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AHR in energy balance regulation

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Abstract

Recent studies on mice genetically modified at the *Ahr* locus and fed on high-fat diet have revealed a novel physiological role for the AHR in energy balance. Globally impaired function of the receptor counteracts the development of obesity by increasing energy expenditure, which appears to occur mostly in the skeletal muscle and brown adipose tissue. On the other hand, global and tissue-specific loss of AHR signaling can have opposite effects on liver fat content and their impact on insulin sensitivity is also context-dependent. As tryptophan metabolites are key AHR activators, these findings suggest that the AHR may act as a protein sensor enabling adequate protein intake from low-protein diets by allowing calorie overfeeding without resultant obesity.

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1. Introduction

One of the most characteristic and conspicuous signs of the acute toxicity of TCDD, an extremely potent agonist of the AHR, is the wasting syndrome, which is especially pronounced in the rat and denotes a profound body weight loss (up to over 50% of initial body weight) before death ensues. It primarily results from reduced feed intake [1,2]. The fact that this dramatic response did not appear to be due to nausea or malaise [3] suggested a specific impact on the regulation of energy balance or body weight. This view was reinforced by the findings that rats treated with sublethal doses of TCDD defended their lowered body weight level against external manipulation attempts by even exhibiting hyperphagia if necessary [4], and that TCDD exposure appeared to permanently imprint peculiar deviations on rats' feeding behavior and responses to feeding regulatory challenges [5–7]. Moreover, female C3H/HeN mice treated with a high dose of TCDD (100 μg/kg, once every 2 weeks for 8 weeks) and fed on a high-fat diet surprisingly exhibited augmented body gain compared with their vehicle-treated controls on the same diet [8]. Thus, it was somewhat disappointing to find out that genetic deletion of the AHR in mice or rats did not markedly influence the growth of the animals, although a transient retardation during the first few weeks of postnatal life was reported in mice [9].

However, more recent studies with congenic and genetically bioengineered mouse models have convincingly demonstrated that the AHR does indeed play an important modulating role in energy homeostasis. Initially, Kerley-Hamilton et al. [10] reported that when fed on high-fat chow ("Western diet") for 28 weeks, male C57BL/6J (B6) mice with a high-affinity AHR (Ahr^{b1} allele) became more obese than their congenic counterparts, B6.D2N-Ahr^{d}/J (B6.D2) mice with a low-affinity AHR (Ahr^{d} allele). The difference in body weight emerged by 17 weeks and broadened thereafter until the end of the study. At termination, the B6 mice had larger gonadal fat pads, greater total volume of lipid vacuoles in the liver and higher plasma cholesterol levels than did B6.D2 mice on the same high-fat diet. Interestingly, however, these differences were not recorded when the mice were fed regular chow, and they did not appear to result from dissimilar feed intake levels. Moreover, in B6 mice high-fat diet repressed hepatic *Cyp1a2* gene expression by approximately 3-fold (data on *Cyp1a1* were lacking) compared with the standard diet, suggesting that the canonical AHR signaling pathway was not activated by the special diet. On the other hand, the expression levels of a wide variety of nuclear receptors were affected by diet and/or genotype in the liver. In most cases, high-fat diet exhibited a depressing influence in B6 mice, but *Pparg* (encoding PPARγ) was induced by 61% in them. On high-fat diet, a genotype difference was recorded for *Ppara* expression [encoding PPARα], which was diminished by 34% in B6 vs. B6.D2 mice [10]. These might have contributed to the outcome because PPARα promotes fatty acid oxidation in the liver [11] while increased expression of PPARγ is involved in liver steatosis [12,13].

2. Impact of global AHR deficiency on diet-induced obesity

Two subsequent studies in which AHR function was affected at the whole animal level corroborated the role of AHR in dietary obesity. Xu et al. [14] showed that on a high-fat diet, both AHR-deficient $Ahr^{-/-}$ and

hemizygous $Ahr^{+/-}$ mice gained significantly less weight than wildtype mice from 8 weeks on. Compared with the mutant strains, the wildtype mice had more epididymal and hepatic fat. The hepatic expression of genes involved in fatty acid translocation (*Cd36*), lipogenesis (*Fas*, *Acc*), β-oxidation (*Cpt1a*, *Acox1*, *Ppara*), gluconeogenesis (*Pck1*, *G6pc*) and glucose oxidation (*Pdk4*) was higher in wildtype animals. These also had higher concentrations of insulin and leptin but lower levels of adiponectin in the serum. The obese wildtype mice displayed glucose intolerance and insulin insensitivity, whereas both were improved in the mutant strains in line with their higher serum adiponectin levels. In mice fed the high-fat diet, there was further evidence of aggravated inflammatory reaction in both the liver and white adipose tissue in the wildtype animals vs. $Ahr^{-/-}$ and $Ahr^{+/-}$ mice. Again, feed intake was not affected, and neither was locomotor activity. However, energy expenditure was higher in high-fat-fed $Ahr^{+/-}$ mice than in wildtype mice on either control or high-fat diet ($Ahr^{-/-}$ mice were not analyzed). This was associated with enhanced expression of the genes for the primary uncoupling protein (*Ucp1*) and for the regulator of mitochondrial biogenesis (*Pgc1a*) in the brown adipose tissue as well as those for *Ppard* (a major regulator of muscle fuel utilization favoring lipid oxidation [15]), *Pgc1a*, *Acox1*, *Cpt1b*, *Ucp2* and *Ucp3* in skeletal muscle, which may account for the increased energy expenditure. It is noteworthy that compared with wildtype mice, the $Ahr^{+/-}$ mice expressed approximately 30% of the AHR mRNA in the liver, and that *Cyp1a1* or other AHR battery genes were not influenced by high-fat diet in wildtype mice [14]. This indicates that even partial elimination of AHR expression can have a substantial influence on energy homeostasis and that high-fat diet does not seem to elicit a general AHR activation as measured by expression of genes regulated by it.

3. Modulation of diet-induced obesity by AHR antagonists

Moyer et al. [16] proved that also pharmacological inhibition of AHR function is capable of preventing obesity in mice fed on a high-fat diet. Initially, they tested two AHR antagonists, α-naphtoflavone (aNF) and CH-223191 (approximate doses 3 and 10 mg/kg/day, respectively; added into diet), and found that during a 5-week exposure, both effectively counteracted the increasing impacts of high-fat diet on body, fat and liver masses. For CH-223191, the outcome was somewhat surprising, because this compound has been reported to be a selective antagonist of dioxin-type AHR agonists [17]. The team next extended the duration of aNF exposure to 26 weeks. In this case, aNF prevented B6 mice from gaining extra weight on high-fat diet, ameliorated hepatic steatosis and reduced serum LDL-cholesterol levels. However, it also decelerated the growth of control mice on regular chow, increased their liver-to-body mass ratio, and caused degenerative changes in their hepatocytes, indicating liver toxicity of the compound. The researchers then showed that the gene for indoleamine 2,3-dioxygenase (*Ido1*), an important tryptophan-metabolizing enzyme, is required for the full development of high-fat diet-induced obesity in mice. Their additional *in vitro* studies revealed that kynurenine rather than kynurenic acid is the probable tryptophan metabolite to activate the AHR. Furthermore, TGFβ1 (an indirect *Ido1* inducer [18] whose hepatic gene expression is enhanced by high-fat diet [19]) and oxidized LDL (a ligand for toll-like receptors 2 & 4 [20]) stimulated AHR activity, but more slowly than kynurenine or TCDD. Finally, antagonism of toll-like receptors 2/4 was shown to prevent oxidized LDL-induced AHR activation, while IDO1 inhibition could only diminish it [16]. The effects of a global reduction in AHR activity caused by genetic or pharmacological means are compiled in Figure 1.

4. Liver-specific AHR deficiency and energy balance

In contrast to the amelioration of hepatic steatosis by global AHR deficiency, targeted knockout of *Ahr* in hepatocytes exacerbated it in B6 mice fed on a high-fat diet, without interfering with body weight gain [21]. This appeared to result from augmented expression of genes involved in *de novo* lipogenesis such as *Srebp1c*, *Scd1*, *Acc1* and *Gpat1*, whereas those related to fatty acid uptake, β-oxidation or gluconeogenesis were not differentially affected from control mice on the same diet. High-fat diet-induced hepatic inflammation was also aggravated in knockout mice, coinciding with reduced induction of *Socs3*, the gene for a negative regulator of STAT3 (a mediator of cytokine signaling [22]). Rescue of hepatic *Socs3* expression largely reversed the deleterious effects of hepatic AHR deficiency; the authors further demonstrated *Socs3* to be transcriptionally regulated by the AHR [21]. In two other studies using B6 mice on regular chow, both cholesterol and fatty acid biosynthesis in the liver were enhanced by hepatocyte-specific ablation of AHR and repressed by AHR activation (instigated by β-naphtoflavone) through coordinated transcriptional regulation; the AHR proved to exert these effects by a non-canonical pathway [23,24]. These findings are illustrated in Figure 2.

5. Constitutively active hepatic AHR and energy balance

In FVB mice fed regular chow, the reverse setting, expression of a constitutively active AHR in the liver (and intestine), also resulted in accumulation of lipids in the liver [25]. While hepatic trigycerides were increased compared with the wildtype control, hepatic cholesterol and plasma triglyceride levels were not. Both body and fat mass decreased in the transgenics, whereas lean body mass was elevated. In the liver, the expression of *Ppara*