Obesity Is Mediated by Differential Aryl Hydrocarbon Receptor Signaling in Mice Fed A Western Diet

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Abstract

Obesity is a growing worldwide problem with genetic and environmental causes and an underlying basis for many diseases. The aryl hydrocarbon receptor (AHR) is a ligand-activated nuclear receptor/transcription factor that is the body's primary defense to environmental toxicant exposures. We used two congenic mouse models that differ at the *Ahr* gene and which encode AHRs with a 10-fold difference in signaling activity. The two *Ahr* mouse strains were fed a Western diet, which differentially affected body size, body fat:body mass ratios, liver size and liver metabolism, blood cell profiles, and liver mRNA and miRNA profiles. A low-fat diet had no significant differential effects. The results suggest that the AHR plays a large and broad role in obesity and associated complications, and importantly, may provide a simple and effective therapeutic strategy in the treatment of obesity.

Introduction

It is estimated that 25-70% of the underlying basis for obesity is gene based (1, 2) making environmental factors a major contributor at 30-75% (3). One of the accepted environmental causes for the worldwide rise in obesity and associated problems is the increased consumption of the high-calorie, high-fat, low-fiber Western diet. A biological entity that tightly links genes and the environment is a nuclear receptor best known for its role in xenobiotic metabolism. The aryl hydrocarbon receptor (AHR) is a ligand-activated nuclear receptor/transcription factor that regulates genes involved in toxicant metabolism and is the body's primary defense to environmental exposures. AHR signaling is also involved in a number of essential non-xenobiotic biological and developmental pathways (4). Upon ligand binding, the AHR translocates to the nucleus where it complexes with the AHR nuclear translocator (ARNT). The AHR/ARNT heterodimer regulates the transcription of genes in the cytochrome P450 *Cyp1* family, some Phase II detoxification genes, as well as thousands of other genes involved in all aspects of cell physiology (5). The AHR is also activated by dietary components, e.g., fats and fat derivatives (6); and there is evidence linking the activated AHR to major diseases including obesity (7).

To identify a possible role for the AHR in obesity, we used two mouse models that differ at the *Ahr* gene **(Fig. 1A)**. The two strains were C57Bl/6 (B6 strain), which naturally bears the high-affinity AHR encoded by the Ahr^{b1} allele and the congenic C57Bl/6.D2 (B6.D2 strain) bearing the low-affinity AHR encoded by the Ahr^{d} allele naturally found in the DBA/2 mouse strain. The two *Ahr* alleles encode AHRs that differ by approximately 10-fold in ligand binding affinities, and in turn, gene induction and gene expression levels, including that of the *Cyp1a1* and *Cyp1b1* xenobiotic genes (8). A distinct advantage of using the B6 and B6.D2 mouse models is that by virtue of the integral role the AHR plays in response to endogenous and environmental agents, any corresponding differences observed in disease states, gene expression profiles, and affected signaling pathways are due to the differing capacities of the corresponding AHRs. We tested the hypothesis that differential AHR signaling activity causes differential effects on body mass, fat metabolism, liver gene expression, and liver physiology. Using the B6 and B6.D2 mouse models, we found that the differential AHR signaling activated by a Western diet drastically affected body size, blood cell profiles, liver gene expression, and liver physiology.

Results

There have been hints that the AHR may be a participant in the regulation of fat metabolism and obesity. For instance, mice exposed to lower levels of polychlorinated biphenyl-77 (PCB-77) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in increased adipocyte

differentiation, *Pparγ* expression, and obesity levels; whereas, higher levels of PCB-77 or TCDD inhibited adipocyte differentiation and *Pparγ* expression (9). The intent of the work reported here was to test more directly the role of the AHR in obesity and fat metabolism but in lieu of exogenous toxicants. Although several studies have examined the relationship between the AHR and fat metabolism using a model system comparing functional AHR signaling to one that is AHR deficient, none have examined the consequences resulting from different levels of AHR signaling activity.

Differential AHR signaling and obesity. We used two congenic mouse models **(Fig. 1A)** that encode AHRs that differ by 10-fold in signaling activity (10). Male mice from B6 and B6.D2 mouse strains were placed into two diet groups (n = 8 per group) and fed low-fat regular chow (18% of kcal from fat) or Western chow, i.e., (42% of kcal from fat) for 27 weeks beginning at 5 weeks of age. By 17 weeks on Western chow, B6 male had significantly greater body mass than did the B6.D2 male mice **(Fig. 1B)**, and at the 27-week conclusion, B6 mice on Western diet were 16% larger than their B6.D2 counterparts **(Table S3)**. **Table S4** displays the average mass of the eight mice in each group over the 27-week span. Except for weeks 20 and 21, when some of the mice were placed in metabolic cages, the low-fat regular diet had no differential effect on the two mouse strains.

In order to determine whether the significant differences in body mass observed between the two mouse strains fed the Western diet were due to metabolic rather than differences in behavioral eating habits, three of the eight mice in the low-fat and Western diet experimental groups at week 20 in the diet regimen were placed in individual metabolic cages to measure food and water intake and urine and feces production. The results presented in **Fig. 1C** show that although there were significant differences in the amount of Western vs. regular chow consumed and of feces generated, there were no significant differences between mouse strains in any of the measured parameters, thus, the difference in body mass between the B6 and B6.D2 were not due to differences in eating and excretion behavior.

The increased body mass seen in the B6 mice relative to B6.D2 mice could be due to an overall proportional increase in body size rather than an increased relative accumulation of body fat. The gonadal fat pad mass to body mass ratio highly correlates to that of the overall body white fat to body mass ratio (11). Relative fat mass to body mass between the two strains was determined by weighing gonadal fad pad masses at the time of sacrifice. Based on the gonadal fat pad mass/body mass ratio, B6 mice relative to B6.D2 mice had a significantly greater white fat mass **(Fig. 1D)**; and B6 mice on Western diet to those on regular diet had a significantly greater white fat mass to body mass ratio while B6.D2 mice remained statistically unchanged **(Fig. 1E)**. Together, these data and the above results suggest that there is an AHR-dependent metabolic basis for the significant increase in fat and body masses observed in the B6 vs. B6.D2 mice fed a Western diet and not due to a difference in eating habits or to a proportional difference in overall body size.

Liver size and metabolism in B6 vs. B6.D2 mice fed a Western diet. The liver is the primary site of dietary fat metabolism and regulator of fat levels in the blood. Several striking results led us to conclude that differential AHR activity had a large impact on liver growth and metabolism **(Table S5)**. For both mouse strains, the Western chow not only had a major impact on body mass after the 27-week diet regimen **(Fig. 2A)** but also on liver mass **(Fig. 2B)**, in that the Western diets caused an approximate two-fold increase in liver mass relative to body mass compared to mice of both strains fed regular chow **(Fig. 2C)**. However, the impact of the Western diet on liver size was greater for B6 mice to that of B6.D2 mice, in that B6 mice had significantly larger livers and liver mass to body mass ratios than did the B6.D2 mice.

The hepatomegaly observed in the mice fed the Western diets was reminiscent of non-alcoholic fatty liver disease, which is most often caused by the accumulation of fat in the liver in obese individuals (12). We investigated whether there was differential fat accumulation in mice fed Western vs. regular chow and whether there were genotypic differences in fat accumulation between strains for each diet. Liver sections were stained with hematoxylin and counter stained with eosin, which can reveal the presence of fat storage vesicles. There were no apparent fat vesicles in B6 and B6.D2 mice fed regular diet **(Fig. 2D,E)** and no significant difference in fat vesicle volume **(Fig. 2H)**. However, B6 mice fed a Western diet had a significantly greater volume of fat storage vesicles than did the B6.D2 strain (*p*-value = 1.54×10^{-8}) **(Fig. 2F-H)**.

Alanine aminotransferase (ALT) levels rise dramatically in acute liver damage; whereas, aspartate aminotransferase (AST) plasma levels is an indicator of extrahepatic tissue damage. B6 mice had significantly greater plasma levels of both AST and ALT compared to that of B6.D2 mice when both strains were fed the Western diet **(Fig. 2I,J)**. B6 mice also had a lower AST/ALT ratio indicating relatively more severe liver damage than in B6.D2 **(Fig. 2K)**. The B6 mice on the Western diet also presented significantly elevated plasma levels of alkaline phosphatase, total protein, and total cholesterol relative to B6.D2 mice **(Fig. 2L-N)**. An increased level of alkaline phosphatase (ALP) **(Fig. 2L)** is another measure of a number of liver anomalies, including obesity (13). Increased total protein levels **(Fig. 2M)** can be associated with liver disease but often remain in the normal range (4.6-6.9 g/dl) typically due to a decrease in plasma albumin concentration and a concomitant increase of plasma globulin levels, including ALT, AST, and ALP. However, there were no significant differences in plasma albumin levels between B6 and B6.D2 mice, and we surmised that the above normal total proteins levels observed in B6 mice was due primarily to the increased globulin levels. Raised plasma levels of total cholesterol **(Fig. 2N)** is associated with the chronic consumption of fatty diets (14).

Blood cell numbers are dependent on *Ahr* genotype and diet. AHR signaling activity is required for normal maintenance of hematopoietic stem cell quiescence (15). The AHR acts as a negative regulator of hematopoiesis, and changes in AHR signaling induced by exogenous toxicants are known to affect stem cell/progenitor cell regulation. Obesity in humans also has a large effect on red blood cell (RBC), white blood cell (WBC), and platelet numbers and/or activity (16-18), hence, we asked whether the Western diets affected blood cell counts and parameters as a result of differential AHR signaling. Blood samples were taken at sacrifice from the B6 and B6.D2 mice at the completion of the 28-week diet regimen, and blood cell counts were determined from blood smears **(Fig. 3** and **Table S6)**. The most notable changes were (i) the significant drop in RBC numbers in B6.D2 mice fed Western diet, (ii) the significant increase in WBC numbers in both strains fed Western diet, (iii) the significant jump in neutrophil numbers in both strains fed Western diet but more so in B6 mice, (iv) the more than doubling of monocyte cell numbers for B6.D2 mice fed Western diet, and (v) the platelet numbers for B6.D2 mice were far outside the normal range (19) independent of diet and were significantly greater relative to that of B6 mice.

Liver mRNA profiles of B6 and B6.D2 mice fed Western diet vs. regular diet. The mRNA levels from liver of B6 and B6.D2 mice fed Western diet were compared to mice of the same strain fed regular diet, i.e., the effect of diet on a given *Ahr* genotype. All differentially expressed genes (*p*-value ≤0.05), for both comparisons (B6W vs. B6R and B6.D2W vs. B6.D2R) are displayed in **Table S7**. The salient observations from the comparisons were the following. One, the affected genes **(Table S8)** and associated biological pathways **(Table S9)** in B6 and B6.D2 mice fed a Western diet were involved primarily in inflammation, immune response, sterol and cholesterol metabolism, and lipid metabolism but at generally lower differential levels in B6.D2 mice to that of B6 mice. Two, there was a relatively high number of

shared genes between B6 and B6.D2 mice in response to a Western diet **(Table S8)**, and not surprisingly, the associated biological pathways were involved primarily in inflammation, immune response, sterol and cholesterol metabolism, and lipid metabolism **(Table S9)**. Of the 5,586 total differentially expressed genes between the B6W/B6R and B6.D2W/D2R comparisons, 1945 genes (34.8%) were shared, of which 1927 (34.5%) were similarly expressed and only 18 (0.3%) were expressed in opposite directions **(Fig. 4A)**. Three, a considerable percentage of the total number of differentially expressed genes by B6 (886 genes, 15.9%) and B6.D2 (2755 genes, 49.3%) mice were unique to that strain **(Fig. 4A, Table S8)**.

The mRNA levels of some genes known to be involved in obesity, lipid and sterol metabolism, inflammation, and blood cell development **(Table S8)**, many of which contained AHR promoter response elements (REs) (20)**,** were impacted by Western diet in B6 and B6.D2, respectively, such as *ApoA4* (↑15.9-fold, ↑10.2-fold), which is involved in innate immunity and fat localization (21); *Anxa2* (↑10.1-fold, ↑10.8-fold; 6 AHR REs), which has a role in hematopoietic stem cell homing (22); and *Hsd3b5* (↓0.03-fold, ↓0.03-fold), a gene associated with hepatic steatosis (23) and which dropped over 30-fold in both strains. Some potentially key genes uniquely differentially expressed in the B6W/B6R group of mice **(Fig. 4B)** included metallothionein (*Mt1*) (↑4.17-fold), a leptin-regulated gene that responds to endoplasmic reticulum (ER) stress brought on by obesity (24) and multiple mRNA forms of *Insig1* (↓0.40 and 0.37-fold; 12 AHR REs). INSIG1 is a key regulator in cholesterol metabolism (25), and in addition to the AHR, the *Insig1* gene is regulated by multiple nuclear receptors including PPARα (15 AHR REs), CAR (2 AHR REs), and PXR. Some uniquely differentially expressed genes in the B6.D2W/B6.D2R group of mice **(Fig. 4C)** included *Hamp2* (↑9.1-fold), which has role in iron metabolism and SMAD phosphorylation (26); and *Creld2* (↓0.3-fold), an ER-stress induced gene (27). Cellular pathways expressed uniquely in B6 mice fed a Western diet relative to those fed a regular diet were associated with inflammation; whereas, genes and pathways unique to B6.D2 mice dealt more with cellular housekeeping chores, including, protein localization and DNA repair **(Fig. 4D** and **E**, **Tables S8** and **S9)**.

Liver mRNA profiles of B6 vs. B6.D2 mice fed a regular and Western diet. Whereas above, we wanted to determine the effect of diet on each of the *Ahr* genotypes, here, we wanted to determine the effect of *Ahr* genotype for each diet. Differential gene expression levels (*p*-value ≤ 0.05) of B6 and B6.D2 mice fed regular diet were compared to each other and B6 and B6.D2 mice fed Western diet were compared to each other **(Fig. 4F, Tables S10** and **S11)**. Several observations were made from the comparisons. One, there were relatively few shared genes between those differentially expressed in B6 vs. B6.D2 mice on regular diet and those differentially expressed in B6 vs. B6.D2 mice on Western diet **(Table S12)**. Of the total 1,876 differentially expressed genes only 73 (3.9%) were shared, of which 48 (2.6%) were similarly expressed and 25 (1.3%) were expressed in opposite directions. Two, there were 712 (38.0%) unique differentially expressed genes in B6 and B6.D2 mice fed regular chow, from which only two statistically significant biological pathways were generated (regulation of nucleobase, nucleoside, nucleotide, and nucleic acid transport and response to wounding) **(Table S13)**. Three, there were 1091 unique genes differentially expressed in B6 vs. B6.D2 mice fed Western chow **(Table S12)**. Thus, the AHR has a huge but distinct impact on gene expression in the liver of B6 and B6.D2 mice that is dependent on fat levels in the diet. The gene lists for all four comparisons are summarized in **Table S14**.

A potentially key differentially expressed gene shared between B6 and B6.D2 fed either the Western or regular diets, respectively, **(Table S14)** was *Erdr1* (↑2.4-fold, ↑1.6-fold)), a gene involved in hematopoietic stem cell regulation (28). Some potentially important genes expressed

uniquely in the B6W/B6.D2W comparison, in which some contained AHR REs **(Fig. 4G)** included *Cyp2d26*, *Gadd45g*, *Bhmt*, *Sqle*, and again, the *Insig1* (↓0.46-fold; 12 AHR REs) gene. *Cyp2d26* (↑42-fold; 2 AHR REs) is a candidate gene for the regulation of triglyceride levels (29); *Gadd45g* (↑2.50-fold; 12 AHR REs) encodes a protein that functions in T cell production (30); the *Bhmt* (↑2.09-fold; 7 AHR REs) gene product is associated with liver steatosis and injury and protects hepatocytes from ER stress and excess lipid accumulation (31); and the obesity-associated *Sqle* (↓0.52-fold; 8 AHR REs) gene encodes a protein that carries out a step in cholesterol biosynthesis (32). Genes relevant to obesity expressed uniquely in the B6R/B6.D2R comparison included *Ppp1r3c*, *Elovl3*, and again, the *Mt1* (↓0.25-fold) gene. The *Ppp1r3c* (↑2.3-fold) gene product is involved in glycogen storage in adipocytes (33); and loss of the *Elovl3* (↑2.2-fold) gene in mice causes reduced adiponectin levels, inhibition of adipose tissue expansion, and resistance to diet-induced obesity (34). The major biological pathways affected in B6 vs. B6.D2 mice fed Western chow were involved in fat metabolism and synthesis, vasculature, and sterol metabolism **(Fig. 4H, Table S13)**.

miRNA profiles of liver. Studies have shown an important role for miRNAs in fat metabolism (35). All statistically significant (p-value ≤0.05) differentially expressed miRNA levels from B6 and B6.D2 mice fed Western and regular diets are listed in **Tables S15-S19**. Those differentially expressed miRNAs with a fold-change of two or greater and those with roles known to be associated with obesity, non-alcoholic fatty liver disease, and adipogenesis, e.g., mmu-miR-130b and mmu-miR-132 are shown in **Table S19B**. However, the majority of the highly differentially expressed miRNAs presented in **Table S19B** have not been described previously as playing a role in obesity and deserve further scrutiny.

Discussion

AHR signaling and human obesity. There are four well-characterized *Ahr* allelic variants in mice, of which, there is a 10-fold difference in the affinity for the AHR ligand between the most responsive/most sensitive allele (Ahr^{b1} of the B6 mouse) and the least responsive/least sensitive allele (Ahr^{d} of the B6.D2 mouse) (36-38). The 10-fold difference in affinity/response/sensitivity corresponds to a 10-fold difference in AHR activity. The difference in affinity is primarily due to the replacement of an alanine at position 375 (high affinity Ahr^{b1}) with a valine (low affinity Ahr^{d}) (39). The results from the mouse model studies should be translatable to humans because the affinity and responsiveness of the mouse Ahr^{d} gene is similar to that of the human *Ahr* gene, in which the human AHR has a valine at the equivalent position of 375 in the mouse AHR (40). In humans, epidemiological studies have shown an association between various polymorphic forms of the AHR and cancer (41) but none to obesity. However, large scale human epidemiological studies examining the AHR and obesity have not been conducted.

Nonetheless, experimental work in mice has revealed a possible link between AHR signaling and obesity and fat metabolism. AHR signaling inhibits lipid synthesis, regulates adipocyte differentiation, and the loss of AHR activity causes an increase in triglyceride synthesis (42). Constitutive AHR signaling in mice caused an increase in the levels and activity of fatty acid transport proteins and CD36 (a cell surface fatty acid receptor and translocase and of which the *Cd36* gene is a transcriptional target of the AHR) inhibition of fatty acid oxidation, an increase in peripheral fat mobilization, and hepatic steatosis (43).

AHR signaling and blood cell numbers. The bone marrow of mice is known to be differentially sensitive to differential toxicant-activated AHR signaling (44). Mouse strains exposed to benzo[a]pyrene carrying the low-affinity Ahr^{d} gene, including B6.D2 mice (45), are much more sickly and prone to dying than are B6 mice carrying the high-affinity Ahr^{b1} gene. Fat-

derived ligands can activate AHR signaling (6), and it may be that fat-derived molecules from the Western diet are differentially activating AHR signaling during hematopoiesis to disrupt various aspects of blood cell differentiation. As with environmental toxicants, Western diet appeared to be generally more detrimental to the blood cells of B6.D2 than to that of B6 mice. For example, B6.D2 mice had much higher levels of monocytes than did B6 mice, and obesity is associated with low grade chronic inflammation that is derived in part from adipose tissue that has been infiltrated with bone marrow-derived, circulating macrophages and monocytes (46). Furthermore, the accumulation of macrophages and monocytes in adipose tissue is strongly correlated with higher total body fat and body mass index (46).

The AHR, other nuclear receptors, and obesity. Understanding the regulatory pathways that govern fat synthesis, accumulation, and catabolism are key to understanding obesity, and nuclear receptors are critical sensors and regulators of fat metabolism. The various nuclear receptors involved in fat metabolism participate in extensive cross-regulatory and cross-signaling interactions among each other and with the AHR We found that the gene expression levels of numerous genes encoding nuclear receptors are differentially affected by diet in B6 and B6.D2 mice and that many of the promoters in genes encoding nuclear receptors possess AHR REs **(Table S20)**.

Probably the most important are the peroxisome proliferator-activated receptors (PPARs), which are stimulated by fatty acid derivatives that act as ligands to promote lipid synthesis and storage in adipocytes (PPARγ, 1 AHR RE, ↑1.61 in B6W/B6R) and to activate oxidation pathways in the liver (PPARα, 15 AHR REs, ↓0.66 in B6W/B6.D2W) and in muscle and brown adipocytes (PPARδ) (47). Other important nuclear receptors include retinoic acid receptors α and β (RARα and RARβ, ↓0.82 in B6W/B6.D2W; ↑1.43 in B6.D2W/B6.D2R), which are activated by retinoic acid (RA) to suppress obesity. RA also serves as a ligand for PPARβ/γ to induce genes involved in the regulation of energy homeostasis and insulin responses (48). The liver X receptors (LXR, 5 AHR REs) sense oxysterols and regulate genes that decrease cholesterol levels and stimulate fatty acid and triglyceride synthesis (↓0.85 in B6W/B6R) (49). Although the closely related nuclear receptors constitutive androstane/active receptor (CAR, 2 AHR REs) and pregnane X receptor (PXR) are known primarily for xenobiotic metabolism (50), activation of CAR significantly reduces serum glucose levels, improves glucose tolerance and insulin sensitivity, and inhibits the expression of lipogenic genes of the liver in mice fed a Western diet (51). On the other hand, the activated PXR induces lipogenesis and suppresses several genes involved in fatty acid oxidation (↑1.22 in B6W/B6R) (52). Members of the retinoid X receptor family (RXRα with 10 AHR REs, RXRβ, and RXRγ with 7 AHR REs), which can form homodimers as well as serve as the heterodimeric binding partners for the PPARs, RARs, LXRs, CARs, and PXRs (53), bind diet-derived lipophilic ligands, and in turn, modulate lipid homeostasis (54). The AHR is also activated by fat derivatives, including eicosanoids and low-density lipoproteins (6, 55), and thus, the observed body size differences between B6 and B6.D2 mice may be due to direct differential activation of AHR signaling via fats.

The AHR as a therapeutic approach to obesity. In summary, we have shown that mice with the high-affinity AHR are more susceptible to obesity than mice with the low-affinity AHR when fed Western diet. The broad binding specificity of the AHR for different ligands suggests the potential for simple preventative and therapeutic strategies, and if AHR signaling activities of similar diversity are found in the human population, then the AHR could serve as a means to combat obesity. The demonstration that obesity may be dependent on AHR signaling may allow easy manipulation via dietary compounds that act as AHR antagonists such as curcumin (56, 57) and resveratrol (58); and commercially available drugs that act as powerful

AHR antagonists, including CH-223191 (59, 60) and 6,2,4-trimethoxyflavone (61). The regulation of AHR levels and activity by siRNA approaches (62) could also prove promising.

Methods

Mice. The mouse strains are available and maintained at The Jackson Laboratory (Bar Harbor, ME; Strain names: C57BL/6J and B6.D2N-*Ahr*^{d}/J; Stock numbers: 000664 and 002921, respectively). The C57BL/6 mouse possesses the high-affinity AHR (*Ahr*^{b1} allele, B6 strain) and the congenic C57BL/6.D2 mouse strain harbors the low-affinity AHR (*Ahr*^{d} allele, B6.D2 strain) **(Fig. 1)**.

Histology. Sections (~5-μm thickness) from formalin-fixed, paraffin-embedded liver samples were stained with hematoxylin and eosin. The histology procedures were carried out by the Pathology Shared Resource at Dartmouth Hitchcock Medical Center.

Plasma chemistry. The plasma chemistry analyses were carried out by the Serology/Clinical Pathology Division of Charles River Laboratory (Wilmington, MA).

Microarrays. The mRNA and miRNA gene expression microarray experiments were carried out by the Dartmouth Genomics & Microarray Laboratory (DGML) using the MouseRef-8 v2.0 Expression BeadChip array (Illumina, San Diego, CA) and Affymetrix GeneChip miRNA 2.0 Array (Affymetrix, Santa Clara, CA), respectively. Four biological replicates per experimental condition were carried out. An intensity-based, modified t-test (63) was used to characterize the significance level of each feature using limma R packages from Bioconductor (64). Experiments are described in greater detail in SI Methods.

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Figure Legends

Fig. 1. B6 mice become more obese than B6.D2 mice on Western diets. The B6 and B6.D2 mouse strains possess the high- and low-affinity *Ahr* genes, respectively, of which there is a 10-fold difference between the most responsive/most sensitive allele (Ahr^{b1}) and the least responsive/least sensitive allele (Ahr^{d}) **(A)**. The B6 and B6.D2 male mice (n = 8 mice/experimental group) were fed low-fat regular chow (Reg) or a Western diet (West) for 27 weeks **(B)**. Consumption and excretion amounts of B6 and B6.D2 male mice on regular vs. Western diets **(C)**. B6 male mice accumulate significantly more fat mass than B6.D2 male mice on Western diet **(D)**. Gonadal fat pads were extracted and weighed from the B6 and B6.D2 male mice fed low-fat regular diet or Western diet in order to determine differential white fat accumulation: ratio = gonadal fat pad mass (g) / body mass (g) **(E)**. Error bars represent Standard Error of the Mean (SEM).

Fig. 2. B6 mice fed Western diets develop significantly larger, more steatotic, and more severely damaged livers than do B6.D2 mice. B6 and B6.D2 male mice (n = 8 mice/experimental group) were fed low-fat regular chow or a Western diet for 27 weeks. At sacrifice, body mass **(A)**, liver mass **(B)**, and body mass:liver mass ratios **(C)** were determined. The collected livers were formalin fixed, sectioned in paraffin, stained with hematoxylin and eosin, and viewed at 200X magnification for B6 mouse fed regular diet **(D)**, B6.D2 mouse fed regular diet **(E)**, B6 mouse fed Western diet **(F)**, and B6.D2 mouse fed Western diet **(G)**. The mean total vacuole area per 10 fields of vision for four mice from each experimental group is plotted **(H)**. Plasma levels of alanine aminotransferase or ALT **(I)**, aspartate aminotransferase or AST **(J)**, AST/ALT ratios **(K),** alkaline phosphatase **(L)**, total protein **(M)**, and total cholesterol **(N)** were determined. Error bars represent SEM.

Fig. 3. Blood cell counts. Blood samples were obtained at sacrifice from B6 and B6.D2 mice regular vs. Western diet **(A)**. Blood cell counts were taken from blood smears (n = 8/experimental group) **(B)**. Error bars represent SEM.

Fig. 4. The most changed RNA levels of genes and corresponding inferred biological pathways of the liver from B6 vs. B6.D2 mice fed regular (R) vs. Western (W) diets. Four mice were selected from each experimental group for microarray analysis. Venn diagrams display the number of differentially expressed genes from the effect of diet on *Ahr* genotype **(A)** and the effect of *Ahr* genotype on diet **(F)**. The twenty genes with the greatest change in differential mRNA expression (*p*-value ≤0.05) that were exclusively expressed in B6W vs. B6R **(B)**, B6.D2W vs. B6.D2R **(C)**, and B6W vs. B6.D2W **(G)** are listed. Genes with cognate AHR promoter response elements (REs) are shaded in gray. The differentially expressed genes were annotated with functional assignments using the Gene Ontology (GO) biological process-FAT database to determine which gene categories were enriched (FDR ≤1.0) for B6W vs. B6R **(D)**, B6.D2W vs. B6.D2R **(E)**, and B6W vs. B6.D2W **(H)**.

References

1. Cardon LR, Carmelli D, Fabsitz RR, & Reed T (1994) Genetic and Environmental Correlations between Obesity and Body-Fat Distribution in Adult Male Twins. *Human Biology* 66(3):465-479.
2. Stunkard A, Foch T, & Hrubec Z (1986) A twin study of human obesity. *JAMA* 256:51-54.
3. Baillie-Hamilton P (2002) Chemical Toxins: A Hypothesis to Explain the Global Obesity Epidemic. *The Journal of Alternative and Complementary Medicine* 8:185-192.
4. Fernandez-Salguero P*, et al.* (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 268(5211):722-726.
5. Trask HW*, et al.* (2009) Microarray analysis of cytoplasmic versus whole cell RNA reveals a considerable number of missed and false positive mRNAs. *RNA* 15:1917-1928.
6. McMillan BJ & Bradfield CA (2007) The aryl hydrocarbon receptor is activated by modified low-density lipoprotein. *Proc Natl Acad Sci U S A* 104(4):1412-1417.
7. La Merrill M, Kuruvilla BS, Pomp D, Birnbaum LS, & Threadgill DW (2009) Dietary fat alters body composition, mammary development, and cytochrome p450 induction after maternal TCDD exposure in DBA/2J mice with low-responsive aryl hydrocarbon receptors. *Environ Health Perspect* 117(9):1414-1419.
8. Thomas RS, Penn SG, Holden K, Bradfield CA, & Rank DR (2002) Sequence variation and phylogenetic history of the mouse Ahr gene. *Pharmacogenetics* 12(2):151-163.
9. Arsenescu V, Arsenescu RI, King V, Swanson H, & Cassis LA (2008) Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. *Environ Health Perspect* 116(6):761-768.
10. Poland A & Glover E (1980) 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of Toxicity with the Ah Locus. *Mol Pharmacol* 17(1):86-94.
11. Rogers P & Webb GP (1980) Estimation of body fat in normal and obese mice. *Br J Nutr* 43(1):83-86.
12. Adams LA, Angulo P, & Lindor KD (2005) Nonalcoholic fatty liver disease. *Canadian Medical Association Journal* 172(7):899-905.
13. Golik A, Rubio A, Weintraub M, & Byrne L (1991) Elevated serum liver enzymes in obesity: a dilemma during clinical trials. *Int J Obes* 15(12):797-801.
14. Turley ML, Skeaff CM, Mann JI, & Cox B (1998) The effect of a low-fat, high-carbohydrate diet on serum high density lipoprotein cholesterol and triglyceride. *Eur J Clin Nutr* 52(10):728-732.
15. Gasiewicz TA, Singh KP, & Casado FL (2009) The aryl hydrocarbon receptor has an important role in the regulation of hematopoiesis: Implications for benzene-induced hematopoietic toxicity. *Chem Biol Interact*.
16. Kullo IJ, Hensrud DD, & Allison TG (2002) Comparison of numbers of circulating blood monocytes in men grouped by body mass index (<25, 25 to <30, >=30). *The American Journal of Cardiology* 89(12):1441-1443.
17. Nijhuis J*, et al.* (2009) Neutrophil Activation in Morbid Obesity, Chronic Activation of Acute Inflammation. 17(11):2014-2018.
18. Anfossi G, Russo I, & Trovati M (2009) Platelet dysfunction in central obesity. *Nutrition, Metabolism and Cardiovascular Diseases* 19(6):440-449.
19. Peters LL (2011) Aging study: Blood hematology in 30 inbred strains of mice. MPD: Peters4. Mouse Phenome Database web site http://phenome.jax.org. (The Jackson Laboratory, Bar Harbor, Maine, USA).
20. Sun YV, Boverhof DR, Burgoon LD, Fielden MR, & Zacharewski TR (2004) Comparative analysis of dioxin response elements in human, mouse and rat genomic sequences. *Nucleic Acids Res* 32(15):4512-4523.

21. Shen L*, et al.* (2007) Hypothalamic Apolipoprotein A-IV Is Regulated by Leptin. *Endocrinology* 148(6):2681-2689.
22. Jung Y*, et al.* (2011) Annexin-2 is a regulator of stromal cell-derived factor-1/CXCL12 function in the hematopoietic stem cell endosteal niche. *Experimental Hematology* 39(2):151-166.e151.
23. Guillen N*, et al.* (May 2009) Microarray analysis of hepatic gene expression identifies new genes involved in steatotic liver. *Physiological Genomics* 37(3):187-198.
24. Sato M*, et al.* (2010) Development of high-fat-diet-induced obesity in female metallothionein-null mice. *The FASEB Journal* 24(7):2375-2384.
25. Kast-Woelbern HR*, et al.* (2004) Rosiglitazone Induction of Insig-1 in White Adipose Tissue Reveals a Novel Interplay of Peroxisome Proliferator-activated Receptor Î³ and Sterol Regulatory Element-binding Protein in the Regulation of Adipogenesis. *Journal of Biological Chemistry* 279(23):23908-23915.
26. Kautz L*, et al.* (2008) Iron regulates phosphorylation of Smad1/5/8 and gene expression of Bmp6, Smad7, Id1, and Atoh8 in the mouse liver. *Blood* 112(4):1503-1509.
27. Oh-hashi K*, et al.* (2009) CRELD2 is a novel endoplasmic reticulum stress-inducible gene. *Biochemical and Biophysical Research Communications* 387(3):504-510.
28. Deneault E*, et al.* (2009) A Functional Screen to Identify Novel Effectors of Hematopoietic Stem Cell Activity. *Cell* 137(2):369-379.
29. Leduc MS*, et al.* (2011) Integration of QTL and bioinformatic tools to identify candidate genes for triglycerides in mice. *Journal of Lipid Research*.
30. Chi H, Lu B, Takekawa M, Davis RJ, & Flavell RA (2004) GADD45[beta]/GADD45[gamma] and MEKK4 comprise a genetic pathway mediating STAT4-independent IFN[gamma] production in T cells. 23(7):1576-1586.
31. Ji C, Shinohara M, Kuhlenkamp J, Chan C, & Kaplowitz N (2007) Mechanisms of protection by the betaine-homocysteine methyltransferase/betaine system in HepG2 cells and primary mouse hepatocytes. *Hepatology* 46(5):1586-1596.
32. Yamamoto S & Bloch K (1970) Studies on squalene epoxidase of rat liver. *J Biol Chem* 245(7):1670-1674.
33. Greenberg CC, Danos AM, & Brady MJ (2006) Central Role for Protein Targeting to Glycogen in the Maintenance of Cellular Glycogen Stores in 3T3-L1 Adipocytes. *Mol. Cell. Biol.* 26(1):334-342.
34. Zadravec D*, et al.* (2010) Ablation of the very-long-chain fatty acid elongase ELOVL3 in mice leads to constrained lipid storage and resistance to diet-induced obesity. *The FASEB Journal* 24(11):4366-4377.
35. McGregor R & Choi M (2011) microRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med* 11(4):304-316.
36. Okey AB, Vella LM, & Harper PA (1989) Detection and characterization of a low affinity form of cytosolic Ah receptor in livers of mice nonresponsive to induction of cytochrome P1-450 by 3-methylcholanthrene. *Mol Pharmacol* 35(6):823-830.
37. Poland A, Palen D, & Glover E (1994) Analysis of the four alleles of the murine aryl hydrocarbon receptor. *Mol Pharmacol* 46(5):915-921.
38. Nebert DW (1989) The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. *Crit Rev Toxicol* 20(3):153-174.
39. Ema M*, et al.* (1994) Dioxin binding activities of polymorphic forms of mouse and human arylhydrocarbon receptors. *J Biol Chem* 269(44):27337-27343.
40. Moriguchi T*, et al.* (2003) Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. *Proc Natl Acad Sci U S A* 100(10):5652-5657.
41. Harper PA, Wong JY, Lam MS, & Okey AB (2002) Polymorphisms in the human AH receptor. *Chem Biol Interact* 141(1-2):161-187.

42. Alexander DL, Ganem LG, Fernandez-Salguero P, Gonzalez F, & Jefcoate CR (1998) Aryl-hydrocarbon receptor is an inhibitory regulator of lipid synthesis and of commitment to adipogenesis. *J Cell Sci* 111 (Pt 22):3311-3322.
43. Lee JH*, et al.* (2010) A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis. *Gastroenterology* 139(2):653-663.
44. Najai AU, Larsen MC, Bushkofsky JR, Czuprynski CJ, & Jefcoate CR (2011) Acute Disruption of Bone Marrow Hematopoiesis by Benzo(a)pyrene Is Selectively Reversed by Aryl Hydrocarbon Receptor-Mediated Processes. *Molecular Pharmacology* 79(4):724-734.
45. Kerley-Hamilton JS*, et al.* (2012) Inherent and Benzo[a]pyrene-Induced Differential Aryl Hydrocarbon Receptor Signaling Greatly Affects Lifespan, Atherosclerosis, Cardiac Gene Expression, and Body and Heart Growth in Mice. *Toxicol Sci* in press.
46. Bourlier V & Bouloumie A (2009) Role of macrophage tissue infiltration in obesity and insulin resistance. *Diabetes & metabolism* 35(4):251-260.
47. Evans RM, Barish GD, & Wang YX (2004) PPARs and the complex journey to obesity. *Nat Med* 10(4):355-361.
48. Berry DC & Noy N (2009) All-trans-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor beta/delta and retinoic acid receptor. *Mol Cell Biol* 29(12):3286-3296.
49. Tontonoz P & Mangelsdorf DJ (2003) Liver X receptor signaling pathways in cardiovascular disease. *Mol Endocrinol* 17(6):985-993.
50. Gao J & Xie W (2010) Pregnane X Receptor and Constitutive Androstane Receptor at the Crossroads of Drug Metabolism and Energy Metabolism. *Drug Metabolism and Disposition* 38(12):2091-2095.
51. Dong B*, et al.* (2009) Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. *Proceedings of the National Academy of Sciences* 106(44):18831-18836.
52. Zhou J*, et al.* (2006) A Novel Pregnane X Receptor-mediated and Sterol Regulatory Element-binding Protein-independent Lipogenic Pathway. *Journal of Biological Chemistry* 281(21):15013-15020.
53. Chan L & Wells R (2009) Cross-Talk between PPARs and the Partners of RXR: A Molecular Perspective. *PPAR Res* 2009:1-9.
54. Chawla A, Repa JJ, Evans RM, & Mangelsdorf DJ (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science* 294(5548):1866-1870.
55. Nebert DW & Karp CL (2008) Endogenous functions of the aryl hydrocarbon receptor (AHR): intersection of cytochrome P450 1 (CYP1)-metabolized eicosanoids and AHR biology. *J Biol Chem* 283(52):36061-36065.
56. Morimoto T*, et al.* (2008) The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J Clin Invest* 118(3):868-878.
57. Ciolino HP, Daschner PJ, Wang TT, & Yeh GC (1998) Effect of curcumin on the aryl hydrocarbon receptor and cytochrome P450 1A1 in MCF-7 human breast carcinoma cells. *Biochem Pharmacol* 56(2):197-206.
58. Beedanagari SR, Bebenek I, Bui P, & Hankinson O (2009) Resveratrol Inhibits Dioxin-Induced Expression of Human CYP1A1 and CYP1B1 by Inhibiting Recruitment of the Aryl Hydrocarbon Receptor Complex and RNA Polymerase II to the Regulatory Regions of the Corresponding Genes. *Toxicol. Sci.* 110(1):61-67.
59. Kim SH*, et al.* (2006) Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. *Mol Pharmacol* 69(6):1871-1878.
60. Zhao B, DeGroot DE, Hayashi A, He G, & Denison MS (2010) CH223191 Is a Ligand-Selective Antagonist of the Ah (Dioxin) Receptor. *Toxicological Sciences* 117:393-403.

61. Murray IA*, et al.* (2010) Antagonism of Aryl Hydrocarbon Receptor Signaling by 6,2',4'-Trimethoxyflavone. *Journal of Pharmacology and Experimental Therapeutics* 332:135-144.
62. Huang T-C*, et al.* (2011) Silencing of miR-124 induces neuroblastoma SK-N-SH cell differentiation, cell cycle arrest and apoptosis through promoting AHR. *FEBS Letters* 585(22):3582-3586.
63. Sartor MA*, et al.* (2006) Intensity-based hierarchical Bayes method improves testing for differentially expressed genes in microarray experiments. *BMC Bioinformatics* 7:538.
64. Smyth GK (2005) limma: Linear Models for Microarray Data. *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, eds Gentleman R, Carey V, Huber W, Irizarry R, & Dudoit S (Springer Verlag, New York), pp 397-420.

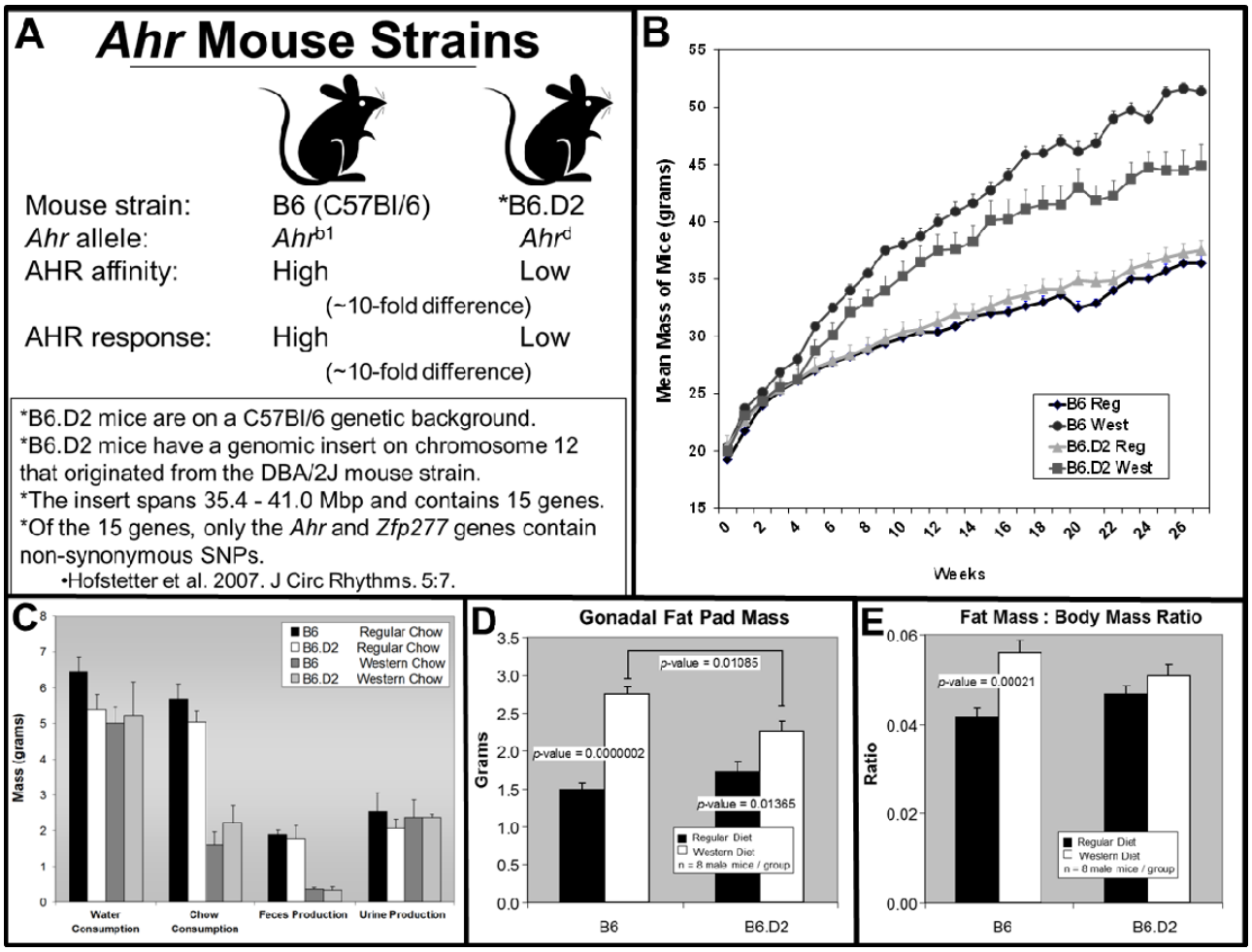

A
Ahr Mouse Strains
Mouse strain: B6 (C57Bl/6) *B6.D2
Ahr allele: Ahrb1 Ahrd
AHR affinity: High Low
(~10-fold difference)
AHR response: High Low
(~10-fold difference)
*B6.D2 mice are on a C57Bl/6 genetic background.
*B6.D2 mice have a genomic insert on chromosome 12 that originated from the DBA/2J mouse strain.
*The insert spans 35.4 - 41.0 Mbp and contains 15 genes.
*Of the 15 genes, only the Ahr and Zfp277 genes contain non-synonymous SNPs.
•Hofstetter et al. 2007. J Circ Rhythms. 5:7.
B
Mean Mass of Mice (grams)
Weeks
B6 Reg
B6 West
B6.D2 Reg
B6.D2 West
C
Mass (grams)
B6 Regular Chow
B6.D2 Regular Chow
B6 Western Chow
B6.D2 Western Chow
Water Consumption
Chow Consumption
Feces Production
Urine Production
D
Gonadal Fat Pad Mass
Grams
p-value = 0.0000002
p-value = 0.01085
p-value = 0.01365
Regular Diet
Western Diet
n = 8 male mice / group
B6
B6.D2
E
Fat Mass : Body Mass Ratio
Ratio
p-value = 0.00021
Regular Diet
Western Diet
n = 8 male mice / group
B6
B6.D2

Fig. 1

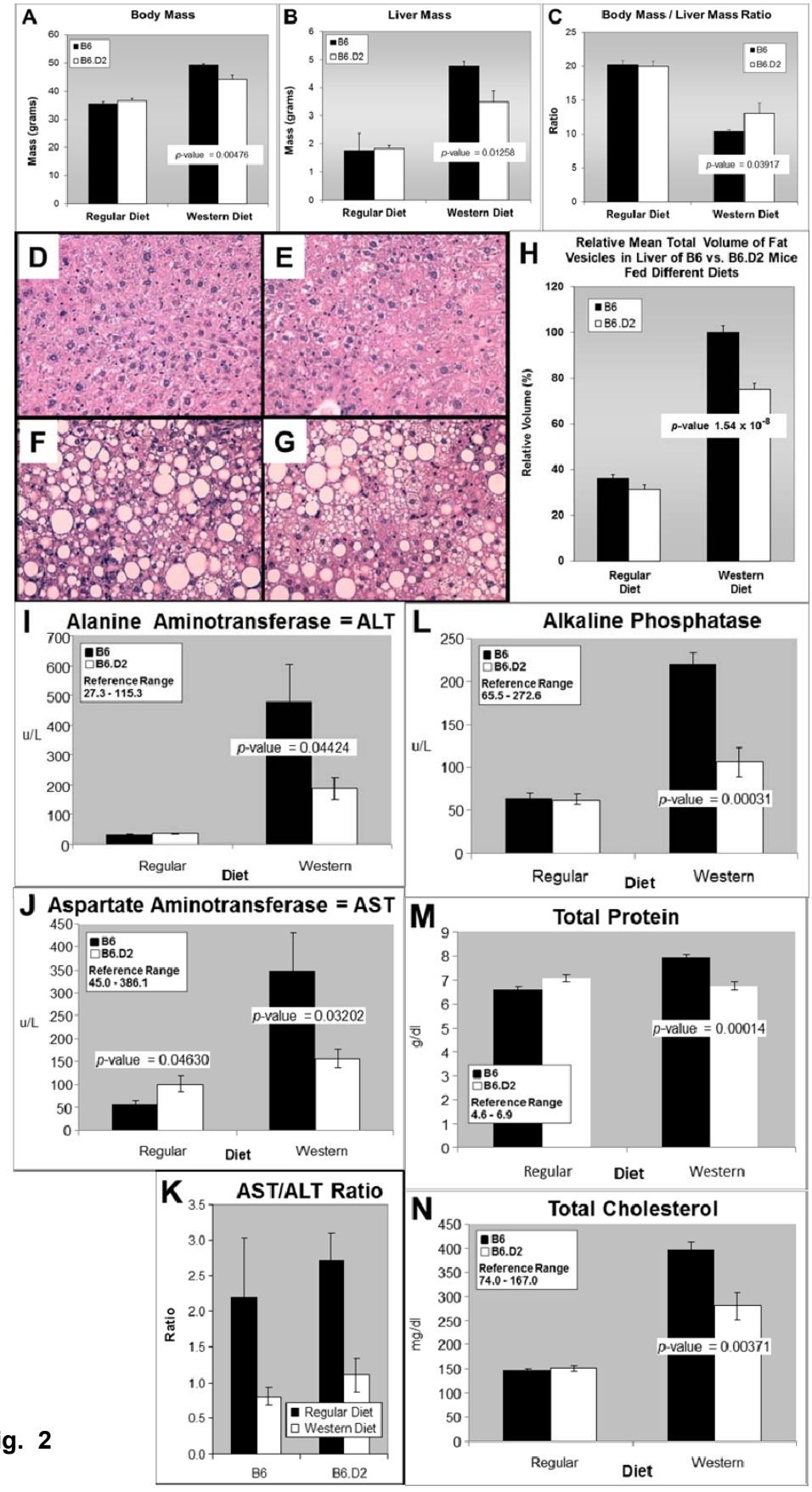
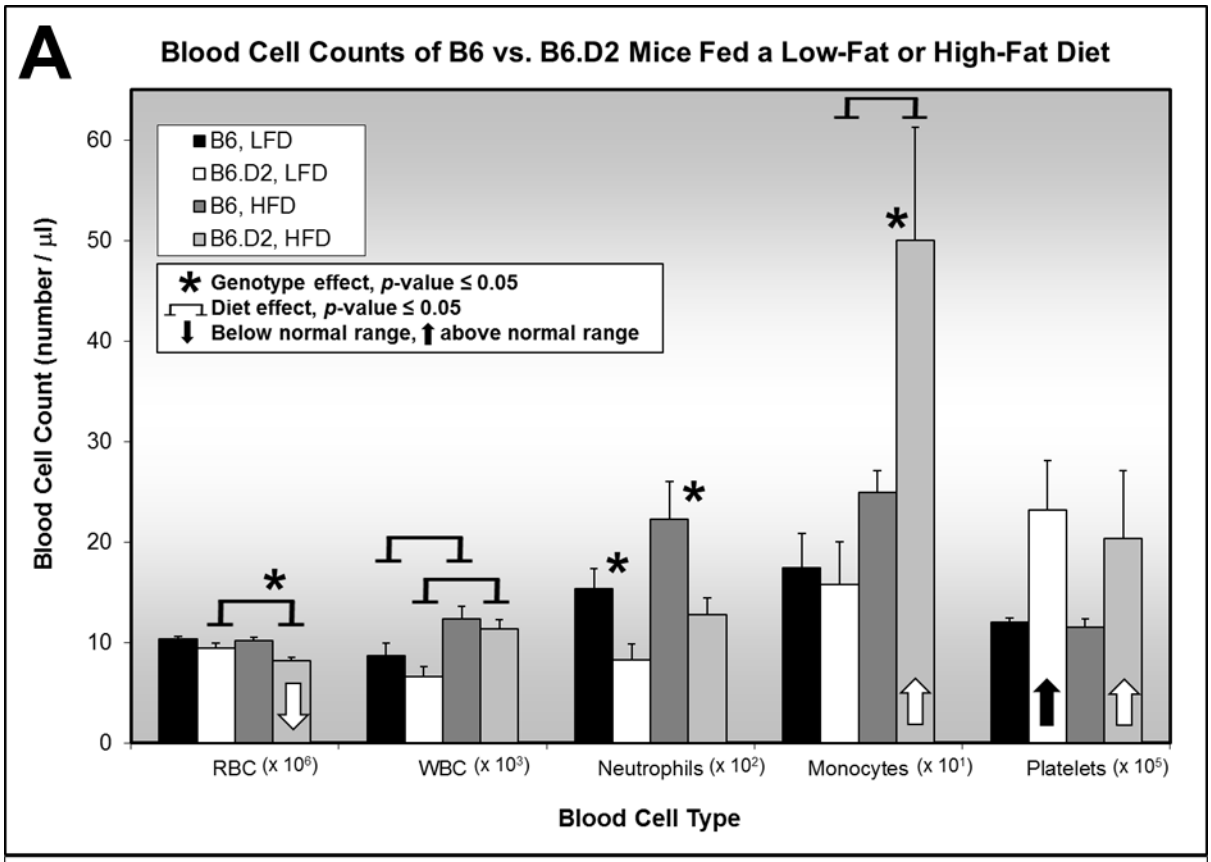

A
Body Mass
Mass (grams)
B6
B6.D2
p-value = 0.00476
Regular Diet
Western Diet
B
Liver Mass
Mass (grams)
B6
B6.D2
p-value = 0.01258
Regular Diet
Western Diet
C
Body Mass / Liver Mass Ratio
Ratio
B6
B6.D2
p-value = 0.03917
Regular Diet
Western Diet
D
E
F
G
H
Relative Mean Total Volume of Fat Vesicles in Liver of B6 vs. B6.D2 Mice Fed Different Diets
Relative Volume (%)
B6
B6.D2
p-value 1.54 x 10-8
Regular Diet
Western Diet
I
Alanine Aminotransferase = ALT
u/L
B6
B6.D2
Reference Range 27.3 - 115.3
p-value = 0.04424
Regular
Diet
Western
L
Alkaline Phosphatase
u/L
B6
B6.D2
Reference Range 65.5 - 272.6
p-value = 0.00031
Regular
Diet
Western
J
Aspartate Aminotransferase = AST
u/L
B6
B6.D2
Reference Range 45.0 - 386.1
p-value = 0.03202
p-value = 0.04630
Regular
Diet
Western
M
Total Protein
g/dl
B6
B6.D2
Reference Range 4.6 - 6.9
p-value = 0.00014
Regular
Diet
Western
K
AST/ALT Ratio
Ratio
Regular Diet
Western Diet
B6
B6.D2
N
Total Cholesterol
mg/dl
B6
B6.D2
Reference Range 74.0 - 167.0
p-value = 0.00371
Regular
Diet
Western

Fig. 2



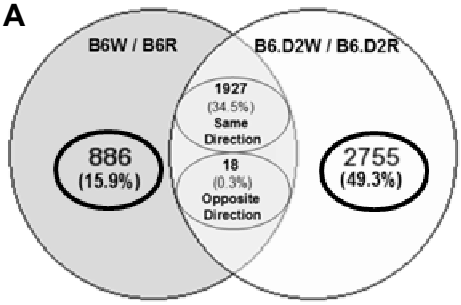
B **Blood Cell Reference Numbers**

Blood Cell Type	[a]Range	[b]6-mo C57Bl/6 Male
RBC	9.3 - 10.8 (x10 ^{6}/µl)	10.8 (x10 ^{6}/µl)
WBC	3.1 - 24.3 (x10 ^{3}/µl)	12.4 (x10 ^{3}/µl)
Neutrophils	5.2 - 24.3 (x10 ^{2}/µl)	14.9 (x10 ^{2}/µl)
Monocytes	5.6 - 38.0 (x10/µl)	18.0 (x10/µl)
Platelets	6.9 - 13.1 (x10 ^{5}/µl)	11.6 (x10 ^{5}/µl)

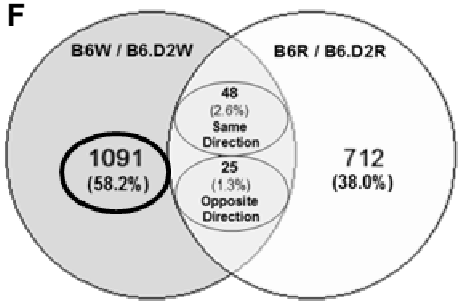
[a]Jackson Laboratory website: http:// phenome.jax.org
[b]Peters, 2011

Fig. 3

A



F



B Genes Unique B6W / B6R

Gene Name	Fold Change	Number AHR REs
Mt1	4.17	0
C1qb	3.03	5
H2-Ab1	2.78	0
Tnfrsf12a	2.63	10
Chac1	2.63	0
Saa1	2.56	0
C1qc	2.38	0
Hspb1	2.33	0
Plk3	2.27	9
Lgmn	2.22	2
Ctsa	0.51	0
Slc38a4	0.51	2
Insig1	0.51	12
Sc5d	0.48	5
Aldoc	0.47	0
LOC100040592	0.46	0
Ppp1r3c	0.43	0
Sucnr1	0.42	0
Insig1	0.40	12
Insig1	0.37	12

C Genes Unique to B6.D2W / B6.D2R

Gene Name	Fold Change	Number AHR REs
Hamp2	9.09	0
Spon2	3.33	3
8430408G22Rik	3.13	4
Lip1	2.94	2
4930572J05Rik	2.94	0
Gsta2	2.86	0
Gstm2	2.86	3
Lbh	2.78	0
Gstm2	2.70	3
Srebf1	2.63	7
Hsp105	0.44	8
Scara5	0.43	0
G6pc	0.42	6
Lpin1	0.40	12
Hamp	0.40	2
Ccbl2	0.34	0
Acot1	0.33	0
Eif4ebp3	0.29	0
Creld2	0.28	0
Creld2	0.27	0

G Genes Unique B6W / B6.D2W

Gene Name	Fold Change	Number AHR REs
Cyp2d26	42.02	2
Gadd45g	2.50	12
Bhmt	2.09	7
Gadd45g	2.05	12
Bax	1.83	0
Gpd1	1.75	7
Clec4f	1.72	0
Bst2	1.69	0
Hmox1	1.60	4
Ccl4	1.57	4
Cyp17a1	0.59	3
Gnat1	0.59	0
Gpam	0.58	4
Sc4mol	0.58	6
Pcsk9	0.57	6
Spp1	0.56	3
Sqle	0.52	8
Esm1	0.50	0
Insig1	0.50	12
Insig1	0.46	12

D Pathways Unique to B6W / B6R

GO:0002026~regulation of the force of heart contraction
GO:0006954~inflammatory response
GO:0009611~response to wounding

E Pathways Unique to B6.D2W / B6.D2R

GO:0046907~intracellular transport
GO:0045184~establishment of protein localization
GO:0015031~protein transport
GO:0033554~cellular response to stress
GO:0008104~protein localization
GO:0006974~response to DNA damage stimulus
GO:0009057~macromolecule catabolic process
GO:0044265~cellular macromolecule catabolic process
GO:0034613~cellular protein localization
GO:0006259~DNA metabolic process
GO:0070727~cellular macromolecule localization
GO:0030163~protein catabolic process
GO:0006886~intracellular protein transport
GO:0006281~DNA repair
GO:0051186~cofactor metabolic process
GO:0006396~RNA processing
GO:0055114~oxidation reduction
GO:0044257~cellular protein catabolic process
GO:0051603~proteolysis in cellular protein catabolism
GO:0006888~ER to Golgi vesicle-mediated transport

H Pathways Unique to B6W / B6.D2W

GO:0055114~oxidation reduction
GO:0051186~cofactor metabolic process
GO:0033554~cellular response to stress
GO:0008610~lipid biosynthetic process
GO:0006732~coenzyme metabolic process
GO:0008202~steroid metabolic process
GO:0001568~blood vessel development
GO:0006974~response to DNA damage stimulus
GO:0001944~vasculature development

Fig. 4