

# Environmental factors act through aryl hydrocarbon receptor activation and circadian rhythm disruption to regulate energy metabolism

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**Abstract:** Disruption of energy metabolism, resulting in metabolic illnesses including diabetes, hyperlipidemia, fatty liver, hypertension and atherosclerosis, will likely shorten human life expectancy over the next several decades. Past work focusing on diet and exercise needs to be continued, but new environmental factors such as exposure to pollutants and the disruption of circadian rhythms in modern life urgently need more attention and understanding. This review focuses on how environmental pollutants acting through the aryl hydrocarbon receptor (AhR) to cause circadian disruption lead to metabolic derangements. AhR-mediated metabolic dysregulation in the whole organism, and dysregulation specific to the liver and adipose tissue, will be explored. Finally, the role of AhR in circadian desynchrony and resultant effects on energy metabolism will be discussed. This review summarizes information vital to future developments that can combat metabolic illnesses.

**Keywords:** aryl hydrocarbon receptor, adipogenesis, glucose metabolism, lipolysis, circadian rhythm, insulin sensitivity

## Introduction

For the first time in modern medical history, life expectancy is decreasing.<sup>1,2</sup> Obesity, insulin resistance and obesity-associated illnesses, including metabolic syndrome, fatty liver, lipid disorders and cardiovascular disease, are major causes of decreasing life span. The Center for Disease Control reports that more than 34.9% of U.S. adults are obese,<sup>3,4</sup> and estimates associated yearly medical costs at \$147 billion dollars.<sup>5</sup> Obesity initiates alterations in adipose tissue, liver and skeletal muscle that affect energy metabolism and ultimately promote a systemic insulin resistance that leads to development of type 2 diabetes mellitus. Not surprisingly, the rapid worldwide increase in obesity has been accompanied by an equally alarming rise in type 2 diabetes, a metabolic disorder characterized by increased serum glucose secondary to decreased insulin sensitivity. The International Diabetes Foundation estimates that 415 million people currently have diabetes with expectations for an increase to 624 million by 2040 (International Diabetes Federation, 2017 <http://www.diabetesatlas.org/>).

Although poor diet and lack of physical activity are the most commonly cited contributors to the obesity and diabetes pandemics, these factors alone cannot explain the alarming rates of increase over the past 40 years. Perhaps the most compelling evidence for alternative causes is an increased body weight in wildlife and domestic animals living in developed countries.<sup>6</sup> Other environmental factors, including exposure to environmental chemicals and alterations in sleep patterns, contribute significantly

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to the emerging problem of obesity, insulin resistance and diabetes and are the focus of this review.

Obesogens are molecules that change glucose, lipid and protein metabolism, as well as produce changes in feeding behavior that lead to development of obesity and its metabolic sequelae.<sup>7,8</sup> Certain persistent organic pollutants (POPs) are environmental obesogens,<sup>9</sup> and epidemiological studies link exposure to these molecules with development of metabolic dysfunction.<sup>10,11</sup> The relationship is clear for diabetes; exposure to air pollution, airborne fine particulate matter and nitrogen dioxide increases the prevalence of diabetes.<sup>12</sup> Serum levels of certain POPs are higher in diabetic patients in the Canary Islands, Spain, with a positive correlation observed between serum dichlorodiphenyldichloroethylene and serum glucose levels.<sup>13</sup> Low levels of insulin are observed in individuals with high serum concentrations of organochlorine pesticides, a type of POPs. Similarly, obesity is more prevalent in children whose mothers were smokers during pregnancy.<sup>14</sup> Lipid disorders are also affected by POPs. Carbon monoxide, nitrogen dioxide and sulfur dioxide exposure deregulates cholesterol metabolism and reduces high-density lipoproteins and apolipoprotein.<sup>15</sup> POPs can create liver dysfunction; children exposed to traffic-related air pollution have increased concentrations of plasma cytokeratin-18, a marker of hepatocellular apoptosis and disrupted liver function.<sup>16</sup> These data suggest a link between exposure to environmental obesogens and metabolic dysfunction. Potential mechanisms for metabolic dysfunction in response to environmental disruptions such as activation of aryl hydrocarbon receptor (AhR) by POPs and circadian rhythm disruption are subsequently explored in this review.

## The AhR

The AhR is an evolutionarily ancient protein that has been studied extensively for its function as a primary mediator of biological responsiveness to xenobiotics, including obesogenic POPs. Industrially produced AhR ligands are toxic chemical contaminants of the global ecosystem, produced as by-products of pesticide production, bleaching and combustion processes. Contamination with these compounds is widespread throughout the biosphere including air, water, fish and mammals. Human studies positively link POPs with obesity and metabolic syndrome.<sup>17–21</sup>

About 90% of human exposure to obesogens occurs through diet, primarily consumption of animal fat (U.S. EPA, 2004, <https://cfpub.epa.gov/ncea/dioxin/recordisplay.cfm?deid=87843>). In addition to toxic pollutants, compounds that elicit AhR activity are also found in natural dietary

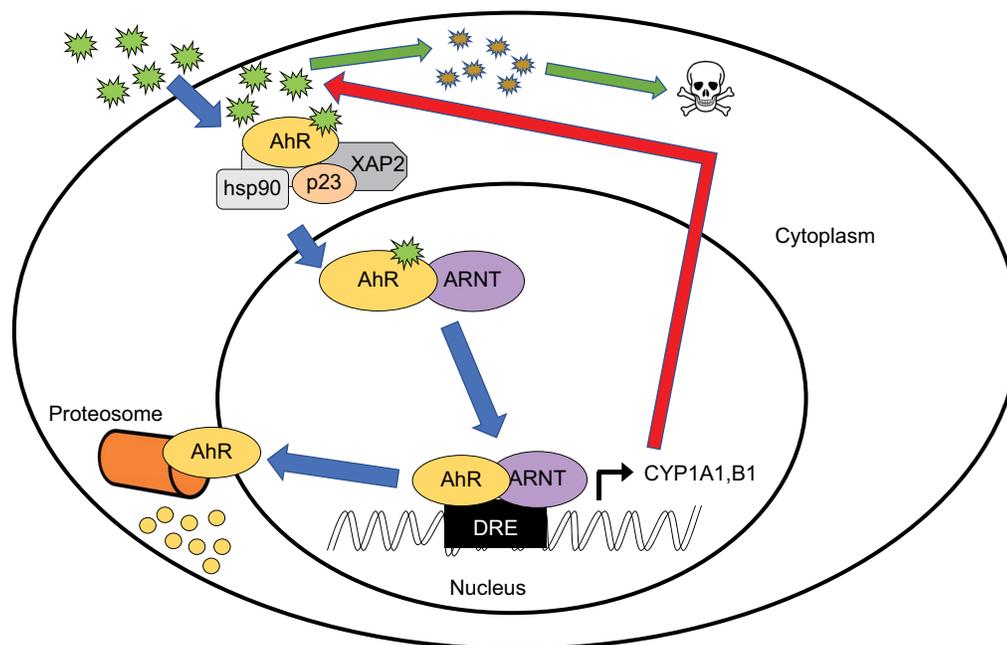
products,<sup>22–25</sup> including indole metabolites from cruciferous plants and flavonoids found in fruits and vegetables.<sup>23</sup> Thus, AhR can be activated by an array of diverse ligands that can be endogenous, naturally occurring and/or anthropogenic.<sup>26–28</sup> AhR may alter metabolic function through its regulation of inflammatory cytokine expression, cell cycle signaling and interaction with the cellular circadian clock.<sup>29–34</sup>

AhR is expressed as a cytoplasmic multiprotein complex.<sup>35–38</sup> Upon activation by high-affinity ligands, a conformational change in the three-dimensional structure of the AhR complex exposes a nuclear localization signal.<sup>39</sup> Ligand-bound AhR translocates to the nucleus, releases ligand and binds to the aryl hydrocarbon nuclear transporter (ARNT). The AhR-ARNT complex binds to specific dioxin response elements (DREs) on the DNA<sup>22–40</sup> and AhR target genes such as cytochrome P450 (*CYP1A1*) are expressed (Figure 1). After target gene activation, AhR is removed from the nucleus and degraded by the 28S proteasome.<sup>39</sup> Expression of AhR is also regulated by an inhibitor, the AhR repressor. While this canonical signaling has been identified as a primary mechanism regulating the toxicological responses through AhR, alternative signaling events are increasingly being understood to mediate the physiological effects of AhR. Both cholesterol and fatty acid synthesis are regulated by endogenous AhR activity that does not depend on binding to the DRE.<sup>41,42</sup> Thus, AhR regulates expression of myriad genes through multiple mechanisms, including many associated with obesity, lipid metabolism and inflammation.<sup>41–44</sup>

## Systemic energy metabolism

AhR activation through POPs and obesogens is linked to the development of type 2 diabetes in humans. Exposure to the strong AhR activator 2,3,7,8-tetrachlorodibenzodioxin (TCDD) is associated with glucose intolerance and hyperinsulinemia; risk of diabetes is positively correlated with TCDD body burden.<sup>45–47</sup> Vietnam veterans exposed to high levels of Agent Orange causing high AhR activation display hyperinsulinemia and increased type 2 diabetes.<sup>48</sup>

Experimental studies in animal models also demonstrate that AhR activation leads to altered energy metabolism and obesity. Exposure to obesogens suppresses gluconeogenesis and glycogenolysis in mice with a functional AhR; mice without significant AhR activity were unaffected.<sup>49</sup> C57BL/6.D2 mice with low-affinity AhR are less susceptible to the negative metabolic impacts of high-fat diet (HFD) exposure compared to C57BL/6 mice with a high-affinity AhR allele. Increased susceptibility to obesity was hypothesized to be a result of altered peroxisome proliferator-activated receptor



**Figure 1** Canonical AhR signaling pathway.

**Notes:** Lipophilic POPs enter into cells and bind to the AhR in the cytoplasm where it is associated with a complex of proteins that include hsp90, p23 and XAP<sub>2</sub>. After binding to POPs, AhR dissociates from its chaperone complex and then translocates into nucleus where it forms a heterodimer with ARNT and binds to DRE elements in the promoters of target genes to induce their transcription, including members of the cytochrome P450 family. While expression of the target genes produce phase I metabolizing enzymes that attack POPs and degrade them (red arrow), they also produce cytotoxic metabolites that may have harmful effects on cells (green arrows), the blue arrows indicate the flow of activity that occurs upon activation of the receptor.

**Abbreviations:** AhR, aryl hydrocarbon receptor; POPs, persistent organic pollutants; ARNT, aryl hydrocarbon nuclear transporter; DRE, dioxin response element.

(PPAR $\alpha$ ,  $\gamma$ , and  $\delta$ ) signaling pathways that control lipid metabolism and homeostasis.<sup>43</sup> AhR activation may inhibit PPAR $\gamma$  function, leading to insulin resistance.<sup>50</sup> Mechanistic studies in primary and immortalized mouse embryonic fibroblasts isolated from wild-type mice treated with TCDD have suppressed adipogenesis, most likely due to decreased activity of PPAR $\gamma$  and stearoyl-CoA desaturase type 1. TCDD had no effect on fibroblasts isolated from AhR mutant mice.<sup>51</sup> AhR-deficient mice are unaffected by TCDD, even at doses that are 10-fold higher than those found to induce pathological effects in wild-type littermates, suggesting that the effect is dependent on AhR.<sup>52</sup>

Mice that express a low-affinity AhR allele are less susceptible to obesity after exposure to HFD, exhibiting differences in fat mass, liver physiology and liver gene expression compared to mice with high-affinity AhR.<sup>43</sup> Similarly, germ line AhR null mice have enhanced insulin sensitivity and improved glucose tolerance.<sup>44</sup> Even mice that express only a single AhR allele (*AhR*<sup>+/-</sup>), which may in some ways be more similar to the mice with the low-affinity allele, are resistant to the harmful effects of HFD-induced obesity.<sup>30</sup> The *Ah*<sup>-/-</sup> mice have increased basal metabolic rates, accompanied by increased expression of thermogenic genes, including uncoupling protein 1 in brown adipose tissue, and elevated

$\beta$ -oxidation in skeletal muscle.<sup>30</sup> Thus, reduced AhR activity may promote enhanced metabolic rate through effects on brown adipose tissue and skeletal muscle.

Fibroblast growth factor 21 (FGF21) is a recently discovered hormone with protective properties against metabolic disease; working independently from the action of insulin, FGF21 promotes glucose uptake, improves lipids metabolism and causes increased energy expenditure and weight loss.<sup>53</sup> FGF21 is an AhR target gene whose promoter contains several DREs.<sup>54,55</sup> In the absence of AhR agonist, liver and blood FGF21 levels are higher in mice with liver-specific knockouts of AhR, suggesting that endogenous AhR activity suppresses FGF21.<sup>55</sup> By contrast, TCDD activation of AhR produced increased liver FGF21 in mice with a normal AhR, but not in AhR null mice. Interestingly, the AhR null mice died within 20 days, while the wild-type control lived to the end of the 30-day experiment, supporting the idea that acute AhR activation and induction of FGF21 is protective.<sup>54</sup> Humans with metabolic illness such as diabetes often have FGF21 resistance,<sup>56</sup> suggesting that FGF21 protection against metabolic dysregulation works only for acute insults and is not effective in long-term exposure. Transgenic mice with constitutively active AhR specific to the liver develop fatty liver, but not obesity or diabetes, when placed on HFD; knockdown of

FGF21 in these AhR mice caused them to respond to HFD in the same way as their wild-type counterparts.<sup>57</sup> In summary, both human and animal studies show a strong correlation between AhR activity and metabolic illness; the exact mechanisms behind this effect are still incompletely understood but may involve FGF21 and PPAR's working through improved glucose uptake and improved lipid and energy metabolism.

## Liver energy metabolism

AhR activation affects systemic energy homeostasis through perturbations of glucose and lipid metabolism.<sup>22,23</sup> AhR activation often disrupts hepatic glucose and lipid metabolism producing disease,<sup>54,56–63</sup> while inhibition of AhR is protective for metabolic illnesses.<sup>28,41,55</sup> Beneficial effects of AhR activity on liver glucose and lipid metabolism have also been shown,<sup>41,42,64</sup> demonstrating the need to examine each experimental design closely. Effects of species, strain, sex and age of animals are likely to be important.<sup>65–67</sup>

AhR activation by dietary intake can lead to fatty liver. Six-week-old male C57BL/6 mice were fed a high methionine (2% methionine) diet for 1 and 2 months to induce hyperhomocysteinemia. They developed elevated levels of the fatty acid transport protein CD36, as well as fatty liver; both the increase in CD36 and the fatty liver were blocked by treatment with CH22319, an AhR antagonist, in drinking water at 10 mg/kg/day showing that the effect was AhR dependent.<sup>58</sup>

Environmental factors affecting metabolic illness include both dietary intake and obesogenic POPs. To study this interaction, male C57BL/6 mice (age unspecified) were fed a low-fat diet (LFD) containing 20% of total calories as the unsaturated fatty acid linoleic acid or HFD containing 40% of total calories from linoleic acid for 4 months. After 2 months of the diet, they were intraperitoneally injected with polychlorinated biphenyls-77 (PCB77) (170  $\mu\text{mol/kg}$ ) or vehicle every 2 weeks for the remaining 2 months. Animals fed with the HFD had larger livers than those fed with LFD. The mice injected with PCB77 had even larger livers. DNA microarray analysis of liver tissue showed alterations in many genes related to fatty acid, triglyceride and cholesterol metabolism when mice were fed HFD, and PCB77 had an interactive effect with diet.<sup>59</sup>

Conclusive demonstration that the environmental factors of diet and obesogenic POPs work together to produce liver failure is provided by a recent study designed to mimic high levels of human exposure to TCDD. Seven-week-old male C57BL/6J mice were fed with LFD (10% fat) or HFD (45% fat) for 14 weeks. After 8 weeks, mice received either vehicle injections or weekly 5  $\mu\text{g/kg}$  TCDD injections for the

final 6 weeks. This dosing strategy produced blood levels of TCDD in the mice similar to those found in humans following industrial accidents with TCDD exposure. The mice fed a HFD and treated with weekly TCDD demonstrated more fatty liver and fatty acid dysregulation than any of the other groups, demonstrating a synergistic effect of HFD and TCDD.<sup>60</sup>

To more specifically determine if the effects of obesogenic POPs act through AhR, wild-type and AhR knockout (AhRKO) animals (Bradfield strain) were treated with 2,3,7,8-tetrachlorodibenzofuran (TCDF) or vehicle. Wild-type 6-week-old male C57BL/6J mice were fed dough pills with TCDF or vehicle for 5 days resulting in a total TCDF dose of 5  $\mu\text{g/kg}$ . AhRKO mice were treated the same, except that the total dose of TCDF was 24  $\mu\text{g/kg}$  given over 5 days. Neither group demonstrated obvious liver problems; on pathological examination, mild liver dysfunction was seen in wild-type TCDF-treated animals. Metabolomics analysis of liver and serum showed that TCDF treatment in wild-type mice caused disruption of hepatic lipogenesis, as well as altered glucose, fatty acid and amino acid metabolism. There was no effect on AhRKO mice who could not process TCDF through the AhR.<sup>49</sup>

By contrast, one study using liver-specific knockdown of AhR demonstrated that AhR activity was protective against hepatotoxicity from HFD. Liver-specific knockout mice were prepared from *AhR<sup>flax/flax</sup>* and C57BL/6J mice with Cre recombinase gene. The final animals had knockout of AhR in liver only. In wild-type mice, HFD induced the expression of suppressor cytokines signal 3 (*Socs3*), which decreases inflammation and protects against HFD-induced obesity. Specific deletion of AhR in the liver inhibited the expression of *Socs3* leading to increased susceptibility to HFD-induced hepatotoxicity. AhR activation promotes *Socs3* expression suggesting that *Socs3* is the target of AhR activation.<sup>64</sup>

Several studies have shown that endogenous AhR suppresses transcription of genes required for cholesterol and fatty acid synthesis through a DRE-independent mechanism. Female C57BL/6J mice aged 10–12 weeks expressing total body AhRs with low or high binding affinity for ligand were examined. Mice with low-affinity AhRs (effectively AhR null mice) had higher transcription levels of enzymes required for fatty acid and cholesterol synthesis than mice of an identical genetic background except that they expressed high-affinity AhRs, indicating that endogenous AhR suppresses fatty acid and cholesterol synthesis. In addition, female C57BL/6J mice aged 10–12 weeks with a liver-specific AhR mutant that cannot bind DRE were injected with the AhR agonist,  $\beta$ -naphthoflavone. Both the wild-type AhR and the mutant

inactive DRE binding AhR demonstrated decreased synthesis of fatty acids and cholesterol compared to AhR null mice. There was no difference between mice that effectively bound to DRE and those that did not, demonstrating that DRE binding is not necessary for the action of AhR in regulating enzymes responsible for fatty acid and cholesterol synthesis.<sup>41,42</sup> These studies indicate that environmental factors such as diet and pollutants act together to affect liver function.

## Adipose tissue and lipid metabolism

Lipophilic obesogenic POPs accumulate in fat-rich tissues and are resistant to metabolism.<sup>61</sup> Thus, it is not surprising that adipose tissue would be a primary target mediating the effects of POPs on metabolism through AhR. Although once considered simply a storage organ for excess nutrients, white adipose tissue is now recognized as a critical endocrine regulator of systemic metabolism. Both obesity and lipodystrophies cause insulin resistance, highlighting the importance of proper adipose tissue mass and function in systemic metabolism.<sup>62,63,68</sup> Adipose tissue regulates food intake, contributes to systemic insulin sensitivity and controls serum lipid levels.

Adipose tissue expansion through the formation of new adipocytes (adipogenesis) promotes insulin sensitivity in the face of obesity. By contrast, adipocyte cell expansion (hypertrophy) leads to lipid overloading and aberrant adipokine production, which likely produces systemic insulin resistance.<sup>69–71</sup> Activation of AhR within adipose tissue generally inhibits adipogenesis, attenuating the production of new adult adipocytes, and impedes lipolysis, reducing the release of free fatty acids into the blood. AhR regulates cell proliferation and differentiation of mesenchymal stem cells and preadipocytes within adipose tissue. AhR activation by TCDD treatment suppresses differentiation of preadipocytes; depletion of nuclear AhR restores preadipocyte differentiation.<sup>72,73</sup> AhR protein is downregulated when preadipocytes differentiate into mature adipocytes.

Together, this evidence suggests that AhR activity negatively regulates adipogenesis, increasing susceptibility to metabolic illnesses. These effects are likely mediated through downregulation of PPAR $\gamma$  and stearoyl-CoA desaturase type 1<sup>51</sup> and higher activity of p42/p44 MAP kinase. AhR stabilizes activity of the retinoblastoma family member, p107, which binds to PPAR $\gamma$ . Ultimately, both MAPK kinase activity and p107 suppress PPAR $\gamma$  activity, thereby inhibiting adipogenesis. Activation of PPAR $\gamma$  with troglitazone, ciglitazone and indomethacin overcomes the inhibition of differentiation imposed by overexpression of AhR.<sup>74,75</sup>

AhR has different effects on several cellular activities to inhibit adipogenesis. TCDD works synergistically with extracellular signal-regulated kinase to decrease PPAR $\gamma$  activity. The AhR antagonist 3V-methoxy-4V-nitroflavone blocks TCDD-induced *Cyp1B1* activation and eliminates TCDD-induced PPAR $\gamma$  inhibition.<sup>76,77</sup> In the C3H10T1/2 (pluripotent mouse mesenchymal stem cell) culture line, TCDD blocks the differentiation effects of inducers; microarray studies suggest that this inhibition of differentiation into adipocytes may be mediated through TCDD effects on cell adhesion-linked signaling pathways.<sup>78</sup> TCDD inhibits differentiation of mouse embryonic fibroblasts into adipocytes by increasing levels of known regulators of cellular differentiation, CCAAT/enhancer-binding protein (C/EBP $\beta$  and C/EBP $\delta$ ), in a tyrosine kinase c-Src-dependent manner.<sup>79</sup> AhR cooperates with nuclear factor (erythroid-derived 2)-like 2 (NRF2) to inhibit adipogenesis, since activation or overexpression of AhR in NRF2-deficient cells decreases adipogenesis.<sup>80</sup>

In toxicological studies, downstream effects of AhR activation can sometimes differ depending on the nature of the AhR ligand. However, suppression of adipogenesis by AhR activation occurs in response to a variety of AhR ligands. Mycelial extract of *Cordyceps militaris* and benzo[a]pyrene (BaP) activate AhR and inhibit adipogenesis in cultured mouse 3T3L1 cells, which have a fibroblast morphology, but can differentiate into an adipocyte phenotype. AhR inhibition of adipogenesis in 3T3L1 cells occurs through decreasing PPAR $\gamma$  and C/EBP $\alpha$ . Dominant-negative inhibition of AhR blocks these effects.<sup>75</sup> Mycelial extract of *C. militaris* inhibits mammalian target of rapamycin complex 1 signaling pathway via inhibition of AKT leading to a decrease in the expression of C/EBP $\beta$  and PPAR $\gamma$ .<sup>81,82</sup> Thus, the inhibitory effects of AhR on adipogenesis are multifactorial and complex.

In a cell line derived from human subcutaneous fat tissue of a non-diabetic donor, obesogenic POPs decreased adipogenesis. These effects were blocked by the AhR antagonist CH223191.<sup>83</sup> BaP inhibits adipogenesis in mesenchymal stem cells derived from human bone marrow by decreasing the expression of fatty acid binding protein 4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH); the AhR antagonist  $\alpha$ -naphthoflavone blocks these effects.<sup>84</sup> The AhR agonist indole-3-carbinol decreases lipid accumulation and expression of NRF2, hormone-sensitive lipase (HSL) and GAPDH in mature adipocytes.<sup>85</sup> Taken together, these data demonstrate the importance of AhR activation by a wide variety of agonists in regulating adipogenesis. Inhibition of

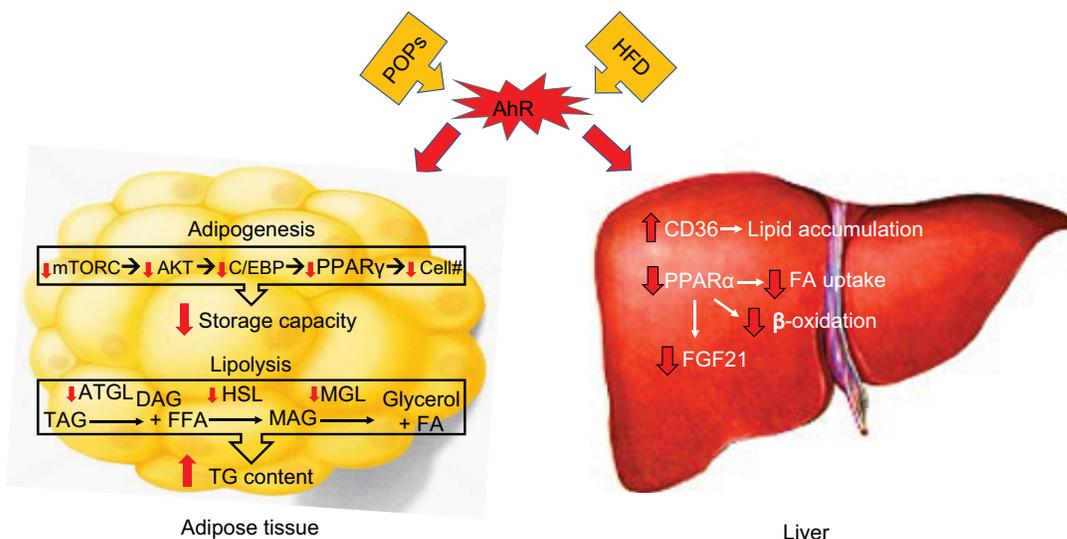
adipogenesis by AhR activation decreases the overall numbers of adipocytes available for storing fat.

AhR also affects the lipolysis function of mature adipocytes. Lipolysis functions as an important regulator of systemic energy homeostasis by providing the body with free fatty acids during fasting and exercise. Defective lipolysis disrupts systemic metabolic processes.<sup>86,87</sup> Adipose triglyceride lipase (ATGL), HSL and monoglyceride lipase (MGL) are key enzymes that regulate this process. ATGL initially hydrolyzes triacylglycerols to produce diacylglycerols (DAGs) and free fatty acids. HSL hydrolyzes DAGs to produce monoacylglycerols (MAGs). Finally, MAGs are broken down to glycerol and non-esterified fatty acids by MGL.<sup>88–90</sup>

Activation of AhR by the agonist  $\beta$ -naphthoflavone inhibits its expression of lipolysis genes in differentiated 3T3-L1 adipocyte cultures (Khazaal and Tischkau, unpublished results) and suppresses lipolysis. BaP suppresses  $\beta$ -adrenoceptor agonist-induced lipolysis in adipose primary explants from mice and humans. In male C57BL/6J mice, weighing 20–22 g (11 weeks), chronic injection of BaP (0.5 mg/kg every 2 days) for 15 days increased body weight and fat mass, although the food intake was similar.<sup>91</sup> However, Hsu et al found that TCDD has no effects on lipolysis in 3T3-L1 cells. This may be because they used the wrong time point. They measured

glycerol content in media of fully differentiated cells treated with TCDD for 3 days or shortly after addition of TCDD.<sup>75</sup>

AhR activation may also increase inflammation in adipose tissue. Administration of obesogenic POPs increases tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) expression in white adipose tissue, which may lead to impaired glucose tolerance in lean and in obese mice.<sup>92</sup> Selective ablation of AhR in adipose tissue abolishes the negative effects of POPs on obesity, inflammation and glucose intolerance.<sup>93</sup> TCDD activation of adipocytes leads to insulin resistance through TNF $\alpha$ .<sup>94</sup> Collectively, these data demonstrate that lipophilic adipogenic POPs accumulate in adipose tissue where they activate AhR. Activated AhR in adipose tissue inhibits adipogenesis, thereby reducing the overall number of fat cells available to store lipids. AhR activation inhibits lipolysis, reducing the ability of adipocytes to break down stored triglycerides into free fatty acids. Finally, AhR activation in adipocytes promotes an inflammatory response in adipose tissue, which contributes to the development of insulin resistance. When combined with long-term exposure to excess energy (HFD), the reduced number of fat cells with impaired ability to breakdown lipids may become over-burdened with lipid, which may subsequently promote local inflammation, leading to insulin resistance (Figure 2).



**Figure 2** AhR regulates body metabolism through actions in the liver and adipose tissue.

**Notes:** AhR activation by POPs and/or high-fat diet directly targets certain genes controlling glucose and lipid metabolism in the liver and adipose tissue, thereby directly contributing to the development of metabolic disorders. In adipose tissue, AhR activation targets adipogenesis and lipolysis, acting to inhibit both processes. In adipose tissue, AhR suppresses mTORC, which decreases AKT activity and inhibits PPAR $\gamma$  levels, ultimately leading to decreased differentiation of pre-adipocytes. The reduction in adipocyte numbers provides fewer adult adipocytes for capturing lipids. Under HFD conditions, adipocytes become hypertrophied and ultimately overwhelmed; lipid then accumulates in other organs and adipose tissue becomes inflamed. In the liver, AhR activation promotes CD36, which enhances lipid uptake by the liver. AhR activation also suppresses PPAR $\alpha$ , leading to decreased  $\beta$ -oxidation and FA metabolism. Finally, AhR may regulate FGF21, which has a role in regulation of systemic metabolism through effects on adipose tissue function.

**Abbreviations:** AhR, aryl hydrocarbon receptor; POPs, persistent organic pollutants; mTORC, mammalian target of rapamycin complex; PPAR, peroxisome proliferator-activated receptor; HFD, high-fat diet; FA, fatty acid; FGF21, fibroblast growth factor 21; C/EBP, CCAAT/enhancer-binding protein; ATGL, adipose triglyceride lipase; DAG, diacylglycerol; HSL, hormone-sensitive lipase; MAG, monoacylglycerol; MGL, monoglyceride lipase; TG, triglycerides; FFA, free fatty acids; TAG, triacyl glycerol.

## AhR, circadian rhythms and metabolism

Alteration of circadian rhythm, specifically inhibition of the clock genes *Period 1 (PER1)*<sup>31</sup> and *Rev-erba* (Sun and Tischkau, unpublished results), may contribute to metabolic dysfunction by AhR activation. Controlled by a master clock in the hypothalamic suprachiasmatic nucleus (SCN), the circadian timing system has emerged as an important regulator of systemic energy metabolism.<sup>95</sup> The SCN provides sympathetic and parasympathetic input to the liver and pancreas<sup>96</sup> to regulate hepatic glucose production and insulin release, respectively.<sup>97</sup> Glucocorticoids and melatonin are hormonal signals that also contribute to clock regulation of systemic metabolism.<sup>98–101</sup>

At the cellular level, circadian clock genes regulate energy balance and metabolism. Mutations or deletion of certain clock genes, including Circadian Locomotor Output Cycles Kaput (*CLOCK*), PERs and cryptochromes, promote obesity, elevated blood glucose and insulin resistance.<sup>102–105</sup> In humans, *PER2* mutations are associated with elevated fasting blood glucose, supporting the link between clock genes and metabolic syndrome.<sup>106</sup> By contrast, liver-specific deletion of the clock gene brain muscle ARNT-like protein 1 (*BMAL1*) produces hypoglycemia, enhanced glucose clearance and loss of rhythmic expression of glucose regulatory genes.<sup>107</sup>

Disrupted circadian rhythms, as commonly occur in human shift workers, are associated with glucose dysregulation, obesity and metabolic syndrome.<sup>108,109</sup> Experimental shift work models support the effects of circadian desynchrony on metabolic disruption.<sup>110,111</sup> Alteration in light/dark cycles in rodent studies suggests that internal desynchrony results from the SCN's rapid shift to align with light/dark cycles, but peripheral clocks becoming uncoupled due to increased food intake during the inactive period.<sup>111,112</sup> This type of internal desynchrony is consistent with the effects of AhR activation on circadian clock function, thereby providing an interesting piece of the mechanism by which both AhR and the clock may interact.

AhR and its partner ARNT as well as core circadian clock genes, *CLOCK* and *BMAL1*, are all members of the PAS domain-containing family of transcriptional regulators.<sup>113</sup> Reciprocal crosstalk between AhR signaling and circadian rhythms has been established.<sup>114</sup> AhR expression is controlled by the circadian clock, likely through *CLOCK/BMAL1* binding to E boxes in the AhR promoter.<sup>115</sup> AhR, ARNT and a number of drug-processing genes are rhythmically expressed;<sup>116–119</sup> AhR rhythmicity is abolished in *CLOCK*-mutant mice.<sup>120</sup> AhR, CYP1A1 and CYP1B1 levels are increased, yet expression is arrhythmic in *PER1* and *PER2*

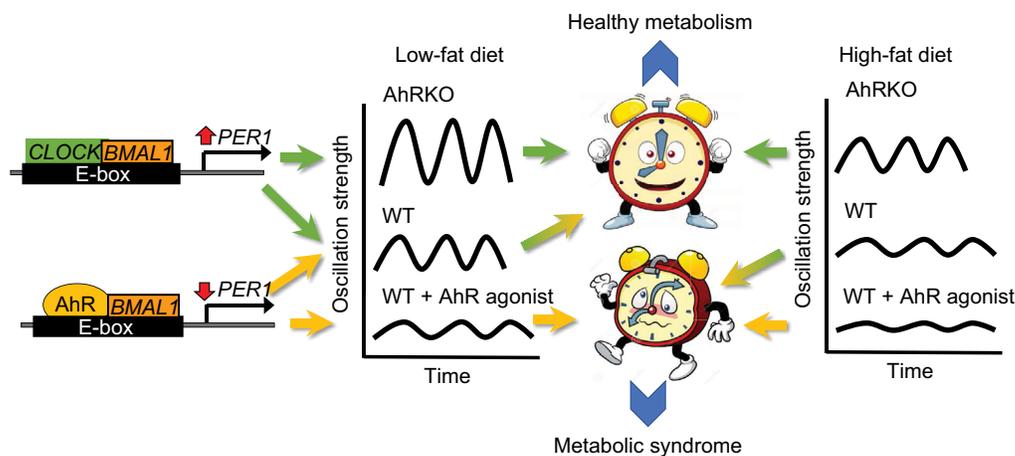
mutant mice.<sup>121–123</sup> Conversely, activation of AhR by TCDD leads to alterations in *PER1*, *PER2* and *BMAL1* expression and reduces the ability of the circadian clock to respond to changes in the light/dark cycle.<sup>114,119,121–128</sup> Moreover, AhR can directly interact with *BMAL1* to attenuate *PER1* expression due to decreased binding of *CLOCK-BMAL1* to E-box of *PER1* promoter.<sup>129</sup> *PER1* expression and rhythm is higher in AhRKO mice than wild-type mice suggesting that these mice had higher response to the light.<sup>31</sup>

Interestingly, like the shift work studies, chronic activation of AhR alters clock function in peripheral metabolically important tissues, while the master clock in the SCN remains aligned with the light/dark cycle.<sup>129</sup> Systemic activation of AhR by treatment with TCDD suppresses the amplitude and delays the peak of *PER1* expression in the liver. Activated AhR can interact with *BMAL1* to suppress E-box derived transcription of the *PER1* gene. *PER1* rhythms in the SCN of these same animals were relatively unaffected by TCDD.<sup>31</sup> It is likely that the *PER1* rhythm was retained in the SCN due to reduced expression of AhR in this tissue and the overriding effects of light in maintaining strong rhythms in the primary clock. However, a shift of liver rhythms creates an internal desynchrony that may underlie the development of metabolic syndrome that is common to both shift work and chronic AhR activation.

Studies in AhR-deficient mice support the cooperation between AhR signaling and the clock in regulation of metabolism. AhR activation suppresses rhythmicity in wild-type AhR-sensitive strains of mice.<sup>129</sup> Mice with germline deletion of one or both *AhR* alleles have increased circadian rhythm amplitude and increased responsiveness to changes in the light/dark cycle indicative of enhanced robustness of their circadian clock.<sup>31,125,126,129–131</sup> Whereas a HFD usually dampens circadian rhythms in metabolically important tissues, AhR-deficient animals retain robust circadian rhythmicity under HFD conditions.<sup>30</sup> Although these studies have not established cause and effect, they support the hypothesis that robust rhythms are important for metabolic health and that AhR interacts with the clock to deregulate energy metabolism.

## Conclusion

AhR activation may contribute to obesity and downstream metabolic disorders. AhR regulates expression of myriad genes,<sup>132</sup> including many associated with obesity, lipid metabolism and inflammation.<sup>43,44</sup> Understanding AhR-dependent mechanisms that contribute to metabolic homeostasis will provide a more holistic understanding regarding



**Figure 3** A healthy circadian clock maintains synchronization of central and peripheral rhythms with the external environment resulting in metabolic homeostasis. AhR can compete with *CLOCK* to form heterodimers with the clock gene, *BMAL1*. Although *CLOCK/BMAL1* act as activators on the E-box of the *PER* promoter, AhR/*BMAL1* suppresses activity at the E-box. Thus, removal of AhR enhances *CLOCK/BMAL1* activity and increases the amplitude of circadian oscillations. When AhR is present, rhythm amplitude is slightly dampened by endogenous activation of AhR. In the presence of AhR agonists, AhR/*BMAL1* activity dominates at the E-box, thereby further dampening the rhythm. HFD can also dampen rhythms, which are strongly associated with metabolic dysfunction. Thus, activation of AhR and HFD interacts to compound the detrimental effects on metabolism. AhR depletion protects against the detrimental effects of HFD by promoting rhythm amplitude and maintaining a healthy clock.

**Abbreviations:** AhR, aryl hydrocarbon receptor; *CLOCK*, Circadian Locomotor Output Cycles Kaput; *BMAL1*, brain muscle ARNT-like protein 1; HFD, high-fat diet; AhRKO, AhR knockout; WT, wild type.

environmental factors that contribute to worldwide pandemics in obesity and metabolic dysfunction. Therapies to address these important global problems are not as simple as fixing diet and encouraging exercise. This review highlights how complex environmental signals, including light/dark cycles and chemical exposure contribute to metabolic health. Altered circadian rhythms, through changes in light/dark cycles, HFD and/or exposure to AhR-activating chemicals, promote metabolic dysfunction. Inhibition of AhR or its downstream signals may provide new targets for combatting these problems. AhR inhibition or silencing protects from the metabolic consequences of rhythm disruption (Figure 3).<sup>30,44,130</sup>

Interestingly, adipose tissue has emerged as an important site regulating whole-body energy metabolism. Many chemicals that activate AhR are lipophilic and aggregate within adipose tissue. However, a recent study demonstrated that altered rhythms in one tissue can lead to disrupted function of other tissues.<sup>133</sup> AhR is highly expressed in adipose tissue and appears to regulate important adipocyte functions, including adipogenesis and lipolysis. As lipolysis is highly controlled by the circadian clock, future studies will examine interactions of AhR with the clock during this physiological process. A better understanding of clock/AhR interactions may provide novel therapeutic approaches to combat obesity and metabolic syndrome.

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## Disclosure

The authors report no conflicts of interest in this work.

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