Dissociation of hepatic insulin resistance from susceptibility of nonalcoholic fatty liver disease induced by a high-fat and high-carbohydrate diet in mice

Akihiro Asai,1,2 Pauline M. Chou,3 Heng-Fu Bu,1,2 Xiao Wang,1,2 M. Sambasiva Rao,3 Anthony Jiang,1 Christine J. DiDonato,2 and Xiao-Di Tan1–4

1Center for Intestinal and Liver Inflammation Research, Ann & Robert H. Lurie Children’s Hospital of Chicago Research Center and Departments of Pediatrics and Pathology, Feinberg School of Medicine, Northwestern University, Chicago; and 4Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois

Submitted 29 August 2013; accepted in final form 30 December 2013

Asai A, Chou PM, Bu H-F, Wang X, Rao MS, Jiang A, DiDonato CJ, Tan X-D. Dissociation of hepatic insulin resistance from susceptibility of nonalcoholic fatty liver disease induced by a high-fat and high-carbohydrate diet in mice. Am J Physiol Gastrointest Liver Physiol 306: G496–G504, 2014. First published January 16, 2014; doi:10.1152/ajpgi.00291.2013.—Liver steatosis in nonalcoholic fatty liver disease is affected by genetics and diet. It is associated with insulin resistance (IR) in hepatic and peripheral tissues. Here, we aimed to characterize the severity of diet-induced steatosis, obesity, and IR in two phylogenetically distant mouse strains, C57BL/6J and DBA/2J. To this end, mice (male, 8 wk old) were fed a high-fat and high-carbohydrate (HFHC) or control diet for 16 wk followed by the application of a combination of classic physiological, biochemical, and pathological studies to determine obesity and hepatic steatosis. Peripheral IR was characterized by measuring blood glucose level, serum insulin level, homeostasis model assessment of IR, glucose intolerance, insulin intolerance, and AKT phosphorylation in adipose tissues, whereas the level of hepatic IR was determined by measuring insulin-triggered hepatic AKT phosphorylation. We discovered that both C57BL/6J and DBA/2J mice developed obesity to a similar degree without the feature of liver inflammation after being fed an HFHC diet for 16 wk. C57BL/6J mice in the HFHC diet group exhibited severe panlobular steatosis, a marked increase in hepatic triglyceride levels, and profound peripheral IR. In contrast, DBA/2J mice in the HFHC diet group developed only a mild degree of pericentrallobular hepatic steatosis that was associated with moderate changes in peripheral IR. Interestingly, both C57BL/6J and DBA/2J developed severe hepatic IR after HFHC diet treatment. Collectively, these data suggest that the severity of diet-induced hepatic steatosis is correlated to the level of peripheral IR, not with the severity of obesity and hepatic IR. Peripheral rather than hepatic IR is a dominant factor of pathophysiology in nonalcoholic fatty liver disease.

nonalcoholic fatty liver disease; insulin resistance; hepatic insulin resistance; steatosis; mice

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is the most common chronic liver disorder in the United States (4, 8). It is a term for a broad continuum of liver illnesses extending from steatosis (simple fatty liver), to nonalcoholic steatohepatitis, to advanced fibrosis, and cirrhosis. Nonalcoholic hepatic steatosis could be an asymptomatic condition, whereas hepatic steatohepatitis is potentially a serious disorder that may progress to substantial liver damage and severe fibrosis. Although patients with simple fatty liver change have a benign clinical course with a very low probability of developing progressive liver dysfunction and cirrhosis, fatty liver disease is associated with inflammatory cardiovascular disease (18). Thus it is important to understand mechanisms underlying the pathogenesis of hepatic steatosis.

Obesity, diabetes, and insulin resistance are important risk factors for the development of hepatic steatosis (31). Insulin resistance plays a critical role in the pathogenesis of NAFLD (3). Global insulin resistance comprises central (i.e., hepatic) and peripheral (i.e., muscle and adipose) tissues (2). Evidence has shown that both hepatic and peripheral insulin resistance occurs in patients with fatty liver disease (19). However, the causal effect of hepatic insulin resistance on steatosis remains largely unknown (31).

High-fat diet is an important component of the etiology of obesity and hepatic steatosis (29). Studies on animals have indicated that large consumption of fatty diets induces fatty changes in the liver (16). High-fat diets are known to increase body weight and body fat and induce insulin resistance in C57BL6 (B6) and DBA/2 (D2) mice (11, 30), two popular inbred mouse strains whose genetic as well as metabolic responsiveness to several diet modifications have been characterized. Liver steatosis has been evident in high-fat diet-fed B6 and D2 mice (12, 14). In addition, a high consumption of saturated fat and fructose has been shown to cause severe hepatic steatosis in B6 mice (14). Together, these studies suggest that B6 and D2 mouse strains can be used to aid in elucidating the underlying pathophysiology and genetic basis of obesity and fatty liver diseases.

It is unclear whether B6 and D2 mouse strains harbor different susceptibility to high-fat diet-induced hepatic steatosis. In the present study, therefore, we examined B6 and D2 mouse strains for their response to a high-fat, high-carbohydrate (HFHC) diet with respect to obesity, insulin resistance, and hepatic steatosis. We found that, despite comparable levels of obesity, B6 and D2 strains differed markedly in the regulation of glucose homeostasis, insulin sensitivity, and levels of hepatic steatosis. Furthermore, we revealed that susceptibility to HFHC diet-induced fatty liver injury correlates with peripheral insulin resistance but not with hepatic insulin resistance in mice. These findings may provide new knowledge for understanding the pathogenesis of NAFLD.

MATERIALS AND METHODS

Reagents. Fructose and sucrose were purchased from Sigma-Aldrich (St. Louis, MO). Human insulin was purchased from Eli Lilly (Indianapolis, IN). Affinity-purified monoclonal antibodies directed against murine phospho-AKT-ser437, phospho-insulin receptor substrate 1 (IRS-1)-ser302, phospho-sterol regulating element binding protein 1c (SREBP-1c)-ser372, pan-AKT, and IRS-1 were purchased...
from Cell Signaling Technology (Cambridge, MA). A murine SREBP-1c antibody was obtained from Abcam (Cambridge, MA).

Animals and treatments. C57BL/6J and DBA/2J mice (male, 8–10 wk old; Jackson Laboratory, Bar Harbor, ME) were housed in cages in a temperature-controlled environment at 22°C. Lighting was maintained to a 12-h:12-h day/night cycle. After 1 wk of being housed with a regular diet, mice were randomly divided into the two diet groups. The “chow” diet group received a regular diet containing (wt/wt) 7% fat, 18% protein, and 75% carbohydrate. The HFHC group received a 58 kcal% fat with cornstarch (Surwit Diet; Research Diets, New Brunswick, NJ) and drinking water that contains carbohydrates (42 g/l) at a ratio of 55% fructose and 45% sucrose by weight using a protocol described by Kohil et al. (14). Animals were given ad libitum access to diets and drinking water.

Before carrying on this project, we compared HFHC diet-induced liver steatosis in B6 and D2 mice through a pilot time course study. To this end, mice were fed HFHC diet for 8, 16, and 24 wk. At each time point, we examined liver histology for the presence and severity of hepatic steatosis. It was found that B6 and D2 mice both developed mild hepatic steatosis after 8 wk of HFHC diet (data not shown). The hepatic steatosis increased to severe grade in B6 mice but remained at mild grade in D2 mice after 16 as well as 24 wk of HFHC diet (data not shown). Thus we chose to analyze the animals after 16 wk of experimental diet throughout the study. Briefly, body weight and food/water consumption were measured weekly for 16 wk. At the end of the 16 wk on experimental diets, mice were euthanized with CO2 inhalation. Liver, blood, and epididymal adipose tissues were collected. Wet mass of the liver and epididymal white adipose tissues was measured. Blood was processed for isolation of plasma by centrifugation at 10,000 g for 10 min at room temperature. Liver tissues and plasma samples were stored at −80°C until used. All animal experiments were conducted in accordance with the National Institutes of Health guidelines and were approved by Institutional Animal Care and Use Committee at Ann & Robert H. Lurie Children’s Hospital of Chicago Research Center.

Histology. The fresh liver specimens were fixed in 10% neutral buffered formalin overnight at room temperature followed by routine histology processing in the Research Histology Core at the Ann & Robert H. Lurie Children’s Hospital of Chicago Research Center. Paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E). For the determination of apoptosis, tissue sections were processed for TUNEL staining using In Situ Cell Death Detection Kit (Roche, Indianapolis, IN). In some experiments, fresh liver tissues were embedded into optimal cutting temperature compound, processed for frozen sections, and stained with Oil Red O staining for determination of fatty changes in tissues. All mice used in these studies underwent histological analysis to monitor pathological changes of hepatic steatosis.

Determination of blood glucose, plasma insulin levels, and systemic insulin resistance. Mice were fasted for 4 h before determinations of whole blood glucose and plasma insulin. Whole blood glucose was determined by clipping tails under appropriate anesthesia followed by measuring glucose levels in blood using the Accu-Check glucose meter (Roche Diagnostics) with a protocol described by the manufacturer. For determination of insulin levels, plasma samples were prepared from whole blood. Plasma insulin concentrations were measured using Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Downers Grove, IL). To evaluate the degree of systemic insulin resistance, the value of homeostasis model of assessment of insulin resistance (HOMA-IR) index was calculated using the following formula: IR = fasting blood glucose (mg/dl) × fasting insulin (μU/ml)/405 (21).

Intraperitoneal glucose tolerance test and insulin tolerance test. Intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (IPITT) were performed (n = 8 per test group for each test) following 16 wk of diet treatments. The protocol was adapted from methods previously described (1, 15). Animals were fasted overnight for IPGTT and 6 h for IPITT, respectively. A bolus of glucose (1 g/kg body wt) or insulin (0.75 U/kg body wt) was injected intraperitoneally. Before and after intraperitoneal injections at various time points, blood samples were collected from mice via a tail-clipping procedure. Whole blood glucose levels were determined as described above. Area under the curve (AUC; mmol-min/l) in the IPGTT and IPITT was calculated using the trapezoidal rule (27). Insulin challenge to determine activity of insulin signaling in liver and adipose tissues by Western blotting. Mice were fasted overnight. They were anesthetized. Insulin (5 U/mouse) was injected into inferior vena cava after laparotomy. After 5 min, mice were euthanized with an overdose of pentobarbital sodium. Liver and epididymal adipose tissues were then removed, frozen with liquid nitrogen, and stored at −80°C until further analysis.

To determine phosphorylation levels of factors related to the insulin pathway, tissue specimens were homogenized in RIPA buffer that contained proteinase and phosphatase inhibitor cocktail (Roche). Protein extracts (12.5 μg) were resolved by 4–12% SDS-PAGE (NuPAGE bis-Tris precast gel) and transferred to PVDF membranes. Membranes were blocked in Tris-buffered saline containing 5% skimmed dry milk or bovine serum albumin and incubated overnight at 4°C with antibodies to phospho-Akt-ser437, phospho-IRS-1-ser302, and phospho-SREBP-1c-ser372 at 1:2,000, 1:1,000, and 1:1,000 dilutions, respectively. Membranes were then extensively washed with Tris-buffered saline containing 0.1% Tween-20. At the end of the final wash, the blots were incubated with secondary antibodies conjugated with horseradish peroxidase in 10 ml of blocking buffer for 1 h at 22°C and washed again followed by treatment with enhanced chemiluminescence reagent (ECL; Pierce Biotechnolog, Rockford, IL) according to the manufacturer’s instructions. Images were captured using the ChemiDec System (Bio-Rad, Hercules, CA). Membranes were reblotted with antibodies against pan-AKT (dilution 1:1,000), IRS-1 (dilution 1:1,000), and SREBP-1c (dilution 1:1,000) followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (dilution 1:5,000) to determine the relative

Table 1. Effect of regular chow or HFHC feeding on body weight, visceral fat, and intake of food and energy in B6 and D2 mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>B6</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>HFHC</td>
<td>Chow</td>
</tr>
</tbody>
</table>
|Initial BW, g|24.10 ± 0.35|24.10 ± 0.28|22.50 ± 0.90|18.90 ± 0.12‡
|Final BW, g|31.70 ± 0.68|48.10 ± 1.10*|29.10 ± 0.60*|42.10 ± 0.68‡
|BW gain, g|7.60 ± 0.56|24.00 ± 0.99*|6.60 ± 0.90|23.40 ± 0.62‡
|Visceral fat/BW, %|2.45 ± 0.44|3.12 ± 0.11*|1.35 ± 0.17*|2.75 ± 0.21‡
|Food intake, Kcal/mouse per day|11.97§|13.50 ± 0.32|11.97§|15.00 ± 0.32‡
|Energy intake from fructose water, Kcal/mouse per day|0|0.95 ± 0.05|0|0.90 ± 0.07

Data are means ± SE, n = 5. *P < 0.05 vs. B6 Chow; †P < 0.05 vs. B6 high-fat, high-carbohydrate diet (HFHC); ‡P < 0.05 vs. D2 Chow; §from Ferguson et al. (9). BW, body weight.
amount of AKT, IRS-1, and SREBP-1c proteins in the membranes. To visualize the targeted proteins, membranes were treated with ECL reagent and scanned with ChemiDoc. The specific band intensities were quantified using Image Lab (Bio-Rad) for integrating the autoradiographic signals.

**Hepatic triglyceride and plasma alanine aminotransferase quantification.** To determine triglyceride (TG) contents in liver tissues, 100 mg of wet liver tissues was homogenized in 1 ml of 5% NP-40 solution and slowly heated up to 100°C and then cooled down to room temperature. This process was repeated twice. The tissue homogenates were centrifuged at 13,000 revolution/min for 2 min at room temperature. The supernatants were processed for measuring TG contents using Triglyceride Quantification Kit (BioVision, Milpitas, CA). The alanine aminotransferase (ALT) activity was measured with Roche/Hitachi Cobas 6000 analyzer (Roche Diagnostics) using plasma samples.

**Statistical analysis.** Statistical analysis was performed with GraphPad Prism 4 software. For all data sets, the unpaired Student’s t-test was performed. Results were deemed significant if P values were <0.05. Data are described as means ± SE.

**RESULTS**

**B6 and D2 mice gained body weight and visceral fat following a 16-wk HFHC diet.** Table 1 shows the effects of HFHC diet-induced obesity in B6 and D2 mice fed an HFHC diet for 16 wk. Male B6 and D2 mice gained ~7.6 g and 6.6 g body wt, respectively, from the baseline after being fed a normal chow diet for 16 wk. In a second cohort of mice fed an HFHC diet, B6 mice gained a mean body weight of 24 g, a 200% increase over the initial body weight, whereas D2 mice gained ~23.4 g, a 225% body wt increase (Table 1). The weight percentage of abdominal adipose tissue in B6 mice was 13% greater than D2 mice after 16 wk of HFHC diet although both increased in a similar degree compared with the normal chow-fed B6 and D2 groups. Furthermore, all mice introduced to the HFHC diet adapted immediately to the special diet and fructose water. Intake of food and fructose/sucrose water during the experiments was measured at 5- to 7-day intervals. Over the course of 16 wk with HFHC diet, D2 mice consumed 11% more calories from the food than B6 mice (13.5 ± 0.32 kcal/B6 mouse per day vs. 15 ± 0.32 kcal/D2 mouse per day, P < 0.05), but there was no difference in the caloric intake from the fructose/sucrose water between B6 and D2 mice. Caloric intakes in mice fed regular chow diet have been well characterized. By comparing our data with one reported by Sohal et al. (28), we found that caloric intakes in either B6 or D2 mice with HFHC diet were ~120% greater than mice fed a regular chow diet. Overall, feeding an HFHC diet caused increases in body weight and epididymal fat pad weight in both B6 and D2 mice, indicating these two strains are very similar in their susceptibility to obesity when fed an HFHC diet.

![Fig. 1. B6 mice develop more severe hepatic steatosis than D2 mice after long-term high-fat, high-carbohydrate (HFHC) diet feeding.](http://ajpgi.physiology.org/)
B6 mice developed greater hepatic steatosis than D2 mice in response to HFHC diet, and liver inflammation did not occur after feeding B6 and D2 mice an HFHC diet for 16 wk. We collected serum and liver tissues from B6 and D2 mice in both normal chow and HFHC diet cohorts at the end of the 16-wk feeding. As shown in Fig. 1A, B6 and D2 mice fed a normal chow diet were confirmed to have normal liver histology. In the cohort of HFHC diet, the liver histology of B6 mice exhibited severe micro and macrovesicular hepatosteatosis in a pattern of panlobular distribution, whereas D2 mice showed mild pericentral lobular hepatosteatosis. The grades of hepatic steatosis were further confirmed with Oil Red O staining (Fig. 1B). However, no signs of liver inflammation (i.e., infiltration of inflammatory cells, hepatocyte ballooning, Mallory bodies in hepatocytes) were determined by examination of H&E-stained slides in HFHC diet-fed B6 and D2 mice (Fig. 1A). With trichrome staining, we did not detect any sign of perisinusoidal liver fibrosis in the cohort of HFHC diet (data not shown). The data indicate that HFHC diet-induced hepatosteatosis is not associated with liver inflammation in these inbred mouse strains. Furthermore, the levels of hepatocyte apoptosis were not increased in either B6 or D2 mice after exposure to HFHC diet for 16 wk compared with the regular chow diet groups (Fig. 1C).

In addition, we measured the liver weight and TG levels in cohorts of B6 and D2 mice that received normal chow as well as HFHC diets to further determine the severity of steatosis. The data of liver weight (Table 2) indicated that the absolute liver weight increased significantly in B6 mice when they received an HFHC diet, and the same trend was observed when the hepatomegaly was expressed as liver-to-body-weight ratio. In contrast, the absolute live weight of D2 mice remained unchanged between a regular chow and HFHC diet. The TG level in the liver of B6 mice fed HFHC diet was 17.8-fold higher than the regular chow diet group (Table 2). Although liver TG content in D2 mice fed HFHC diet was 4.5-fold greater compared with the regular chow diet group, it was 70% lower than B6 mice fed the HFHC diet (P < 0.001).

Finally, we measured serum ALT levels in each group as an indirect measure of hepatocyte injury. The data (Table 2) showed that feeding an HFHC diet for 16 wk did not alter plasma ALT levels in either B6 or D2 mice. Together, the results indicate that B6 mice developed a more severe fatty liver after 16 wk of exposure to an HFHC diet compared with D2 mice. However, HFHC diet-induced obesity and fatty liver change were not associated with active liver damage and inflammation in these inbred mice.

### Table 2. Effect of regular chow or HFHC feeding on liver weight, liver triglyceride content, and plasma ALT level in B6 and D2 mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chow</th>
<th>B6 HFHC</th>
<th>D2 HFHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight, g</td>
<td>1.34 ± 0.10</td>
<td>2.48 ± 0.21*</td>
<td>1.67 ± 0.06*</td>
</tr>
<tr>
<td>Liver weight/body weight, %</td>
<td>4.18 ± 0.21</td>
<td>5.00 ± 0.31</td>
<td>5.77 ± 0.11*</td>
</tr>
<tr>
<td>Liver triglyceride, mg/dl per 100 mg tissue</td>
<td>209.8 ± 7.70</td>
<td>3729.5 ± 304*</td>
<td>253 ± 42</td>
</tr>
<tr>
<td>Plasma ALT, IU/l</td>
<td>55 ± 5.00</td>
<td>80 ± 12</td>
<td>79 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± SE, n = 6. *P < 0.05 vs. B6 chow; †P < 0.05 vs. B6 HFHC; ‡P < 0.05 vs. D2 chow; §P < 0.001 vs. B6 HFHC. ALT, alanine aminotransferase.
contrast, blood glucose levels at all time points after glucose injection except 30 min were similar between regular chow diet and HFHC diet groups, indicating that the physiological function of regulating blood glucose levels in this mouse strain remained normal after exposure to HFHC diet for 16 wk (Fig. 2B). By calculating AUC after glucose injection (\( \text{AUC}_{\text{glc}} \), mmol-min/l), B6 mice were revealed to develop significantly higher glucose intolerance than D2 mice under HFHC diet feeding (\( P < 0.001 \), Fig. 2C).

We also carried out IPITT on these animals. It was found that, although HFHC diet caused insulin resistance in both B6 (Fig. 3A) and D2 mice (Fig. 3B), AUCglc in B6 mice was significantly higher compared with AUCglc in D2 mice (Fig. 3C). This indicates that the long-term HFHC diet induced a more severe impairment of insulin response of cellular glucose uptake in B6 mice than in D2 mice, rather than impairment of insulin production from \( \beta \)-cells in response to baseline hyperglycemia attributable to the HFHC diet.

In addition, we measured levels of HFHC diet-induced peripheral insulin resistance by examining sensitivity to insulin-induced AKT activation in adipose tissues. In B6 mice fed HFHC diet, insulin-induced AKT phosphorylation was markedly reduced in the adipose tissue compared with the chow group (Fig. 4). In contrast, D2 mice fed HFHC diet showed only mild impairment of insulin-induced AKT phosphorylation in the adipose tissue. Taken together, the data suggest that HFHC feeding for 16 wk caused severe peripheral insulin resistance in B6 but not D2 mice. Their degree of peripheral insulin resistance correlated with the severity of their hepatic steatosis.

Hepatic insulin resistance is induced in B6 and D2 mice fed a 16-wk HFHC diet. We examined whether HFHC diet induced alteration of hepatic insulin resistance specifically in B6 and D2 mice by determining expression and phosphorylation of IRS-1, AKT, and SREBP-1c in livers. IRS-1 represents subreceptor activity of the insulin pathway. AKT plays a pivotal role in regulation of gluconeogenesis in hepatocytes, whereas SREBP-1c is a main regulator of hepatic lipogenesis. To examine whether a long-term HFHC diet disrupts activation of the insulin signaling pathway in the liver, B6 and D2 mice in both regular chow and HFHC diet groups were subjected to intravenous administration of insulin followed by measurement of phosphorylation of AKT, IRS-1, and SREBP-1c with Western blotting. First, in a pilot study, we confirmed that phosphorylation levels of hepatic AKT were negligible in fasted B6 and D2 mice without insulin stimulation (data not shown). We then found that B6 and D2 mice fed a regular chow diet had strong AKT phosphorylation in the liver tissues at 5 min after insulin stimulation (Fig. 5A). Feeding an HFHC diet for 16 wk resulted in marked impairment of insulin-stimulated phosphorylation of AKT in both B6 and D2 mice (Fig. 5A). Densitometric analysis indicated a 57% reduction in
insulin-triggered AKT phosphorylation in B6 mice and an 84% reduction of that in D2 mice after exposure to HFHC diet. Total liver AKT expression was comparable between groups. In contrast to AKT, feeding an HFHC diet showed a decrease in total hepatic IRS-1 expression in both strains but had no effect on insulin-induced phosphorylation of IRS-1 in livers (Fig. 5B). Expression and insulin-induced phosphorylation of SREBP-1c in livers were not disrupted by the HFHC diet in either B6 or D2 mice (Fig. 5C). These data indicated that severity of hepatic steatosis did not correlate with alteration of hepatic insulin resistance.

DISCUSSION

The aim of the present study was to compare susceptibility of fatty liver disease between two common inbred mouse strains and further determine the association between the susceptibility of HFHC diet-induced fatty liver change and insulin resistance. B6 and D2 mice are the most popular strains for diet-induced and genetically modified mouse models of fatty liver disease. However, a detailed investigation of their susceptibility to fatty liver disease has not been established. The focus of the present study was to determine whether insulin resistance correlates with the susceptibility of NAFLD.

Several lines of evidence implicate that NAFLD has remarkably diverse etiologies that are involved with dysregulation of lipid metabolism and gene-environment interactions (7, 22). However, the exact contributions of genetic factors for disease development and its mechanisms remain unclear. Inbred mice, such as the B6 and D2 strains, are powerful tools that can be used to investigate the mechanisms and pathophysiology underlying the development of human diseases. Recently, over
8.2 million single nucleotide polymorphisms have been reported across a range of different inbred strains of mice (10). The impact of this genomic variance to disease susceptibility remains unclear. B6 and D2 mice have previously been shown to develop fatty livers after a prolonged fat-rich diet (12, 24). In the present study, we further investigated whether a genomic basis controls the susceptibility to HFHC diet-induced fatty liver disease, by applying a mouse model using B6 and D2 strains. The results indicated that both strains consumed more calories on an HFHC diet than on a normal chow diet, and both strains showed a higher feed efficiency (i.e., weight gained/calories consumed) on the HFHC diet vs. the normal chow diet. B6 and D2 mice both developed increased adiposity to a similar extent after 16 wk on an HFHC diet regardless of genetic background. Together, our findings suggest that development of higher adiposity in response to an HFHC diet is independent of single nucleotide polymorphism alterations between B6 and D2 mice. However, we did find that B6 mice have more profound hepatic steatosis than D2 mice in response to the HFHC feeding. The development of hepatic steatosis by dietary fat and fructose is independent from the degree of their body weight gain, which highlights the impact of genetic variance on development of hepatic steatosis in B6 and D2 mice.

It has been shown that feeding methionine-choline-deficient (MCD) diet can induce nonalcoholic steatohepatitis, the advanced stage of NAFLD in mice. Recently, Rangnekar et al. (25) reported that D2 mice develop more severe hepatic steatosis than B6 mice after treatment with MCD diet for 10 wk. In contrast, we revealed that B6 mice are more susceptible to HFHC diet-induced hepatic steatosis. Collectively, these findings suggest that genetic factors that regulate HFHC diet-induced hepatic steatosis may differ from those that govern fatty liver changes induced by an MCD diet in mice.

Insulin resistance is a pathophysiological condition in which cells fail to respond to insulin. It occurs both systemically in...
Hepatic insulin resistance has been postulated to be a major contributing factor of steatosis although its cause-effect relation remains debatable (31). Interestingly, we found that B6 and D2 mice developed hepatic insulin resistance at similar levels although B6 mice have more severe hepatic steatosis than D2 mice under HFHC diet feeding. This suggests that hepatic insulin resistance is dissociated from the genetic variances that control hepatic steatosis. However, future investigations are required to delineate molecular mechanisms by which HFHC diet induces more severe hepatic steatosis in B6 mice than in D2 mice.

NAFLD is an obesity-driven chronic liver disease. Hepatic steatosis occurs in most obese individuals; however, only a small subset of patients with NAFLD develops steatohepatitis, which can further progress to fibrosis and cirrhosis. Although prolonged simple steatosis is speculated to result in inflammatory reactions and loss of metabolic competency in hepatocytes, the exact mechanisms that drive steatosis to steatohepatitis remain unknown. It has been shown that prolonged treatment of high-fat diets can induce simple fatty liver in rodents. However, evidence has shown that high-fat diet alone is usually unable to induce hepatocyte injury and liver inflammation. This suggests that evolution of steatosis to steatohepatitis requires additional hits or factors. Previously, Kohli et al. (14) reported that prolonged high-fat diet plus fructose intake was able to induce steatohepatitis with significant liver fibrosis in B6 mice (14), suggesting that fructose plays a role in induction of inflammation in high-fat diet-induced fatty liver. Interestingly, in our study using B6 and D2 mice, we found steatosis but no inflammatory phenotype in the liver after feeding an HFHC diet for 16 wk. Recently, several studies have shown that intestinal microbiota contributes to the development of steatosis and inflammation in the liver during high-fat diet feeding (13, 17). As gut microbiota in mice at our research facility are expected to differ from ones at other institutions, it would be interesting to further explore the roles of intestinal microbiota in the development of nonalcoholic steatohepatitis-like phenotype in HFHC diet exposure.

In summary, we revealed a significant difference of NAFLD susceptibility between two common inbred mouse strains. The HFHC diet induced greater steatosis on B6 mice than D2 mice, in correlation with greater peripheral insulin resistance in the B6 genetic background. In contrast, both strains similarly developed severe hepatic insulin resistance. Our findings indicated that peripheral insulin resistance, not hepatic, correlated with the susceptibility of NAFLD.

ACKNOWLEDGMENTS

The authors thank Dr. Rohit Kohli for advice on the animal model.

GRANTS

This work was supported in part by grants from National Institutes of Health, including R21AA020494 (to X.-D. Tan), R01DK064240 (to X.-D. Tan), and R01NS060926 (to C. J. DiDonato), Merit Review Award (to X.-D. Tan) from the US Department of Veterans Affairs, and the Excellence in Academic Medicine Award from Illinois Department of Public Aid (to X.-D. Tan).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


