ORIGINAL ARTICLE

Impact of obese levels on the hepatic expression of nuclear receptors and drug-metabolizing enzymes in adult and offspring mice

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**KEY WORDS**

Diet-induced obesity; Overweight; High-fat diet; Drug-metabolizing enzymes; Nuclear receptors; Ontogenic expression

**Abstract**

The prevalence of obesity-associated conditions raises new challenges in clinical medication. Although altered expression of drug-metabolizing enzymes (DMEs) has been shown in obesity, the impacts of obese levels (overweight, obesity, and severe obesity) on the expression of DMEs have not been elucidated. Especially, limited information is available on whether parental obese levels affect ontogenic expression of DMEs in children. Here, a high-fat diet (HFD) and three feeding durations were used to mimic different obese levels in C57BL/6 mice. The hepatic expression of five nuclear receptors (NRs) and nine DMEs was examined. In general, a trend of induced expression of NRs and DMEs (except for \textit{Cyp2c29} and \textit{3a11}) was observed in HFD groups compared to low-fat diet (LFD) groups. Differential

**Abbreviations:** 4-HA, adult mice fed with 4 weeks HFD; 4-LA, adult mice fed with 4 weeks LFD; 7-ER, 7-ethoxyresorufin; 8-HA, adult mice fed with 8 weeks HFD; 8-LA, adult mice fed with 8 weeks LFD; 18-HA, adult mice fed with 18 weeks HFD; 18-LA, adult mice fed with 18 weeks LFD; AhR, aryl hydrocarbon receptor; BMI, body mass index; CAR, constitutive androstane receptor; CHZ, chlorzoxazone; CYP2E1, cytochrome P450 2E1; DIO, diet-induced obesity; DMEs, drug-metabolizing enzymes; EFV, efavirenz; \textit{Gapdh}, glyceraldehyde-3-phosphate dehydrogenase; HFD, high-fat diet; HNF4\textalpha, hepatocyte nuclear factor 4 alpha; LFD, low-fat diet; MDZ, midazolam; MPA, mobile phase A; MPB, mobile phase B; NADPH, nicotinamide adenine dinucleotide phosphate; NAFLD, non-alcoholic fatty liver disease; NRs, nuclear receptors; O-4-HA, offspring from parental mice fed with 4 weeks HFD; O-4-LA, offspring from parental mice fed with 4 weeks LFD; O-8-HA, offspring from parental mice fed with 8 weeks HFD; O-8-LA, offspring from parental mice fed with 8 weeks LFD; O-18-HA, offspring from parental mice fed with 18 weeks HFD; O-18-LA, offspring from parental mice fed with 18 weeks LFD; PBS, phosphate-buffered saline; PPAR\textalpha, peroxisome proliferator-activated receptor alpha; PXR, pregnane X receptor; RSF, resorufin; RT-qPCR, real-time quantitative PCR; SD, standard deviation; SULT1A1, sulfotransferase 1A1; UGT1A1, uridine diphosphate glucuronosyltransferase 1A1.

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1. Introduction

Globally, overweight and obesity, which are defined as a body mass index (BMI) greater than 25 and 30 kg/m², respectively, have become a serious epidemic health issue. In 2016, overweight and obesity affected more than 39% of adult population (1.9 billion) and 18% of children (340 million) worldwide, according to the updated data from the World Health Organization. Strikingly, the incidence of overweight and obesity continues rising at an alarming rate.

The prevalence of overweight and obesity elevates the incidence of many diseases, including type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), cardiovascular diseases, and cancer. For instance, the incidence rate of NAFLD in the general population is 20%–30%, whereas this prevalence increases dramatically to 80%–90% in the obese adult population. Overweight and obese populations consume on average more medications than non-obese individuals. Notably, it is well-documented that overweight and obese patients are more vulnerable to drug-induced toxicity as a consequence of altered drug metabolism in both adult and pediatric patients. Thus, it is critical to elucidate mechanisms contributing to the dysregulated drug metabolism in overweight and obese individuals.

Evidence from a few clinical studies has shown that changed activities of drug-metabolizing enzymes (DMEs) may be recognized for altering the drug metabolism in obese populations. For example, induced cytochrome P450 2E1 (CYP2E1) activity was found to be associated with the high-risk of acetaminophen-induced liver injury in obese adults. However, these researches were confined to simply comparing the differential activities of drug-metabolizing enzymes between (morbidly) obese and non-obese populations. There is a clue indicating that the degrees of obesity could impact the activity of DMEs in overweight and obese patients. Thus, far less information is available in overweight adults on whether overweight alters activities of DMEs as well as the differential effects of obese levels (overweight, obesity, and severe obesity). Especially, the impacts of overweight and obesity on the expression of DMEs in children at younger ages have not been elucidated. Importantly, solutions for these questions have great significance for precision medicine as well as the animal models. As male mice are more sensitive than female mice to HFD, only male mice were recruited in most previous studies. Limited information is available on the consequence of an HFD-consumption on the expression of DMEs in female mice.

The goal of this work is to determine the impact of obese levels on hepatic expression of NRs and DMEs. In this study, C57BL/6 mice were fed with a low-fat diet (LFD) or HFD for different feeding durations to mimic different obese levels. The effects of HFD-feeding durations were comprehensively investigated on the expression of NRs and DMEs as well as the enzyme activities of selected DMEs in the liver of adult mice. Given the fact that childhood obesity is mostly owing to a family-based dietary style, the impacts of family-based consumption of an HFD were also studied on the ontogenic expression of NRs and DMEs in the liver of offspring mice. In addition, the consequences of HFD-feeding durations of parental mice on the hepatic expression of NRs and DMEs in the offspring were demonstrated in the present study. The generated knowledge may help to develop optimal dosage selections for overweight and obese populations in health care practice.

2. Materials and methods

2.1. Chemicals and reagents

Efavirenz (EFV) was purchased from TCI America Inc. (Portland, OR, USA). Chlorozoxaze (CHZ), 6-OH-CHZ, 7-ethoxyresorufin (7-ER), and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Midazolam (MDZ) and resorufin (RSF) were purchased from Sigma—Aldrich, Inc. (St. Louis, MO, USA). Formic acid and acetonitrile were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Phosphate-buffered saline (PBS) was
obtained from Research Products International Corp. (Mount Prospect, IL, USA).

2.2. Animal experiments

Male and female C57BL/6 mice (8-week old) were purchased from the Jackson laboratory- (Bar Harbor, ME, USA) and used for breeding. All mice were housed and bred under the standard conditions in the Animal Care Service Facility at the University of Connecticut (Storrs, CT, USA) in accordance with the animal care guidelines provided by the American Association for Animal Laboratory Sciences. All mice were allowed ad libitum access to food and water. The use of animals in the present study was approved by the Institutional Animal Care and Use Committee at the University of Connecticut.

A flow chart for the experimental design of the current study is shown in Supporting information Fig. S1. Briefly, both male and female mice (4–5 weeks old) were maintained on either a LFD containing 10 kcal% from fat (D12450L, Research Diets, New Brunswick, NJ, USA) or an HFD containing 60 kcal% from fat (D12492, Research Diets). Compositions of the diets are shown in Table 1. After 4, 8, or 18 weeks of diet treatment, some mice were sacrificed and liver tissues were collected to study the impacts of obese levels on the expression of NRs and DMEs in adult mice. The remaining mice with a LFD or HFD were mated and were still maintained on a LFD or HFD, respectively, to assess the effects of parental obese levels on the expression of NRs and DMEs in offspring mice. Offspring younger than 21 days were fostered by the maternal mice. After weaning at day 21, the offspring had free access to food and water. All offspring were maintained on the same diet as their parental mice. Liver S9 was obtained by homogenizing liver tissue in 1 L of ice-cold acetonitrile/methanol (1:1, v/v) followed by centrifugation at 15,000 rpm (Centrifuge 5415R, Eppendorf, Hamburg, Germany) for 30 min. Protein concentrations were measured using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). To determine the enzyme activities of CYP1A1, 2B10, 2E1, and 3A, 7-ER, EFV, CHZ, and MDZ were used as probe substrates and the formation of RSF, 8-OH-EFV, 100 μmol/L 7-ER, or 15 μmol/L MDZ, respectively. After 10 min of incubation, the reactions were terminated by adding 100 μL of ice-cold acetonitrile/methanol (1:1, v/v). After 10 min of centrifugation, some aliquots of supernatant were injected into a SYNAPT G2-S QTOFMS system (Waters Corp., Milford, MA, USA) for metabolite analysis. Chromatographic separation of metabolites was performed on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 mm; Waters Corp.).

2.3. RNA isolation and real-time quantitative PCR (RT-qPCR)

Total RNAs were isolated from frozen liver tissues using a TRIzol reagent (Life Technologies, Guilford, CT, USA) according to the manufacture’s protocols. The concentrations of total RNAs were quantified spectrophotometrically at 260 nm and the purities were confirmed by 260/280 using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The cDNAs were synthesized from total RNAs using an iScript Reverse Transcription Supermix (Bio−Rad Laboratories, Hercules, CA, USA) according to the manufacture’s protocols. RT-qPCR was performed using a CFX96 Real-Time System (Bio−Rad Laboratories). The mRNA expression of aryl hydrocarbon receptor (Ahr) and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) was determined using an TaqMan assay used for RT-qPCR.

Table 2. TaqMan assay used for RT-qPCR.

| Gene     | Assay ID          |
|----------|-------------------|
| Nr1i3 (Car) | Mm01283978_m1    |
| Nr1i2 (Por) | Mm01344139_m1    |
| Hnf4a    | Mm00433964_m1    |
| Ppara    | Mm00449039_m1    |
| Cyplal   | Mm00487218_m1    |
| Cyplia2  | Mm00487224_m1    |
| Cyp2e1   | Mm00491127_m1    |
| Cyp2b10  | Mm01972453_s1    |
| Cyp2c9   | Mm00725580_s1    |
| Cyp3a11  | Mm00731567_m1    |
| Cyp3a16  | Mm00655824_m1    |
| Ugt1a    | Mm02603337_m1    |
| Sult1a1  | Mm00467072_m1    |
| Gapdh    | Mm99999915_g1    |

Table 1. Composition of diets.

| Formula         | LFD (# D12450L) | HFD (# D12492) |
|-----------------|-----------------|----------------|
| Fat (lard), kcal% | 10              | 60             |
| Protein, kcal%  | 20              | 20             |
| Carbohydrate, kcal% | 70              | 20             |
| Energy density, kcal/g | 3.82             | 5.21           |

All values were obtained from Research Diets product data sheets (https://researchdiets.com/opensource-diets/dio-series-diets).

Universal SYBR Green Supermix (Bio−Rad Laboratories) with specific primers. The sequences of the primers were as follows: Ahr (sense), 5′-ACCAGAACTGAGGGTTTGG-3′; Ahr (anti-sense), 5′-CTCCCATCGTATAGGGGAC-3′; Gapdh (sense), 5′-GGTAAGGCTTTGAAC-3′; Gapdh (anti-sense), 5′-GAGTGGAGCTATAACTTGAAC-3′. The mRNA expression of other genes was measured using a TaqMan gene expression assays (Thermo Fisher Scientific). The identification numbers of each gene are listed in Table 2. Relative mRNA expression levels were calculated by normalizing examined genes against Gapdh using the 2−ΔΔCt method. In the study of ontogenic expression, the differences of mRNA expression were also expressed as 2−ΔΔCt compared to Gapdh as described in a previous study.54

2.4. Enzyme activities in mouse liver S9

Liver S9 was obtained by homogenizing liver tissue in 1 × PBS (pH 7.4) followed by centrifugation at 9800 rpm (Centrifuge 5415R, Eppendorf, Hamburg, Germany) for 30 min. Protein concentrations were measured using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). To determine the enzyme activities of CYP1A1, 2B10, 2E1, and 3A, 7-ER, EFV, CHZ, and MDZ were used as probe substrates and the formation of RSF, 8-OH-EFV, 6-OH-CHZ, and 1-OH-MDZ were detected, respectively.55−58 Incubations were performed in 1 × PBS (pH 7.4), containing 1 μg/mL S9, 1 mMol/L NADPH, and the probe substrate (10 μmol/L 7-ER, 30 μmol/L EFV, 100 μmol/L CHZ, or 15 μmol/L MDZ, respectively). After 10 min of incubation, the reactions were terminated by adding 100 μL of ice-cold acetonitrile/methanol (1:1, v/v). After 10 min of centrifugation at 15,000 rpm (Centrifuge 5415R, Eppendorf, Hamburg), a 2 μL aliquot of supernatant was injected into a SYNAPT G2-S QTOFMS system (Waters Corp., Milford, MA, USA) for metabolite analysis. Chromatographic separation of metabolites was performed on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 mm; Waters Corp.). Mobile phase A (MPA) consisted of 0.1% formic acid in water and mobile phase B (MPB) consisted of 0.1% formic acid in acetonitrile. The gradient for aqueous extraction began at 5% MPB and held for 0.5 min, followed by 1.9 min linear gradient to 95% MPB, held for 2 min, and decreased to 5% MPB for column equilibration. The flow rate of the mobile phase was 0.5 mL/min and the column temperature was maintained at 50 °C. The G2-S QTOFMS system was operated in a resolution mode (for RSF, 8-OH-EFV, and 1-OH-MDZ) or sensitive mode (for 6-OH-CHZ) with electrospray ionization. The source and desolvation temperatures were...
set at 150 °C and 500 °C, respectively. Nitrogen was applied as the cone gas (50 L/h) and desolvation gas (800 L/h). The capillary and cone voltages were set at 0.8 kV and 40 V. The data were acquired in a positive (for 1-OH-MDZ) or negative (for RSF, 8-OH-EFV, and 6-OH-CHZ) ionization mode. QTOFMS was calibrated with sodium formate and monitored by the intermittent injection of lock mass leucine encephalin (m/z = 556.2771) in real time. QuanLynx software (Waters Corp.) was used for quantification of the concentrations of metabolites.

Enzyme reactions were validated to be linear over a 10-min incubation period. The linear ranges of detection for RSF, 8-OH-EFV, 6-OH-CHZ, and 1-OH-MDZ were 1–500, 1–1000, 1–1000, and 1–1000 ng/mL, respectively.

2.5. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS 25 (IBM, Armonk, NY, USA). A comparison between two groups was performed using an unpaired Student’s t-test. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Consequences of obese levels on adult mice

An experiment illustrated in Fig. 1A was performed to investigate the consequences of obese levels on adult mice. An HFD-induced obesity model was used in the current study as it is more closely imitated the status of human obesity than other genetically modified models.23,26 Both male and female C57BL/6 mice (4–5 weeks) were fed with a LFD (10 kcal% from fat) or HFD (60 kcal% from fat) for a series of durations (4, 8, or 18 weeks) to mimic different obese levels in humans. Body weight of the mice was monitored during the study. At the end of

Figure 1 Impacts of HFD-feeding durations on the body weight gain, liver weight, and liver appearance of adult mice. (A) A schematic study design. Male and female mice (4–5 weeks old) were fed with either a LFD or HFD for 4, 8, or 18 weeks ($n = 3–7$ per group). Body weight gain (B) and liver weight (C) of both male and female adult mice after different feeding durations under a LFD or HFD were measured. (D) Representative liver images from each group. Data are depicted as mean ± SD. Each dot represents a value from one mouse. Statistical significance between two groups was determined by an unpaired Student’s t-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, HFD versus the corresponding LFD groups.
each feeding duration, livers were weighted and collected to
determine the expression of NRs and DMEs.

In this study, adult mice fed with a LFD for 4, 8, or 18 weeks
were referred to as 4-LA, 8-LA, or 18-LA, respectively; adult
mice fed with an HFD for 4, 8, or 18 weeks were referred to as 4-
HA, 8-HA, or 18-HA, respectively.

3.1.1. Impacts of HFD-feeding duration on body weight and
liver of adult mice
As seen in Fig. 1B, consumption of an HFD led to a higher
 gained body weight in both genders compared to the corre-
sponding LFD groups. Moreover, along with extended feeding
duration, the gained body weight increased in both male and
female adult mice. Whereas, lower gained body weight was
observed in females compared to males in the same feeding
duration groups (Fig. 1B).

Consistent with the heavier gained body weight of mice with a
longer feeding duration, liver weight increased along with feeding
duration extended (Fig. 1C). However, significantly heavier liver
weight was only found in male 18-HA mice compared to the male
LFD groups (Fig. 1C). A slightly lighter liver weight was seen in
the male 4-HA group (Fig. 1C). No difference in liver weight was
observed between the HFD groups and the corresponding LFD
groups in females (Fig. 1C).

Representative liver photos from each group are shown in
Fig. 1D. Consistent with the trend of the body weight gain, more
severe fat accumulation was observed in the liver of 18-HA mice
than that of 4-HA mice. There was less fat accumulation in the
liver of females in comparison with the male groups fed with same
weeks of HFD.

Taken together, these results suggest that HFD-feeding dura-
tions affect the levels of obesity in both genders and males are
more susceptible to HFD than females in mice.
3.1.2. Effects of HFD-feeding duration on the hepatic expression of NRs and DMEs in adult mice

To explore whether obese levels affect the expression of NRs and DMEs in mouse liver, the hepatic mRNA expression of 5 NRs (Car, Pxr, Hnf4α, Ppara, and Ahr) and 9 DMEs (Cyp1a1, 1a2, 2b10, 2c29, 2e1, 3a11, 3a16, Ugt1a1, and Sult1a1), as well as the enzyme activities of CYP1A, 2B, 2E1, and 3A, were measured in this study (Fig. 2).

Compared to the corresponding LFD groups, HFD-fed male mice were found to have a comparatively higher mRNA expression of Car (~2.0-fold) and Ppara (~1.6-fold) in all three HFD groups (Fig. 2A and D). The mRNA expression of Pxr was only increased in the 4-HA group in comparison with the related LFD group in males (Fig. 2B). For the mRNA expression of Hnf4α, consumption of an HFD for 4 weeks resulted in a 2.0-fold induced expression in males compared to the male 4-LA group (Fig. 2C). Interestingly, the upregulated effects of HFD on Hnf4α were attenuated in longer feeding duration groups as no difference was seen between 8-LA and 8-HA groups, while a 40% reduced expression in the 18-HA group compared to the related LFD group (Fig. 2C). For the mRNA expression of Ppara, consumption of an HFD for 4 weeks resulted in a 2.0-fold induced expression in females compared to the related LFD group (Fig. 2C). In females, increased mRNA expression of Hnf4α and Ppara was observed in the mice fed with an HFD for 8 weeks but not 4 or 18 weeks versus the corresponding LFD groups (Fig. 2C and D). For the mRNA level of Ahr, only an induced expression was observed in female mice with an HFD, but not in males (Fig. 2E). However, there was no difference in the mRNA expression of Car and Pxr between the LFD and HFD groups for all three feeding durations in the liver of female mice (Fig. 2A and B).

Gene-specific and gender-different effects of HFD on the mRNA expression of DMEs were also found (Fig. 2F–K). For Cyp1a1, only the longest (18 weeks) feeding duration of HFD led to a slightly induced expression in males, whereas the shorter (4 and 8 weeks) but not the longest feeding durations of HFD increased its expression in females (Fig. 2F). Compared to the related LFD groups, elevated mRNA expression of Cyp1a2 and 2e1 was only observed in the male 4-HA group (Fig. 2G and I). However, the mRNA expression of Ugt1a1 was higher in the 8-HA and the 18-HA groups but not the 4-HA group in males (Fig. 2J). Notably, consumption of an HFD for all three durations resulted in significantly higher mRNA expression of Cyp2b10 in males not in females (Fig. 2H). Moreover, the induction extent of Cyp2b10 was bigger in the groups with 4 weeks HFD (46-fold) than that of 8 and 18 weeks. In females, consistent with the increased mRNA expression of Hnf4α and Ppara, the mRNA expression of Cyp1a2, 2e1, Ugt1a1, and Sult1a1 was only higher in the 8-HA group not another two groups compared to the corresponding LFD groups. No difference in the mRNA expression of Cyp2c29, 3a11, and 3a16 was seen between the LFD and the HFD groups for all three feeding durations (data not shown).

The enzyme activities of CYP1A, 2B, 2E1, and 3A were tested with four random selected male livers from the mice fed with a LFD or HFD for 8 weeks. As seen in Fig. 2L, the enzyme activity of CYP2B (8-OH-EFV) was 1.7-fold higher in the liver of 8-HA mice, while no difference in the enzyme activities of CYP1A (RSF), 2E (6-OH-CHZ), and 3A (1-OH-MDZ) compared to the LFD group. These results were consistent with the mRNA expression of corresponding DMEs.

Taken together, these findings indicate that the obese levels of the DIO model, which were built by maintained for different durations of an HFD, affect the expression of NRs and DMEs in mice liver and these effects are gene and gender specific.

3.2. Consequences of consumption of an HFD on offspring mice

Given that parental factors, e.g., dietary style and genetic background, are highly associated with childhood obesity, the consequences of family-based consumption of an HFD were next investigated on offspring. As depicted in Fig. 3A, parental mice were mated after fed with a LFD or HFD for 8 weeks. Offspring mice were sacrificed at different ages (postnatal days 5, 10, 15, 20, 30, or 60) and body weight and liver weight were measured. Liver tissues were collected to determine the expression of NRs and DMEs.

In this study, offspring from parental mice fed with a LFD or HFD for 8 weeks were referred as O-8-LA or O-8-HA, respectively.
3.2.1. Impacts of consumption of an HFD on the body weight and liver weight of offspring mice

As seen in Fig. 3B, consumption of an HFD resulted in increased body weight of offspring mice compared to the related LFD group at postnatal day 5. Moreover, the body weight of 60-day male offspring and 20-day female offspring in the HFD groups was higher than that of age-matched LFD groups (Fig. 3C). However, decreased liver weight was observed in the O-8-HA mice at 30-day age in both male and female mice (Fig. 3C).

3.2.2. Effects of consumption of an HFD on the hepatic expression of NRs in offspring mice

Generally, consumption of an HFD led to increased mRNA expression of NRs in offspring older than 20 days compared to the age-matched LFD groups (Fig. 4). The mRNA expression of Car was higher in both days 30 and 60 offspring from HFD-fed mice than that of O-8-LA mice (Fig. 4A). The mRNA expression of Pxr and Hnf4a in the liver of 30-day offspring was also induced by HFD (Fig. 4B and C). For Ppara, elevated expression was only observed in O-8-HA mice at day 60 compared to the related LFD groups (Fig. 4D). Higher expression of Ahr was seen in the O-8-HA mice at day 60 for males and day 30 for females compared to the LFD groups (Fig. 4E).

3.2.3. Effects of consumption of an HFD on the ontogenic expression of DMEs in offspring livers

It is well-established that liver is not fully developed in newborns and children and undergoes a period of maturation, during which many DMEs display distinct ontogenic expression patterns and the metabolic function is not comparable to that of adult. Fig. 5 shows that altered ontogenetic expression of DMEs in the offspring livers by consumption of an HFD.

Compared to the age-matched LFD groups, the relative mRNA expression of Cyp1a1 in the O-8-HA mice was decreased at day 5 in both genders, whereas increased at days 30 and 60 in females (Fig. 5A, upper panel); however, the alteration may contribute little to drug metabolism due to the low basal expression level of Cyp1a1 in mouse liver (Fig. 5A, lower panel). The fold change of Cyp1a2 mRNA expression was significantly higher in the O-8-HA groups at days 30 and 60 for both males and females compared to age-related O-8-LA groups (Fig. 5B, upper panel). Moreover, consumption of an HFD altered the ontogenic pattern of Cyp1a2 expression (Fig. 5B, lower panel). In LFD groups, the ontogenic pattern of Cyp1a2 was adolescent-enriched, which had the highest expression level at days 15 and 20 after birth; however, in the HFD groups, it was an adult-enriched pattern, which had a trend to keep growing with age (Fig. 5B, lower panel). Similarly, the relative expression of Cyp2el was increased in the days 30 and 60 male offspring from the HFD-fed mice and its ontogenic expression pattern changed from an adolescent-enriched pattern to an adult-enriched pattern in both males and females; whereas, a decreased expression of Cyp2el was seen in the female offspring at day 20 by consumption of an HFD (Fig. 5C). The relative expression of Cyp3a16 (a neonatal-enriched pattern) was decreased in 15-day male and 60-day female offspring, as well as increased at 20-day male offspring, by consumption of an HFD compared to the age-related LFD groups (Fig. 5D). Higher relative mRNA expression of Ugt1a1 at day 30 and lower relative mRNA expression of Sult1a1 at days 20 and 30 were seen in the O-8-HA groups compared to the corresponding LFD groups (Fig. 5E and F, upper panel). For Ugt1a1 and Sult1a1, no dramatically altered mRNA expression levels were observed after birth as other DMEs (Fig. 5E and F; lower panel). Both the relative expression levels and ontogenic patterns of Cyp2b10, 2c29,
and 3a11 showed no difference between the HFD groups and the related LFD groups (data not shown).

The enzyme activities of CYP1A, 2B, 2E, and 3A in the liver of four random selected 30-day male O-8-HA or O-8-LA mice are shown in Fig. 5G. Comparable levels of enzyme activities of CYPs were found between the HFD groups and the corresponding LFD groups.

Overall, these results suggest that family-based consumption of an HFD alters the hepatic expression of DMEs in offspring at day 5 or older than 15 days and changes the ontogenic patterns of DMEs in mouse liver.

### 3.3. Consequences of obese levels of parental mice on offspring mice

Then, whether obese levels of parental mice were associated with the impacts on offspring mice was assessed. As depicted in Fig. 6A, parental mice were mated after fed with a LFD or HFD for different durations (4 or 18 weeks) to mimic different obese levels. Based on the result in the offspring from mice fed with an HFD for 8 weeks, the livers of offspring mice older than postnatal day 15 were collected in this study.
Obese levels affect drug-metabolizing enzymes in mouse liver

In this study, offspring from parental mice fed with a LFD for 4 or 18 weeks were referred to as O-4-LA or O-18-LA, respectively; offspring from parental mice fed with an HFD for 4 or 18 weeks were referred to as O-4-HA or O-18-HA, respectively.

3.3.1. Impacts of HFD-feeding durations of parental mice on the body weight and liver weight of offspring

For the offspring from mice fed with a LFD or HFD for 4 weeks, the body weight of O-4-HA mice was higher than that of the O-4-LA mice at postnatal days 20 and 60 (Fig. 6A). Consistent with the increased body weight at day 20, the liver weight of 20-day O-4-HA mice was heavier than that of 20-day O-4-LA mice (Fig. 6B).

Due to the impaired fertility on mice by consumption of an HFD, few offspring were obtained from the mice fed with an HFD for 18 weeks. Thus, only data of male O-18-LA and O-18-HA mice at day 60 after birth were analyzed here. As seen in Fig. 6C, the body weight of day 60 male offspring in the HFD group is higher than that of the LFD group. However, there is no difference in the body weight and liver weight of male offspring (postnatal day 60) derived from parental mice fed with a LFD or HFD for 18 weeks. Data are depicted as mean ± SD. Each dot represents a value from one mouse. Statistical significance between two groups was determined by an unpaired Student’s t-test. “*” P < 0.01, “***” P < 0.001, HFD versus the age-matched LFD groups.

3.3.2. Effects of HFD-feeding durations of parental mice on the hepatic expression of NRs in offspring mice

In the liver of 20-day O-4-HA mice, the mRNA expression of Ppara was significantly increased in comparison with the related LFD groups (Fig. 7F). There was no difference in the expression of Car, Pxr, and Ahr between the O-18-HA and O-18-LA mice at day 60.

3.3.3. Effects of HFD-feeding durations of parental mice on the hepatic expression of DMEs in offspring mice

In the liver of 15-day offspring from 4-HA mice, decreased mRNA expression of Cyp2c29 but not the other DMEs was observed compared to the related LFD groups (Fig. 8). Consistent with the reduced expression of Car and Ahr in the male O-4-HA group at day 20, the mRNA expression levels of Cyp1a1, 1a2, 2c29, 2e1, 3a11, Ugt1a1, and Sult1a1 were decreased as well in comparison with the O-4-LA group at day 20 (Fig. 8). Thirty-day old offspring in the O-4-HA group had a higher hepatic mRNA expression of Cyp1a1, 1a2, 2e1, and Ugt1a1 in males, as well as Cyp1a1 and 2b10 in females, than that of the corresponding O-4-LA groups. Strikingly, reduced hepatic expression of Cyp2c29, 3a11, and Sult1a1 was found in the O-4-HA mice at day 60 compared to the age-related O-4-LA mice (Fig. 8).

There was no difference in the hepatic mRNA expression of DMEs between the O-18-LA group and the O-18-HA group (data not shown).

In summary, the data indicate that obese levels of parental mice are associated with the consequences of consumption of an HFD on offspring mice.

4. Discussion

The present study demonstrated that obese levels were associated with the consequences of consumption of an HFD on the hepatic expression of NRs in a mouse model. Maintenance on an HFD (family-based dietary style) altered the ontogenetic expression of NRs and DMEs in mouse liver. Furthermore, obese levels of parental mice contributed to the differential effects of HFD were observed on the hepatic expression of NRs and DMEs in offspring. To the best of our knowledge, this is the first study systematically demonstrating...
the impacts of obese levels on hepatic expression of NRs and DMEs in both adults and offspring in mice.

Animal models are extremely helpful in understanding obesity. However, a standard threshold for obesity in animals like BMI in humans has not been developed\textsuperscript{45}. In this study, the feeding durations of 4, 8, and 18 weeks were selected to mimic different levels of obesity in male mice based on previous researches. In most studies, male mice maintained on an HFD for 4 weeks were considered as a short-term HFD, which featured with obviously higher bodyweight than that of LFD groups in males\textsuperscript{46}; HFD feeding for 8 weeks was considered to sufficiently build a DIO model\textsuperscript{47}; mice fed with an HFD for over 12 weeks were commonly used as obesity-induced disease models, which could be referred to as severe obesity\textsuperscript{50,51}. Some researchers have attempted to evaluate the degree of obesity by comparing body weight of the experimental groups to the control groups\textsuperscript{52}\textsuperscript{-54}. The values that are 10\%–25\% (mostly 20\%) greater body weight than the control groups as overweight or moderate obesity and greater than 40\% as severe obesity\textsuperscript{52,53,55,56}. In the present study, the body weight of male mice after 8 weeks of HFD was 19\% higher than the related LFD groups, which were considered as moderate obesity (Supporting information Table S1). After consumption of an HFD for 18 weeks, the body weight of males was 54\% higher and that of females was 19\% heavier than the gender-matched LFD groups, which were considered as severe obesity and moderate obesity, respectively (Table S1). Though this classification is rough, it is reasonable to mimic different stages of obesity and/or overweight as greater differences in body weight gained between LFD and HFD groups were observed in the longer duration groups, indicating that obese levels became more severe (Fig. 1B). In the further study, a more precise method, such as dual-energy X-ray absorptiometry, may be used to evaluate the specific status of individual animals and standard assessment of obese levels in animal models is desiderated.

A number of researches have reported that obesity could alter the expression of DMEs and may subsequently change the metabolism of drugs\textsuperscript{19,29}. However, it is difficult to draw conclusions out of these studies because the differential experimental designs. It is also unclear whether obese levels (overweight, obesity, and severe obesity) are factors that contribute to the controversial results in the previous studies. Here, feeding duration, which referred to different obese levels, affected the impacts of an HFD on the hepatic expression of DMEs in an adult mouse model was demonstrated (Fig. 2). In general, consumption of an HFD led to an increased expression of \textit{Cyp1a1}, \textit{1a2}, \textit{2b10}, and \textit{2e1} as well as an elevated expression of \textit{Ugt1a1} and \textit{Sult1a1}, whereas no effects on the expression of \textit{Cyp2c29}, \textit{3a11}, and \textit{3a16} in adult mice (Fig. 2). Similar to our findings, clinical studies also suggested trends of higher clearance values mediated by most DMEs (except for \textit{CYP3A4}) in obese as compared with non-obese individuals, indicating an increase in the enzyme activities of DMEs\textsuperscript{19}. Furthermore, when corrected for body weight, for example, \textit{CYP1A2}-mediated clearance in obese \textit{versus} non-obese subject showed both higher and lower values in different studies\textsuperscript{19}. This phenomenon can be explained by our data. As seen in Fig. 2G, consumption of an HFD for 4 weeks resulted in a significant increased expression of \textit{Cyp1a2}, while mice fed with an HFD for 18 weeks showed a heavier body weight but a trend of decreased

Figure 7  Effects of HFD-feeding durations of parental mice on the hepatic expression of NRs in offspring. After being fed with either a LFD or HFD for 4 or 18 weeks, mice were mated. The offspring were sacrificed at different ages after birth (n = 3–6 per group). The liver tissues were collected to determine the mRNA expression levels of NRs. (A)–(E) The mRNA expression of NRs in the liver of offspring derived from LFD- or HFD-fed parental mice for 4 weeks. (F) The mRNA expression levels of NRs in the liver of offspring derived from LFD- or HFD-fed parental mice for 18 weeks. The mRNA expression levels were measured using the RT-qPCR method and calculated using the 2^{-\Delta \Delta Ct} method with normalizations to \textit{Gapdh}. Data are depicted as mean ± SD. Each dot represents a fold change in gene expression of one mouse. Statistical significance between two groups was determined by an unpaired Student’s t-test. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \), HFD versus the age-matched LFD groups.
Figure 8  Effects of HFD-feeding durations of parental mice on the hepatic expression levels of DMEs in offspring. (A)–(H) The mRNA expression levels of DMEs in the liver of offspring derived from LFD- or HFD-fed parental mice for 4 weeks (n = 5–6 per group). The mRNA expression levels were determined using the RT-qPCR method and calculated using the 2^{−ΔΔCt} method with normalizations to Gapdh. Data are depicted as mean ± SD. Each dot represents a fold change in gene expression of one mouse. Statistical significance between two groups was determined by an unpaired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001, HFD versus the age-matched LFD groups.
expression of Cyp1a2. Thus, varied results may be obtained due to individuals at different obese levels were recruited in different studies. Consistent with the trend of an induced expression of Cyp2e1 in the HFD mice versus the LFD counterparts in this study, Tomankova et al. reported an increased activity of CYP2E1 in monosodium glutamate-induced obese mice. However, studies, which conducted in ob/ob mice, observed a decreased activity of CYP2E1. For CYP3A11, studies using male ICR mice or CD1 mice fed with an HFD demonstrated a significantly decrease of Cyp3a11 expression, whereas no difference was observed in the current study. As species, strain, and gene background of animal models are well-documented factors affecting the consequences of obesity, data may be not comparable between studies conducted by different study groups. Though our data provide a new insight into the role of obese levels in the drug metabolism, these impacts should be confirmed in different animal models even in humans in the future studies. Experiments related to in vivo enzyme activities of DMEs will also be performed in future.

Different effects by genders on the expression of NRs and DMEs by HFD consumption were observed. Consistent with previous studies, male mice were more susceptible to an HFD than female mice in the current study as heavier body weight gain and more severe lipid accumulation in the liver were seen in males. It’s also well documented that the basal expression of DMEs has sexual dimorphism. The phenomenon that differential effects of an HFD consumption for same durations on males and females may be due to the sexual dimorphism and lipid accumulation. However, how these two factors work and the underlying molecular mechanisms have not been investigated in this study.

In the current study, the enzyme activities of DMEs in one group from adult or offspring mice were measured in vitro (Figs. 2L and 5G). The enzyme activities of DMEs in male mice were investigated as male mice appeared to have more significantly phenotypic alterations (including body weight and liver appearance) than female mice (Fig. 1). For offspring, day-30 offspring mice were selected as representative over others for two reasons. One reason is that mice at day-30 could provide sufficient liver samples to conduct the experiments of enzyme activities. The other reason is that the alteration of most drug-metabolizing enzymes and nuclear receptors occurred at day 30 in this study. A discrepancy in the expression at levels of mRNAs and enzyme activities was observed (Figs. 2 and 5). Generally, compared to the related mRNAs expression, relatively decreased enzyme activities were found in the current study. For instance, consumption of an HFD for 8 weeks led to a 13-fold increase of Cyp2b10 expression at mRNA level, whereas only a 1.7-fold reduction at the level of enzyme activity in comparison with the related LFD groups (Fig. 2H and L). Compared to the 30-day O-8-LA group, the expression of Cyp1a2 was 2.7-fold higher at mRNA level in the 30-day O-8-HA group, but no statistical difference at the level of enzyme activity (Fig. 5B and G). Actually, inconsistence effects at the levels of mRNA and enzyme activities/proteins occurred in many cases. Factors involving in multiple levels can influence to the correlation of mRNA-protein/enzyme activities, including post-transcriptional regulation (e.g., microRNAs and long non-coding RNAs), post-translational modification (e.g., protein phosphorylation and sulfation), and RNA modifications (e.g., m6A and m5C). In the current study, it is unclear what factors contribute to the discrepancy in the expression of DMEs at mRNA and protein levels. Further studies will be conducted to address this question.

It’s well-established that nuclear receptor PPARα plays vital roles in the hepatic metabolism of fatty acids and obesity. Recently, increasing evidences show that NRs, including PXR, CAR, and HNF4α, are also associated with the development of obesity. Given that PXR, CAR, HNF4α, and PPARα are all key transcriptional regulators of DMEs, it is reasonable to assume that altered expression of these nuclear receptors is an underlying mechanism contributing to the changed expression of DMEs by an HFD. In adult male mice, significantly increased expression of Car and Pxr was observed in all HFD groups as well as elevated expression of Hnf4α and Ppara was found in the female mice fed with an HFD for 8 weeks, which was accompanied by increased expression of Cyp1a2, 2el, Ugt1a1, and Sult1a1 (Fig. 2). Similar phenomena were seen in offspring groups. For instance, higher expression levels of nuclear receptors (Car, Pxr, Hnf4α, and Ppara) were demonstrated in the HFD groups at days 30 and/or 60 compared to the age-related LFD groups, while the expressions of DMEs, including Cyp1a1, 2a2, 2e1, Ugt1a1, and Sult1a1, were also induced in these groups (Figs. 4 and 5). Consistent with our results, Yoshinari and colleagues reported increased Car levels as well as Cyp2b10 in db/db mice. Reduced Car and Pxr levels were accompanied by decreased expression of DMEs, e.g., Cyp3a11, 2b10, and Ugt1a1 in a study using male CD1 mice fed with an HFD. These findings indicate that altered expression of DMEs may due to the changed levels of nuclear receptors by consumption of an HFD. The deeper molecular mechanism on how HFD or obesity influences expression of nuclear receptors and subsequently alters the expression of DMEs needs to be investigated in the future.

The consequences of maternal consumption of an HFD on the body composition as well as the hepatic expression of DMEs in the offspring have been addressed in several previous studies. In previous studies, a temporary HFD-fed maternal mouse model was used. However, childhood obesity is highly associated with the family-based life style and obese parents. Thus, the present study also aims to combine these two factors together to assess the impacts of obese levels of parental mice on the expression of drug processing genes in the offspring mice. For the O-8-HA mice (offspring from parental mice fed with an HFD for 8 weeks), induced expression levels of Cyp1a1, 2a2, 2e1, Ugt1a1, and Sult1a1 were observed only at days 30 and/or 60 compared to the corresponding LFD groups (Fig. 5). However, in the livers of 20-day O-4-HA mice (offspring from parental mice fed with an HFD for 4 weeks), decreased expression of Cyp1a1, 2a2, 2e1, Ugt1a1, and Sult1a1 was also found (Fig. 8). Moreover, induced expression of Cyp2b10 at day 20, as well as decreased expression of Cyp2c29 and 3a11 at days 20 and 60, was seen in the livers of O-4-HA mice versus the O-4-LA groups, whereas no changes were found in the O-8-HA and O-18-HA groups compared to the corresponding LFD groups (Fig. 5). These results together suggest parental obese levels affect the expression of drug processing genes in the offspring liver. Due to the severely impaired fertility by an HFD consumption, limited offspring mice were obtained especially for the O-18-HA group. The sample size in the further study will be enlarged. Strikingly, an HFD consumption for 4 or 8 weeks was observed to increase the expression of most NRs and DMEs in the adult mice (Fig. 2). To explore how obese levels of parental mice and the family-based dietary style influence the expression of NRs and DMEs in the offspring, RNA-sequencing experiments are under conducting.
5. Conclusions

The current study demonstrates that an HFD tends to induce the expression of most DMEs and NRs and obese levels influence the impacts of an HFD on expression of DMEs and NRs in the mouse liver. Consumption of an HFD can alter the ontogenic expression in the liver of offspring. Furthermore, our results indicate that obese levels of parental mice affect the hepatic expression of DMEs and NRs in offspring. These findings provide a new insight into the drug metabolism in overweight and obese populations.

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Author contributions

Pei Wang, Lirong Zhang, and Xiao-bo Zhong designed the study. Pei Wang, Xueyan Shao, Yifan Bao, Junjie Zhu, and Liming Chen performed the experiments. Pei Wang and Xiao-bo Zhong analyzed the data and wrote the manuscript. Lirong Zhang and Xiaoqiao Ma revised the manuscript. Xiao-bo Zhong obtained the funding and supervised the whole study. All the authors approved the final version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.10.009.

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Obese levels affect drug-metabolizing enzymes in mouse liver 183
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