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Cyp1b1 deletion and retinol deficiency coordinately suppress mouse liver lipogenic genes and hepcidin expression during post-natal development

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ABSTRACT

Cyp1b1 deletion and gestational vitamin A deficiency (GVAD) redirect adult liver gene expression. A matched sufficient pre- and post-natal diet, which has high carbohydrate and normal iron content (LF12), increased inflammatory gene expression markers in adult livers that were suppressed by GVAD and *Cyp1b1* deletion. At birth on the LF12 diet, *Cyp1b1* deletion and GVAD each suppress liver expression of the iron suppressor, hepcidin (*Hepc*), while increasing stellate cell activation markers and suppressing post-natal increases in lipogenesis. *Hepc* was less suppressed in *Cyp1b1*−/− pups with a standard breeder diet, but was restored by iron supplementation of the LF12 diet. *Conclusions*. The LF12 diet delivered low post-natal iron and attenuated *Hepc*. *Hepc* decreases in *Cyp1b1*−/− and GVAD mice resulted in stellate activation and lipogenesis suppression. Endothelial BMP6, a *Hepc* stimulant, is a potential coordinator and *Cyp1b1* target. These neonatal changes in *Cyp1b1*−/− mice link to diminished adult obesity and liver inflammation.

1. Introduction

Cytochrome P450 1b1 (*Cyp1b1*) is an atypical P450, demonstrating extra-hepatocyte expression and the capacity to metabolize endogenous substrates, such as estradiol, polyunsaturated fatty acids, and retinol (Chambers et al., 2007; Jennings et al., 2014; Larsen et al., 2015; Li et al., 2014). *Cyp1b1* is expressed in many types of support cells, including mesenchymal progenitor cells, endothelia, pericytes, macrophage, and stellate cells (Choudhary et al., 2003; Piscaglia et al., 1999; Tang et al., 2009). *Cyp1b1* acts in vascular cells to restrain local oxidative stress (Palenski et al., 2013) and metabolize estradiol for estrogen receptor-independent signaling mechanisms (Jennings et al., 2014; Malik et al., 2012; White et al., 2012). *Cyp1b1* may exert developmental control over liver homeostasis, in part through changes in hypothalamic signaling (Bushkofsky et al., 2016; Larsen et al., 2015). *Cyp1b1* deletion (*Cyp1b1*−/− mice) suppresses diet-induced obesity (DIO) by preventing adiposity from a post-weaning high fat diet and alters liver gene expression compared to wild type (WT) controls.

In adult *Cyp1b1*−/− male mice, we have characterized three clusters of diet-selective gene responses. These clusters demonstrate functional links to [1] growth hormone signaling through HNF4α, [2] leptin suppression of fatty acid synthesis genes, including stearoyl-coenzyme A desaturase 1 (*Scd1*), and [3] suppression of postprandial inflammation derived from a high carbohydrate diet (Bushkofsky et al., 2016; Larsen et al., 2015). Overexpression of *Scd1* in *Cyp1b1*−/− mice restores adiposity and DIO on a post-weaning high fat diet (Li et al., 2014).

Cyp1b1−/− mice that retain DIO (*R-Cyp1b1*−/−) have been bred

Abbreviations: BD, standard breeder diet; Cyp1b1, cytochrome P450 1b1; DIO, diet-induced obesity; E, embryonic day; GVAD, gestational initiation of vitamin A deficient diet; HFD, high fat diet; LF12, novel low fat diet with 12 percent kcal from fat; LF12 + Iron, LF12 diet supplemented with ferrous sulfate; LFD, low fat diet; PN, post-natal day; *R-Cyp1b1*−/−, a substrain of *Cyp1b1*−/− mice that are resistant to DIO suppression; RA, retinoic acid; RE, retinyl ester; VA, Vitamin A; VAD diet, vitamin A deficient LF12 diet; WT, wild type, C56Bl/6J.

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from outlier mice in the original colony. We show that these mice do not show the characteristic liver gene expression signature (Larsen et al., 2015). However, when *R-Cyp1b1*−/− mice were backcrossed with WT C57Bl/6J mice, the progeny reverted to the original DIO suppression phenotype. Here, we use *R-Cyp1b1*−/− mice to identify changes in neonatal liver gene expression responses that link neonatal development to the adult gene regulation that determines the DIO response.

Developmental expression of *Cyp1b1* at embryonic day (E)9.5 is temporally localized to the hindbrain and the foregut, where liver development is initiating. *Cyp1b1* expression corresponds to a key period of retinoid regulation of morphogenic patterning genes and overlaps with expression of the retinoid-responsive transcription factor, *Hoxb1*, in the hindbrain and the foregut (Chambers et al., 2007, 2009; Huang et al., 1998; Stoilov et al., 2004). This connection between retinol and *Cyp1b1* has led us to investigate neonatal gene expression patterns that may redirect adult liver function.

CYP1B1 metabolizes retinol to the bioactive retinoic acid (RA) (Chambers et al., 2007, 2009). Vitamin A (VA)/retinol is obtained from the diet, absorbed in chylomicra, and stored as retinyl ester (RE) in stellate cells of the liver (Bonet et al., 2012; Harrison, 2012). Stellate cells comprise less than five percent of the total liver and are located between endothelia and hepatocytes in the space of Disse (Si-Tayeb et al., 2010). Retinol plays key roles in development, reproduction, and immunity that largely result from conversion to RA (Clagett-Dame and DeLuca, 2002; Napoli, 2012; See et al., 2008; Smith et al., 1987). Direct RA administration can decrease adiposity and improve glucose tolerance (Bonet et al., 2012).

Systemic retinol deficiency in mice requires initiation of a VA-deficient diet to the mother during mid-gestation, with continuation to maturity in the progeny (McCarthy and Cerecedo, 1952; Smith et al., 1987) (GVAD treatment). A VA-deficient diet can only be obtained by avoidance of unprocessed plant constituents that have appreciable retinoid sources (corn, grasses, etc) (Ross, 2010). The VA-deficient diet historically and currently used in these experiments contained cottonseed oil as a fat source (12 percent kcal from fat) and other defined carbohydrate, protein, and essential nutrient components (LF12 diet; Supplementary Table 1). When GVAD was combined with a post-weaning high fat diet (HFD), the typical obesity response is suppressed, much as seen with *Cyp1b1* deletion. Here, we show that the growth hormone- and leptin-associated liver responses to *Cyp1b1* deletion were largely unaffected by GVAD. We have recently shown that administration of the LF12 diet in gestation to the mother and post-weaning to the progeny produces major increases in a set of inflammatory markers (Maguire et al., 2017). This appears to be a postprandial response to high dietary carbohydrate that is suppressed by GVAD treatment. The gene expression responses overlapped extensively with changes seen with deficiency of the nuclear co-repressor, *Nr0b2/Shp* (Kim et al., 2014). We show that these changes also overlap with changes produced by *Cyp1b1* deletion with the standard post-weaning low fat diet (Bushkofsky et al., 2016; Larsen et al., 2015).

We hypothesize that changes in liver development prior to weaning contribute to this shared adult obesity suppression response and to the overlapping gene expression changes. To study mice at birth and weaning under more defined conditions, we have moved from the standard breeder diet (BD) to the defined LF12 diet. Compared to the BD, the LF12 maternal diet has an increase in the balance of carbohydrate to fat and lacks the iron supplementation typically included to meet the demands of the progeny (Supplementary Table 1). We examined pups at birth and weaning with respect to genes that were changed similarly by retinol deficiency (GVAD) or *Cyp1b1* deletion. Further insight into the relationship between effects of *Cyp1b1* on neonatal and adult gene expression was provided by comparisons of normal *Cyp1b1*−/− with variant *R-Cyp1b1*−/− mice that lack the distinctive adult obesity suppression.

The overlapping gene expression responses in perinatal and neonatal livers exhibited an unusual signature: GVAD and *Cyp1b1* deletion produced parallel changes, but the effect of *Cyp1b1* deletion was reversed when combined with GVAD. This pattern was seen for suppression of multiple genes that convert acetyl CoA to lipogenic products, a response that is likely to contribute to adult obesity suppression. This response was preceded by stimulation of a cluster of stellate cell activation markers and by extensive suppression of *Hamp/Hepc*. *Hamp/Hepc* generates hepcidin, a 25 amino acid peptide that suppresses iron transfer into the circulation (Ganz, 2013) and *Hamp2*, a highly expressed gene duplication product that shares the *Hepc* locus. *Hamp2* produces hepcidin 2, which has different activities that may include effects on metabolism (Lou et al., 2004). The unusual shared features of these responses points to a novel regulatory process that is impacted by retinol and *Cyp1b1*. This concept is reinforced by the fact that these responses are selectively absent in *R-Cyp1b1*−/− mice. The selective loss of many typical adult *Cyp1b1* deletion responses in the *R-Cyp1b1*−/− variants is evaluated as a basis to link neonatal and adult regulation.

The unexpected *Hepc* findings have led us to adopt a new perspective on the relationship between *Cyp1b1* and energy control through iron as a mediator. A dramatic loss of *Hepc* in *Cyp1b1*−/− and GVAD mice was first considered as a toxicity risk, based on the iron overload seen in adult *Hepc*-deficiency conditions when exposed to a normal adult diet (Ganz, 2013). The present work with perinatal and neonatal mice shows a distinct situation for iron/*Hepc* regulation during pregnancy, where regulation is primarily linked to iron deficiency caused by the developmental demands of the progeny. We establish, here, that *Cyp1b1* deletion in mice fed the LF12 diet show exceptionally low *Hepc* expression, due to the need to enhance iron intake. These mice show remarkably high *Hepc* stimulation by elevated dietary iron. Differences between the BD and LF12 diets at constant iron content reveal a strong dependence of *Hepc* on other dietary components, notably carbohydrate and fat. This is complemented by the finding that carbohydrate metabolism and lipogenesis are also integrated through *Cyp1b1* metabolism with iron homeostasis.

2. Materials and methods

2.1. Animal care and husbandry

An in-house colony of wild type C57BL/6J (WT) (Jackson Labs, Bar Harbor, ME), *Cyp1b1*−/− (Buters et al., 1999), and an inbred substrain of *Cyp1b1*−/− mice that are resistant to DIO suppression, obtained by breeding the most obese mice, which led to a higher proportion of obese progeny, *R-Cyp1b1*−/− (Larsen et al., 2015) mice, were maintained in the AAALAC-accredited University of Wisconsin School of Medicine and Public Health facility. Mice were provided food and water *ad libitum* and maintained in a controlled 12-h light/dark cycle. All protocols were approved by the School of Medicine and Public Health Animal Care and Use Committee (ACUC, Protocol number M005635). Nulliparous females aged 8–12 weeks were time mated, such that the presence of a vaginal plug was designated embryonic day (E)0.5. Prior to mating and until dietary administration, dams were maintained on a standard breeder diet (BD, Product Number 2019, Harlan Teklad, Madison, WI).

Offspring were examined at birth [post-natal day (PN)1], weaning (PN21), and 14 weeks of age.