Cyp1b1 affects external control of mouse hepatocytes, fatty acid homeostasis and signaling involving HNF4 α and PPAR α .

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Key words: Cyp1b1, obesity, liver, HNF4 α , PPAR α , and growth hormone.

Abbreviations: Cyp1b1, Cytochrome P450 isoform 1b1; PPAR γ , peroxisome proliferator activated receptor gamma; WT, wild-type; LFD, low fat diet; HFD, high fat diet; DIO, diet induced obesity; Cyp, cytochrome P450; PPAR α , peroxisome proliferator activated receptor alpha; CAR (Nr1i3), constitutive androstane receptor; PXR, pregnane X receptor; HNF4 α , hepatic nuclear factor isoform 4 alpha; GH, growth hormone; ER α -ko, estrogen receptor alpha knockout; Shp, small heterodimeric partner; Krt23, keratin isoform 23; NASH, nonalcoholic steatohepatitis; Acot1, acyl-CoA thioesterase isoform 1; Igfbp1, insulin-like growth factor binding protein isoform 1; Klf6, Kruppel-like factor isoform 6; Mup, major urinary protein; Plin4, perilipin isoform 4; G0S2, G0/G1 switch gene 2; Cidec, cell death-inducing DFFA-like effector c; Vldlr, very low density lipoprotein receptor; EGF, epidermal growth factor; LIF, leukemia inhibitory factor; LIFR, leukemia inhibitory factor receptor; Ppp1r3c, protein phosphatase 1, regulatory subunit 3C; Ppp1r3g, protein phosphatase 1, regulatory (inhibitor) subunit 3G; Scd1, stearoyl-Coenzyme A desaturase isoform 1; Pdk4, pyruvate dehydrogenase kinase, isozyme 4; Me1, malic enzyme 1, NADP(+)-dependent, cytosolic; FA, fatty acid, Hsd3b, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase cluster; Nr4a1, nuclear receptor subfamily 4, group A, member 1; Gadd45g, growth arrest and DNA-damage-inducible, gamma; Serpina7, serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7; Marco, macrophage receptor with collagenous structure; EGFR, epidermal growth factor receptor; Elvol5, ELOVL fatty acid elongase isoform 5; DR1, direct repeat 1; RXR, retinoid X receptor; VMH, ventromedial hypothalamus; Cd36, CD36 antigen; Lgals, lectin, galactose binding, soluble 1; Vnn1, vanin 1; Gsta2, glutathione S-transferase, alpha 2; Ly6d, lymphocyte antigen 6 complex, locus D; Cdh1, cadherin 1; Nudt7, nudix (nucleoside diphosphate linked moiety X)-type motif 7; Pex11a, peroxisomal biogenesis factor 11 alpha; Slco1a1, solute carrier organic anion transporter family, member 1a1; Nupr1, nuclear protein transcription regulator 1; Ccl2, chemokine (C-C motif) ligand 2; Spp1, secreted phosphoprotein 1; Gas6, growth arrest specific 6; Cd34, CD34 antigen; Cd14, CD14 antigen; Cd9, CD9 antigen; Aim1l, absent in melanoma 1-like; Aldh1a1, aldehyde dehydrogenase family 1, subfamily A1; Gpi1, glucose phosphate isomerase 1; Btg3, B cell translocation gene 3; Ephx1, epoxide hydrolase 1, microsomal; Saa1, serum amyloid A 1; Srebp2, sterol regulatory element binding protein 2; Slc25a47, solute carrier family 25, member 47; Aox1, aldehyde oxidase 1; Cpt1a, carnitine palmitoyltransferase 1a, liver; Fitm1, fat storage-inducing transmembrane

protein 1; Angptl4, angiopoietin-like 4; Afp, alpha fetoprotein; Igf2, insulin-like growth factor 2; Fasn, fatty acid synthase; Mgst1, microsomal glutathione S-transferase 1; Stat4a, signal transducer and activator of transcription 4a; Enho, energy homeostasis associated; H19, H19 imprinted maternally expressed transcript; GHRH, growth hormone releasing hormone.

Abstract

Cytochrome P450 1b1 (Cyp1b1) is expressed in endothelia, stellate cells and pre-adipocytes, but not hepatocytes. Deletion alters liver fatty acid metabolism and prevents obesity and hepatic steatosis. This suggests a novel extra-hepatocyte regulation directed from cells that express Cyp1b1. To characterize these mechanisms, microarray gene expression was analyzed in livers of normal and congenic Cyp1b1-ko C57BL/6J mice fed either low or high fat diets. Cyp1b1-ko gene responses indicate suppression of endogenous PPAR α activity, a switch from triglyceride storage to mitochondrial fatty acid oxidation and decreased oxidative stress. Many gene responses in Cyp1b1-ko are sexually dimorphic and correspond to increased activity of growth hormone mediated by HNF4 α . Male responses stimulated by GH pulses are enhanced, whereas responses that decline exhibit further suppression, including Cyp regulation by PPAR α , CAR and PXR. These effects of Cyp1b1 deletion overlap with effects caused by deletion of the small heterodimeric partner, a suppressor of these nuclear factors. Redirection of gene expression associated with liver fat homeostasis in Cyp1b1-ko mice that directs hypothalamic control of GH and leptin. Cyp1b1-ko suppresses neonatal Scd1 and delays adult maturation of dimorphic GH/HNF4 α signaling. Alternatively, deletion may diminish hypothalamic metabolism of estradiol, which establishes adult GH regulation.

Introduction

Experiments with Cytochrome P450 1b1 (Cyp1b1) null (Cyp1b1-ko) transgenic mice demonstrate the role of Cyp1b1 in toxicity and carcinogenesis caused by polycyclic aromatic hydrocarbons [1]. Cyp1b1 is essentially absent in mouse hepatocytes, but is expressed in non-parenchymal sinusoidal cells, including endothelia and stellate cells that are each adjacent to hepatocytes [2-4]. Cyp1b1 deletion in endothelia decreases vasculogenesis *in vivo* and capillary morphogenesis *in vitro* [3]. Cyp1b1 loss of function mutations in humans cause congenital glaucoma and provide a risk factor for liver adenomas [5,6].

During the differentiation of mesenchymal progenitor cells to adipocytes, Cyp1b1 expression increases in concert with peroxisome proliferator activated receptor γ (PPAR γ), a key regulator of triglyceride synthesis [7]. To further characterize these mechanisms, gene expression was analyzed in livers of C57BL/6J (WT) and congenic Cyp1b1-ko mice fed either low or high fat diets (LFD or HFD, respectively). This HFD eventually causes diet-induced obesity (DIO), severe steatotic liver toxicity and glucose intolerance, which are each prevented by Cyp1b1 deletion [8,9]. The near absence of Cyp1b1 expression in mouse hepatocytes indicates that this signaling does not arise from substrates within these cells. Possibilities include the liver non-parenchymal cells, neuronal control from the hypothalamus or developmental re-direction.

Liver Cyp expression patterns are particularly susceptible to diet changes [10]. We show, here, that Cyp1b1 deletion has a striking impact on the expression of the 60 forms of Cyps in the liver. Although obesity changes are retained in both sexes, these Cyp changes occur almost exclusively in males. Many of these Cyps respond to the endogenous activity of nuclear receptors, such as the peroxisome proliferator activated receptor α (PPAR α), the constitutive androstane receptor (CAR), the pregnane X receptor (PXR) and hepatocyte nuclear factor 4 α (HNF4 α). Each is selectively activated by fatty acid or cholesterol derivatives [11-13]. HNF4 α also plays a major role in liver development [13]. A liver-specific deletion of HNF4 α in mice enhances hepatic steatosis [14] and substantially affects Cyp expression [15].

Endocrine control of hepatocytes is principally determined by insulin, glucagon, growth hormone (GH), epinephrine and corticosteroids to maintain euglycemia. Adiponectin, leptin and

other cytokines from adipose tissue modulate these signals, particularly to engage fat metabolism. Leptin activity increases fatty acid oxidation and is directed through activation of sympathetic neurons in the hypothalamus [16]. Local signaling to hepatocytes from the sinusoids derives from fenestrated endothelia, Kupfer macrophage and stellate cells. Cyp1b1 deletion substantially affects the morphology and oxidant signaling in endothelia and pericytes, which are present in the liver as stellate cells [3,17]. Cyp1b1 metabolism can impact hepatocytes through endogenous substrates, notably estradiol that imprints effects on fat homeostasis [18].

Cyp1b1 deletion principally affects pathways that control fatty acid metabolism and the associated oxidative stress processes [9]. We address the hypothesis that extra-hepatocyte Cyp1b1 metabolism controls diet-dependent signaling. The typical expression of Cyp1b1 in mesenchymal progenitors, vascular cells and astroglia indicates that this control may include effects on hepatocyte development. We have adopted the strategy that such diverse system influences may be better captured by a mechanism neutral approach: we resolve gene deletion effects according to the selectivity of two diverse, but relevant treatments (LFD versus HFD) and compare male and female liver gene responses.

The onset of adiposity occurs with different timing in males and females. We show that extension of the HFD for an additional 15 weeks in females produces a similar adiposity, liver physiology and HFD gene expression response as detailed for males. The NASH/obesity effects are similarly suppressed in male and female Cyp1b1-ko mice, much like the pro-obesity effects from the deletions of either $ER\alpha$ or aromatase [19,20]. Key gene changes should, therefore, appear in both sexes. Here, we show that Cyp1b1 extensively affects many gene responses directed by the lipid-responsive receptors HNF4 α , PPAR α , CAR and PXR and their control through pulsatile GH signaling and the small heterodimeric partner (Shp), a nuclear factor that represses this regulation [13,21,22]. Other responses that occur in both sexes correspond to suppression of leptin-controlled genes involved in fatty acid synthesis and suppression of inflammatory gene markers. These regulatory processes are sexually dimorphic and highly sensitive to post-weaning hepatocyte maturation. We show that many of the effects of Cyp1b1 deletion on liver gene expression result from altering this developmental program.

Materials and Methods

Ethics Statement. Mice were maintained in the AAALAC-accredited University of Wisconsin School of Medicine and Public Health Animal Care Facility. Experimental protocols were approved by the University of Wisconsin School of Medicine and Public Health Animal Care and Use Committee (ACUC; protocol number: M00682), in strict accordance to the EU Directive 2010/63/EU.

Mouse breeding and maintenance. Mice were provided food and water ad libitum and were maintained on a 12-hour light/dark cycle. The mice were fed a 10 percent fat/70 percent carbohydrate (LFD, D12450B) or 60 percent fat/20 percent carbohydrate (HFD, D12492) diet from Research Diets Inc. for 11 or 36 weeks post-weaning, as indicated.

Microsomal isolation and western blotting. Individual animal liver microsomes were isolated using differential centrifugation. Liver microsomes (25 µg) were separated by SDS-PAGE on a 10% gel (4% stacking) under reducing conditions and transferred to nitrocellulose membranes. Proteins were quantified repeatedly with a BCA assay (Pierce). Membranes were blocked with 5% nonfat dry milk in Tris buffered saline with 0.1% Tween-20 (TBST) for 1 hour at room temperature. Membranes were then incubated with Cyp1a2 (1:5000), Cyp2b (1:500), Cyp2c (1:1500), Cyp3a (1:2000), Cyp4a (1:1000), Mgst (1:5000), Ephx1 (1:5000) and Scd1 (1:400) overnight at 4°C in TBST with 0.02% sodium azide. Membranes were incubated with IgG horseradish peroxidase labeled secondary antibodies (1:10,000) for 1 hour at room temperature in 5% nonfat dry milk TBST. Membranes were exposed to ECL for 1 minute and developed on autoradiography film using an AGFA Curix 60 CP film processor. Band intensity was measured using Image J software and expressed as a percent of total peak area.

RNA isolation. RNA was isolated from liver samples using Qiagen shredder columns, followed by Qiagen RNeasy purification, according to the manufacturers specifications. RNA quality was assessed spectrophotometrically using a Thermo Scientific Nanodrop, followed by agarose gel electrophoresis.

Microarray analyses. Microarray analyses. Microarray experiments from male WT and Cyp1b1-ko mice, fed either the LFD or HFD for 11 weeks post weaning, were performed with three replicate liver mRNA samples, isolated from individual mice. Methods used for microarray preparation, cy3 or cy5 labeling of the cDNA probe, sample hybridization, washing, and scanning of arrays were carried out as previously described using the Agilent Technologies 4x44k platform [23]. Samples were prepared according to the manufacturer's instructions for dual-color or one-color labeling. Dual color labeling was used such that competitive binding of each of the treatment samples (Cy3-labeled) is co-hybridized with a pooled control sample of three LFD WT controls (Cy5-labeled). The one-color Cy3-labeling method was employed using a modified Agilent Technologies 4x44k platform for subsequent female liver mRNA expression analyses at weeks 11 and 36 weeks post weaning (WT, Cyp1b1-ko mice, HFD and LFD) and for male liver mRNA at 5 weeks post weaning (WT, HFD and LFD). In this study liver mRNA was examined from two individual mice in each group that were typical of the group phenotype. Microarray results were analyzed by EDGE3 software using Limma analysis, which assesses significance based on ANOVA statistics [23,24]. For n=2 multi-group analyses, only genes with EDGE $p < 0.01$ were considered. In order to make comparisons between the male group microarray analyses and later the female group analyses, the Agilent Technologies platform was redesigned to lower background and extend the response range (Cy3 100-800,000). Cy3 outputs from the two Agilent platforms were compared based on application of equivalent samples to both platforms. Both common liver genes and reference ribosomal protein RNA genes showed a constant conversion factor for Cy3 values between 100-20,000 and a calibrated decline for Cy3 values between 20,000-100,000. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE53910 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53910>).

Gene response classification. Responsive genes were identified by a fold change of ± 2 and a p -value < 0.05 . A cut-off of Cy5 > 200 to measure binding efficiency was used to minimize false responses. Genes were sorted into 5 clusters according to the effects of diet and Cyp1b1 deletion: Type A: Cyp1b1-ko effects independent of diet; Types B and C: Cyp1b1-ko effects selective to HFD or LFD, respectively; Type HF: effects of HFD, independent of Cyp1b1-ko; Type D: HFD effect reversal in Cyp1b1-ko.

Results

Effects of diet change and Cyp1b1 deletion on adiposity.

In order to test whether Cyp1b1 metabolism of endogenous substrates has a role in fatty acid homeostasis, WT and Cyp1b1-ko mice were administered defined diets that were either high in carbohydrate (LFD) or fat (HFD), while maintaining similar caloric content (**Fig. 1A**) [9]. The HFD mediated increased body weight, which derived from the accumulation of epididymal and inguinal adipose mass in males and females, respectively (**Fig. 1B**). This DIO was significantly suppressed in Cyp1b1-ko mice compared to WT mice (males $p < 0.0001$; females $p = 0.03$). The decreased gonadal fat correlated with the attenuated body weight gain ($R^2 = 0.85$). There was no significant effect of Cyp1b1 deletion on caloric intake or on general growth [9].

Liver enlargement, derived from lipid droplet accumulation, is associated with steatosis, which typically appears between diet weeks 11 (dw11) and 36 (dw36) on the HFD [25]. This increase in liver mass was prevented in Cyp1b1-ko mice (**Fig. 1C**). In WT mouse livers, increased lipid droplet accumulation, visualized by lipid ghosts in H&E stained images (**Fig. 1D**), paralleled the reported increased liver triglyceride measurements [9]. Collectively, the increases in body weight, liver mass and lipid droplets in males after dw11 on the HFD were only slightly less than in females after dw36. This difference arises because DIO in females is delayed by several weeks compared to males [9]. Serum triglyceride levels were scarcely affected by either change, but Cyp1b1 deletion prevented the typical increases in serum cholesterol, glucose, insulin and leptin produced by HFD [9]. Keratin 23 (Krt23), the most sensitive marker of the hepatitis phase of NASH [26], increased gene expression 9-fold in the livers of WT female mice on the HFD, but not in the Cyp1b1-ko animals (**Fig. 1E**).

Many genes in the male liver respond to both the diet change and to Cyp1b1 deletion.

Liver gene expression was examined by microarray at dw11 in male mice in the four treatment groups (WT: LFD and HFD; Cyp1b1-ko: LFD and HFD). We focused on Cyp1b1-ko mice that were representative of those with more highly suppressed adiposity. Individual gene expression is represented as one of four ratios, based on the mean expression values per treatment (**Fig. 2A**). The WT mice on the LFD (WT/LFD) undergo two comparisons, as the control for Cyp1b1-ko

mice on the LFD (expression ratio designated KO/LFD) and for WT mice on the HFD (expression ratio designated WT/HFD). To determine the effect of Cyp1b1-ko on the HFD, they were compared to the WT mice on the HFD (expression ratio designated as KO/HFD).

In **Fig. 2B**, we present a hierarchical clustering of 240 gene changes that occur for the 12-individual male mice in these four treatment groups (Cy5>2000, FC>2.0, p<0.01). The clustering follows similar patterns when genes with lower expression are examined (Cy5>200). The hierarchical pattern has been divided into 5 stimulation clusters (1-5) and 6 suppression clusters (6-11) (see **Supplementary Table 1**). The association of Cyps within the same sub-families is apparent. In cluster 3, four Cyp2c forms and Cyp2e are each highly stimulated by the switch from LFD to HFD. In **cluster 4**, Cyp7b1, two hydroxysteroid dehydrogenase (Hsd3b) forms and seven major urinary proteins (Mups) share strong stimulatory responses to Cyp1b1 deletion. In **cluster 9**, ten Cyp forms (two 2a forms, Cyp2b9, two 4a forms, four 3a forms and Cyp39) show suppressed expression in Cyp1b1-ko mice on each diet. **Cluster 9** also includes PPAR γ and several genes that are stimulated by PPAR α (Cd36, Lgals and Vnn1). **Cluster 11** includes metallothioneins (Mt1 and Mt2) and several other markers of oxidant stress (Gsta2, Cidec/Fsp27 and Ly6d). Their general suppression suggests that oxidative stress is low in Cyp1b1-ko mice compared to the WT mice.

Liver Cyps are highly responsive to diet and Cyp1b1 deletion in males.

In male mice, the effect of Cyp1b1 deletion on the liver is most clearly demonstrated by the many changes in Cyp expression. Thus, more Cyps are responsive to Cyp1b1 deletion (18) than to the diet change in the WT mice (5) (**Table 1**). Cyp4a forms are selectively induced by PPAR α -specific ligands, the Cyp2a and Cyp2b forms are stimulated by CAR-specific ligands, while several of the Cyp3a forms are stimulated by PXR-specific ligands [27]. The basal attenuation of these Cyps suggests a shared suppression of the endogenous activation of these receptors in Cyp1b1-ko mice that is independent of dietary fat. By contrast, 15 highly expressed Cyp forms do not respond to either diet or Cyp1b1-ko changes (including six 2d forms, two 2a forms and two 4f forms) (data not shown, see complete array file from GEO Series accession number GSE53910).

Division of liver gene responses for Cyp1b1 male mice according to selectivity of diet.

Gene expression changes were processed using the Limma algorithm on the Agilent EDGE microarray analysis website to determine fold-change and significance between treatment groups. Limma analysis utilizes a linear model and empirical bayes method for determining significant gene changes in large data sets (microarrays). The Limma algorithm allows for a large number of comparisons and produces reliable expression changes when the number of arrays is small by borrowing information across genes [28]. Criteria were set for fold-change, p-value and Cy5 array binding ($FC > 2.0$, $p < 0.05$, $Cy5 > 200$). Most of these triplicate comparisons met a more stringent criterion ($p < 0.01$). The responses were resolved into five groups based on diet selectivity and Cyp1b1-ko responses after dw11, designated as Groups *A*, *B*, *C*, *D* and *HF*. These groups represent changes characterized by Cyp1b1-ko diet independence (*A*), Cyp1b1-ko HFD selectivity (*B*), Cyp1b1-ko LFD selectivity (*C*) and response in WT mice to the diet change but not to Cyp1b1-deletion (*HF*), respectively. A fifth group represents a reversal of gene expression between WT and Cyp1b1-ko mice on the HFD that is referred to as Group *D* (**Fig. 3A**). Therefore, there is a total of 5 Groups designated as Group: *A*, *B*, *C*, *D* and *HF* based on diet selectivity and Cyp1b1-ko responses. The inter-relationship of these male groups is diagrammed in **Fig. 3B**. These differences were compared to expression differences seen for the same genes in females at dw36.

Cyp genes are distributed across these groups according to their constitutive regulation by lipid-controlled receptors. Group *A* genes (*127 significant genes*) exhibit similar Cyp1b1-ko responses on both diets and include many Cyp genes that are regulated by PPAR α and PXR. By contrast, Group *B* genes (*99 significant genes*) only respond to Cyp1b1 deletion on the HFD and do not contain any Cyp genes. The Group *C* genes (*329 significant genes*) only respond to Cyp1b1 deletion on the LFD and only include a few Cyps. Group *D* genes (*20 significant genes*) include CAR-regulated Cyp2a and Cyp2b genes and all show significant responses to the diet change in WT mice that are reversed in Cyp1b1-ko mice. Group *HF* genes (*166 significant genes*) respond significantly to the HFD in WT mice, but not to Cyp1b1 deletion and include a cluster of Cyp2c and Cyp2e1 genes.

In **Fig. 4A**, we show typical expression changes for genes that are assigned to Groups A, B, C, D and HF, respectively. Selected genes are presented in **Table 2**, while the full set of responses for each group is shown in **Supplementary Table 2**. The acyl CoA transferase, *Acot1*, typifies **Group A genes** by showing similarly large decreases on each diet for *Cyp1b1*-ko mice compared to WT mice. There is no effect of the diet as the magnitude and direction of gene expression is similar between the LFD and HFD in Group A. The Igf1 binding protein, *Igfbp1*, typifies **Group B genes** by showing a much larger suppression on the HFD than on the LFD. The transcription factor *Klf6* is representative of **Type C genes** with a substantially lower expression on the LFD in *Cyp1b1*-ko mice that is not further enhanced by the HFD. Typically for genes in this group, the WT/HFD expression moves in the same direction as *Cyp1b1*-ko/LFD, suggesting a functional connection. A typical **HF gene**, such as the transcription factor *Nr4a1*, shows a pattern that differs from that of *Klf6* by showing no suppression in the *Cyp1b1*-ko mice on the LFD. A typical **Group D gene**, like the phospholipid hydrolase *Cdh1*, exhibits a reversal of the WT/HFD response in *Cyp1b1*-ko mice on the HFD. There are an appreciable number of genes that adopt intermediate expression patterns; we have focused on the statistically significant differences.

Genes within the same sub-families exhibit highly correlated expression changes for the individual mice across the four treatments (**Fig. 4B**). This is particularly evident for several Cyp sub-families (*Cyp2a4/Cyp2a5* $R^2=0.94$; *Cyp4a10/Cyp4a14* $R^2=0.82$; *Cyp2b9/Cyp2b13* $R^2=0.95$; *Cyp2c29/Cyp2c39* $R^2=0.84$), the five Mup forms and six 3β -hydroxysteroid dehydrogenases (*Hsd3b*) (*Mup3/Mup4* $R^2=0.97$; *Hsd3b4/Hsd3b5* $R^2=0.97$).

A DAVID Gene Ontology analysis provided insight into functional linkages within each group. We focused on the pathways with the highest enrichment (**Supplementary Table 3**) [29, 30]. The most prominent effects featured Cyps found in three of the groups, as noted above. We have, however, identified appreciable additional mechanistic coherence within these various groups that validates the assignments. Select gene functions are summarized in **Supplementary Table 4**. The distinctive functional features of genes within each group are discussed below. The above sorting is based on male gene expression. Each gene group has a different pattern of conservation in female mice, which will be addressed.

Cyp1b1-sensitive genes in Groups A and B control energy homeostasis in different ways.

In males, many genes that respond to Cyp1b1 deletion similarly on each diet (Group A) are regulated by PPAR α . Several of these genes may contribute to the loss of liver lipid droplets [31]. These include suppressions of CD36, Perilipin/Plin 4, G0S2, Cidec and Vldl receptors. Thus, CD36 and Vldlr mediate uptake of fatty acids and lipoproteins, respectively, while Plin4 and Cidec coat lipid droplets and G0S2 inhibits triglyceride lipases [32-35]. PPAR γ promotes triglyceride synthesis [36] and is also extensively suppressed in Group A. Over 30 responses to the PPAR α stimulant, WY-14643, in male mice corresponds to reverse constitutive changes in Cyp1b1-ko mice [9]. These 30 genes include three that contribute to peroxisome function (Acot1, Nudt7 and Pex11a) and three Cyp4a forms [9]. Thus, Cyp1b1-ko in some way prevents a normal constitutive PPAR α activation in these ad libitum fed mice. Additionally, two highly expressed hepatocyte plasma membrane receptors for EGF and LIF along with adropin, a novel-circulating regulator of fat metabolism [37], exhibit diet-independent stimulation. Cyp7b1 and the bile acid transporter, Slco1a1, which contribute to bile acid regulation, are also stimulated in Cyp1b1-ko mice (**Tables 1 and 2**, respectively). Elevation of the phosphatase stimulant, Ppp1r3c, and the decline in the corresponding inhibitor, Ppp1r3g [38], in male Cyp1b1-ko livers are compatible with the observed increased glycogen synthesis that we have reported previously [9].

Several genes that respond to the HFD further decline in the male Cyp1b1-ko mice on the HFD (Group B) and divert fatty acid metabolism away from triglyceride storage and into mitochondrial oxidation. These include stearyl-CoA desaturase 1 (Scd1), pyruvate decarboxylase kinase regulatory subunit 4 (Pdk4) and malate enzyme (Me1) [39]. The suppression of these genes are further reduced in Cyp1b1-ko mice compared to WT mice on the HFD.

The array and qPCR measurements were closely correlated between individual mice across the four treatments for three genes (PPAR γ : $R^2=0.61$, Cd36: $R^2=0.86$, Scd1: $R^2=0.40$). The decreased expression of these key genes in Cyp1b1-ko livers was maintained in qPCR determinations of all available mice in the four treatment groups [9].

Group C responses in Cyp1b1-ko mice link to inflammation or male sexual dimorphism.

Group C genes that respond selectively to Cyp1b1 deletion on the LFD exhibit two notable subgroups. About 30 percent of the Group C genes exhibit parallel Cyp1b1-ko/LFD and WT/HFD responses (**Fig. 3B, Table 3**). Selection of genes from group C based on this dual response, predominantly identified suppression of gene expression associated with either oxidative stress or an inflammatory response, including markers of contributing hematopoietic cells. Decreases in stress and inflammation signaling in Cyp1b1-ko mice on the LFD are indicated by two transcription factors (Nupr1/p8 and Klf6), a set of chemokines (Ccl2, Spp1 and Gas6), the stem cell marker Cd34, the monocyte marker Cd14 and the lymphocyte marker Ly6d.

Group C also captures increased expression of six Hsd3b genes and nine Mups in Cyp1b1-ko mice. Each also shows increased expression in the HFD compared to the LFD in both WT and Cyp1b1-ko mice. These genes exhibit closely correlated expression in the individual mice across the treatment groups, which is also highlighted by the hierarchical clustering in **Fig. 2B**. Thus, Hsd3b4 expression correlates closely with other genes in cluster 5, including Mup4 ($R^2=0.96$, **Fig. 4B**), Cyp7b1 ($R^2=0.77$) and Slco1a1 ($R^2=0.83$). These genes each exhibit strong male selectivity in their expression.

HF Genes respond to the HFD in WT mice and Cyp1b1-ko mice.

The Group HF genes for WT males (dw11) show significant differences in expression between LFD and HFD, but are distinguished by their insensitivity to Cyp1b1 deletion. This group is comprised of 166 genes (**Supplemental Table 4**). The HFD decreases expression of nuclear factors Nur77/Nr4a1 [40], and Gadd45g [41], but increases Nr0b2/Shp, a direct suppressor of Hnf4 α [42], and the CAR receptor (NR1i3) (**Table 2**). This HF Group also includes stimulation of a set of Cyp2c genes: 2c29, 2c37, 2c39 and 2c54 (**Table 2**). These HF responses were also dependent on the age of the mice, as they are largely absent in male livers at dw5, prior to the onset of the HFD-induced obesity.

The HF change represents a difference between a high carbohydrate diet and a high fat diet. The ad libitum feeding and morning sacrifice of the mice suggests that the LFD/high carbohydrate

diet may deliver a post-prandial glucose stimulus, which has been reported to cause liver inflammation and oxidative stress [43]. Thus, the HFD/LFD gene differences relating to inflammation and oxidative stress most likely correspond to an increased glucose inflammatory response on the LFD. Similarly, the parallel expression change seen for the combination of Cyp1b1-ko mice with this diet corresponds to an anti-inflammatory reversal of this post-prandial response.

Gene responses to HFD and Cyp1b1-ko in dw36 females.

Due to the delayed onset of obesity in females, lipid droplet accumulation after dw36 on the HFD resembles that of males at dw11. The dw36 Cyp1b1-ko females also exhibit suppression of DIO relative to their WT counterparts (**Fig. 1B and 1C**). A modest proportion of responses of dw11 Cyp1b1-ko males are retained in females at dw36, corresponding to each of the diet-based groups (**Table 4**). In addition to inflammatory markers, there are notable conserved responses relating to lipid homeostasis. These Group A genes include increases in Cyp7b1, the adipose suppressor, adropin/Enho, EGFR and LIFR, with shared decreases in steatosis stimulants Cd36, Lgals1, Aim11, Plin 4 and G0S2. The Group B suppressions of Scd1 and Elovl5 and serum regulator Igfbp1 are notable conserved changes. However, the extensive suppressions among CyPs linked to PPAR α , CAR and PXR in male Cyp1b1-ko mice are essentially absent in the females (**Supplementary Tables 2 and 6**).

Interestingly, the HF responses that are independent of Cyp1b1-ko, as well as the Group C responses that are matched by HFD/LFD changes, are each substantially conserved in the females (**Table 5**). About 60 percent of the responses to the HFD are conserved in females at dw36, including: Aldh1a1, Gpi1, Cd9, Cyp2c54, Gadd45g and Nr1i3. For the Group C/HF parallel responses, 18 exhibited similar responses, most notably inflammatory markers Ly6d, Cd34, Nupr1, and Btg3 (**Table 5**). The match in HF genes between males at dw11 and females at dw36 validates the DIO timing discrepancy used for the older females. As in males, these responses are age dependent.

The female livers at dw36 exhibit multiple changes due to the switch from LFD to HFD that are paralleled in Cyp1b1-ko mice on the LFD (**Table 6**). These HFD-induce gene changes are not

seen in females at dw11, thus confirming the need to adjust to the later response period in females. A reversal of gene expression corresponding to the Group D designation in males is much more common in comparing WT and Cyp1b1-ko dw36 females. The most striking HFD response of this type is the steatohepatitis marker, Krt 23 (**Fig. 1E**) [26]. Six other contributors to hepatitis-associated inflammation include soluble epoxide hydrolase (Ephx1), serum amyloid1 (Saa1), inhibin E (Inhbe), glypican 1 (Gpc1), Slc25a47/Hdmcp and Gsta1 (**Table 7**).

The five cholesterol synthesis genes are each suppressed by the HFD in WT females, but the changes are reversed in Cyp1b1-ko mice. These are representative of responses seen for each of the fourteen genes in this pathway that share regulation by Srebp2. Cholesterol biosynthesis genes are not affected in males at dw11. **Table 7** also shows three genes associated with fatty acid synthesis (Scd1, Pdk4 and Me1) that have much lower expression on the HFD, but are not further suppressed when Cyp1b1-deletion is combined with this diet. However, in dw11 HFD fed males, Cyp1b1 deficiency enhances the suppression of these genes (**Table 2**). In females, the HFD suppression is greater and the Cyp1b1-ko affects less.

Another notable difference in the dw36 females is that PPAR α is stimulated in WT mice by the change to HFD, which is not seen in dw11 WT males. Cyp1b1-ko dw36 females on the LFD, but not the HFD, share this PPAR α target gene stimulation. Several PPAR α target genes associated with fatty acid oxidation (Acot1, Aox1, Aox3, Cpt1a, Cyp4a10 and Cyp4a14) also show this stimulatory response pattern. PPAR α -regulated genes Fitm1 (controls lipid droplet morphology) and Angptl4 (inflammatory marker) also follow this pattern of stimulation. The dw11 males exhibit suppression of PPAR α target gene expression, without any change in PPAR α expression.

Linkage of Cyp1b1 deletion to enhanced Hnf4 α activation and dimorphic GH signaling.

Many of the genes that respond to Cyp1b1 deletion in Group A also exhibit sexual dimorphism in their liver gene expression. This dimorphism derives from male/female differences in the temporal profile of serum GH and the associated signaling to HNF4 α [13,44] (**Figure 5A**). Responses in male Cyp1b1-ko mice frequently reverse the effects seen in liver-specific Hnf4 α knockout (Hnf4 α -ko) mice [15] (**Table 8**). Comparison of Cyp1b1-ko microarray expression with the previous liver Hnf4 α -ko expression data indicates an inverse relationship, depicted in **Fig. 5B**.

Hnf4 α deletion lowers Cyp7b1, Egfr, Hsd3b forms, and Mup1 and Mup2 genes, which are each stimulated in Cyp1b1-ko mice. The male selective expression of these genes derives from their greater stimulation by Hnf4 α in males [13]. Genes that are induced by the PPAR α agonist, WY-14,643, and decreased in Cyp1b1-ko mice include: Acot3, Cd36, Vnn1, Serpina7 and Marco [9]. Each of those PPAR α target genes is increased in the liver of Hnf α -ko mice (**Table 8**).

These previously reported studies of the GH/Hnf4 α dimorphic signaling were completed at dw5 (13,43). The reported expression differences between male- and female-selective genes seen on the LFD at dw5 are substantially attenuated 6 weeks later. Female-selective genes that are suppressed at dw5 exhibit large increases in expression at dw11 compared to reference genes. Male selective genes that are elevated at dw5 exhibit decreases at dw11 compared to reference genes. Five Cyps follow this response and are sexually dimorphic at dw5: male selective Cyp7b1 and Cyp4a12 and female selective Cyp2a4, Cyp3a41 and Cyp4a10 (**Fig. 5C**). This pattern of change extends to other Cyp4a, Cyp3a and Cyp2a forms. Cyp4f14 is representative of a Cyp that is highly expressed, but insensitive to these changes. In Cyp1b1-ko mice, these maturation changes in liver Cyp expression are largely prevented. Thus, the Cyp1b1-ko mice at dw11 retain expression at the dw5 levels (**Fig. 5D**). For example, male selective Hnf4 α -promoted Cyp7b1 expression declines in WT mice but not in Cyp1b1-ko mice. This developmental intervention explains why so many Cyp1b1-ko expression differences at dw11, relative to WT, are inversely proportional to the effect of Hnf4 α deletion at dw5 (**Fig. 5B**) The gene changes in Cyp1b1-ko mice that we have linked to loss of constitutive PPAR α regulation [9] are similarly caused by this attenuation of GH/HNF4 α activity in the liver between dw5 and dw11. This expression transition for GH/Hnf4 α -regulated genes between dw5 and dw11 in male mice also diminishes their apparent sexual dimorphism at dw11, while it is maintained in Cyp1b1-ko mice.

Cyp1b1 deletion linkage to Small Heterodimeric Partner (Shp).

Many of the gene changes in Cyp1b1-ko mice also overlap with responses to deletion of Nr0b2/Shp, which also suppresses obesity [42]. This nuclear factor heterodimerizes with HNF4 α and suppresses the activity. A recent report shows that Shp deletion in male mice produces over 1000 changes in liver gene expression [42]. Based on the relationship between Shp and Hnf4 α , we compared the microarrays of these gene expression changes with those produced in dw11

male Cyp1b1-ko mice. We found that 80 gene changes in Shp-ko mice matched changes in the same genes produced by Cyp1b1-ko (27 Group A, 5 Group B and 52 Group C genes (**Table 9**). The magnitude of the Cyp1b1-ko- and Shp-ko- [42] induced changes from these separate studies are highly correlated ($R^2=0.78$ $p<0.001$, **Fig. 5E**). The Shp-ko matches to Group A genes, in most cases, relate inversely to the effects of Hnf4 α deletion. The overlap with Group C genes includes 17 markers indicative of inflammation and oxidative stress (**Table 9**).

Cyp1b1 effects on gene expression at weaning.

The pulsatile release of GH from the pituitary in the adult is established by hypothalamic imprinting at the time of birth (43). We, therefore, tested whether Cyp1b1-deletion intervened at this time in the hepatocyte gene development profile by measuring liver gene expression at weaning. We found evidence for major selective gene changes, but without an effect on overall liver development: the extensive post-natal decreases in alpha-fetoprotein (Afp) and the peptide hormone, Igf2, are delayed, leading to large increases at post-natal day (pnd) 21 (**Fig. 6A**). Genes involved in fatty acid synthesis increase dramatically from nearly absent levels between birth and weaning. The increases in fatty acid synthase (Fasn) and Scd1 are greatly suppressed in Cyp1b1-ko mice at pnd 21 (**Fig. 6B**). The HNF4 α -ko-responsive genes that correlate with Cyp1b1-ko dw11 LFD genes in **Fig. 5B** are comparable in Cyp1b1-ko and WT mice at weaning. Thus, the effects of Cyp1b1 deletion are realized only several weeks post-weaning (**Supplemental Table 5**), when responses to GH/HNF4 α signaling becomes evident [13,44].

Gene expression changes correlate poorly with protein changes.

Liver oxidative stress has been linked to increased protein turnover through autophagy [45]. P450 cytochromes exhibit appreciable changes in turnover, mediated by both autophagy and lysosomal degradation [46]. LFD exhibits markers of inflammation, suggesting that altered protein turnover may cause such differences between mRNA expression and protein levels. An initial examination showed major discrepancies when compared to mRNA expression suggesting that Cyp1b1-associated signaling also targets protein turnover (**Supplementary Fig. 1**) [47]. Scd1 protein is highly suppressed in Cyp1b1-ko mice on the LFD, even though mRNA is unaffected. The suppression of Scd1 provides a stimulatory signal for autophagy [48]. Microsomal epoxide hydrolase (Ephx1) and microsomal glutathione transferase (Mgst1) are each

associated with protection from oxidative stress. Although their mRNA expression was unaffected, each protein was suppressed in Cyp1b1-ko livers on both diets. CYP1A2 protein exhibited increased mRNA expression, but nevertheless showed decreased expression in Cyp1b1-ko mice. These affects appear to be gene selective, since changes in the protein levels of CYP2B, CYP2C and CYP4A match the mRNA expression changes. In each case, the antibodies recognize several forms. However, we show that genes within the same family share similar diet and Cyp1b1 deletion effects.

Discussion

Although Cyp1b1 is not expressed in mouse hepatocytes, systemic deletion suppresses the typical obesity and liver steatosis caused by administration of a HFD to male mice for 11 weeks post-weaning (dw11, **Fig. 1**) [9]. This response is delayed by approximately 4 weeks in female mice, which, however, still exhibit suppression of obesity and steatosis with Cyp1b1 deletion. Loss of Cyp1b1 metabolism of endogenous substrates produces greater changes in liver gene expression than the diet change. Restoration of human Cyp1b1 to Cyp1b1-ko mice restores the DIO [49]. The liver gene expression profiles in males Cyp1b1-ko livers show decreased activities for the lipogenic gene, *Scd1*, and the lipid-activated nuclear receptors, PPAR α , CAR and PXR. Cyp1b1-ko mice show a decrease in lipid accumulation in favor of glycogen storage and fatty acid oxidation, as well as a suppression of the hepatitis marker, *Krt23*, after an extended exposure to the HFD [9,27].

Gene expression changes in males have been effectively resolved into functionally different groups based on diet sensitivity and Cyp1b1-ko responses. Diet-independent effects of Cyp1b1 deletion (Group A) largely include changes linked to suppression of GH-stimulated HNF4 α activity. Cyp1b1-ko HFD-selective responses (Group B), including *Scd1* suppression, may be associated with leptin signaling from the hypothalamus [9]. Depletion of *Scd1* alone causes a loss of DIO that depends on leptin [50]. Cyp1b1-ko LFD-selective responses (Group C) include a large set of markers of diminished inflammatory and oxidative stress response processes. A fourth group of genes respond in WT mice to the switch from LFD to HFD, but are insensitive to Cyp1b1 deletion (HF genes).

The Group C inflammatory/oxidative stress marker genes typically show a parallel decline in WT mice for the switch from the LFD to the HFD. The ad libitum feeding used here, with an early morning sacrifice, delivers a postprandial glucose increase that increases liver inflammation. Thus, the group C/HF combination selects inflammatory marker genes (Cd14, Ly6D, Ccl2) that respond to a surge of blood glucose, which is suppressed in Cyp1b1-ko mice, via lower basal blood glucose levels and possibly distinct anti-inflammatory effects.

The pattern of responses is very different between males and females, even though the overall liver and adipose changes are similar. We compared females fed the diet for 36 weeks to males fed the diets for 11 weeks (dw36 versus dw11). WT females responded much later to DIO and, at dw36, showed many comparable characteristics to the younger WT males (**Fig. 1**) that are substantiated by conservation of 60 percent of the HF gene responses (**Table 5**). A proportion of Cyp1b1-dependent responses are retained (**Table 4**) and includes several Group A changes that are linked to lipid homeostasis.

There is, however, a large shift in females towards diet-induced responses that are suppressed in Cyp1b1-ko mice. This is most evident for a set of increases that may derive from the onset of NASH and an effective reversal in Cyp1b1-ko mice (Krt23, Ephx1) (**Table 7**). This pattern is also seen for a broad suppression of cholesterol biosynthetic genes, suggesting inhibition of the central regulator, SREBP2, by an increase in dietary fat that is reversed in Cyp1b1-ko mice. Suppression of Scd1 and several other important Group B responses in Cyp1b1-ko mice on the HFD are retained in both sexes. These decreases have been associated with increased leptin activity through the hypothalamus, which may be enhanced in these females due to prolonged HFD feeding, but are still further suppressed in Cyp1b1-ko mice. There is also retention of suppression by many LFD-associated inflammatory markers designated to Group C (**Table 6**).

There is a major shift in PPAR α activity in Cyp1b1-ko mice on the LFD. The strong suppression of activity in males switches to stimulations in females that parallel the increase in receptor activity (**Table 7**). There are also major increases in PPAR α stimulation of recognized target genes in females on the HFD that are not seen in the males.

The majority of HFD-selective suppression responses in Cyp1b1-ko mice, including Scd1, Elovl5, Pdk4 and Me1, indicate a shift to increased fatty acid oxidation. The decreases in PPAR α activity correspond to expression changes that decrease lipid droplet formation (G0S2, Perilipin/Plin 4 and Cidec/Fsp27) or peroxisome activity (Acot1 and Pex11a). The decreased PPAR γ gene expression is also associated with decrease lipid droplet formation. Opposing changes in glycogen synthase modulators, Ppp1r3c and Ppp1r3g, favor glycogen synthesis. These gene responses account for the switch from lipid droplets to glycogen accumulation for Cyp1b1-ko mice on a HFD [9].

The Group A gene responses include many of the Cyps that exhibit sexually dimorphic liver expression. Their expression depends on HNF4 α and on GH signaling, which is differentially delivered in males and females. GH in males is delivered in short pulses every 4 hours that activates Hnf4 α via a synchronized Stat4a phosphorylation/dephosphorylation cycle [51]. Females exhibit a more constant GH blood level [51]. The male-selective expression at dw5 decreases after liver specific deletion of Hnf4 α . At dw11, the stimulatory effects of the pulsatile GH stimulus in male mice on Cyp7b1, Hsd3b4, Mup 4, Enho, Egfr, and Lifr are substantially elevated in Cyp1b1-ko mice. Other GH-regulated genes that exhibit low expression at dw5 in male mice showed increased expression after Hnf4 α deletion. This group includes many genes regulated by PPAR α , PXR and CAR and most of the responsive Cyps (**Table 1**). At dw11, these receptor-mediated genes, including these Cyps, showed decreased expression in male Cyp1b1-ko mice. Overall the effects of Cyp1b1 deletion correlate inversely with the effects of Hnf4 α deletion (**Fig 5B**).

Acot1 is representative of genes that are stimulated by PPAR α , but suppressed by Hnf4 α deletion and in Cyp1b1-ko mice at dw11. Analysis of the Acot1 promoter identifies a DR1 PPAR α site that also binds HNF4 α and may, therefore, compete for the site with the heterodimeric RXR partner [52]. Deletion of the Hnf4a suppressor, Shp/Nr0b2, also correlates with many of these Group A responses: in this case with a direct proportionality (**Fig 5E**).

We initially had difficulty envisaging how Cyp1b1-ko could so substantially increase or decrease the responses to the GH pulses. However, we noticed that that the differences in GH-linked and

sexual dimorphism gene expression were much less at dw11. In males, the GH/Hnf4 α stimulation declined while the female-selective suppressions exhibited large increases (**Fig. 5C**). The remarkable 60-fold Cyp1b1-ko suppression of Cyp4a14 corresponded to a similarly extreme stimulation between dw5 and dw11, which also removed much of the female selectivity at dw11. This pattern appears for a majority of the Group A genes that we have examined. Typically, the Cyp1b1-ko expression at dw11 matches the WT expression at dw5, as if the decline in GH/Hnf4 α signaling is absent in these mice (**Fig. 5D**). In support of this proposed mechanism, serum GH pulses in male mice indeed decline as the mice mature during this period [53,54]. Clearly validation of these changes is necessary, including measurement of male Cyp1b1 expression levels at dw5 and litter paired analyses of both GH maturation changes and key gene expression changes at dw5 and dw11.

Cyp1b1 deletion only minimally affects genes controlled by this GH network at weaning. However, Cyp1b1 deletion substantially impacts other genes in this fetal/neonatal period. Scd1 and Fasn increase dramatically after birth, but are suppressed post-weaning by Cyp1b1-deletion and in the adult (**Fig. 6, Table 7**). The normal neonatal disappearance of the gene combination of Igf2 and H19 caused by DNA methylation is substantially delayed in Cyp1b1-ko mice (**Fig. 6**). This change has been linked to polyunsaturated fatty acid levels in humans [55]. The loss of Afp between birth and weaning is also appreciably delayed leading to extensive perinatal expression. Afp sequesters estradiol and potentially diminishes perinatal effects of estradiol on development [56].

Cyp1b1 can exert a physiological impact through metabolism of estradiol, as has been described for the impact of Cyp1b1 on blood pressure [57]. Estradiol suppresses obesity, as evidenced by the increased obesity in ER α -ko and aromatase-ko mice [19,20]. The similar responsiveness in males indicates that the functional estradiol is generated locally by aromatase near the site of ER α regulatory activity. Cyp1b1 is highly expressed in the hypothalamus and deletion has the capacity to increase local estradiol levels. Activating ER α has previously been shown to suppress obesity in male mice [58,19]. Obesity is suppressed both by the direct tissue effects of GH and by leptin effects in hypothalamus. Leptin acts via the VMH, which is also impacted by neonatal estradiol [59,60]. The suppression of Scd1 and several other genes in Group B may

arise from increased hypothalamic leptin activity in Cyp1b-ko mice [9]. Estradiol also regulates the development of cells in the hypothalamus that generate GHRH, which subsequently control adult the male pulsatile GH signaling [51,58,60].

The attenuation of GH/HNF4 α signaling to the liver between pw5 and pw11 coincides with the onset of weight differences between LFD and HFD. In this period, the pulsatile release of GH in males appreciably declines in association with changes in GHRH in the hypothalamus (53,54). GH suppresses adiposity and, therefore, this attenuation of GH effects between dw5 and dw11 is likely to be a major contributor to the onset of DIO in males exactly at this time. The retention of dw5 liver responses to GH pulses in Cyp1b1-ko mice suggests that this is a major contributor to the suppression of DIO in these mice. Future experiments will focus on testing the hypothesis that this maturation change is connected to obesity. This effect of Cyp1b1 deletion may have origins in the neonatal gene expression changes brought about by Cyp1b1 metabolism.

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Figure 1

Figure 2 Changes between 3 states defined by diet/genotype

B

Figure 3

A

B

Figure 4

A

Figure 6

Table 1. Responses of liver P450 Cytochrome genes to Cyp1b1 deletion and diet changes.

	LFD-KO ^a	HFD ^a	HFD-KO ^b
Gene	(KO/WT)	(HFD/LFD)	(KO/WT)
Cyp1a2	2.5*	2.1	2.2
Cyp2a4	-5.0**	2.2*	-2.7**
Cyp2a5	-4.5**	2.3**	-3.0**
Cyp2b13	-6.6*	3.5	-30.3**
Cyp2b10	-1.5	4.2	-12.4*
Cyp2b9	-7.4*	4.1	-41.1**
Cyp2c29	1.6	2.2*	n.c.
Cyp2c37	2.0	4.4*	n.c.
Cyp2c39	n.c.	3.0*	n.c.
Cyp2c40	2.1*	1.5	n.c.
Cyp2c54	3.5	7.4**	n.c.
Cyp2c55	-2.1*	-1.5	-1.6
Cyp2c70	3.0**	n.c.	2.8**
Cyp2e1	2.6	3.9*	n.c.
Cyp3a11	-2.2*	n.c.	-2.1
Cyp3a16	-4.0**	-1.8	-2.6*
Cyp3a41a	-4.0**	-1.7	-2.4*
Cyp3a44	-3.4**	-1.8	-2.1
Cyp4a10	-11.7**	1.7	-11.2**
Cyp4a12	2.5	n.c.	1.7
Cyp4a14	-43.4**	n.c.	-66.3**
Cyp4a31	-5.1**	2.0	-6.4**
Cyp4v3	2.2*	1.7	2.0*
Cyp7a1	-2.6	2.3	-1.8
Cyp7b1	11.4**	3.4	-4.6*
Cyp8b1	1.5	2.1	n.c.
Cyp17a1	-3.5**	-1.9	-2.5*
Cyp39a1	-5.7**	n.c.	-9.3**

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning.

^a Expression levels are calculated relative to WT LFD.

^b Expression levels are calculated relative to WT HFD.

n.c. represents no change (FC<±1.5).

*p<0.05, **p<0.01

Table 2. Expression of liver genes involved in metabolism divided according to responses to Cyp1b1-ko and diet responses.

	LFD-KO ^a	HFD ^a	HFD-KO ^b
	(KO/WT)	(HFD/LFD)	(KO/WT)
Group A			
Acot1	-4.4**	1.5	-4.7**
Acot4	-2.7**	1.5	-3.7**
Akr1c19	-3.0**	n.c.	-2.7**
Cd36	-7.6**	-1.7	-2.5**
Cidec	-4.7	-1.9	-4.8
Cyp4a10	-11.7**	1.7	-11.2**
Cyp7b1	13.0**	4.0	5.0*
Egfr	2.4**	n.c.	2.6*
Enho/Adropin	2.2	2.0	3.9
Fgf21	-3.1*	-2.9*	-2.9*
G0s2	-2.5*	n.c.	-2.6*
Gsta2	-5.6**	-1.8	-2.8*
Gstp1	2.1**	n.c.	3.1**
Hamp2	-2.2*	-1.5	-5.5**
Lgals1	-4.8**	n.c.	-5.0**
Lifr	4.2**	2.3*	3.5**
Lpin2	-1.7	n.c.	-2.4**
Nudt7	2.1**	n.c.	3.1**
Mgst3	-4.7**	n.c.	-4.6**
Nqo2	-2.0*	n.c.	-1.8*
Pex11a	-2.4**	n.c.	-2.1*
Plin4	-3.5*	n.c.	-5.5*
Pparg	-4.5**	n.c.	-4.6**
Ppp1r3c	3.8*	1.6	2.4
Ppp1r3g	-4.6*	-2.4	-5.9**
Selenbp1	2.4**	n.c.	2.5**
Vldlr	-9.1**	n.c.	-2.5*
Vnn1	-10.0**	2.3	-6.8**
Group B			
Elovl5	n.c.	-1.7	-2.2*
Igfbp1	-1.7	-3.5	-5.1*
Me1	n.c.	-1.7	-7.5**
Osgin1	1.5	-1.5	2.7*
Pdk4	-2.0	-3.1*	-3.7**
Scd1	n.c.	-3.9*	-6.0**
Socs2	1.5	-2.7	5.0*
Group C			
Ccl2	-3.5*	-3.1*	-1.8
Cd34	-2.5*	-2.7*	-1.5
Gas6	-2.2**	-2.2**	-1.6**

Hes6	1.9**	n.c.	2.1
Hsd3b5	15.3*	6.6	2.3
Klf6	-2.9**	-2.4**	-1.5
Map4k4	-3.2**	-2.8**	-1.8
Mup3	4.7*	3.4	1.5
Nupr1	-5.1**	-5.5**	n.c.
Slco1a1	5.2*	4.2	1.7
Spp1	-2.6*	-2.0	n.c.
Group D			
Abcc3	-3.2**	2.4	-5.5**
Aldh1a1	-1.8*	3.1*	-1.7
Aldh3a2	-2.6**	2.4*	-2.4**
Asns	1.8	-3.1*	11.2**
Cdh1	n.c.	-2.5**	2.8**
Cyp2a4	-5.0**	2.2*	-2.7**
Cyp2b13	-6.6*	3.5	-30.3**
Cyp2b9	-7.4*	4.0	-41.1**
Hpgd	n.c.	2.5**	-2.5**
Ntrk2	1.6	-14.6**	13.5**
Rgs16	5.0	15.0*	-16.0*
Group HF			
Acacb	n.c.	-3.2*	-1.9
Cyp2c29	1.6	2.2*	n.c.
Cyp2c37	n.c.	4.1*	n.c.
Cyp2c39	n.c.	2.9*	n.c.
Cyp2c54	3.48	7.41**	n.c.
Gadd45g	n.c.	-3.9**	-1.5
Icam1	n.c.	-3.6*	1.8
Lpl	-1.5	-3.3*	1.5
Nr0b2/Shp	n.c.	2.4**	n.c.
Nr4a1/Nurr77	n.c.	-3.5**	n.c.
Nr1i3/CAR	n.c.	3.0*	-1.5
Rbp1	n.c.	-2.3*	n.c.
Relb	-1.8	-3.0**	n.c.
Scd2	-2.3	-4.5*	n.c.
Slco1b2	1.5	2.5*	n.c.
Sult1b1	n.c.	3.0**	n.c.
Tubb2a	n.c.	-2.8*	n.c.

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning.

^a Expression levels are calculated relative to WT LFD.

^b Expression levels are calculated relative to WT HFD.

n.c. represents no change (FC<±1.5).

*p<0.05, **p<0.01

Table 3. Group C/HF genes

Gene	WT HFD/ LFD	(KO/WT) LFD
Anxa5	-2.3*	-2.4*
Aox3	2.4**	3.1**
Atf3	-3.1*	-2.9*
Bhmt	1.9*	2.1**
Btg2	-3.0*	-2.7*
Btg3	-3.0*	-3.5*
Ccl2	-3.1*	-3.5*
Cd14	-3.2*	-3.0*
Cd34	-2.7*	-2.5*
Cd52	-1.9**	-2.7**
Cd63	-2.6*	-3.1*
Cgref1	-2.7*	-3.0**
Cx3cl1	-2.7*	-2.6*
Cxadr	-2.5*	-3.0**
Ddr1	-2.9*	-3.6*
Dio1	2.6**	2.1**
Gas6	-2.2**	-2.1**
H2-Ab1	-1.5	-2.0**
Hist1h1c	-1.9	-2.5*
Il17rb	-2.0*	-2.8**
Itk	-2.2	-2.8*
Klf6	-2.4**	-2.9**
Ly6d	-4.3**	-6.1**
Map4k4	-2.8**	-3.2**
Mgp	-2.2*	-3.5**
Mmd2	-4.6**	-3.9**
Myc	-5.6*	-4.4*
Nupr1	-5.5**	-5.1**
Prodh	1.9*	2.2*
Pvr	-2.3*	-2.5*
Renbp	-2.4**	-2.3**
Rhoc	-2.5**	-2.8**
Sgce	-2.1**	-2.6**
Sirpa	-2.1*	-2.0*
Slc17a3	2.1**	2.7**
Spp1	-2.0	-2.6*
Stmn1	-2.0	-2.6*
Uap1l1	-3.5**	-3.5*
Vwf	-2.0	-2.5*
Fgf21	-2.9*	-3.1*

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning.

Expression levels are calculated relative to WT LFD.

n.c. represents no change ($FC < \pm 1.5$).

* $p < 0.05$, ** $p < 0.01$

Table 4. Female dw36 conserved genes.

	LFD-KO ^a	WT ^a	HFD-KO ^b
	(KO/WT)	(HFD/LFD)	(KO/WT)
Group A			
Cyp7b1	3.2	1.7	2.8
Enho	6.8	2.7	4.0
Egfr	3.3	-2.0	4.4
Lifr	2.4	n.c.	3.8
F11	2.9	1.4	2.2
Serpina3k	2.5	n.c.	1.9
Cd36	-4.1	1.4	-4.9
Lgals1	-2.8	2.1	-3.9
Aim1l	-5.9	n.c.	-3.1
Cxcl10	-3.2	n.c.	-2.3
Cxcl9	-2.2	n.c.	-2.9
Plin4	-2.8	3.4	-2.3
G0s2	-2.3	n.c.	-1.8
Tiam2	-6.5	-1.5	-3.3
Serpina7	-2.8	-3.1	-2.4
Group B			
Scd1	-1.4	-4.7	-2.3
Elov15	n.c.	-1.4	-1.7
Igfbp1	1.3	-4.3	-2.9
Me1	-1.6	-6.2	-1.8
Group C			
Saa4	3.4	n.c.	1.6
Hsd3b5	-2.7	n.c.	n.c.
H2-Ab1	5.2	n.c.	n.c.
Ly6d	-5.2	-1.8	-2.4
Nupr1	-2.8	-3.7	n.c.
Cd34	-2.2	-3.3	2.3

Data represents fold change.

Female (n=2) mice were fed the diets for 36 weeks post-weaning.

^a Expression levels are calculated relative to WT LFD.

^b Expression levels are calculated relative to WT HFD.

n.c. represents no change (FC<±1.5).

Table 5. Male dw11 HF genes-conservation in female dw 36 mice.

Stimulation			Suppression		
HFD/LFD			HFD/LFD		
Gene	WT Male dw 11	WT Female dw 36	Gene	WT Male dw 11	WT Female dw 36
Aldh1a1	2.0	2.9	Acacb	-3.2	-1.9
Apol7a	2.1	<i>n.c.</i>	Apcs	-2.4	-2.1
Arrdc3	3.5	2.1	Arhgef16	-3.1	-2.3
Car8	2.5	<i>n.c.</i>	Btg3	-3.0	-3.1
Chpt1	2.1	1.6	Capg	-2.0	<i>n.c.</i>
Cyp2c54	7.4	2.3	Car2	-2.2	-2.5
Cyp2e1	3.9	1.7	Cd34	-2.7	-3.3
<i>Cyp51</i>	2.1	-1.6	<i>Cdt1</i>	-2.2	1.5
Dars	2.0	<i>n.c.</i>	Cd9	-4.3	-6.6
Dbp	3.7	87.0	Cdkn3c	-2.0	<i>n.c.</i>
Dnaja1	2.3	-1.7	Cdt1	-2.2	+1.5
Herpud1	3.4	1.7	Chka	-2.5	-10.7
Hmgcs1	2.2	-2.0	Cotl1	-2.0	-1.8
Hpgd	2.0	1.5	Fndc3b	-2.5	-2.5
Nrli3	3.0	1.5	Gadd45g	-4.8	-3.4
Oat	2.2	2.2	Gpi1	-2.0	-3.7
			Gram24	-2.4	-2.1
			Icam1	-3.1	<i>n.c.</i>
			Igh-Vj	-6.1	-3.2
			Lpl	-3.3	-1.8
			Ly6d	-4.3	-1.8
			Nr4a1	-3.5	<i>n.c.</i>
			Nupr1	-5.5	-3.7
			Rbp1	-2.5	-1.9
			Scd2	-4.5	-7.9
			Socs3	-2.1	-1.4
			Tff3	-6.4	-3.6
			Tmem51	-2.4	-2.0
			Slc6a8	-3.0	-2.8
			Slc7a7	-2.4	-2.7

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning.

Female (n=2) mice were fed the diets for 36 weeks post-weaning.

Expression levels are calculated relative to WT LFD males and females, respectively.

n.c. represents no change ($FC < \pm 1.5$).

Table 6. Group C/HF genes compared between dw11 males and dw36 females.

Gene	Male HFD/ LFD	Male (KO/WT) LFD	Female HFD/ LFD	Female (KO/WT) LFD
Anxa5	-2.3*	-2.4*		
Aox3	2.4**	3.1**	3.0	1.8
Atf3	-3.1*	-2.9*	-1.6	-1.7
Bhmt	1.9*	2.1**		
Btg2	-3.0*	-2.7*	-1.4	-1.6
Btg3	-3.0*	-3.5*	-3.1	-2.3
Ccl2	-3.1*	-3.5*		
Cd14	-3.2*	-3.0*		
Cd34	-2.7*	-2.5*	-3.3	-2.2
Cd52	-1.9**	-2.7**	-1.3	-2.8
Cd63	-2.6*	-3.1*	-1.3	-1.4
Cgref1	-2.7*	-3.0**		
Cx3cl1	-2.7*	-2.6*		
Cxadr	-2.5*	-3.0**	-2.0	-1.4
Ddr1	-2.9*	-3.6*	-2.0	-1.4
Dio1	2.6**	2.1**	1.6	1.4
Gas6	-2.2**	-2.1**	-1.4	-1.6
H2-Ab1	-1.5	-2.0**	-1.2	-2.7
H2-Dmb1	-1.4*	-2.2**	-1.2	-1.8
Hist1h1c	-1.9	-2.5*		
Hsd3b3	2.0**	2.3**		
Il17rb	-2.0*	-2.8**		
Il33	-1.9	-2.7*		
Itk	-2.2	-2.8*		
Klf6	-2.4**	-2.9**		
Ly6d	-4.3**	-6.1**	-1.8	-5.3
Map4k4	-2.8**	-3.2**	-2.0	-1.3
Mgp	-2.2*	-3.5**	-1.3	-1.5
Mmd2	-4.6**	-3.9**	n.c.	-2.1
Mup1	3.8	7.5**		
Myc	-5.6*	-4.4*		
Npr2	2.3*	2.2*		
Nupr1	-5.5**	-5.1**	-3.7	-2.8
Prodh	1.9*	2.2*		
Pvr	-2.3*	-2.5*		
Renbp	-2.4**	-2.3**		
Rhoc	-2.5**	-2.8**		
Sgce	-2.1**	-2.6**	-2.6	-1.4
Sirpa	-2.1*	-2.0*	n.c.	-1.6
Slc17a3	2.1**	2.7**		
Slc2a2	2.0*	2.1*		
Spp1	-2.0	-2.6*		

Stmn1	-2.0	-2.6*		
Tmem19	1.8*	2.4**		
Uap111	-3.5**	-3.5*		
Vwf	-2.0	-2.5*		
Fgf21	-2.9*	-3.1*		
Lifr	2.2*	4.7**		
S100a4	-2.1**	-2.1**		

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning, *p<0.05, **p<0.01.

Female (n=2) mice were fed the diets for 36 weeks post-weaning.

Expression levels are calculated relative to WT LFD males and females, respectively.

n.c. represents no change (FC<±1.5).

Table 7. Female dw36 responses to HFD typically reversed by Cyp1b1-ko.

	LFD-KO ^a	WT ^a	HFD-KO ^b
	(KO/WT)	(HFD/LFD)	(KO/WT)
OxS/ Inflammation			
Krt 23	n.c.	9.1	-5.0
Gsta1	-1.7	3.2	-4.0
Gpc1	n.c.	4.4	-2.5
Slc25a47	2.3	4.2	-2.4
Inhbe	1.7	3.8	-1.7
Ephx1	1.3	3.1	-2.3
Saa1	-2.4	2.6	-2.2
PPARα			
Ppara	2.2	3.2	n.c.
Vnn1	-1.4	2.9	-2.8
Angptl4	6.6	9.5	-1.7
Cyp4a10	1.9	5.4	-1.5
Acot1	3.6	5.1	-1.7
Aox1	1.6	2.7	-1.8
Cpt1a	2.1	3.7	-1.4
FA-Oxidation			
Fitm1	n.c.	7.9	-4.2
Hmgcs2	1.4	2.6	-2.0
Ech1	n.c.	2.6	-1.6
Lipid Synthesis			
Ppar γ	n.c.	2.2	-2.4
Hes6	-1.9	-4.2	2.8
Cholesterol			
Aacs	-1.6	-6.4	2.3
Fdps	n.c.	-4.5	2.1
Sqle	n.c.	4.2	2.6
Mvd	n.c.	-3.5	1.9
Hmgcr	n.c.	-3.5	1.9
Fatty Acid Synthesis			
Elovl6	-2.5	-14.9	n.c.
Acacb	n.c.	-4.6	1.7
Fasn	-2.3	-11.1	n.c.
Scd1	-1.4	-4.1	-2.3
Pdk4	-1.9	-3.3	-1.5
Me1	-2.3	-6.2	-1.6
Fabp4	-3.5	-2.8	1.7

Data represents fold change.

Female (n=2) mice were fed the diets for 36 weeks post-weaning.

^a Expression levels are calculated relative to WT LFD.

^b Expression levels are calculated relative to WT HFD.

n.c. represents no change (FC< $\pm$ 1.5).

Table 8. Responses to HNF4 α deletion compared to effects of Cyp1b1 deletion.

Gene Symbol	KO LFD^a	HNF4α-KO^{[15]b}
Acot3	-10.7**	8.9**
C8b	2.9**	-6.5**
C9	2.3**	-21.6**
Cd36	-6.7**	10.9**
Cidec	-4.7	-1.3**
Cyp17a1	-3.4**	3.9**
Cyp2a4	-5.0**	2.8**
Cyp2a5	-4.5**	2.1**
Cyp2b10	-1.5	1.9**
Cyp2b13	-6.6*	3.7**
Cyp2b9	-7.4*	8.5**
Cyp2c40	1.3	-2.0**
Cyp39a1	-5.7**	2.9**
Cyp4a14	-43.4**	2.4**
Cyp7a1	-2.6	-1.2*
Cyp7b1	11.4**	-23.7**
Cyp8b1	1.5	11.3**
Egfr	3.1	-9.3**
Gdf15	-3.3**	1.6**
GhR	1.3	-5.0**
Hamp2	-2.3*	5.7**
Hsd17b2	2.1**	-2.7**
Hsd3b2	1.8*	-12.4**
Hsd3b5	15.4*	-11.1**
Marco	-3.0	2.4**
Mug2	1.8*	-8.1**
Mup1	7.5**	-8.1**
Mup3	1.8	37.6**
Mup4	4.7*	-12.7**
Nudt7	2.4**	-6.5**
Prodh	2.2*	-2.0**
Rarres1	2.6**	-17.1**
Selenbp1	2.4**	-3.7**
Serpina7	-3.5**	7.1**
Spp1	-2.6*	8.0**
Vldlr	-9.1**	10.7**
Vnn1	-10.9**	2.2**

Data represents fold change.

Male (n=3) mice were fed the diets for 11 weeks post-weaning and compared to HNF4 α -ko [15].

^a Expression levels are calculated relative to WT LFD.

^b Expression levels are calculated male liver-specific HNF4 α -ko relative to male WT [15].

*p<0.05, **p<0.01

Table 9. Responses to SHP deletion compared to effects of Cyp1b1 deletion.

	SHP-KO	Cyp1b1-KO Male^a		Cyp1b1-KO Female^b	
Gene Symbol	WD FC^{[42]e}	LFD FC^c	HFD FC^d	LFD FC^c	HFD FC^d
Type A					
Ccl4	-9.5**	-5.5**	-2.4*	-2.2	1.0
Ubd	-8.5*	-5.8**	-5.0**	-2	-1.1
Gsta2	-6.7**	-5.6**	-2.8*	-1.2	-3.1
Serpina7	-4.4**	-3.5**	-9.4**	-1.9	-1.6
Mgst3	-3.7**	-4.7**	-3.1**	-1.5	-1.5
Cxcl10	-3.5**	-3.8*	-3.6*	-2.4	-1.5
Pparg	-3.1**	-4.5**	-4.6**	-1.3	-2.2
Lgals1	-2.9**	-5.8**	-5.2**	-2.8	-3.9
Gdf15	-2.9**	-3.3**	-2.9*	-4	-1.8
Fgf21	-2.4**	-3.1*	-2.9*	-1.3	-1.8
Adora1	-1.7*	-2.4**	-2.5**	1.1	-3.1
Cyp1a2	1.5*	2.5*	2.2	-1.4	1.5
Serpina11	1.8	2.3**	2.8**	1.9	2.2
Nudt7	2.0	2.4**	2.4**	-1.1	1.9
F11	2.3**	2.2**	2.0**	2.9	2.2
C8b	2.9**	2.9**	2.7**	1.4	1.9
Cyp7b1	5.1**	11.4**	4.6*	3.2	2.8
Cidec	-11.6**	-4.7	-4.8	-1.7	-1.3
Ms4a7	-6.7**	-4.9**	-2.4*	-1.6	-1.4
Cd83	-3.9*	-2.3*	-2.3*	1.0	-1.4
Cyp17a1	-3.0*	-3.4**	-2.5*	-1.1	1.1
Osbp13	-2.6**	-14.1**	-7.9**	1.1	-1.3
Unc5b	-1.9	-3.6**	-2.4**	1.2	1.0
Akr1c19	-1.7	-3.1**	-2.7**	-1.0	1.2
Plxna2	-1.6	-2.3**	-2.5**	1.4	-1.2
Aadat	1.6	2.2**	2.1*	1.3	1.0
Selenbp2	3.3*	3.4**	2.2**	-1.0	1.4
Type B					
Tnfrsf19	-2.3*	-1.5	-2.5**	1.3	-1.5
Il1b	-1.7*	-1.5	-1.7*	1.0	-1.2
Hmgn2	2.3**	1.4	2.2**	1.9	1.7
C8a	1.5*	1.8**	2.2**	1.6	1.4
Hapln4	1.9**	1.4	2.6**	-1.1	1.1
Type C					
Ly6d	-15.7**	-6.1**	-1.6	-5.2	-2.4
Trem2	-12.4	-3.3*	-1.2	-1.9	-1.3
Gal3st1	-12.1**	-3.3*	-2.1	1.5	-1.1
Atf3	-10.9**	-2.9*	-1.1	-1.8	1.0
Anxa2	-7.1*	-2.9*	-1.1	-1.5	-1.0

Nrg1	-5.8**	-7.2**	-1.4	1.7	-1.1
Serinc2	-5.2**	-2.2*	-1.2	1.3	-2.2
Nupr1	-5.0**	-5.1**	-1.3	-2.8	1.1
Cd52	-4.8**	-2.7**	-1.4	-2.8	-1.0
H2-Eb1	-3.8	-2.2**	-1.7*	-2.1	-1.1
Sprr1a	-3.4**	-2.9**	-1.3	1.7	-1.1
5430435G22Rik	-3.2	-2.3**	-1.4	-1.9	1.2
Cd14	-3.2**	-3.0*	-1.3	-1.8	-1.4
Slamf9	-3.1*	-2.5**	-1.0	-1.6	-1.3
Gpx7	-2.7	-2.4**	-1.3	1.7	1.2
Gas6	-2.5**	-2.1**	-1.7**	-1.9	-1.1
Tmsb4x	-2.2**	-2.3**	-1.2	-1.9	1.2
Spp1	-2.2**	-2.6*	-1.2	-1.2	-1.7
Als2cr4/ Tmem237	-2.1**	-2.1**	-1.4	1.6	-1.2
Trib3	-1.9*	-2.7*	-1.7	1.8	-1.1
Csnk1e	-1.7*	-2.5**	-1.7	1.5	1.3
Fgf1	1.7**	2.3**	1.2	1.5	1.1
Slc17a3	1.8**	2.7**	1.8**	1.1	1.4
Hes6	2.0	2.6**	1.7	-1.9	2.8
Slc30a10	2.0	2.2*	1.4	-1.5	-1.3
Scnn1a	2.3**	2.1**	1.2	1.4	1.5
Serpina3k	2.7**	2.5**	1.5**	2.5	1.9
Avpr1a	3.8**	2.3*	1.5	-1.2	1.7
Hsd3b5	243.3**	15.4*	2.3	5.2	1.3
Sdcbp2	-61.5**	-4.5*	-1.2	1.7	1.0
Cd63	-5.7**	-3.1*	-1.4	-1.4	1.1
Uap1l1	-5.7**	-3.5*	1.2	-1.1	1.0
Spats2l	-3.7**	-2.1*	-1.4	1.1	-1.0
Slc41a3	-3.5**	-2.1*	-1.0	1.2	1.1
Anxa5	-3.2**	-2.4*	-1.3	-1.3	-1.3
Tmsb10	-3.2	-2.9*	-1.2	-1.2	1.2
Cpxm1	-3.1*	-3.0**	-2.0	-1.1	1.2
Iqgap1	-2.8**	-2.2*	-1.0	-1.3	-1.2
Rhoc	-2.3	-2.8**	-1.1	1.2	1.2
Serpina3g	-2.2	-2.1*	-1.4	-1.2	-1.4
Ddit3	-2.1**	-2.6*	-1.7	-1.1	-1.2
Cxadr	-2.1**	-3.0**	-1.6	-1.4	-1.2
Cdc42ep5	-2.1**	-2.9**	-1.3	1.4	-1.1
Renbp	-2.0**	-3.2**	1.2	1.3	1.1
Ank3	-1.7	-3.5**	-1.9**	1.2	-1.2
AI317395	1.5**	2.7**	1.6	-1.0	-1.0
Gulo	1.6**	2.1*	1.6	1.3	1.3
Bach2	1.6**	3.0**	2.0*	1.2	-1.0
Hsd3b2	1.6	2.2**	1.2	1.2	-1.0
Rnase4	1.8	2.1**	1.4*	1.0	1.2
Npr2	2.1**	2.2*	1.5	1.1	-1.2
Mup4	5.9	4.7*	2.0	-1.0	1.3

Data represents fold change.

^aMale (n=3) mice were fed the diets for 11 weeks post-weaning and compared to HNF4 α -ko [15], *p<0.05, **p<0.01.

^bFemale (n=2) mice were fed the diets for 36 weeks post-weaning and compared to HNF4 α -ko [15].

^c Expression levels are calculated relative to WT LFD

^d Expression levels are calculated relative to WT LFD

^eExpression levels are calculated male liver-specific HNF4 α -ko relative to male WT [15], *p<0.05, **p<0.01.