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Inhibition of the aryl hydrocarbon receptor prevents Western diet-induced obesity. Model for AHR activation by kynurenine via oxidized-LDL, TLR2/4, TGFβ, and IDO1

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ABSTRACT

Obesity is an increasingly urgent global problem, yet, little is known about its causes and less is known how obesity can be effectively treated. We showed previously that the aryl hydrocarbon receptor (AHR) plays a role in the regulation of body mass in mice fed Western diet. The AHR is a ligand-activated nuclear receptor that regulates genes involved in a number of biological pathways, including xenobiotic metabolism and T cell polarization. This study was an investigation into whether inhibition of the AHR prevents Western diet-based obesity. Male C57Bl/6J mice were fed control and Western diets with and without the AHR antagonist α-naphthoflavone or CH-223191, and a mouse hepatocyte cell line was used to delineate relevant cellular pathways. Studies are presented showing that the AHR antagonists α-naphthoflavone and CH-223191 significantly reduce obesity and adiposity and ameliorates liver steatosis in male C57Bl/6J mice fed a Western diet. Mice deficient in the tryptophan metabolizing enzyme indoleamine 2,3-dioxygenase 1 (IDO1) were also resistant to obesity. Using an AHR-directed, luciferase-expressing mouse hepatocyte cell line, we show that the transforming growth factor β1 (TGFβ1) signaling pathway via PI3K and NF-κB and the toll-like receptor 2/4 (TLR2/4) signaling pathway stimulated by oxidized low-density lipoproteins via NF-κB, each induce luciferase expression; however, TLR2/4 signaling was significantly reduced by inhibition of IDO1. At physiological levels, kynurenine but not kynurenic acid (both tryptophan metabolites and known AHR agonists) activated AHR-directed luciferase expression. We propose a hepatocyte-based model, in which kynurenine production is increased by enhanced IDO1 activity stimulated by TGFβ1 and TLR2/4 signaling, via PI3K and NF-κB, to perpetuate a cycle of AHR activation to cause obesity; and inhibition of the AHR, in turn, blocks the cycle's output to prevent obesity. The AHR with its broad ligand binding specificity is a promising candidate for a potentially simple therapeutic approach for the prevention and treatment of obesity and associated complications.

1. Introduction

One of the accepted causes for the worldwide rise in obesity and associated problems is the increased global consumption of the high-calorie, high-fat, high-carbohydrate, high-salt, low-fiber Western diet. In 2011–2012, nearly 35% of adults aged were obese (Ogden et al., 2014). Obesity is a contributor to inflammation (De Nardo and Latz, 2011), diabetes and metabolic syndrome (Wang et al., 2011b), cardiovascular disease (Poirier et al., 2006), and cancer (van den Brandt et al., 2000). It has been estimated that 25–70% of obesity is gene based (Maffeis, 2000), and twins studies suggest that 25–40% of individual differences in obesity are genetic (Stunkard et al., 1986). A few genes have been identified that influence obesity, such as the *Leptin* (*Ob*) (Zhang et al., 1994) and *Adiponectin* (Yamauchi et al., 2001) genes; however, it is clear that many genes are involved in the complex interactions that have given rise to the global obesity explosion (Woods et al., 1998). Furthermore, non-genetic factors are a major contributor

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to obesity in that a heightened exposure to environmental toxicants or obesogens (Grun and Blumberg, 2006) is strongly associated with the sharp increase in obesity and associated diseases (Baillie-Hamilton, 2002), in which the aryl hydrocarbon receptor (AHR) may be playing a large role (Wang et al., 2011a; Kerley-Hamilton et al., 2012; Xu et al., 2015).

The AHR is a ligand/toxicant-activated nuclear receptor that regulates hundreds of genes (Kerley-Hamilton et al., 2012) and many cellular pathways, and mice with the *Ahr* gene deleted suffer several developmental and metabolic anomalies (Fernandez-Salguero et al., 1995; Lahvis et al., 2000; Quintana et al., 2008). Upon agonist binding, the AHR translocates to the nucleus where it complexes with the AHR nuclear translocator (Hoffman et al., 1991). The AHR is best known for the induction by environmental toxicants of genes in the cytochrome P450 *Cyp1* family and several Phase II detoxification genes (Nebert et al., 1993; Hankinson, 1995). Like all cellular signaling pathways, the AHR is known to interact with many other signaling pathways (Puga et al., 2005), including TGFβ (Gaido et al., 1992). In summation, the AHR plays vital roles in vascular patterning, organ modeling, extracellular matrix deposition, cell proliferation, apoptosis, and the cardiac system.

In addition to exogenous ligands, such as dioxin and benzo[*a*]pyrene, several tryptophan (Trp) catabolites have been identified as endogenous AHR ligands (Zelante et al., 2014). One such Trp catabolite is L-kynurenine (Kyn), an AHR agonist that activates AHR-directed, naive T cell polarization to the anti-inflammatory Treg phenotype (Veldhoen et al., 2009; Mezrich et al., 2010; Nguyen et al., 2010; Nguyen et al., 2013). Trp is an essential amino acid of which 95% is metabolized in a tissue-specific manner by the rate-limiting enzymes tryptophan 2,3-dioxygenase 2 (TDO2) and indoleamine 2,3-dioxygenase (IDO1 and IDO2) (Mangge et al., 2013).

Low-density lipoproteins (LDLs) have also been identified as an endogenous activator of AHR signaling (McMillan and Bradfield, 2007), although not as an AHR-binding ligand. LDLs are one of several types of lipoprotein particles in forms by which diet-derived fats are transported in the blood. An LDL particle is composed of an apolipoprotein B protein, 50–60 ancillary proteins, and ~5000 fat molecules that includes variable amounts of cholesterol, phospholipids, and triglycerides (Dashty et al.,

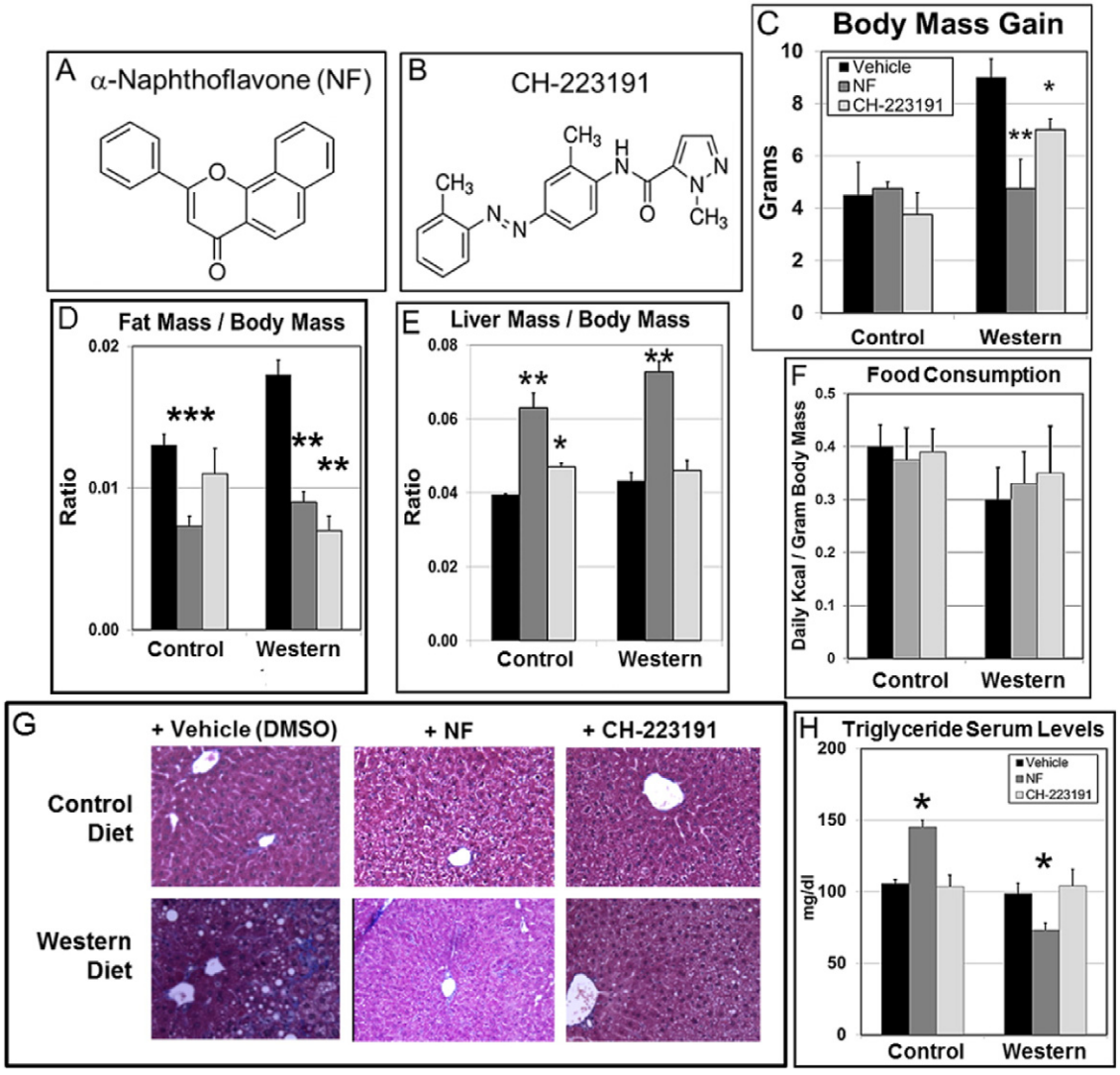

Fig. 1. AHR antagonists prevent diet-based obesity, adiposity, and hepatic steatosis. Chemical structure of (A) α-naphthoflavone (NF) and (B) CH-223191. A comparison of the effects of NF and CH-223191 on B6 male mice (n = 4/experimental group) fed *ad libitum* at weaning control and Western diets ± NF (~3 mg/kg/day) or ± CH-223191 (~10 mg/kg/day) on (C) total body mass gain and (D) gonadal fat mass to total body mass ratio. (E) Food consumption for each experimental group was determined over a 7-day period at week 3 during the 5-wk diet regimen. (F) Total liver mass to total body mass ratio at the conclusion of the 5-wk diet regimen. (G) Representative liver sections stained with Masson's trichrome and (H) plot of triglyceride serum levels of the same experimental groups. *p*-values to the corresponding vehicle-treated control group: *, ≤0.05; **, ≤0.02; ***, ≤0.002. Error bars represent standard error of the mean (SEM).