Deficiency of aryl hydrocarbon receptor in adipocytes augments the development of diet-induced obesity

Nika Larian, Nicki A. Baker, Robin Shoemaker, Victoria English, Lisa A. Cassis
Department of Pharmacology and Nutritional Sciences, University of Kentucky

Abstract

Objectives: Ligands of the aryl hydrocarbon receptor (AhR) have been shown to regulate adipocyte differentiation and promote expression of proinflammatory adipokines. We investigated the effect of adipocyte-specific deletion of AhR on the development of diet-induced obesity.

Methods/Results: AhR floxed mice ($AhR^{fl/fl}$) with loxP sites flanking exon 2 were bred to hemizygous transgenic male Cre mice under control of an adiponectin promotor (AdQ) to generate adipocyte AhR-deficient mice (AhR^{AdQ}) and their littermate controls ($AhR^{fl/fl}$). AhR mRNA abundance was not detectable in adipocytes differentiated from the stromal vascular fraction of AhR^{AdQ} mice (AhR^{AdQ}, not detectable; $AhR^{fl/fl}$, 86.79 ± 44.39 ; ΔΔ Ct ; P < 0.05). Male, 8-week old $AhR^{fl/fl}$ and AhR^{AdQ} mice were fed a high fat (HF, 60% kcal from fat) diet for 12 weeks. Both groups of mice fed a HF diet became obese, but body weight was significantly increased in AhR^{AdQ} mice compared to $AhR^{fl/fl}$ after 12 weeks (AhR^{AdQ}, 50.34 ± 3.64 ; $AhR^{fl/fl}$, 43.13 ± 5.52 grams; P<0.05). Similarly, HF-fed adipocyte AhR deficient mice exhibited increased fat mass and decreased lean mass compared to HF-fed controls. Excess adiposity was deposited subcutaneously in HF-fed AhR^{AdQ} mice, and was associated with increased adipocyte size (AhR^{AdQ}, 1.93 ± 0.06; $AhR^{fl/fl}$, 1.3 ± 0.36μ^2/cell number; P<0.05) compared to HF-fed controls. Increased adiposity in HF-fed adipocyte-specific AhR deficient mice was associated with pronounced elevations in mRNA abundance of F4/80, a macrophage marker, in epididymal adipose tissue (AhR^{AdQ}, 3.52 ± 0.68; $AhR^{fl/fl}$, 1.62 ± 0.67; ΔΔ Ct; P<0.05).

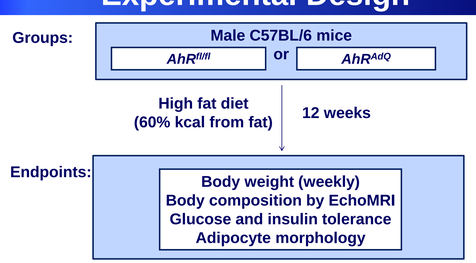
Conclusions: Deficiency of AhR in adipocytes augments the development of obesity, indicating a role for endogenous AhR ligand(s) in the regulation of body weight, adiposity, and adipose inflammation.

Introduction

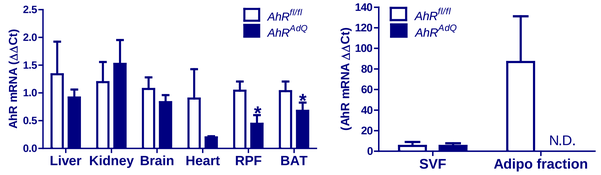
- The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates toxic and carcinogenic effects of environmental contaminants (Beischlag et al. 2008). There are also several putative endogenous ligands of the AhR.
- Ligands of the AhR have been shown to regulate adipocyte differentiation (Arsenescu et al. 2008), promote expression of proinflammatory adipokines (Arsensecu et al. 2008; Kim et al. 2012), and alter glucose homeostasis (Baker et al. 2013b).
- Antagonism of the AhR has been demonstrated to prevent AhR-mediated disruptions in glucose homeostasis (Baker et al. 2013a).
- Whole body AhR deficiency in mice has been reported to promote triglyceride synthesis (Alexander et al. 1998).

We hypothesized that adipocyte AhRs contribute to the regulation of body weight and adiposity during the development of obesity.

Experimental Design



Development of a mouse model with adipocyte-specific AhR deletion



Characteristics of $AhR^{fl/fl}$ mice fed standard diet.

Parameter/Genotype	$AhR^{fl/fl}$	AhR^{AdQ}
Body Weights (g)	24.6 ± 0.4	23.6 ± 0.5
Lean mass (% body weight)	81.0 ± 0.5	77.2 ± 1.2*
Fat mass (% body weight)	8.9 ± 0.3	11.2 ± 0.9*
Epididymal fat (g)	0.89 ± 0.12	1.27 ± 0.07*
Retroperitoneal fat (g)	0.16 ± 0.02	0.26 ± 0.03*
Subcutaneous fat (g)	0.56 ± 0.08	0.74 ± 0.03

Data are mean ± SEM from n = 6-8 mice/group.
*, P<0.05 compared to $AhR^{fl/fl}$.

Figure 1. (Top left) AhR mRNA abundance in liver, kidney, brain, heart, retroperitoneal fat (RPF), and brown adipose tissue (BAT). Data are mean ± SEM from n = 5 mice/genotype. *, P<0.05 compared to $AhR^{fl/fl}$. (Top right) AhR mRNA abundance in the preadipocyte enriched stromal vascular fraction (SVF) versus differentiated mature adipocytes (Adipo fraction) from $AhR^{fl/fl}$ and AhR^{AdQ} mice fed standard mouse diet. Data are mean ± SEM from n = 3 mice/genotype. (Bottom) Characteristics of $AhR^{fl/fl}$ and AhR^{AdQ} mice fed standard murine diet.

Adipocyte AhR deficiency promotes the development of obesity

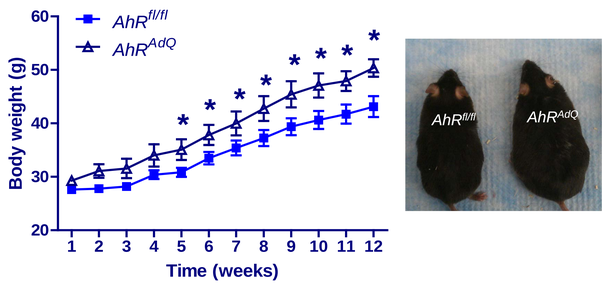


Figure 2. (Left) Body weights of mice in each treatment group during 12 weeks of HF feeding. Data are mean ± SEM from n = 6-8 mice per group. *, P<0.05 compared to $AhR^{fl/fl}$. (Right) A representative HF-fed mouse from each genotype.

HF-fed adipocyte AhR-deficient mice have increased adiposity

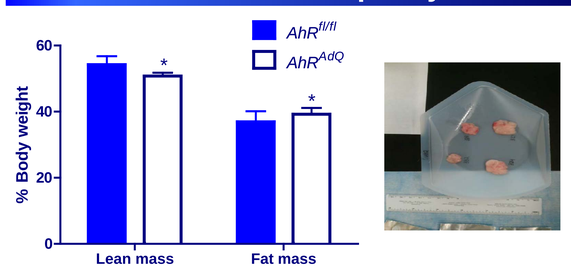


Figure 3. (Left) Lean mass and fat mass as a percentage of body weight after 12 weeks HF feeding. Data are mean ± SEM from n = 6-8 mice/group. (Right) Subcutaneous adipose tissue removed from representative $AhR^{fl/fl}$ (left) and AhR^{AdQ} (right) mice (n = 2/group).

Adipocyte size is increased in HF-fed adipocyte AhR-deficient mice

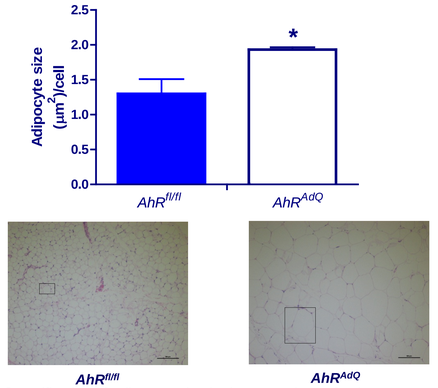


Figure 4. (Top) Quantification of adipocyte size in tissue sections of subcutaneous adipose tissue. *, P<0.05 compared to $AhR^{fl/fl}$. Data are mean ± SEM from n = 3 mice/genotype. (Bottom) Representative sections of subcutaneous adipose tissue from mice of each genotype.

F4/80 mRNA abundance in adipose tissue is elevated in HF-fed adipocyte AhR-deficient mice

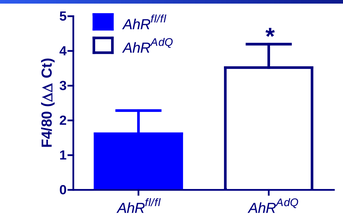


Figure 5. Increased adiposity in HF-fed adipocyte-specific AhR deficient mice was associated with significant elevations in mRNA abundance of F4/80, a macrophage marker, in epididymal adipose tissue. *, P<0.05 compared to $AhR^{fl/fl}$. Data are mean ± from n = 4 mice per group.

Impaired glucose homeostasis with HF-feeding is augmented in mice with adipocyte AhR-deficiency

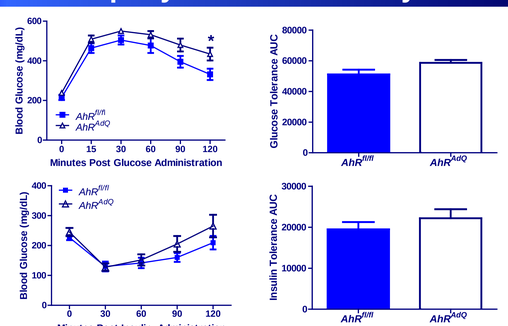


Figure 6. (Top left) Blood glucose concentrations following intraperitoneal administration of glucose and (top right) corresponding area under the curve (AUC) (p=0.06). (Bottom left) Blood glucose concentrations following intraperitoneal administration of insulin and (bottom right) corresponding AUC. *, P<0.05 compared to $AhR^{fl/fl}$ within time point. Data are mean ± SEM from n = 6-7 mice per group.

Conclusions

- Mice with adipocyte AhR deficiency exhibit increased body weight during the development of obesity compared to wild-type controls.
- HF-fed adipocyte AhR-deficient mice have increased adiposity associated with increased fat mass and decreased lean mass.
- Excess adiposity in HF-fed AhR-deficient mice is associated with increased adipocyte size.
- Increased adiposity in HF-fed adipocyte-specific AhR deficient mice is associated with elevated mRNA abundance of F4/80 in epididymal adipose tissue.
- Obese adipocyte AhR-deficient mice exhibit more pronounced impairments in glucose homeostasis.

Taken together, these data suggest a role for endogenous AhR ligand(s) acting at adipocyte AhRs in the regulation of body weight, adiposity, and adipose inflammation.

Clinical and Nutritional Implications

- The endogenous ligand(s) of AhR is unknown.
- Putative endogenous ligands include nutritional metabolites such as tryptophan degradation products and fatty acid metabolites.
- Future studies will focus on the role of prospective ligands to regulate adipocyte differentiation and proliferation.
- Nutritionally derived endogenous ligands of the AhR found to regulate adipocyte function and body weight may be effective therapeutic targets for the treatment of obesity and metabolic disorders.

Acknowledgements

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