

# Effects of Aryl Hydrocarbon Receptor Deficiency on PCB-77-Induced Impairment of Glucose Homeostasis during Weight Loss in Male and Female Obese Mice

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**BACKGROUND:** Lipophilic polychlorinated biphenyls (PCBs) accumulate with obesity, but during weight loss, liberated PCBs act as ligands of the aryl hydrocarbon receptor (AhR) to negatively influence health. Previous studies demonstrated that PCB-77 administration to obese male mice impaired glucose tolerance during weight loss. Recent studies indicate higher toxic equivalencies of dioxin-like PCBs in exposed females than males.

**OBJECTIVES:** We compared effects of PCB-77 on weight gain or loss and glucose homeostasis in male vs. female mice. We defined effects of AhR deficiency during weight gain or loss in male and female mice exposed to PCB-77.

**METHODS:** Study design was vehicle (VEH) or PCB-77 administration while fed a high-fat (HF) diet for 12 wk, followed by weight loss for 4 wk. The following groups were examined: male and female C57BL/6 mice administered VEH or PCB-77, female *AhR*<sup>+/+</sup> and <sup>-/-</sup> mice administered VEH or PCB-77, and male *AhR*<sup>+/+</sup> and <sup>-/-</sup> mice administered PCB-77. Glucose tolerance was quantified during weight gain (week 11) and loss (week 15); liver and adipose AhR and IRS2 (insulin receptor substrate 2) mRNA abundance, and PCB-77 concentrations were quantified at week 16.

**RESULTS:** PCB-77 attenuated development of obesity in females but not males. During weight loss, PCB-77 impaired glucose tolerance of males. AhR-deficient females (VEH) were resistant to diet-induced obesity. Compared with VEH-treated mice, HF-fed *AhR*<sup>+/+</sup> females treated with PCB-77 has less weight gain, and *AhR*<sup>-/-</sup> females had greater weight gain. During weight loss, *AhR*<sup>-/-</sup> females but not *AhR*<sup>-/-</sup> males treated with PCB-77 exhibited impaired glucose tolerance. In *AhR*<sup>-/-</sup> females administered PCB-77, IRS2 mRNA abundance was lower in adipose tissue compared with VEH-treated mice.

**CONCLUSION:** Male and female mice responded differently to PCB-77 and AhR deficiency in body weight (BW) regulation and glucose homeostasis. AhR deficiency reversed PCB-77-induced glucose impairment of obese males losing weight but augmented glucose intolerance of females. These results demonstrate sex differences in PCB-77-induced regulation of glucose homeostasis of mice. <https://doi.org/10.1289/EHP4133>

## Introduction

Accumulating evidence supports a role for environmental toxicants in the development of obesity, type 2 diabetes (T2D), and other metabolic conditions. A variety of basic research, including studies from our laboratory, have demonstrated that exposures to environmental toxicants, including dioxin-like polychlorinated biphenyls (PCBs) that are ligands of the aryl hydrocarbon receptor (AhR), impair glucose homeostasis (Jackson et al. 2017). These findings may be related to the observed bioaccumulation of these lipophilic toxicants in adipose tissue (U.S. EPA 2016; Airaksinen et al. 2011; Arrebola et al. 2013; Chevrier et al. 2000; Cok et al. 2008; Jackson 2017; Kim et al. 2011; Kodavanti et al. 1998; Lanting et al. 1998; Levitt 2010; Ludwicki and Górczlyk 1994; Obana et al. 1981; Oberg et al. 2002; Pestana et al. 2014; Peterson et al. 2015; Shen et al. 2009; Westrand and Norén 1998; Witt and Niessen 2000), which is a site of insulin resistance in T2D. Kim et al. (2011) found that total persistent organic pollutants (POPs) were higher in a sample of 71 obese individuals than in 18 lean women, suggesting that body burdens of

lipophilic toxicants may be increased in obese subjects with expanded adipose mass (Kim et al. 2011). Moreover, sequestration of lipophilic PCBs and other environmental toxicants in adipose tissue of obese subjects may have therapeutic implications, as studies suggest that lipophilic toxicants are liberated from adipose lipid stores during weight loss and can deleteriously influence health (Kim et al. 2011; Baker et al. 2015). As two-thirds of adults in the United States are overweight and/or obese (Flegal et al. 2016), and weight loss is a lifestyle modification with proven health benefits, negative influences of liberated toxicants may mitigate the health benefits of weight loss.

Anniston, Alabama had a production facility responsible for a large portion of the U.S. PCB production from the 1930s to 1970s. Unfortunately, Agency for Toxic Substances and Disease Registry investigations demonstrated high concentrations of PCBs in the environment and in local Anniston residents (ATSDR 2000). Initial data from a human health survey from the Anniston community demonstrated significant associations between elevated serum PCB levels and higher diabetes prevalence in women (Silverstone et al. 2012). The 2014 follow-up of the Anniston Community Health Survey, consisting of 388 surviving participants from the 2005–2007 baseline study, demonstrated significantly higher average total dioxin toxic equivalencies in serum of females compared with males (29.8 vs. 17.0 pg/g lipid, respectively) (Yang et al. 2018). While initial results from the Anniston health survey suggested a link between PCB exposures and T2D in females, health consequences of sex differences in levels of and responsiveness to PCBs are unclear, as most experimental studies using preclinical models have been conducted in males (Baker et al. 2013, 2015; Gadupudi et al. 2016; Murphy et al. 2016; Sipka et al. 2008; Wahlang et al. 2016).

Coplanar PCBs, similar to many other dioxins and dioxin-like compounds, are ligands of AhR (Arsenescu et al. 2008; Baker et al. 2013, 2015; Denison and Heath-Pagliuso 1998; Kim et al. 2012), a transcription factor that has been reported to mediate

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sex-dependent responses that can differ depending on species (Lee et al. 2015; Nault et al. 2017; Pohjanvirta et al. 1993, 2012; Prokopec et al. 2015). For example, while male rats were twofold more resistant than females to acute lethality of dioxin (Pohjanvirta et al. 1993), the opposite was found in mice, where, depending on murine strain, female mice were reported to be 10-fold more resistant to dioxin lethality than males (Pohjanvirta et al. 2012). In addition to sex differences in response to AhR ligands such as PCBs, effects of AhR deficiency on ligand-induced or dietary manipulation has been reported to differ between sexes (Kurita et al. 2016; Moyer et al. 2017). However, the role of AhR as a mediator of sex differences in relationships between PCB exposures and the development of T2D is unknown.

Previous studies from our laboratory demonstrated that PCB-77 promoted glucose intolerance in lean male mice (Baker et al. 2013). In obese male mice exposed to PCB-77, the benefits of weight loss to improve glucose homeostasis were attenuated upon liberation of PCB-77 from adipose tissue stores (Baker et al. 2013, 2015). Additionally, deficiency of AhR in adipocytes augmented the development of obesity (Baker et al. 2015). In this study, we defined effects of PCB-77 on body weight (BW) and glucose homeostasis in male and female mice during the development of obesity and then during weight loss. To determine the role of AhR, we examined effects of PCB-77 administration in male and female whole-body *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice during the development of obesity, and then determined effects of AhR deficiency on glucose homeostasis during weight loss in both sexes.

## Materials and Methods

### Chemicals

We purchased 3,3',4,4'-Tetrachlorobiphenyl (PCB-77) from Accu-Standard (Catalog # C-077N, 99.5% purity).

### Animal Treatments and Sample Collection

All experimental procedures met the approval of the Animal Care and Use Committee at the University of Kentucky. Mice were treated humanely and with regard for alleviation of suffering. Mice (three to five/cage) were housed in microisolator, polystyrene cages with a 14 h light/10 h dark cycle. Room temperature was at a range of 20–21°C, and humidity ranged from 30–70%. Whole-body AhR-heterozygous (*AhR*<sup>+/-</sup>) mice on a C57BL/6 background, originally purchased from the Jackson Laboratory (Stock # 002727), were a generous gift from H. Swanson (University of Kentucky). Female *AhR*<sup>+/-</sup> were bred to male *AhR*<sup>+/-</sup> to produce AhR whole-body wild type (*AhR*<sup>+/+</sup>) and deficient (*AhR*<sup>-/-</sup>) mice. Male and female *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> littermate controls (~2–4 months of age) were randomly assigned to study groups in all experiments. All mice were given *ad libitum* access to food and water. There were no overt differences observed in appearance or health between genotypes at the beginning of the study. The study utilized a total of 90 mice, of which *n*=61 were *AhR*<sup>+/+</sup>, and *n*=29 were *AhR*<sup>-/-</sup>. Of the AhR wild type mice, *n*=30 were female, of which half were orally gavaged with vehicle (VEH; tocopherol-stripped safflower oil) or PCB-77 [50 mg/kg, oral (Baker et al. 2013, 2015)]. For *AhR*<sup>+/+</sup> males, *n*=15 mice were administered VEH, and *n*=16 mice were orally gavaged with PCB-77 as a model AhR ligand demonstrated previously to impair glucose homeostasis of lean mice (Baker et al. 2013). This dose of PCB-77 (50 mg/kg) was demonstrated previously (Baker et al. 2013) to result in plasma levels (48 h after the last dose) that are comparable with

those observed in subjects from the Anniston Community Health Survey exhibiting a higher risk of T2D (Silverstone et al. 2012). To examine the effect of AhR deficiency on PCB-induced glucose impairment, AhR-deficient mice were administered PCB-77 (50 mg/kg, 5 mL/kg; oral; *n*=9 female; *n*=10 male). During the 12-wk period of weight gain, mice of each sex and genotype were administered VEH or PCB-77 as four separate once/day divided doses administered during weeks 1, 2, and then again during weeks 9 and 10. We chose this dosing regimen for PCB-77 administration based on previous results demonstrating impairment of glucose tolerance in obese mice losing weight following 4 wk of weight loss (5 wk after the last dose of PCB-77) (Baker et al. 2013, 2015). To promote the development of obesity, mice were fed a high-fat diet (HF; 60% kcal as fat, D12492; Research Diets Inc.) *ad libitum* for 12 wk.

To induce weight loss, mice of each genotype were fed a low-fat diet (LF; 14.6% kcal as fat, D12450B; Research Diets) from week 13 to 16. BWs were quantified weekly. At study end point, mice were anesthetized [ketamine/xylazine, 10/100 mg/kg, by intraperitoneal (i.p.) injection] for exsanguination and tissue harvest [liver, subcutaneous adipose, retroperitoneal adipose, epididymal adipose (EF), interscapular brown adipose tissue, soleus muscle, spleen, and heart].

### Measurement of Body Composition

Body composition (fat and lean mass) of conscious mice was determined by nuclear magnetic resonance spectroscopy [EchoMRI™ (magnetic resonance imaging)] as described previously (Baker et al. 2015). Briefly, the EchoMRI™-5000 Whole Body Composition (Echo Medical System) was calibrated with a known standard, and then conscious mice were gently placed inside a plastic tube containing holes for breathing for restraint in a horizontal plane. Three sequential scans, approximately 2 min in duration, were conducted for each mouse. Measurements were obtained at baseline (week 1), during week 12 (weight gain phase), and at study end point (week 16, weight loss phase).

### Glucose Tolerance Tests

Glucose tolerance tests (GTT) were performed during the development of obesity (week 11 of HF feeding) and during weight loss (week 15, 3 wk after switching mice to a LF diet). Mice were fasted for 6 h prior to the quantification of GTT. Mice were injected i.p. with D-glucose (Sigma; 15% in saline, 10 μL/g of BW), and blood glucose concentrations were quantified at 0, 15, 30, 60, 90, and 120 min. Blood was collected from the tail vein, and the glucose concentration was measured using a handheld glucometer (Accu-Chek Aviva Plus; Roche Diagnostics). Total area under the curve (AUC) was calculated by adjusting the baseline to each individual mouse's fasting blood glucose concentration at time 0. The total incremental area (between times 0 and 120 min) above baseline was calculated using GraphPad Prism (GraphPad Software, Version 7.02) and represented as arbitrary units.

### Quantification of PCB-77 in Tissues

At week 16 (end of weight loss phase), epididymal (EF) or ovarian fat and liver samples (20–40 mg) from mice of each treatment and genotype (*n*=three to four mice/group) were weighed and homogenized in deionized water (DIH<sub>2</sub>O), acetonitrile containing 1% acetic acid, and 50 μL of internal standard (5 μM <sup>13</sup>C-labeled d6-PCB-77). Homogenates were vortex-mixed and centrifuged at 4,000 rpm for 5 min. Samples were transferred to fatty dispersive 2 mL solid-phase extraction columns (Agilent Bond Elut QuEChERS; Agilent Technologies), vortex-mixed and centrifuged at 13,000 rpm for 4 min. The

supernatants were transferred to 4-mL glass vials, dried under nitrogen, and reconstituted in 100  $\mu$ L isooctane and vortex-mixed. Gas chromatographic analysis of PCB-77 and hydroxy-PCB-77 was performed using an Agilent Triple Quadrupole GC/MS gas chromatography–mass spectrometry (GC-MS/MS) 7000C system equipped with a multimode inlet and a HP-5ms ultra inert (UI) column (30 m length, 0.25 mm internal diameter, 0.25  $\mu$ m film thickness) in multiple reaction monitoring mode. Relative quantitation was measured by comparing peak area of the sample to peak area of an internal standard of known concentration.

### Quantification of Mono-Hydroxylated PCB-77 Metabolites in Plasma

At week 16 (end of weight loss phase), plasma (50  $\mu$ L) from mice of each treatment and genotype were homogenized in DIH<sub>2</sub>O. Cold acetonitrile and 50  $\mu$ L of internal standard (5  $\mu$ M <sup>13</sup>C-labeled 4-OH-PCB-159) were added to the homogenates. Samples were vortex-mixed for 5 min, sonicated for 30 s, and centrifuged at 15,000 rpm for 5 min. Supernatants from each sample were transferred to clean 4-mL glass vials. The remaining pellets were washed twice with 1 mL of cold acetonitrile and 1 mL of a 50:50 solution of acetonitrile:water. Samples were vortex-mixed for 5 min, sonicated for 30 s, and centrifuged at 15,000 rpm for 5 min. The supernatants for each sample were pooled and dried under nitrogen. Ethereal diazomethane (100  $\mu$ L) was added to each vial and left for 3 h at 4–8°C. Samples were then dried under nitrogen and reconstituted in dichloromethane (100  $\mu$ L). OH-PCB-77 was quantitated by reference to the OH-PCB-159 internal standard using gas chromatography (see above).

### Extraction of RNA and Quantification of mRNA Abundance

At week 16 (end of weight loss phase), total RNA was extracted from tissues (liver, periovarian for females, EF for males) using the Maxwell RSC<sup>®</sup> simplyRNA Tissue Kit (Promega Corporation) according to the manufacturer's instructions. RNA concentrations were determined using a NanoDrop<sup>™</sup> 2000 spectrophotometer (Thermo Scientific). We performed two-step reverse transcription polymerase chain reaction using the random decamer method for reverse transcription. cDNA was synthesized from 0.8  $\mu$ g total RNA with qScript cDNA SuperMix (Quanta Biosciences) in the following reaction: 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min. The cDNA was diluted to 4 ng/ $\mu$ L and amplified with an iCycler (Bio-rad CTX96 system) and PerfeCTa SYBR Green Fastmix for iQ (Quanta Biosciences). Using the difference from the reference gene (18S for liver;  $\beta$ -actin for adipose) and the  $\Delta\Delta$ Ct method, the relative quantification of gene expression in each sample was calculated. We used 18s rRNA as a reference gene, and Ct values were statistically analyzed between groups by analysis of variance (ANOVA) to confirm suitability for use as a reference gene across the sample set. The PCR reaction was 94°C for 5 min, 40 cycles at 94°C for 15 s, 58°C or 60°C (based on tested primer efficiency) for 40 s, 72°C for 10 min, and 100 cycles from 95°C to 45.5°C for 10 s. Primer sequences were as follows: *AhR*, forward 5'-AGT-AAAGCCATCCCGCTGAAGG-3', reverse 5'-CATCAAAGA-AGCTCTTGGCCC-3'; *IRS-2* (insulin receptor substrate 2), forward 5'-GCTGGTAGCGCTTCACTCTG-3', reverse 5'-GGACCTTGC-GTTTGCATCTC-3'; *CYP1A1* (cytochrome P450 1A1), forward 5'-AGTCAATCTGAGCAATGAGTTTGG-3', reverse 5'-GGC-ATCCAGGGAAGAGTTAGG-3'; *PEPCK* (phosphoenolpyruvate carboxykinase), forward 5'-CCACAGCTGCTGCAGAAC-3', reverse 5'-GAAGGGTCGCATGGCAA-3'; *TNF $\alpha$*  (tumor necrosis factor- $\alpha$ ), forward 5'-CCCACTCTGACCCCTTATCTC-3', reverse 5'-TCACTGTCCAGCATCTTGT-3'.

### Statistical Analysis

Data are represented as mean + standard error of the mean. Data were tested for normality using the Shapiro-Wilk test, and equal variance was tested using the Brown-Forsythe test. Outliers were not detected. If data did not pass normality using these approaches, data were logarithmically transformed. Two-way ANOVA (SigmaPlot, version 13.0; Systat Software Inc.) was used to define effects of PCB-77 treatment and *AhR* deficiency in male and female mice. Glucose tolerance tests and BWs were also analyzed using two-way ANOVA with repeated measures over time. Statistical significance was defined as  $p < 0.05$ .

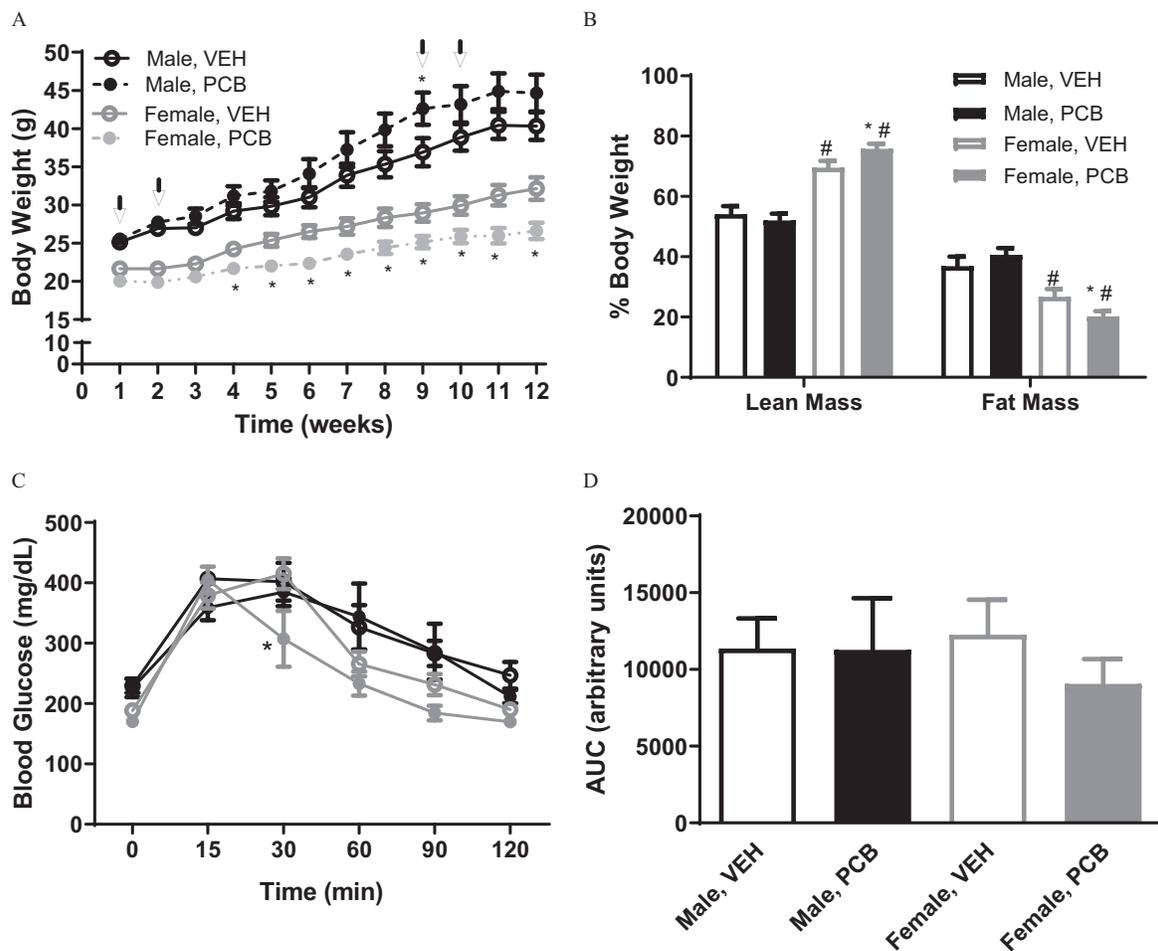
## Results

### PCB-77 Effects on Body Weight, Fat Distribution, and Glucose Homeostasis in Male and Female Mice Fed a High-Fat Diet

We defined effects of PCB-77 on the development of obesity and glucose homeostasis in age-matched male and female C57BL/6 mice fed a HF diet. Male mice fed a HF diet (VEH and PCB-77) were significantly heavier and had higher fat mass and less lean mass compared with age-matched females (Figure 1A,B;  $p < 0.05$ ). Compared with those treated with VEH, male mice administered PCB-77 did not differ in fat or lean body mass or glucose tolerance (Figure 1A–D). Male mice administered PCB-77 were slightly larger than those administered VEH; however, this difference was only statistically significant at week 9 (Figure 1A). In contrast, female mice administered the same dose of PCB-77 had significantly lower BW in response to the HF diet from weeks 4–12 (Figure 1A;  $p < 0.05$ ). As a percentage of BW, lean mass was significantly higher in HF-fed females administered PCB-77 compared with HF-fed VEH controls at week 9, while fat mass percentage was significantly lower in PCB-77-treated females (Figure 1B;  $p < 0.05$ ). Despite the significantly lower BW and fat mass of PCB-77-treated HF-fed females, glucose homeostasis, quantified at week 11 of HF feeding, was not significantly different from VEH female controls (Figure 1C,D;  $p > 0.05$ ).

### Effects of PCB-77 on Glucose Tolerance during Weight Loss in Male and Female Mice

Previous studies demonstrated that when obese male mice were made to lose weight, glucose tolerance was impaired in PCB-77-treated mice compared with VEH controls (Baker et al. 2013, 2015). These effects were associated with higher serum concentrations of PCB-77 when obese male mice lost weight. We contrasted effects of PCB-77 on glucose homeostasis in obese male and female mice experiencing weight loss. When male and female obese mice were fed a LF diet to induce weight loss, both sexes lost weight, and sex differences in BW, fat, and lean mass remained evident (Figure 2A,B;  $p < 0.05$ ). At week 16, there were no significant differences in BW, fat, or lean mass between mice administered VEH or PCB-77, regardless of sex (Figure 2A,B;  $p > 0.05$ ). During weight loss (week 15), glucose tolerance was significantly impaired in male mice administered PCB-77 compared with VEH controls (Figure 2C,D;  $p < 0.05$ ). In contrast, there was no significant effect of PCB-77 on glucose tolerance in female mice experiencing weight loss (Figure 2D;  $p > 0.05$ ), despite differences in blood glucose concentrations at 15 and 30 min during the glucose tolerance test (Figure 2C;  $p < 0.05$ ). Moreover, the AUC for blood glucose concentrations following a glucose tolerance test was higher for males than females, regardless of treatment (Figure 2D;  $p < 0.05$ ).



**Figure 1.** Effect of polychlorinated biphenyl (PCB)-77 on the development of obesity, body composition, and glucose homeostasis in male and female mice fed a high-fat (HF) diet. Male and female mice were administered vehicle (VEH) or PCB-77 on weeks 1, 2, 9, and 10 during a HF diet for 12 wk. (A) Body weight (BW) of HF-fed male and female mice (arrows indicate PCB dosing). (B) Lean and fat mass as a percentage of BW measured on week 9. (C) Blood glucose concentrations over time following a glucose bolus [intraperitoneal (i.p.)] measured on week 11. (D) Area under the curve (AUC) for data in (C). Data are mean  $\pm$  standard error of the mean (SEM) from  $n=6-10$  mice/sex/treatment. \* $p < 0.05$  compared with VEH within sex; # $p < 0.05$  compared with male within treatment. A repeated measures two-way analysis of variance (ANOVA) tested sex and PCB-77 treatment on the development of BW (A), and glucose homeostasis (C). A two-way ANOVA tested sex and PCB-77 treatment on lean and fat mass (B), and AUC for glucose tolerance tests (D).

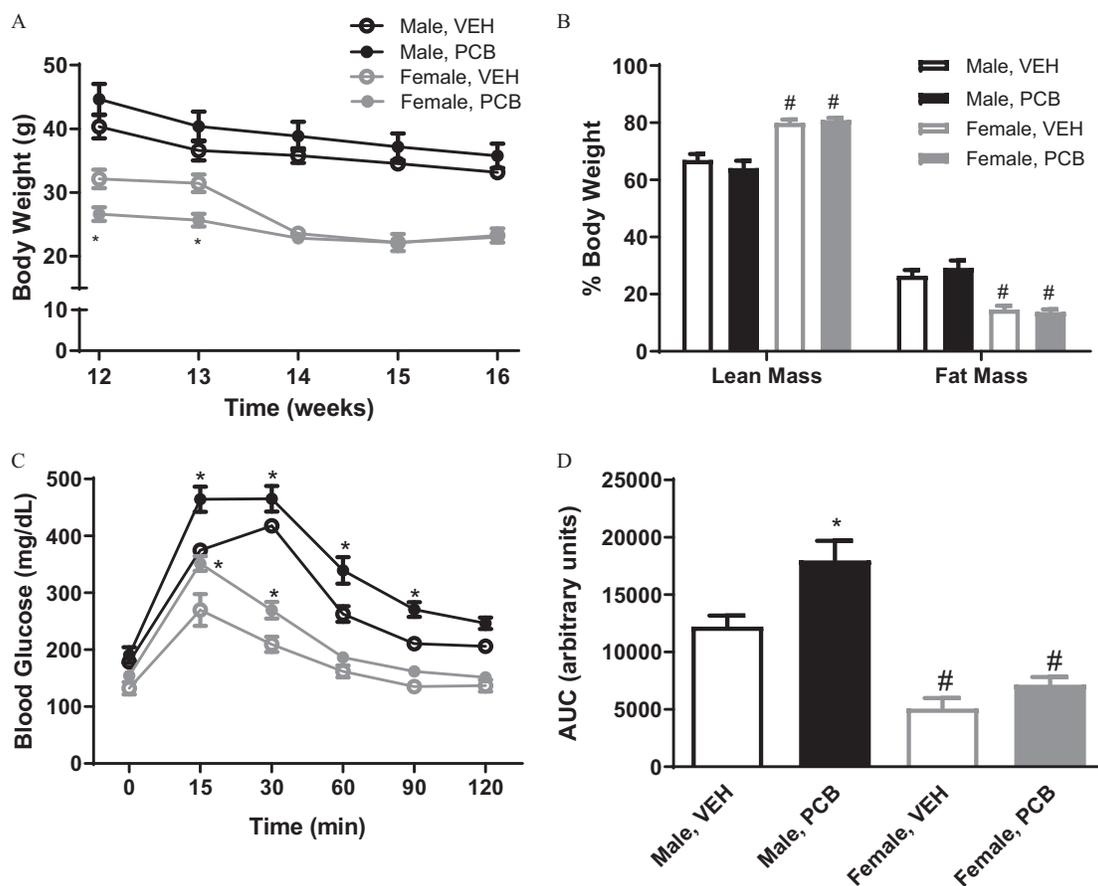
### Effects of AhR Deficiency on Body Weight and Glucose Homeostasis in Male and Female Mice Fed a High-Fat Diet and Administered PCB-77

Since effects of PCB-77 on glucose homeostasis of obese female mice have not been previously defined, in this study, we included groups of  $AhR^{+/+}$  and  $AhR^{-/-}$  female mice administered either VEH or PCB-77, and examined the development of obesity and weight loss–induced regulation of glucose homeostasis. Moreover, we contrasted effects of AhR deficiency on PCB-77-induced regulation of the development of obesity and during weight loss in female compared with male mice.

Female  $AhR^{-/-}$  mice administered VEH weighed less than  $AhR^{+/+}$  females administered VEH each week of the weight gain phase of the study (Figure 3A;  $p < 0.05$ ). In wild type females, administration of PCB-77 resulted in less BW gain compared with HF-fed VEH controls (Figure 3A;  $p < 0.05$ ). In contrast,  $AhR^{-/-}$  females administered PCB-77 had significantly more BW gain compared with HF-fed VEH controls (Figure 3A;  $p < 0.05$ ). BWs of HF-fed females were significantly less than males regardless of treatment or genotype (Figure 3A;  $p < 0.05$ ). In both male and female mice, BW gain was similar in HF-fed  $AhR^{+/+}$  and  $AhR^{-/-}$  mice administered PCB-77 (Figure 3A).

At week 11 of HF feeding, lean mass (as a percentage of BW) of HF-fed female  $AhR^{-/-}$  mice administered VEH was significantly higher than VEH-treated  $AhR^{+/+}$  mice, while fat mass of  $AhR^{-/-}$  mice was significantly lower than VEH-treated  $AhR^{+/+}$  mice (Figure 3B;  $p < 0.05$ ). Compared with VEH groups within each genotype, mice administered PCB-77 had higher lean and lower fat mass than  $AhR^{+/+}$  females, but PCB-77 had the opposite effect (lower lean mass, higher fat mass) on  $AhR^{-/-}$  females (Figure 3B). Lean mass (as a percentage of BW) of HF-fed mice was higher in PCB-77-treated females compared with PCB-77-treated males, regardless of genotype, while fat mass of females of either genotype administered PCB-77 was significantly lower than males (Figure 3B;  $p < 0.05$ ).

During the weight gain phase (week 11), there were no significant differences in blood glucose concentrations or AUC between  $AhR^{+/+}$  and  $AhR^{-/-}$  females, regardless of treatment (Figure 3C,D). Moreover, glucose tolerance of HF-fed male mice administered PCB-77 was not significantly influenced by AhR deficiency (Figure 3D;  $p > 0.05$ ). However, PCB-77-treated obese males had a higher AUC for blood glucose than did females, regardless of genotype, suggesting that they were less glucose tolerant (Figure 3C,D;  $p < 0.05$ ).



**Figure 2.** Effect of a low-fat (LF) diet on body weight (BW), body composition, and glucose homeostasis of polychlorinated biphenyl (PCB)-77-treated male and female mice. Male and female mice were administered vehicle (VEH) or PCB-77 and fed a high-fat (HF) diet for 12 wk, and then switched to a LF diet for 4 wk. (A) Weekly BWs of LF-fed male and female mice. (B) Lean and fat mass as a percentage of BW measured on week 16. (C) Blood glucose concentrations over time following a glucose bolus [intraperitoneal (i.p.)] on week 15. (D) Area under the curve (AUC) for data in (C). Data are mean  $\pm$  standard error of the mean (SEM) from  $n = 6-10$  mice/sex/treatment. \* $p < 0.05$  compared with VEH within sex; # $p < 0.05$  compared with male within treatment. A repeated measures two-way analysis of variance (ANOVA) tested sex and PCB-77 treatment on the development of BW (A), and glucose homeostasis (C). A two-way ANOVA tested sex and PCB-77 treatment on lean and fat mass (B), and AUC for glucose tolerance tests (D).

### Effects of AhR Deficiency during Weight Loss on PCB-Induced Regulation of Glucose Tolerance in Obese Male and Female Mice

Male and female obese mice lost weight when switched to a LF diet with no differences between genotypes or between VEH compared with PCB-77 administered females at week 16 (Figure 4A;  $p > 0.05$ ). However, male mice continued to have higher BWs than females, regardless of genotype or treatment (Figure 4A;  $p < 0.05$ ). In female mice of each genotype and treatment group experiencing weight loss, the percent fat and lean mass was not significantly different at week 16 (Figure 4B). However, even though BWs were not significantly different between PCB-77-treated  $AhR^{+/+}$  and  $AhR^{-/-}$  male mice at week 16, male  $AhR$ -deficient mice administered PCB-77 had significantly higher lean and lower fat mass compared with  $AhR^{+/+}$  mice administered PCB-77 (Figure 4B;  $p < 0.05$ ).

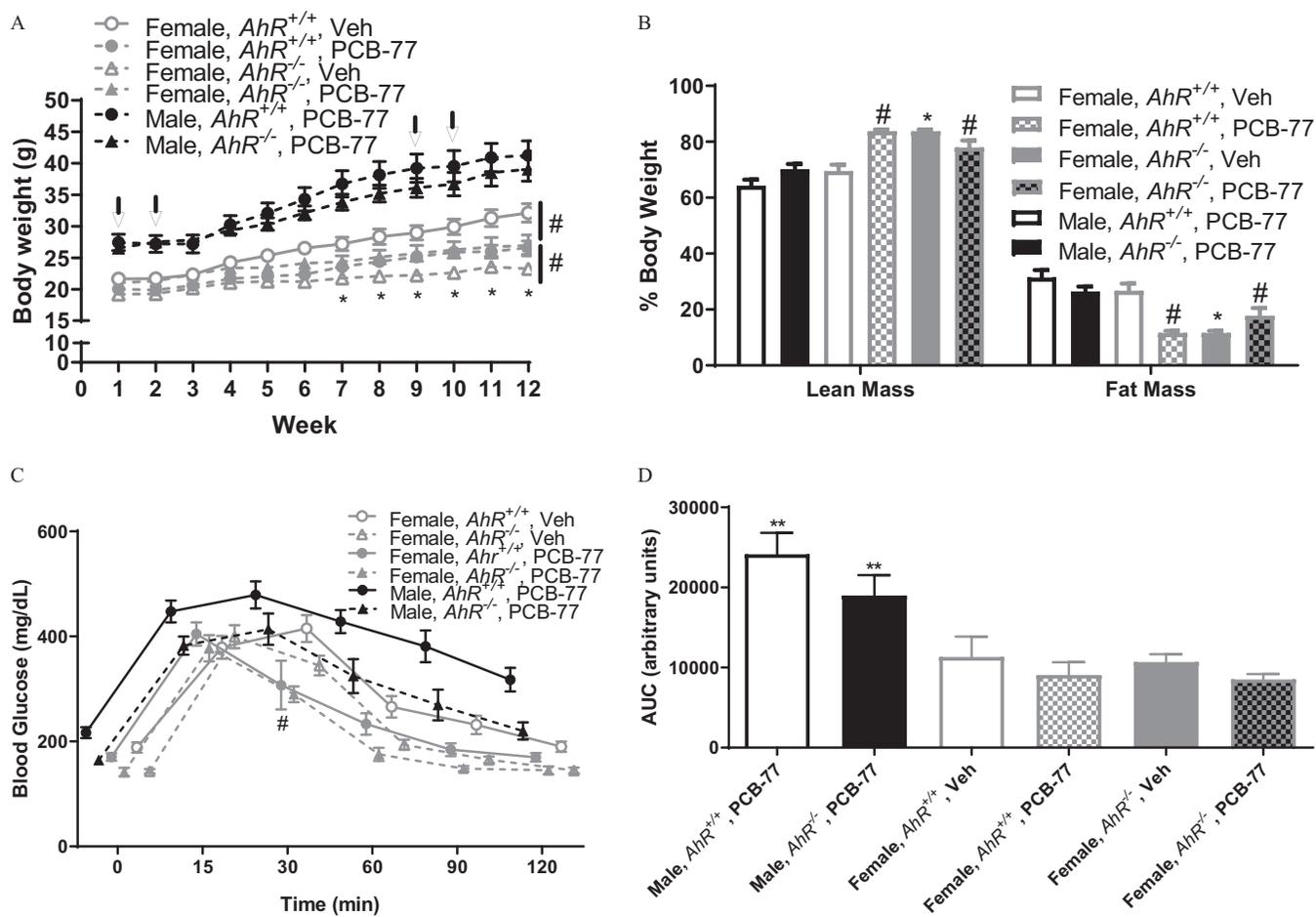
Following weight loss (week 15), the AUC for glucose tolerance was not significantly different between  $AhR^{+/+}$  females administered PCB-77 compared with VEH  $AhR^{+/+}$  controls (Figure 4C,D;  $p > 0.05$ ). In contrast, PCB-77-treated  $AhR^{-/-}$  females exhibited significantly higher AUC for blood glucose compared with  $AhR^{-/-}$  VEH controls, and compared with  $AhR^{+/+}$  females administered PCB-77, suggesting lower glucose tolerance (Figure 4D;  $p < 0.05$ ). In contrast,  $AhR^{-/-}$  male

mice administered PCB-77 exhibited significantly lower AUC for blood glucose than did  $AhR^{+/+}$  males administered PCB-77, suggesting improved glucose tolerance (Figure 4C,D;  $p < 0.05$ ). In addition, female  $AhR^{+/+}$  mice administered PCB-77 had better glucose tolerance compared with  $AhR^{+/+}$  males administered PCB-77, as determined by a lower AUC for glucose tolerance (Figure 4C,D;  $p < 0.05$ ).

We quantified plasma and tissue concentrations of PCB-77 (liver, adipose) or hydroxy-PCB-77 concentrations (plasma) at week 16 (Table 1). Although liver and adipose concentrations of PCB-77 were not significantly different between sexes,  $AhR$  deficient mice possessed significantly greater levels of PCB-77 in adipose compared with  $AhR^{+/+}$  mice, regardless of sex (Table 1;  $p < 0.05$ ). Plasma concentrations of hydroxy-PCB-77 were modestly, but not significantly higher in male mice compared with females, regardless of genotype (Table 1;  $p > 0.05$ ).

### Measures of Insulin Receptor Activity in Livers and Adipose from PCB-77-Treated AhR-Deficient Female Mice Experiencing Weight Loss

We quantified mRNA abundance of genes indicating responsiveness to PCB-77 [ $AhR$ , cytochrome P4501A1 ( $CYP1A1$ )], gluconeogenesis ( $PEPCK$ ) and insulin sensitivity ( $IRS2$ ,  $TNF\alpha$ ) in livers and adipose from PCB-77-treated male and female mice of each genotype following weight loss at week 16. There was a



**Figure 3.** Effect of aryl hydrocarbon receptor (AhR) deficiency on the development of obesity, body fat distribution, and glucose homeostasis in female mice administered vehicle (VEH) or polychlorinated biphenyl (PCB)-77, and in male mice administered PCB while being fed a high-fat (HF) diet. Female *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice were administered VEH or PCB-77 and fed a HF diet for 12 wk. PCB dosing occurred on weeks 1, 2, 9, 10 during a HF diet for 12 wk. Male *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice were administered PCB-77 and fed a HF diet for 12 wk. PCB dosing occurred on weeks 1, 2, 9, 10 during a HF diet for 12 wk. (A) Body weight (BW) of HF-fed female *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice administered VEH or PCB-77, and male *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice administered PCB-77 (arrows indicate PCB dosing). (B) Lean and fat mass as a percentage of BW measured on week 9. (C) Blood glucose concentrations over time following a glucose bolus [intraperitoneal (i.p.)] measured on week 11. (D) Area under the curve (AUC) for data in (C). Data are mean  $\pm$  standard error of the mean (SEM) from  $n = 7$ – $10$  mice/sex/genotype. \* $p < 0.05$  compared with *AhR*<sup>+/+</sup> within sex; # $p < 0.05$  compared with VEH within genotype; \*\* $p < 0.05$  compared with female within treatment. A repeated measures two-way analysis of variance (ANOVA) tested AhR deficiency and PCB-77 treatment on the development of BW (A), and glucose homeostasis (C). A two-way ANOVA tested AhR deficiency and PCB-77 treatment on lean and fat mass (B), and AUC for glucose tolerance tests (D).

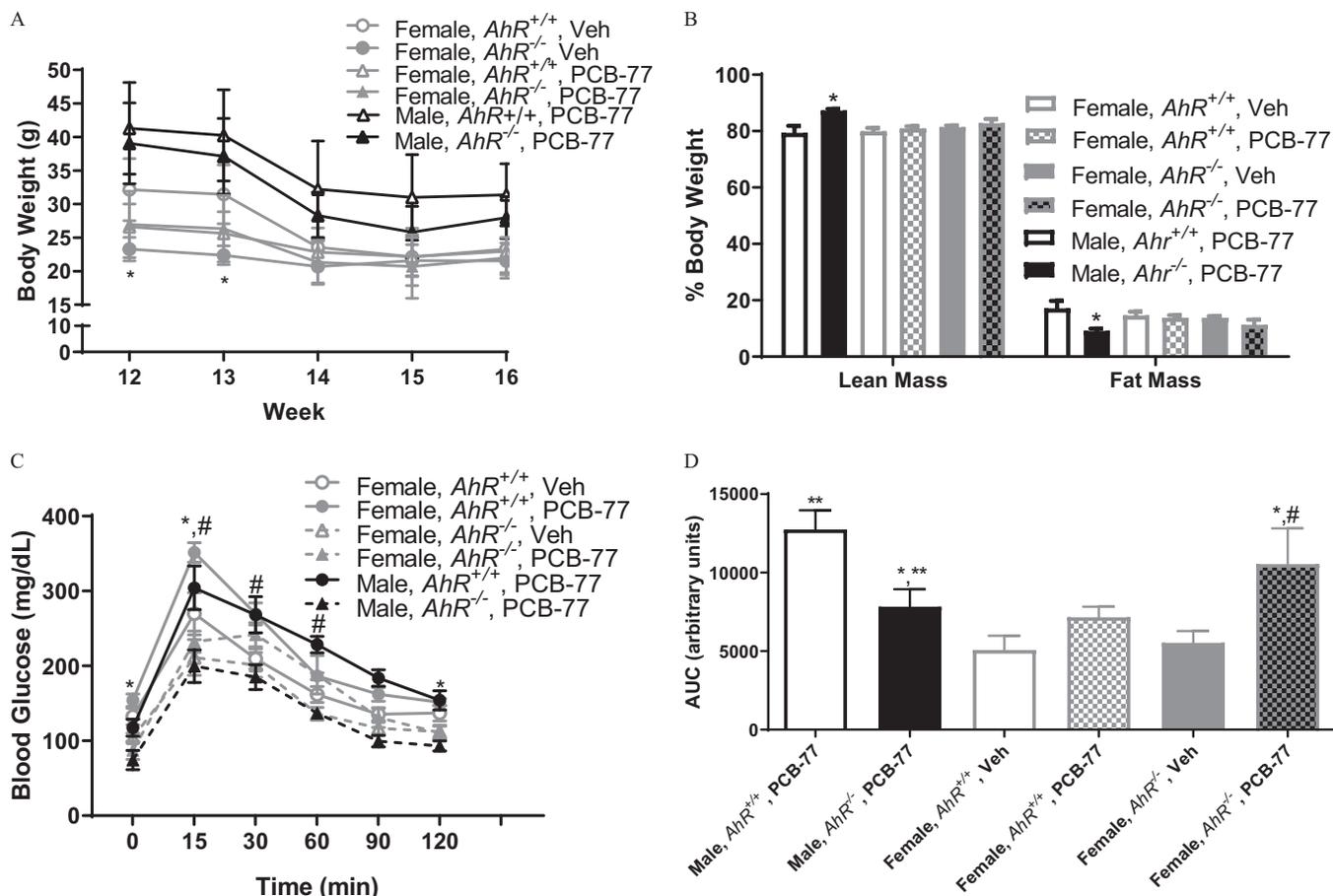
trend (nonsignificant) for lower mRNA abundance of CYP1A1 in liver (Figure 5A;  $p > 0.05$ ), with significantly lower CYP1A1 mRNA abundance in adipose tissue (Figure 5B;  $p < 0.05$ ) from male, but not female *AhR*<sup>-/-</sup> compared with *AhR*<sup>+/+</sup> mice. However, there was also a trend for lower CYP1A1 mRNA abundance in both liver and adipose tissues of female *AhR*<sup>-/-</sup> compared with *AhR*<sup>+/+</sup> females (Figure 5A,B).

As anticipated, AhR mRNA abundance was significantly lower in tissues (liver, adipose) from AhR-deficient mice, regardless of sex (Figure 6;  $p < 0.05$ ). In liver, AhR mRNA abundance was significantly higher in female *AhR*<sup>+/+</sup> mice administered PCB-77 compared with *AhR*<sup>+/+</sup> PCB-77-treated males (Figure 6A;  $p < 0.05$ ). In contrast, AhR mRNA abundance was not significantly different in adipose tissue from *AhR*<sup>+/+</sup> male and female mice (Figure 6B;  $p > 0.05$ ).

Expression levels of PEPCK, an enzyme important in gluconeogenesis, were not different in livers from male vs. female mice administered PCB-77, or by AhR deficiency (Figure 5A;  $p > 0.05$ ). In contrast, while there were no significant differences in PEPCK mRNA abundance in adipose tissue of male

compared with female *AhR*<sup>+/+</sup> mice, AhR deficiency resulted in significantly lower PEPCK mRNA abundance in adipose tissue from female but not male mice (Figure 5B;  $p < 0.05$ ).

mRNA abundance of IRS2, a molecular adaptor important in insulin receptor signaling (Copps and White 2012), was significantly higher in livers from female *AhR*<sup>+/+</sup> compared with male *AhR*<sup>+/+</sup> PCB-77-treated mice (Figure 6A;  $p < 0.05$ ). However, sex differences in IRS2 mRNA abundance were not evident in adipose tissue from *AhR*<sup>+/+</sup> male vs. female mice (Figure 6B;  $p > 0.05$ ). AhR-deficient male mice administered PCB-77 had modestly, but not significantly, higher IRS2 mRNA abundance in livers compared with wild type controls, while female AhR-deficient PCB-77 treated mice had modestly, but not significantly, lower liver IRS2 mRNA abundance compared with wild type female controls (Figure 6A;  $p > 0.05$ ). Similarly, adipose tissue of female, but not male *AhR*<sup>-/-</sup> mice administered PCB-77 had significantly lower IRS2 mRNA abundance compared with wild type controls (Figure 6B;  $p < 0.05$ ).



**Figure 4.** Effect of aryl hydrocarbon receptor (AhR) deficiency during weight loss on body weight (BW), body fat distribution, and glucose homeostasis in female mice administered vehicle (VEH) or polychlorinated biphenyl (PCB)-77, and in male mice administered PCB while being fed a high-fat (HF) diet. Female *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice were administered VEH or PCB-77 and fed a HF diet for 12 wk, and then switched to a low-fat LF diet for 4 wk. Male *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice were administered PCB-77 and fed a HF diet for 12 wk, and then switched to a LF diet for 4 wk. (A) Weekly BWs of LF-fed female *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice administered VEH or PCB-77, and male *AhR*<sup>+/+</sup> and *AhR*<sup>-/-</sup> mice administered PCB-77. (B) Lean and fat mass as a percentage of BW measured on week 16. (C) Blood glucose concentrations over time following a glucose bolus [intraperitoneal (i.p.)] on week 15. (D) Area under the curve (AUC) for data in (C). Data are mean ± standard error of the mean (SEM) from *n* = 7–10 mice/sex/genotype. \**p* < 0.05 compared with *AhR*<sup>+/+</sup> within sex; #*p* < 0.05 compared with VEH within genotype; \*\**p* < 0.05 compared with female within treatment. A repeated measures two-way analysis of variance (ANOVA) tested AhR deficiency and PCB-77 treatment on the development of body weight (BW) (A), and glucose homeostasis (C). A two-way ANOVA tested AhR deficiency and PCB-77 treatment on lean and fat mass (B), and AUC for glucose tolerance tests (D).

## Discussion

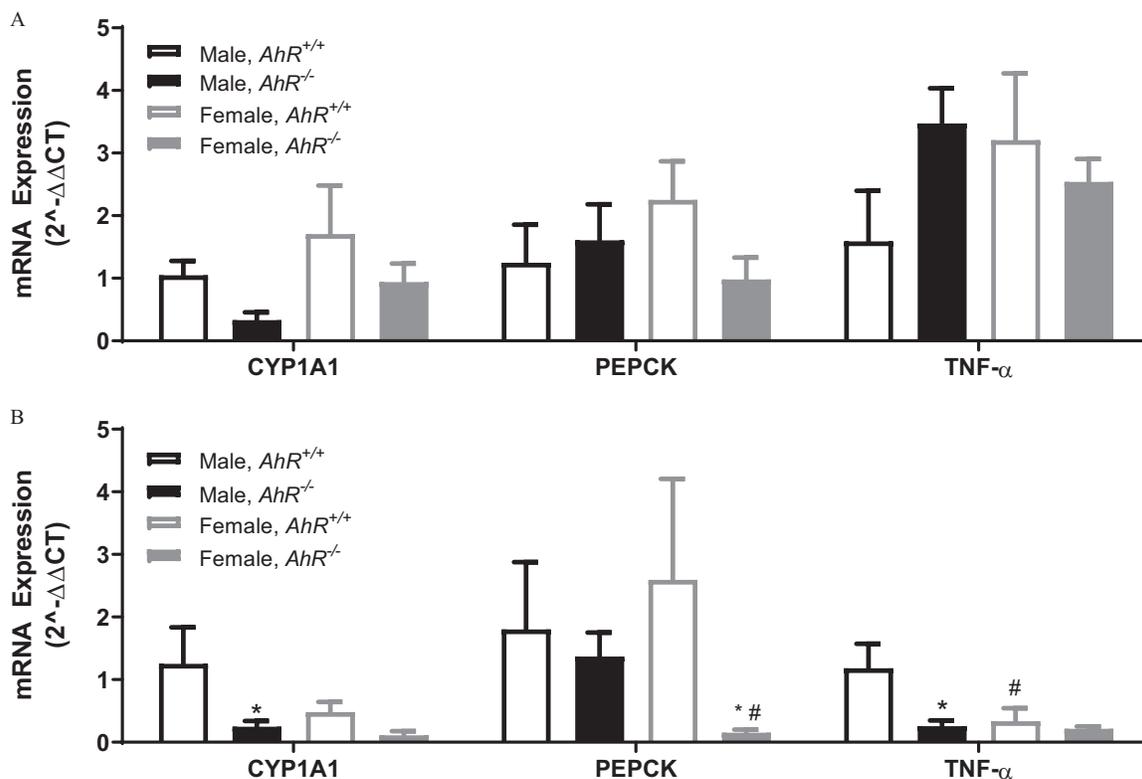
There has been a growing interest in the environmental contribution to the etiology of diabetes, and increasing evidence suggests an association between exposure to POPs, like PCBs, and the development of T2D (Lee et al. 2010, 2011). Recent results from the Anniston Community Health Survey demonstrated a statistically significant association of serum PCB levels with higher diabetes prevalence overall, especially among females (Silverstone et al. 2012). Follow-up studies in participants from this population demonstrated higher serum dioxin toxic equivalencies in

females compared with males (Yang et al. 2018). Results from the present study demonstrate sex differences in the ability of PCB-77 to regulate BW and impair glucose tolerance in obese mice and in obese mice exhibiting weight loss. The inclusion of studies examining effects of weight loss in obese male and female mice previously exposed to PCB-77 is relevant to higher body burdens of lipophilic toxicants in obese subjects (Dirinck et al. 2011; Kim et al. 2011; Lim et al. 2011; Myre and Imbeault 2014) and potential harmful effects of liberated toxicants during weight loss. Moreover, while several studies have examined sex differences in responses to AhR stimulation (Lee et al. 2015; Nault et al.

**Table 1.** Polychlorinated biphenyl (PCB) and metabolite concentrations detected in tissues on week 16 after weight loss phase.

Tissues (PCB-77)	Male		Female	
	<i>AhR</i> <sup>+/+</sup>	<i>AhR</i> <sup>-/-</sup>	<i>AhR</i> <sup>+/+</sup>	<i>AhR</i> <sup>-/-</sup>
Liver (nmoles/mg)	N.D.	0.029 ± 0.016	0.001 ± 0.001	0.007 ± 0.002
Adipose (nmoles/mg)	<0.001	0.147 ± 0.087*	<0.001	0.143 ± 0.060*
Plasma (μM; Hydroxy-PCB-77 metabolites)	5.59 ± 3.34	7.79 ± 1.68	2.36 ± 2.36	3.38 ± 3.38

Note: AhR whole-body wild type (*AhR*<sup>+/+</sup>; *n* = 3 female, *n* = 4 male) and deficient mice (*AhR*<sup>-/-</sup>; *n* = 4 female, *n* = 4 male). \**p* < 0.05 compared with *AhR*<sup>+/+</sup> mice. A two-way analysis of variance (ANOVA) tested sex and AhR deficiency examining either PCB-77 or hydroxy-PCB-77 metabolites in liver, adipose, and plasma, respectively, in male and female mice. AhR, aryl hydrocarbon receptor; N.D., not detected.



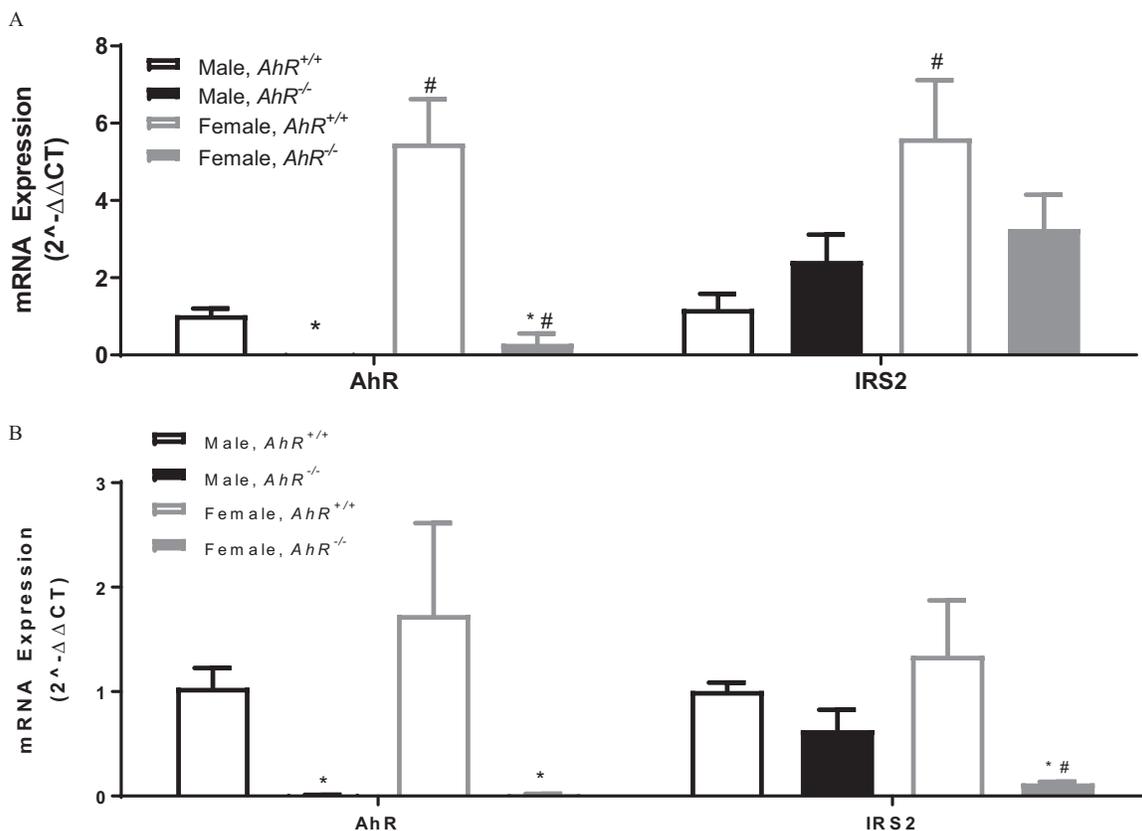
**Figure 5.** *CYP1A1* (cytochrome P450 1A1), *PEPCK* (phosphoenolpyruvate carboxykinase), and *TNFα* (tumor necrosis factor-α) mRNA abundance in (A) liver, and (B) adipose of polychlorinated biphenyl (PCB)-77-treated male and female mice at week 16 (end of weight loss phase). Data are mean ± standard error of the mean (SEM) from  $n = 3-5$  mice/genotype and are normalized to PCB-treated male of aryl hydrocarbon receptor (*AhR*)<sup>+/+</sup> mice. \* $p < 0.05$  compared with *AhR*<sup>+/+</sup> within sex; # $p < 0.05$  compared with male within genotype. A two-way analysis of variance (ANOVA) tested sex and *AhR* deficiency on gene expression in male and female mice (A,B).

2017; Prokopec et al. 2015), we focused on the role of AhR as a mediator of effects of PCB-77 to impair glucose homeostasis in males and females during the development of obesity and during weight loss in obese mice. Results suggest that AhR deficiency abrogated harmful effects of PCB-77 to impair glucose homeostasis during weight loss of obese male mice but promoted PCB-77-induced impairment of glucose homeostasis as well as insulin receptor activity in female obese mice experiencing weight loss.

We observed sex differences in the ability of PCB-77 to regulate the development of obesity between male and female mice, with modestly higher BW gain of PCB-77-treated males, but lower BW gain of PCB-77 treated HF-fed females. Recent studies examined effects of 2,3,7,8-tetrachlorodibenzodioxin (TCDD), an AhR ligand, on the development of obesity in male vs. female mice (Brulport et al. 2017). In contrast to our findings, chronic TCDD exposures resulted in modest but significantly higher BWs of both male and female mice fed a HF diet (with added cholesterol); however, males and females exhibited differential sex-dependent regulation of genes involved in adipose tissue lipolysis and insulin resistance. Specifically, male obese mice exposed to TCDD had higher mRNA abundance of lipolysis and insulin-resistant promoting genes in adipose tissue, with no effect of TCDD on expression levels of these genes in adipose tissue of obese females (Brulport et al. 2017). Differences in diet composition, duration of toxicant and/or diet exposure, and the toxicant examined may have contributed to diverging results on BW regulation between these studies. Both studies suggest sex-dependent responses to AhR ligands during the development of obesity, but sex differences in BW gain may be influenced by the ligand under study. Moreover, our results demonstrate that lower

BW gain of HF-fed female mice administered PCB-77 did not translate into significant improvements of glucose tolerance, suggesting that the BW-reducing effects of PCB-77 in females were not beneficial for glucose homeostasis.

Previous studies in our laboratory demonstrated that when obese male mice exposed to PCB-77 during weight gain experienced weight loss, the benefits of weight loss to improve glucose tolerance were mitigated (Baker et al. 2013, 2015). These effects were associated with liberation of PCB-77 from adipose stores upon weight loss of obese male mice. Results from the current study confirm findings of impaired glucose tolerance in male PCB-77-treated obese mice upon weight loss. Moreover, results from this study extend previous findings by demonstrating that female obese mice losing weight may be resistant to effects of PCB-77 to impair glucose homeostasis. A limitation of this study is that because we had previously examined effects of VEH vs. PCB-77 administration using the same study design in male wild type mice (Baker et al. 2013), we did not perform a side-by-side comparison between VEH and PCB-77 administration in both sexes of *AhR*<sup>+/+</sup> and <sup>-/-</sup> mice. An explanation for the observed sex differences is that female mice may be less sensitive to the toxic effects of PCB-77 than males (Pohjanvirta et al. 2012). Specifically, evidence suggests sex differences in AhR responsiveness, depending on species (Lee et al. 2015; Nault et al. 2017; Prokopec et al. 2015). Sex differences may arise from variability in rates of PCB metabolism, including AhR-mediated stimulation of *CYP1A1* expression, an enzyme responsible for hydroxylating PCBs and increasing their water solubility and eventual elimination (Mise et al. 2016). However, in this study, mRNA abundance of *CYP1A1* was similar in adipose and livers from male compared with female mice administered PCB-77,



**Figure 6.** Liver and adipose tissue of aryl hydrocarbon receptor (AhR) and IRS2 (insulin receptor substrate 2) mRNA abundance in polychlorinated biphenyl (PCB)-77-treated AhR-proficient and AhR-deficient males and females at week 16 (end of weight loss phase). (A) AhR and IRS2 mRNA abundance in livers from male and female mice of each genotype. (B) AhR and IRS2 mRNA abundance in adipose tissue from male and female mice of each genotype. Data are mean  $\pm$  standard error of the mean (SEM) from  $n = 3-5$  mice/genotype. \* $p < 0.05$  compared with  $AhR^{+/+}$  within sex; # $p < 0.05$  compared with male within genotype. A two-way analysis of variance (ANOVA) tested sex and AhR deficiency on gene expression in liver and adipose tissue from male and female mice.

and there were no significant differences in levels of PCB-77 or hydroxy-PCB-77 metabolites between males and females. As levels of PCB-77 and its metabolite were quantified at one time point, which was several weeks after the last dose of PCB-77, it is difficult to make conclusions regarding the impact of metabolism of PCB-77 on the observed sex differences within the present study. As anticipated, AhR-deficient male and female mice had higher levels of parent compound PCB-77 in all tissues analyzed, but these effects were not statistically significant. However, in AhR-deficient mice exhibiting lower CYP1A1-mediated metabolism of PCB-77, there were again no sex differences in levels of the toxicant that would explain differences in glucose homeostasis between males and females.

An interesting finding of the present study was sexual dimorphism of AhR-mediated regulation of PCB-77-induced impairment of glucose homeostasis during weight loss. In males, AhR deficiency abrogated effects of PCB-77 to impair glucose tolerance during weight loss, while in females, AhR deficiency augmented glucose intolerance, manifested as longer elevations in blood glucose concentrations over time compared with wild type females. Since liver and adipose tissue are key insulin-responsive organs, we quantified measures of insulin receptor activity to define mechanisms for these differences. Tissues from insulin-resistant and diabetic individuals exhibit disruption in IRS-dependent signaling, implicating the dysregulation of IRS1 and IRS2 in the initiation and progression of metabolic disease (Coppa and White 2012). Although IRS1 predominately regulates insulin sensitivity in muscle, IRS2 has a more prominent role in

liver and adipose tissues (Previs et al. 2000). Results from this study suggest that AhR activation is protective in females for regulation of insulin receptor signaling. Indeed, liver AhR mRNA abundance in females exhibited similar patterns as IRS2 mRNA expression, with higher levels of AhR and IRS2 mRNA in livers from wild type females compared with males, and lower levels in livers from AhR-deficient females. Similar findings of lower IRS2 mRNA abundance were observed in adipose tissue of AhR-deficient females, supporting impaired insulin receptor signaling. The precise targets of AhR to differentially regulate insulin receptor signaling proteins between male and female mice are unclear. Moreover, since both testosterone and estrogen interact with AhR (Bonefeld-Jørgensen et al. 2001; Ohtake et al. 2003; Wu et al. 2013), future studies should address the role of sex hormones as mediators of sex differences in AhR regulation of insulin sensitivity. Finally, since AhR deficiency abolished effects of PCB-77 on glucose homeostasis during weight loss in males but augmented effects of PCB-77 in females, these results suggest that PCB-77 may exert AhR-independent effects to regulate glucose homeostasis in females.

## Conclusion

These results demonstrate that male and female mice responded differently to PCB-77 and AhR deficiency in the regulation of BW and glucose homeostasis. Results suggest that PCB-77 impaired glucose homeostasis during weight loss in obese male mice through an AhR-mediated mechanism, mitigating the

beneficial effects of weight loss on glucose homeostasis. In contrast, rather than improved glucose homeostasis, AhR-deficient females administered PCB-77 had significant impairments of glucose tolerance during weight loss. Moreover, livers from AhR-deficient females exposed previously to PCB-77 had lower mRNA expression of IRS2, indicating potential impairment of insulin receptor signaling. These results suggest sex differences in the responsiveness of mice to PCB-77 and to mechanisms by which PCB-77 regulates BW and glucose homeostasis during the development of obesity and during weight loss.

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