutral amino acid transporter 2
SOD1	Sod1	Superoxide dismutase 1
SOD3	Sod3	Superoxide dismutase 3
Opn	**Spp1**	**Osteopontin**
TCF7L2 variant 1	**Tcf7l2**	**Transcription factor 7-like 2**
Tnf	Tnf	Tumor necrosis factor alpha
Tubb5	Tubb5	Tubulin beta-4A chain
Txnrd2	Txnrd2	Thioredoxin reductase 2
UCP1	Ucp1	Uncoupling protein 1
Vcam1	**Vcam1**	**Vascular cell adhesion protein 1**
XBP1	Xbp1	X-box binding protein 1

Table 4.11 | Genes on 2nd codeset. Underline indicates a putative housekeeping gene and Bold indicates gene is not included in the other codeset.

Common Name	Gene Symbol	Gene Name
Acc1	Acaca	Fatty acid synthase
Adipoq	Adipoq	Adiponectin
Adipor1	Adipor1	Adiponectin receptor 1
AFP	**Afp**	**Alpha-fetoprotein**
Ahr	Ahr	Aryl hydrocarbon receptor
Hif1b	Arnt	Aryl hydrocarbon receptor nuclear translocator
Bmal1	Arntl	Brain and muscle ARNT-like protein 1
ATF6	Atf6	Activating transcription factor 6
Atg7	Atg7	Autophagy related protein 7
B2m	B2m	beta-2 microglobulin
Cat	Cat	Catalase
MCP1	Ccl2	Monocyte chemotractant protein-1
CHRNA7	**Chrna7**	**Cholinergic receptor, nicotinic, alpha 7**
Cltc	Cltc	Clathrin heavy chain 1
CPT-1A	Cpt1a	Carnitine palmitoyltransferase 1A (liver)
Creb1	Creb1	CAMP responsive element binding protein 1
Il8ra	**Cxcr1**	**Interleukin 8 receptor, alpha**
IL8rb	**Cxcr2**	**Interleukin 8 receptor, beta**
Cyb5r1	Cyb5r1	NADH-cytochrome b5 reductase 1
Cycs	**Cycs**	**Cytochrome complex, somatic**
Cyp1a1	Cyp1a1	Cytochrome P450, family 1, subfamily A, polypeptide 1
Cyp1b1	Cyp1b1	Cytochrome P450, family 1, subfamily B, polypeptide 1
Cyp3a11	Cyp3a11	Alpha-methyl acyl-CoA racemase
CHOP	Ddit3	C/EBP homologous protein (CHOP)
DKK1	**Dkk1**	**Dickkopf-related protein 1**
Dnmt1	Dnmt1	DNA methyl transferase 1
Dnmt3a	Dnmt3a	DNA methyl transferase 3a
Dnmt3b	Dnmt3b	DNA methyl transferase 3b
Dpp4/CD26	Dpp4	Dipeptidyl peptidase 4
eEF2	Eef2	Eukaryotic elongation factor 2
Eif3f	Eif3f	Eukaryotic translation initiation factor-3
Estrogen receptor 1	Esr1	Estrogen receptor alpha
fasn	Fasn	Fatty acid synthase

Table 4.11 (continued)

Fndc5	**Fndc5**	**Fibronectin type III domain-containing protein 5**
FoxA2	Foxa2	forkhead box protein A2
Foxo1	Foxo1	forkhead box protein O1
G6pc	G6pc	Glucose-6-phosphatase, catalytic
G6pd2	**G6pd2**	**Glucose-6-phosphate dehydrogenase 2**
G6pdx	**G6pdx**	**Glucose-6-phosphate dehydrogenase X-linked**
Gapdh	Gapdh	Glyceraldehyde 3-phosphate dehydrogenase
GBE1	**Gbe1**	**Glycogen-branching enzyme**
glucokinase	Gck	Glucokinase
Gckr	**Gckr**	**Glucokinase regulatory protein**
GLP1R	**Glp1r**	**Glucagon-like peptide 1 receptor**
Gpx1	Gpx1	Glutathione peroxidase 1
Gstm4	Gstm4	Glutathione S-transferase mu 4
Gusb	Gusb	Beta-glucuronidase
Gyg	**Gyg**	**Glycogenin**
GYS1	Gys1	Glycogen synthase 1
GYS2	Gys2	Glycogen synthase 2
Hat1	Hat1	Histone acetyltransferase 1
Hdac1	Hdac1	Histone deacetylase 1
Hdac3	Hdac3	Histone deacetylase 3
Hdac4	Hdac4	Histone deacetylase 4
HGF	Hgf	Hepatocyte growth factor
Hif1a	Hif1a	Hypoxia-inducible factor 1-alpha
Hk1	**Hk1**	**Hexokinase-1**
Hk2	**Hk2**	**Hexokinase-2**
Hk3	**Hk3**	**Hexokinase-3**
Hnf4a	**Hnf4a**	**Hepatocyte nuclear factor 4 alpha**
Hprt1	Hprt	Hypoxanthine-guanine phosphoribosyltransferase
Hsd11b1	**Hsd11b1**	**Hydroxysteroid 11-beta dehydrogenase 1**
Hsd11b2	**Hsd11b2**	**Hydroxysteroid 11-beta dehydrogenase 2**
Icam1	Icam1	Intercellular adhesion molecule 1
IDE	Ide	insulin degrading enzyme
Ifng	Ifng	Interferon-gamma
Igf1	Igf1	Insulin-like growth factor 1
IGF1R	Igf1r	Insulin-like growth factor 1 receptor
Igf2	Igf2	Insulin-like growth factor 2
Igfbp1	Igfbp1	Insulin-like growth factor-binding protein 1
IGFBP2	Igfbp2	Insulin-like growth factor-binding protein 2
Il10	Il10	Interleukin-10

Table 4.11 (continued)

Il1a	Il1a	Interleukin-1a
Il1b	Il1b	Interleukin-1b
Il6	Il6	Interleukin-6
Insr	Insr	Insulin receptor
Irs1	Irs1	Insulin receptor substrate 1
Irs2	Irs2	Insulin receptor substrate 2
LDHA	Ldha	Lactate dehydrogenase A
LDHB	Ldhb	Lactate dehydrogenase A
Lep	Lep	Leptin
Lipe	Lipe	Hormone-sensitive lipase
LRP1	Lrp1	Low density lipoprotein receptor-related protein 1
Mecp2	**Mecp2**	**Methyl CpG binding protein 2**
c-MET aka HGFR	Met	Met protooncogene
mtor	Mtor	Mammalian target of rapamycin
Nfe2l2	Nfe2l2	Nuclear factor (erythroid-derived 2)-like 2
NOX1	**Nox1**	**NADPH oxidase 1**
NPY1R	**Npy1r**	**Neuropeptide Y receptor type 1**
NQO1	Nqo1	NAD(P)H dehydrogenase [quinone] 1
Lxra	**Nr1h3**	**Liver X receptor alpha**
GCR	Nr3c1	Glucocorticoid receptor
MCR	**Nr3c2**	**Mineralocorticoid receptor**
Hnf6	**Onecut1**	**Hepatocyte nuclear factor 6 alpha**
PEPCK	Pck1	Phosphoenolpyruvate carboxykinase
Pgk1	Pgk1	Phosphoglycerate kinase 1
Phkb	**Phkb**	**Phosphorylase kinase, beta subunit**
Phkg	**Phkg1**	**Phosphorylase kinase, gamma 1**
Plin2	Plin2	Perilipin 2
Ppara	Ppara	Peroxisome proliferator-activated receptor alpha
Pparg	Pparg	Peroxisome proliferator-activated receptor gamma
Ppargc1a	Ppargc1a	Pparg coactivator 1-alpha
PRDM16	**Prdm16**	**PR domain containing 16**
Prdx1	Prdx1	Peroxiredoxin-1
Pygl	**Pygl**	**Glycogen phosphorylase**
RCAN1	Rcan1	Regulator of calcineurin 1
Rxra	Rxra	Retinoid X receptor alpha
SIRT1	Sirt1	Sirtuin 1
FATP1	Slc27a1	Fatty acid transport protein 1
FATP4	Slc27a4	Fatty acid transport protein 4

Table 4.11 (continued)

GLUT1	Slc2a1	Glucose transporter 1
GLUT2	Slc2a2	Glucose transporter 2
GLUT3	**Slc2a3**	**Glucose transporter 3**
GLUT4	Slc2a4	Glucose transporter 4
SNAT1	Slc38a1	Sodium-coupled neutral amino acid transporter 1
SNAT2	Slc38a2	Sodium-coupled neutral amino acid transporter 2
SOD1	Sod1	Superoxide dismutase 1
SOD3	Sod3	Superoxide dismutase 3
Sox9	**Sox9**	**SRY box-9**
Sry	**Sry**	**Sex determining region of chromosome Y**
Tsp1	**Thbs1**	**Thrombospondin 1**
Tnf	Tnf	Tumor necrosis factor alpha
Tubb5	Tubb5	Tubulin beta-4A chain
Txnrd2	Txnrd2	Thioredoxin reductase 2
UCP1	Ucp1	Uncoupling protein 1
WNT3	**Wnt3**	**Wingless-related MMTV integration site 3**
WNT7a	**Wnt7a**	**Wingless-related MMTV integration site 7a**
XBP1	Xbp1	X-box binding protein 1
Zhx2	**Zhx2**	**Zinc fingers and homeoboxes protein 2**

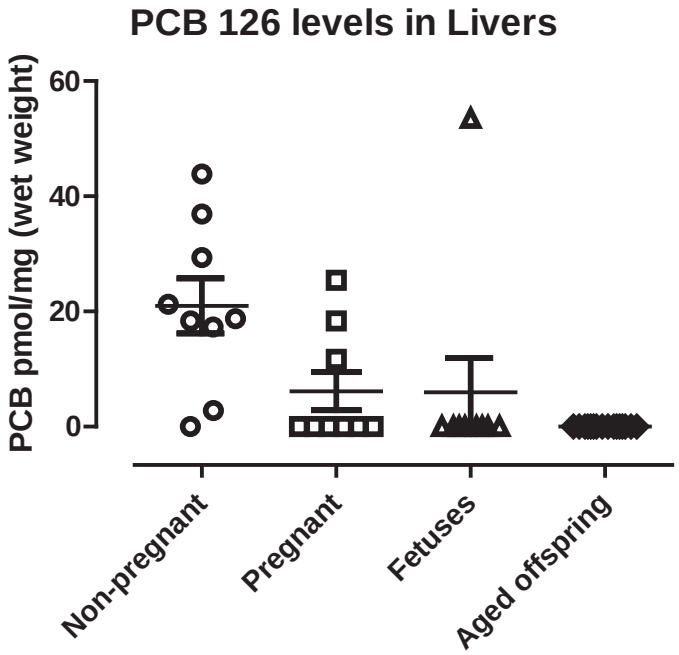


Figure 4.1. Measurement of hepatic PCB 126 levels. Graph illustrates quantity of PCB 126 parent compound in the livers of non-pregnant, pregnant females, fetuses and 4 month old offspring. Lines represent mean and the standard error of the mean. Aged offspring n=16; all others n=9.

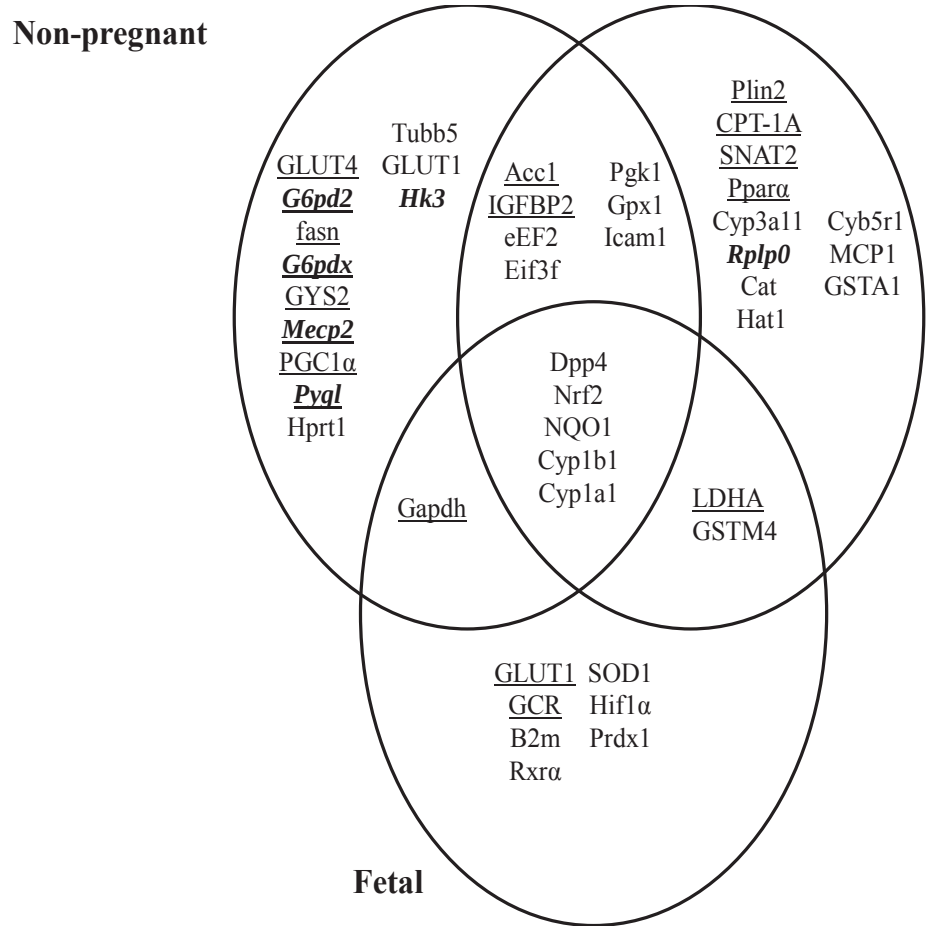


Figure 4.2 Venn diagram of genes differentially expressed in non-pregnant female mice, dams and E18.5 pups. Genes are arranged in ascending order beginning with the genes most down regulated in PCB exposed animals and ending in those genes that are most upregulated. Further, downregulated genes are underlined. Bold italics indicates that genes were included in only one of the two codesets.

CHAPTER 5

DISCUSSION

5.1. General Discussion

5.1.1. IUGR Biweekly exposure to 1 µmole/kg polychlorinated biphenyl (PCB) 126 had no effect on the initial measurement of pup weight, which was at postnatal day (PND) 3, suggesting that gestational exposure to the pollutant did not restrict fetal growth. I also mated the animals for 7 days to ensure copulation, but by doing so I introduced a confounding factor in that it was impossible to determine the length of gestation which can clearly have an effect on offspring size. Also, because we did not weigh the pups on PND 0, I cannot conclude for certain that birth weights were not different due to PCB exposure. I corrected for these factors in AIM 3 by 1) allowing coitus for a single night, thus all conceptions were on the same day; and 2) measuring body weights at embryonic day 18 (E18) thereby nullifying variations in gestation length. Embryonic day 18 fetal weights were not different between groups, confirming the finding in AIM 1, that biweekly exposure to 1µmole/kg PCB 126 does not cause intrauterine growth restriction in my mouse model. This of course is not to say that PCB exposure does not growth restrict the developing fetus as I only used one congener at a specific dosage. Nonetheless, there are findings in human studies that support that prenatal PCB exposure does not cause low birth weight [254].

Although the DOHaD hypothesis was predicated on changes of fetal growth and subsequent disease risk, it is now well accepted that fetal milieu modulation can affect offspring health irrespective of alterations in fetal growth. Comparable fetal growth in

between treatment groups means that changes in divergent phenotypes with aging are not contingent upon decreased birth weight. This adds to the impact of the presented research in that most births are of normal birth weight, and the majority of obese and diabetic individuals had normal birth weights.

5.1.2 Body composition AIM 1 investigated the role of perinatal PCB exposure on offspring body weight and composition. EchoMRI was first performed at 7 weeks of age at which time females from PCB exposed offspring showed dose-dependent increases in percent fat and reductions in percent lean mass while body weights were comparable between treatment groups. This metabolically unfavorable profile was no longer statistically significant at 6 months of age even though the trend was still there regarding the 1 μmole/kg dose (T-Test $p=0.089$ and $p=0.104$ for percent lean and percent fat, respectively). However, this decline in statistical significance continued. These data are consistent with published data in humans that demonstrate increased adiposity in young and adolescent females exposed prenatally to PCBs [208, 239]. Moreover, the one investigation of prenatal PCB exposure on adult female obesity found no association, again corroborating the present data on a lack of effect of perinatal PCB exposure on aged females [242]. Interestingly, ovariectomized rodents exhibit similar changes in body composition as was observed in females born to PCB exposed dams at 7 weeks of age. Particularly, ovariectomized rats have increased percent body fat and decreased percent lean mass [350, 351]. PCBs can modulate estrogen receptor activity [352, 353]. Because the effect on body composition in the females was only observed at the 7 week time point, when PCB body burden would have been greatest compared to the later time points, it is possible that the effects on body composition could have been secondary to

PCB disruption of estrogen activity thereby precipitating body composition profiles similar to those resulting from ovariectomization.

The male offspring born to PCB-exposed dams displayed dose-dependent attenuation of lean mass and only trends toward decreased body weights. Statistical comparison between vehicle exposed and the 1 μmole/kg PCB 126 groups only, showed significant reductions in body weight in addition to reductions in grams of lean mass for the duration of the study with no difference in fatness between groups meaning the reduction in lean mass in the male offspring is attributed solely to diminished lean mass. The lack of association between perinatal PCB exposure and obesity in male offspring is also supported by human studies that find significant associations only in female offspring. The diminished lean mass is a novel finding that warrants further investigation. The rate of childhood obesity is increasing alarmingly in America and perhaps perinatal PCB exposure is a contributing factor. Cross-fostering studies should be conducted to determine whether in utero or lactational exposure precipitates these phenotypes. Perhaps, supplementation of maternal or infant diet with protein can mitigate the reduction of lean mass. Further, muscle biopsies can be taken for histological purposes to evaluate whether the reduction in lean mass is due to decreased muscle fiber number or size.

5.1.3 Glucose tolerance AIM 2 investigated the role of perinatal coplanar PCB 126 exposure on offspring glucose tolerance. Interestingly the male offspring born to PCB-exposed dams exhibited impaired glucose tolerance upon an oral glucose tolerance test. This impairment was not observed, however, at 3 months of age or later time points, suggesting disrupted glucose homeostasis was due to direct exposure of PCBs as aging

would indeed decrease the body burden. The fact that impaired glucose tolerance was not observed late in life also excludes a possibility of developmental programming of glucose intolerance as would be predicted by the DOHaD Hypothesis. Animal studies have shown impaired glucose tolerance mediated by insulin resistance in male mice exposed to coplanar PCBs 77 and 126 [214]. Unfortunately an insulin tolerance test was not performed at this time so although likely, it is uncertain that PCB-induced impairment in glucose homeostasis was caused by insulin resistance. Initially, it was thought that this impairment was a result of diminished lean mass, but in the present study, the body composition phenotype persisted while the impairment in glucose tolerance did not.

Experimentally, there were some factors that may compromise the glucose tolerance results in the males. The male vehicle exposed offspring fought excessively and many had to be single-housed because of this. The stress of fighting and being single-housed could have impacted the glucose tolerance results. The PCB-treated males experienced delayed and more mild aggression resulting in unmatched housing conditions between groups. Additionally, some of the vehicle treated males exhibited severe disruption of glucose homeostasis with 3 hour fasted glucose levels above 300 mg/dL. This observance, as I understand it via personal communication, is a very rare one. Maternal ingestion of the safflower oil vehicle apparently disrupted glucose homeostasis in male offspring. Several studies use corn oil as a vehicle, while others lace a cookie with PCB. Perhaps these methods of PCB exposure should be used in future experiments in order to circumvent perinatal safflower oil-induced ablation of glucose homeostasis.

Female offspring exhibited impaired glucose tolerance only upon aging, with the first observance of glucose intolerance being at 6 months of age. This impairment persisted for the duration of the study which is suggestive of developmental programming. However, this impairment was most pronounced at the first time point of the glucose tolerance test, at 15 minutes. This spike in blood glucose at 15 minutes and a rapid recovery is not characteristic of a glucose tolerance curve with insulin resistance or impaired insulin secretion. A glucose tolerance test with i.p. administered glucose revealed comparable blood glucose regulation. These data suggest that PCB-mediated attenuation of glucose tolerance was not due to glucose-stimulated insulin secretion. Further, insulin tolerance tests demonstrated similar rates of decline of blood glucose between vehicle and perinatal PCB-exposed female offspring, thus confirming the absence of insulin resistance. An early event in glucose appearance may have been mediating the effects such as increased rate of gastric emptying, portal vein shunting by the ductus venosus, impaired first pass hepatic glucose uptake, delayed hepatic glucose production inhibition, or incretin dysfunction. The pyruvate tolerance test demonstrated no difference in hepatic glucose output via gluconeogenesis. The glucagon-like peptide (GLP) -1 receptor antagonism study is inconclusive as it was not controlled for properly due to low number of animals remaining and so should be repeated with larger sample size. In addition to repeating the GLP-1 receptor antagonism study, GLP-1 receptor agonist Exendin-4 should be used to further evaluate the involvement of this incretin in the observed phenotype. Future studies should also use a different vehicle because it is likely safflower oil had an effect on the female offspring because of the profound effects on males. A paraceptamol gastric emptying test could be performed to investigate if

different rates of gastric emptying are causing the increase in blood glucose at the early 15 minute time point. Cross fostering experiments again would aid in determining the significance of in utero versus postnatal exposure. Because poor diet is prevalent in developed countries and causal for obesity and diabetes, I propose weaning the a subset of the offspring onto a Western Diet determine if this exacerbates either PCB-induced obesity or impaired glucose tolerance.

5.1.4 Hepatic gene expression Absolute quantification of mRNA of liver tissue from vehicle and PCB treated pregnant and non-pregnant dams, E18 fetuses and 4 month old male and female offspring was measured using NanoString nCounter technology. The goal was not to make a direct comparison between PCB-induced effects during pregnancy versus those female mice that were not impregnated so as to investigate the effect of pregnancy on PCB-induced modulation of hepatic gene expression, therefore, two-way ANOVA's were not performed. The goal was to uncover possible mechanisms involved in disruption of glucose homeostasis as a result of maternal PCB exposure. Insulin-like growth factor binding protein 2 (IGFBP2) has been shown to play a role in both development of obesity and glucose tolerance evidenced by attenuated development of age and diet associated obesity and glucose intolerance in human-IGFBP2 overexpressing mice [333]. The decreased IGFBP2 gene expression was observed in PCB exposed female mice, indicating susceptibility to both obesity and perturbed glucose regulation. Further, euglycemic clamp studies reveal that IGFBP2 improves hepatic insulin sensitivity [334]. Moreover, increased dipeptidyl peptidase 4 (DPP4) expression in female livers, fetal livers, and the interaction of Dpp4 with gender reveal susceptibility of females to the glucoregulatory effects of incretins (reviewed in [322] and [323]).

DPP4 degrades incretins, and by reducing systemic levels, their beneficial effects may be compromised. These female specific changes in gene expression illustrate a role of PCBs in both obesogenicity and oral glucose intolerance. Finally, aged offspring displayed increased expression of endoplasmic reticulum stress response proteins DNA damage-inducible transcript 3 (CHOP) and autophagy related protein 7 (Atg7). Obesity causes chronic ER stress in liver and other tissues in humans and animal models [340-342]. Recent evidence demonstrates a role of ER stress in propagating metabolic derangements, and mitigation of ER stress has been shown to improve insulin sensitivity and glycemic control [341, 343-347]. The putative mechanism is that ER stress signaling causes c-Jun N-terminal Kinase (JNK) activation which attenuates insulin signal transduction and consequently insulin sensitivity [354-356]. However, the present study only showed increases in two ER stress-related gene transcripts. The activities of many ER stress proteins such as X-box-binding protein-1 (Xbp1) and activating transcription factor (ATF) 6 are not transcriptionally regulated, therefore future studies should utilize immunohistochemical techniques to further elucidate the extent of PCB-programmed ER stress, as well as Western blotting for phosphorylated JNK and components of insulin signaling.

A role of dioxins in gut homeostasis is emerging. The immune system of the gut plays a major role in whole body immunity as many foreign particles are ingested yet do not elicit immune response. Also, the gut is home to over a trillion commensal bacteria whose presence in the blood stream can cause many disorders including sepsis and death. The AhR is expressed abundantly in intestinal epithelial cells and may play a role in oral tolerance and gut barrier maintenance [357, 358]. Moreover, toll-like receptors that

recognize bacterial components have been shown to be involved in energy homeostasis [359]. Research also shows that the composition of the gut microbiome can predispose the host to obesity [360, 361]. Just this year, Choi et al. has shown that PCB ingestion alters the gut microflora to one which is metabolically unfavourable; the study also showed that exercise can attenuate the observed negative changes in bacterial composition [362].

5.1.5 Limitations One limitation of the presented research is that only one toxicant was used. Humans are exposed to a plethora of dietary xenobiotic compounds. Toxic equivalency factors are AhR binding affinities relative to dioxin and are used to provide an estimation of AhR-mediated toxicity. However, some xenobiotics do not bind the AhR but still mediate toxicological effects. Another limitation is that my exposure model is not consistent with the human experience in that humans have chronic low level exposure over many years that produce reported body burdens while my model used an acute exposure model in order to decrease body burden variability and expedite the findings. Because my dissertation is focused on perinatal effects of PCBs on offspring body composition and glucose tolerance, experimental animals were not exposed to PCBs after weaning. However, feeding the offspring a Western diet containing PCBs would more precisely mimic dietary exposure seen in humans and perhaps the results would provide for better extrapolation.

Appendix

Pilot Study

Introduction: The experiments presented in this dissertation investigates the effects of maternal PCB exposure on offspring body composition and glucose tolerance and further, investigates changes in hepatic gene expression. Before experiments were performed, there was a selection of dosage that occurred. Here I present data regarding the selection of our dose and the frequency of dosing. The dosing was selected based upon systemic inflammation occurring as a result of PCB exposure. Further, a preliminary study investigated the effects of maternal PCB exposure on fecundity.

Materials and Methods: The experiments were performed in accordance with an approved Institutional Animal Care and Use Committee protocol at the University of Kentucky. ICR/CD-1 mice were purchased from Taconic. In selection of my dose, mice were oral gavaged with either tocopherol-stripped safflower oil (Dyets # 403952 Bethlehem, PA) or PCB 126 (3, 3', 4, 4', 5-pentachlorobiphenyl from AccuStandard C-126N, New Haven, CT). Concentrations of 0, 1, and 5 μmole/kg PCB 126 was administered to female ICR mice via oral gavage and serum was collected either 24 hours or one week post exposure in separate cohorts. Serum cytokine levels were measured using Bio-Plex Pro Mouse Cytokine 23-Plex Panel (Bio-rad M60-009RDPD). Based upon the findings regarding PCB-induced inflammation, a preliminary study was conducted to investigate their effects on fecundity. Naïve females were purchased from Taconic and acclimated for one week prior to PCB exposure. Female mice were orally gavaged with either vehicle (tocopherol-stripped safflower oil) or PCB 126 at concentrations of 1 and 5 μmole/kg beginning 2 weeks prior to mating. This exposure

was repeated weekly for a total of 5 doses. The frequency was such that mice were exposed twice prior to mating, once at the commencement of mating, and twice again during gestation. Mice were not exposed during lactation. Oneway ANOVA was performed to test for statistical significance.

Results

Exposure to PCB 126 resulted in an acute inflammatory response with proinflammatory cytokines, TNF-α, Il-1 and Il-6, being elevated 24 hours after exposure but not at one week post exposure although these effects were not statistically significant (Figure S.1A-C). Because the inflammatory response appeared to be mitigated after one week, I exposed mice weekly to PCB. Weekly PCB exposure had dire effects on fecundity and rearing with PCB exposure affecting the number of pregnancies, and PND 2 litter number, size and mean body weight (Table S.1). On PND 28, at which time mice were weaned, there was a marked effect on body size specifically in the male offspring (Figure S.1D).

Discussion

This weekly dosing appeared to cause overt toxicity which was not my goal. My goal is to investigate subtle changes in PCB exposure on offspring metabolic parameters. These effects on offspring health were not conducive to subsequent experimentation due to its confounding effect on survival. As a result, PCB exposure was lessened in frequency. Also the 5 μmole/kg dose was removed from any further experimentation.

Table S.1 │Pilot Study: Weekly PCB Exposure on Fecundity and Survival

Dose of PCB 126 (µmole/kg)	#[a] Pregnancies	Postnatal Day 2 Mean (SEM)		
		# litters	Pups/litter	Body weight
0	19/20	19	10 (0.485)	2.207 (0.056)
1	17/19	6	3.5* (0.428)	1.967 (0.163)
5	3/18[b]	1	5 (0.000)	2.560 (0.000)
P	-	-	0.002	0.032

*, ** significantly different from control, $p<0.05$ and $p<0.01$, respectively.

a: Number of pregnancies based upon litter delivery

b: One of these pregnancies is based on body weight gain and not litter delivery

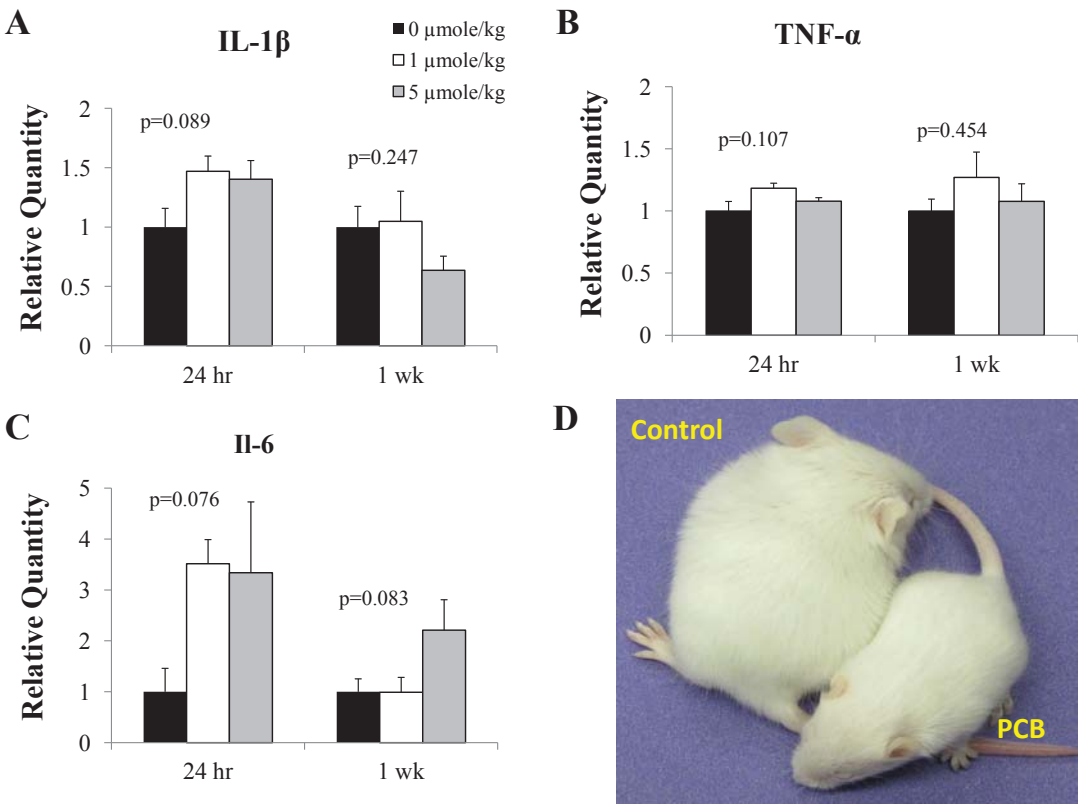


Figure S.1 Effect of PCB exposure on inflammation and offspring size. In selection of a dose, PCB 126 or vehicle was administered to female ICR mice via oral gavage. Serum cytokine levels were measured 24 hours after exposure and one week post exposure in separate cohorts. A-C show the amount of inflammatory cytokines in serum as fold change to vehicle exposed animals. The p values were determined via Oneway ANOVA. D is a picture showing the size difference among male offspring between treatment groups at weaning, which was at PND 28.

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Journal Publications:

1. Toborek M, Seelbach MJ, **Rashid CS**, Andras IE, Chen L, Park M, Esser KA. Voluntary exercise protects against methamphetamine-induced oxidative stress in brain microvasculature and disruption of the blood-brain barrier. (2013) Mol Neurodegener. Jun 24;8(1):22
2. **Rashid CS,** Carter LG, Hennig B, Pearson KJ. Perinatal polychlorinated biphenyl 126 exposure alters offspring body composition. (2013) Journal of Pediatric Biochemistry 3;47-53.

3. Sturgill TL, Strong D, **Rashid C**, Betancourt A, Horohov DW. Effect of propionibacterium acnes-containing immunostimulant on interferon-gamma (IFNγ) production in the neonatal foal. (2011) Vet Immunol Immunopathol. May 15;141(1-2):124-7.
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Manuscripts:

1. **Rashid CS**, Carter LG, Jarrell AL, Platt K, Yiannikouris F, Thatcher S, Cassis LA, Pearson KJ. Maternal PCB exposure affects gene expression in livers from dam, fetus, and aged offspring. (manuscript in preparation)
2. Epstein RI, **Rashid CS**, Jarrell AL, Carter LG, Platt K, Pearson KJ. Evidence of altered energy homeostasis in foreskins of infants born to smoking mothers. (manuscript in preparation)

Book Chapter:

The Neurology of AIDS, Third Edition. Edited by Howard E. Gendelman, Igor Grant, Ian Paul Everall, Howard S. Fox, Harris A. Gelbard, Stuart A. Lipton and Susan Swindells

3.2. *Endothelial cell biology and HIV-1 infection*, Michal Toborek, Ibolya E. Andras, **Cetewayo S. Rashid**, Yu Zhong, and Shinsuke Nakagawa

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2011 **Rashid CS**, Seelbach MJ, Chen L, András IE, Hennig B, Esser KA, Toborek M.
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Guest Lecture:

NFS 311— Nutritional Biochemistry—Fall 2012—"Epigenetics and Nutrition"

References:

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