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Original Research

Obesity and fatty liver are prevented by inhibition of the aryl hydrocarbon receptor in both female and male mice

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ABSTRACT

Inhibition of the aryl hydrocarbon receptor (AHR) prevents Western diet–induced obesity and fatty liver in C57Bl/6J (B6) male mice. The AHR is a ligand-activated nuclear receptor that regulates genes involved in xenobiotic metabolism and T-cell differentiation. Here, we tested the hypothesis that AHR antagonism would also prevent obesity and fatty liver in female mice and that B6 mice (higher-affinity AHR) and congenic B6.D2 mice (lower-affinity AHR) would differentially respond to AHR inhibition. Female and male adult B6 and B6.D2 mice were fed control and Western diets with and without α-naphthoflavone (NF), an AHR inhibitor. A nonlinear mixed-model analysis was developed to project asymptote body mass. We found that obesity, adiposity, and liver steatosis were reduced to near control levels in all female and male B6 and B6.D2 experimental groups fed Western diet with NF. However, differences were noted in that female B6.D2 vs B6 mice on Western diet became more obese; and in general, female mice compared with male mice had a greater fat mass to body mass ratio, were less responsive to NF, and had reduced liver steatosis and hepatomegaly. We report that male mice fed Western diet containing NF or CH-223191, another AHR inhibitor, caused reduced mRNA levels of several liver genes involved in metabolism, including *Cyp1b1* and *Scd1*, offering evidence for a possible mechanism by which the AHR regulates obesity. In conclusion, although there are some sex- and *Ahr* allelic-dependent differences, AHR inhibition prevents obesity and liver steatosis in both males and females regardless of the ligand-binding capacity of the AHR. We also present evidence consistent with the notion that an AHR-CYP1B1-SCD1 axis is involved in obesity, providing potentially convenient and effective targets for treatment.

Abbreviations: AHR, aryl hydrocarbon receptor; B6, C57BL/6J mouse strain; B6.D2, B6.D2N-*Ahr*^{d}/J mouse strain; CH, CH-223191; NF, α-naphthoflavone; MRI, magnetic resonance imaging; SEM, standard error of the means; qPCR, quantitative polymerase chain reaction.

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1. Introduction

Obesity is a growing and increasingly challenging problem with a prevalence in the United States of 16.9% for 2- to 19-year-olds and 34.9% for adults 20 years and older [1]. It is well established that obesity [2] and associated complications [3,4] develop differently between males and females and that gene expression profiles in liver and adipose tissue are dissimilar in male and female mice [5]. Given that the aryl hydrocarbon receptor (AHR) has a large role in obesity in male mice [6-8], we asked whether the AHR carries out a similar role in obesity for females.

The AHR is a ligand-activated nuclear receptor/transcription factor [9] (Fig. 1A). In the cytoplasm, the ligand-free AHR is in a complex with 2 HSP90 molecules, at least 1 p45 protein identified as an immunophilin [10,11], and the co-chaperone p23 protein [12]. In canonical AHR signaling, ligand binding alters the complex, allowing the translocation of the AHR to the nucleus and partnering with the aryl hydrocarbon nuclear translocator. The nuclear AHR/aryl hydrocarbon nuclear translocator heterodimer binds to core CACGC DNA motifs called *dioxin response elements* present in the regulatory regions of several thousand genes [13] to activate transcription. Because of the AHR's ligand-binding promiscuity, several AHR ligands, such as α-naphthoflavone (NF) (Fig. 1B), have been identified that act as antagonists to inhibit AHR signaling [14].

We first showed the involvement of the AHR in obesity using 2 male mouse models that differ at the *Ahr* gene [6]. The C57Bl/6 mouse (B6 strain) expresses an AHR possessing a generally higher-affinity ligand-binding domain encoded by the Ahr^{b1} allele, and the congenic C57Bl/6.D2 mouse (B6.D2N-Ahr^{d}/J mouse strain [B6.D2]) expresses an AHR more similar to that of the human AHR with a lower-affinity, ligand-binding domain encoded by the Ahr^{d} allele naturally found in the DBA/2 mouse strain [15]. The 2 AHRs differ by approximately 10-fold in 2,3,7,8-terachlorodibenzo-*p*-dioxin binding affinity [16], and in turn, AHR-based gene induction and gene expression levels of the *Cyp1a1* and *Cyp1a2* xenobiotic-metabolizing genes are correspondingly affected [17]. It should be noted, however, that the AHR displays a wide range of allelic-, ligand-, and species-specific binding affinities and activation [18-20]. Using male mice, we found that the B6 and B6.D2 mouse strains when fed Western diet were differentially affected in body size, body fat to body mass ratios, liver gene expression, and liver physiology [6].

We extended those studies in B6 male mice to show that NF and the more specific AHR antagonist CH-223191 (CH) [14,21,22] effectively reduced obesity and hepatosteatosis [7]. We also investigated how Western diet may activate the AHR. Based on in vivo and in vitro studies, we proposed a model that linked Western diet to AHR activation via Toll-like receptor 2/4 and TGFβ signaling pathways stimulated by diet-derived compounds [7]. These studies and recent work with B6.$Ahr^{-/-}$ mice, which were shown to be resistant to obesity [7,8,23], indicated key roles for the AHR in fat metabolism and deposition as well as a possible target for AHR-based therapeutic and preventative approaches.

A major question is how the Western diet–activated AHR causes obesity and, in turn, how inhibition of AHR signaling then prevents obesity. An explanation may lie in recent studies showing that deletion of the canonical AHR-regulated *Cyp1b1* gene prevents obesity in mice fed a high-fat diet [24-26]. Here, we initiate an investigation into AHR-based obesity by determining liver mRNA levels of the *Cyp1b1* gene along with several other AHR-regulated genes involved in metabolism.

As most of the reported work on obesity has been carried out using male mice, we tested the hypothesis that the outcomes observed in male mice regarding the role of the AHR in obesity and steatosis would be similarly observed in females. Specifically, our objectives were to determine whether the AHR plays a role in diet-based obesity in females and whether the AHR antagonist NF could act as an obesity preventative in mice with different AHR ligand-binding affinities. Congenic strains of female and male mice harboring different *Ahr* alleles encoding proteins with different ligand-binding affinities were fed control and Western diets with and without the AHR antagonist NF. The Western diet was high in animal fat and cholesterol to best reflect the typical human Western diet vs a low-fat, low-cholesterol control diet. Although several significant differences are described, we found that the AHR played parallel roles in the deposition and distribution of fat in male and female mice and that both sexes responded fully to AHR antagonism regardless of ligand-binding strength in preventing Western diet–based obesity and hepatosteatosis. These studies strengthen the contention that the AHR offers a potentially

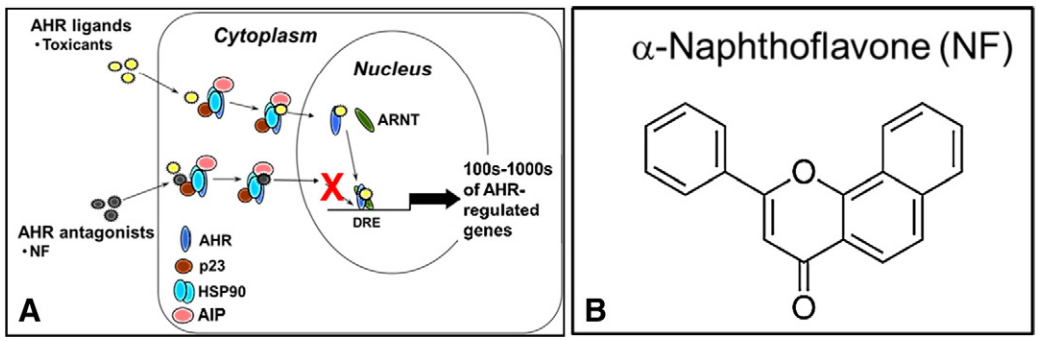

Fig. 1 – The AHR signaling pathway (A) is best known for the induction of xenobiotic metabolizing genes via activation by environmental toxicants. AHR activation can be inhibited by AHR antagonists such as NF (B) by decreasing AHR-directed transcription.