A high-fat high-energy diet influences hepatic CYP3A expression and activity in low-birth-weight developing female rats

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Background: **The objective of this study was to investigate the effects of a high-fat, high-energy (HFHE) diet on the hepatic expression of CYP3A in low-birth-weight developing female rats.**

Methods: **Pregnant rats were divided into nourished and undernourished groups. The offspring of the nourished rats were defined as the normal-birth-weight (NBW) group, and those of undernourished rats were defined as the low-birth-weight (LBW) group. According to their birth weights and diets, the rats were subdivided into the following four groups: NBW-normal diet (NN) group; NBW-HFHE (NH) group; LBW-normal diet (LN) group; and LBW-HFHE (LH) group. Liver samples were isolated on days 3, 7, 14, 21, 28, 56 and 84 after birth.**

Results: **The CYP3A1 mRNA levels in the LH group on days 3, 56 and 84 were significantly higher than those of the NN group ($P<0.05$). CYP3A1 expression was significantly higher in the LH group than that in the NH group on days 21, 28 and 84 ($P<0.05$). *CYP3A1* mRNA expression was higher in the LH group than that in the LN group on days 3 and 21 ($P<0.05$). No zonal CYP3A1 expression pattern was observed in the LH developmental group. The LH group had significantly higher mean activity than the LN group on days 7, 14, 28 and 56.**

Conclusion: **Our results indicated that an HFHE diet can result in alterations of CYP3A expression in a developmental LBW rat model.**

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Key words: **CYP3A;**
development;
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Introduction

Nutrition is a major intrauterine environmental factor that alters fetal genome expression and may have lifelong consequences.[1] Previous studies have indicated that maternal undernutrition lead to low birth weight (LBW) in the offspring;[1-3] LBW is a risk factor for overweight or obesity, which may be due to over-feeding LBW children "catch up" with their peers. Children who are born small but grow heavy (or tall) show the highest insulin resistance and the highest levels of cardiovascular risk factors.[2]

Researchers have also postulated that in LBW offspring, rather than a thrifty gene or genotype being responsible for increased risk in later life, the observed relationship may be due to rapid weight gain caused by neonatal over-feeding or over-nutrition of LBW babies. It has been suggested that over-nutrition during critical periods of life may lead to overweight and obesity later in life.[2-4]

Hepatic cytochrome P-450 3A (CYP3A) plays a critical role in drug metabolism and protects the body against potentially harmful environmental insults. The expression patterns of the human and rodent cytochrome P450 3A isoforms are subject to developmental influences.[5] It has been suggested that alterations in the expression or activity of CYP3A isoforms is a key predictor of drug responsiveness and toxicity.[6] CYP3A1 and CYP3A2 are the rat orthologues of the human enzyme CYP3A4,[7,8] and murine CYP3A has different expression levels

in female and male livers.[9] Our previous research indicated that the effect of LBW on CYP3A mRNA expression and enzyme activity in developing female rats was higher on day 3.[10] Furthermore, differences in the zonal expression and mean activity of CYP3A1 were observed between normal-birth-weight and normal diet (NN) and normal-birth-weight and high-fat, high-energy diet (NH) developing female rats.[11] However, the developmental changes of CYP3A expression and activity in LBW and high-fat, high-energy (HFHE) diet situations remain unclear. We proposed that CYP3A expression and activity may be different under conditions involving both a LBW and HFHE diet compared with conditions of either a low birth weight or a high-fat and high-energy diet.

In this study, we investigated the effects of a high-fat and high-energy (HFHE) diet on CYP3A (CYP3A1 and CYP3A2) expression and activity at different ages in LBW female rats for investigating developmental changes in CYP3A under different physiological and pathophysiological conditions.

Methods

Animals, feeding protocol and sample preparation

All of the procedures applied in this study were approved and performed in accordance with the guidelines established by the Animal Ethics Committee of the Zhejiang University School of Medicine. Twelve-week-old pregnant Sprague-Dawley rats (250-300 g) were obtained from the Zhejiang Medical Science Academy (Hangzhou, China). The experiments were performed when the rats were 2 days pregnant, and their pups were assessed in this study. The rats were housed in a 22±1ºC environment at a humidity of 60% and were maintained under a 12-h light:12-h dark cycle. The number of live pups produced by each dam varied from 6 to 15. When the number of live pups for a dam was greater than 8, only 8 pups were permitted to live with their dam after random deletion to ensure that each pup was adequately breast-fed.

The rats were housed individually in standard rat cages. The pregnant rats were randomly divided into two groups: nourished and under-nourished. The rats in the nourished group were fed *ad libitum*, whereas the rats in the undernourished group were fed with 50% of the intake of the nourished rats, as determined by the amount of food consumed by the nourished group from day 2 of pregnancy until day 23 (parturition). Both groups were fed the same standard commercial rat diet containing (per 100 g) protein (22.5 g), carbohydrates (57.0 g), fat (3.9 g), cellulose (8.0 g), minerals (1.0 g), vitamins (mixed, 5.0 g) and water (2.5 g), with a metabolizable energy content of 1.583 (KJ/100 g). Water was provided to both groups *ad libitum*. All of the rats were fed daily, in the morning, and their consumption was determined 24 h later.

Pup body weight was determined at birth. The offspring of nourished rats weighing between 5.2 and 7.2 grams were included into the NBW group. The offspring of undernourished rats weighing below 5.2 grams were included into the LBW group. The normal diet groups were fed with the same diet as the pregnant rats, while the HFHE diet contained (per 100 g) protein (21.3 g), carbohydrates (53.7 g), fat (16.4 g), cellulose (8.0 g), minerals (1.0 g), vitamins (mixed, 5.0 g) and water (2.5 g) and had a metabolizable energy content of 1.974 (KJ/100 g). HFHE diet was provided by the Zhejiang Medical Science Academy (Hangzhou, China). According to their birth weights and diets, the rats were randomly subdivided into the four following groups: NN group, NH group, LBW-normal diet group (LN group) and LBW-HFHE group (LH group). In the NH and LH groups, the dams and their offspring were fed with the HFHE diet after birth, while in the NN and LN groups, the dams and their offspring were fed with the same diet as during the pregnancy period.

All of the pups were housed with their dams in the same cage for 21 days (3 weeks) after birth, until weaning. Following weaning, all of the pups were housed individually in standard rat cages and fed with the same diet as their dams. The dams and their pups in each group were fed with the same diet throughout the experiment.

Offspring were sacrificed via exsanguination under ether anesthesia. Their livers were isolated on days 3, 7, 14, 21, 28, 56 and 84 after birth in each group (n=6-8 per group) and subsequently subjected to analyses. The body and liver weights of pups were recorded. A portion of the right hepatic lobe was fixed in 10% neutral-buffered formalin for immunohistochemical examination. The remainder of the liver tissue was frozen in liquid nitrogen and subsequently stored at -80ºC until RNA and microsome extraction. The samples of female and male offspring were collected the same way. In this study, we used only female samples.

RNA extraction and real-time PCR

RNA extraction, the design of gene-specific primers and real-time PCR were performed as previously reported.[10,12,13] Total RNA was extracted from liver tissue using the Total RNA Miniprep Kit (Axygen Biosciences, CA, USA) according to the manufacturer's protocol. Gene-specific primers were designed according to the sequences deposited in GenBank.[14]

PCR amplifications were performed twice in duplicate using a 7500 Applied Biosystems instrument. To analyze the results of the real-time PCR experiments, we quantified the target gene transcripts relative to those of the reference gene *GAPDH* using the ΔC_T method ($2^{C_T\text{-}C_T[target]}$), as described in the Bio-Rad real-time PCR protocol.

Immunohistochemistry (IHC)

Immunohistochemical analysis of the CYP3A1 and CYP3A2 proteins in paraffin-imbedded liver sections was performed as previously reported.[10,12,13] The CYP3A1 and CYP3A2 proteins were detected using a two-step immunohistochemical technique with the DAKO Envision system. The sections were stained with peroxidase-diaminobenzidine and then counterstained with hematoxylin. Images were captured with a digital camera mounted on an Olympus microscope.

Samples were analyzed by calculating the percentage of positive cells in five fields under an Olympus microscope. Protein expression was evaluated using a score corresponding to the sum of the scores for the 1) staining intensity (0, negative; 1, weak; 2, intermediate; 3, strong) and 2) percentage of positive hepatocytes (0, 0% positive; 1, <25% positive; 2, ≥25 and ≤50% positive; 3, >50% positive). Using the summed scores (maximum sum score was 6), the samples were classified as "-" (0), "+" (1-2), "++" (3-4) or "+++" (5-6). A score greater than 2 was regarded as positive.[15] Each sample was scored in duplicate by two blinded observers.

CYP3A activity assay using DFB

The CYP3A activity assay using DFB ([3-[(3,4-difluorobenzyl) oxy]-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl] furan-2(5H)-one]) was performed as previously described.[10,11] Glutathione reductase, oxidized glutathione and *β*-NADPH were obtained from Sigma Chemicals (St Louis, MO, USA). The benzyloxy-substituted lactone cyclooxygenase-2 inhibitor DFB and its fluorescent metabolite, DFH ([3-hydroxy-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl] furan-2(5H)-one]), were kindly provided by Merck Frosst Canada Ltd. (Pointe Claire, Que, Canada). All other chemicals were of ACS (American Chemical Society) grade or higher. Rat liver microsomes were prepared via calcium precipitation as previously described.[15] The microsomal protein concentration was determined using the BCA assay (provided by the Beyotime Institute of Biotechnology, Jiangsu, China). Microsome samples were added to each well of a flat-bottom 96-well polystyrene plate. The substrate DFB was then added to the wells. The plate was pre-incubated in an incubator. At this point, a background fluorescence reading of the plate was performed to eliminate any interference at the selected wavelengths on the cytofluorimeter. The reaction was then started by adding *β*-NADPH. The plate was incubated at 37°C. At the end of the incubation, oxidized glutathione and glutathione reductase were added and the plate was left in the dark at room temperature for 15 min. Finally, the reaction was quenched with acetonitrile in Tris buffer and the samples' fluorescences were read on the cytofluorimeter, as described above. The final production of DFH was quantified using a standard curve prepared from pure DFH. The production of DFH was employed as a measure of microsomal CYP3A activity.[16]

Statistical analysis

Statistical analyses were performed using R software, version 2.6.0. The differences in mRNA and protein expression levels between groups were determined using an independent samples *t* test or the Mann-Whitney *U* test, as appropriate. For multiple comparisons, the Dunnett test was applied to statistically examine the differences in mean values. Pearson or Spearman's rho (for data points with a skewed distribution) correlation coefficient was used to compare correlations between groups, as appropriate. A *P* value of less than 0.05 was considered statistically significant for all tests.[10]

Results

Body and hepatic weight and hepatic index

The average body weight in the LH group was significantly higher than that in the LN group after postnatal day 7. The average hepatic weight in the LH group was also higher than that in the LN group after day 7, with significant differences on days 7, 14, 28, 56, and 84. The hepatic index (liver weight per unit of body weight) varied with age during development in all four groups. The mean hepatic index in the LH group was higher than that in the other three groups (NN, NH, LN) after 28 days. The mean hepatic index on day 56 was significantly higher in the LH group than that in the LN, NH and NN groups.

CYP3A1 and CYP3A2 mRNA expression

CYP3A1 mRNA expression

CYP3A1 expression varied with age in every group (Fig. 1). The timing of the peak CYP3A1 expression level varied between groups, with the peak for the NN group being observed on day 28, the NH group on day 14, the LN group on day 21 and the LH group on day 56.

CYP3A1 mRNA expression was significantly higher

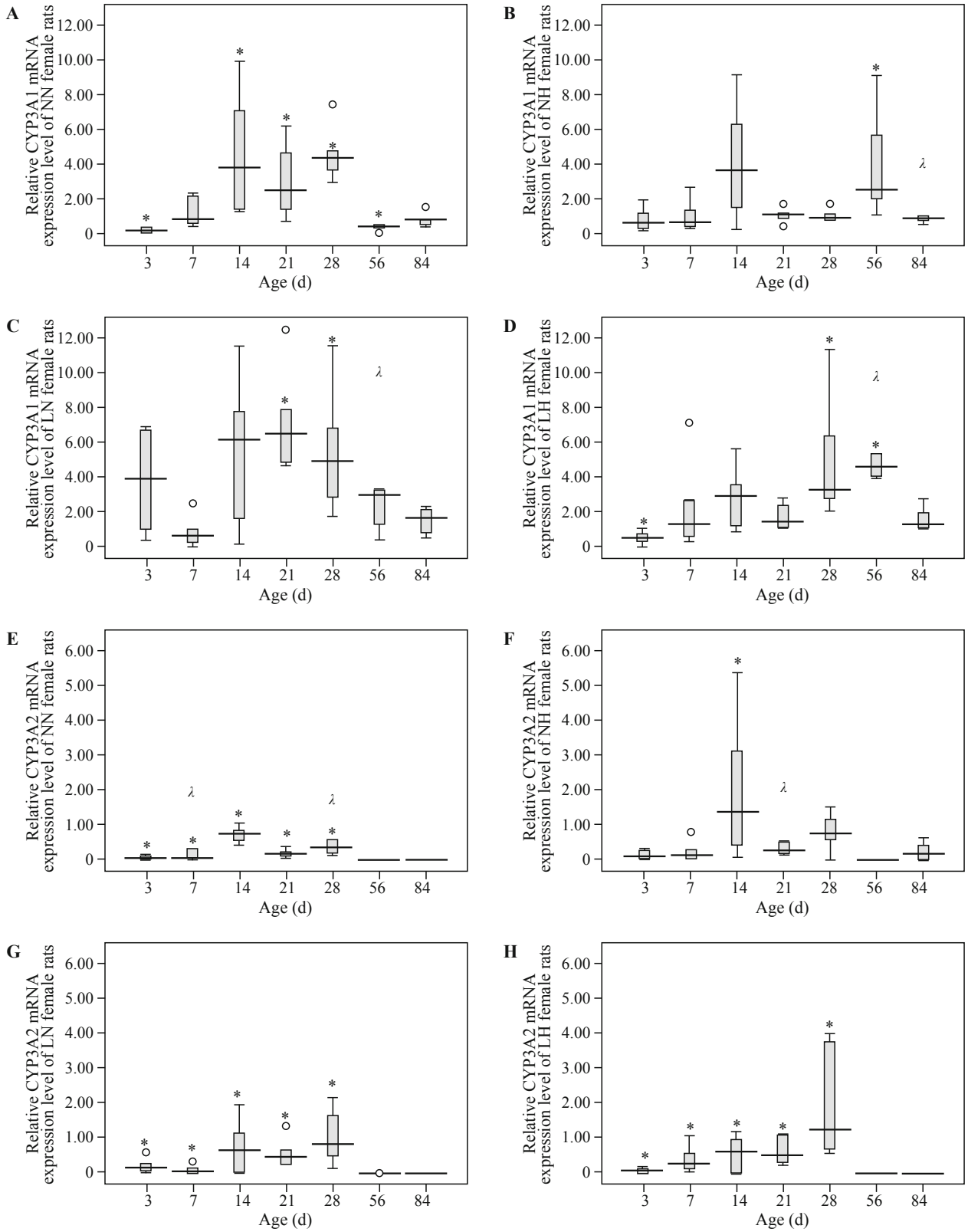

Fig. 1. Ontogeny of relative CYP3A1 (**A, B, C, D**) and CYP3A2 (**E, F, G, H**) mRNA expression levels in the NN (**A&E**), NH (**B&F**), LN (**C&G**) and LH (**D&H**) groups (n=6/group) measured using real-time PCR. The values were normalized against GAPDH. The data are presented as box-and-whisker plots: the bottom and top of boxes represent the 25th and 75th percentiles, respectively; and the solid horizontal line within each box represents the median value. The whiskers correspond to the 10th and 90th percentiles. ○ denotes each data point that is more than 1.5 times the interquartile range; λ denotes outliers that are more than 3 times the interquartile range; *: P<0.05, compared with the day 84 group. To make the scale in the same legend we did not include two outliners in the graph (NH 3 day 33.95, NH 21 day 13.07). NN: normal-birth-weight, normal diet group; NH: normal-birth-weight, high-lipid diet group; LN: low-birth-weight, normal diet group; LH: low-birth-weight, high-lipid diet group.

in the LH group on days 3, 56 and 84 than that in the NN group (P<0.05) (Supplementary Table 1). CYP3A1 expression was higher in the LH group than that in the NH group after day 21, with significant differences on days 21, 28 and 84 (P<0.05). CYP3A1 expression on days 3 and 21 was higher in the LH group than that in the LN group (P<0.05) (Supplementary Table 1).

CYP3A2 mRNA expression

CYP3A2 expression varied with age in all groups. However, the observed trend had a different pattern with that of CYP3A1 (Fig. 1). Almost all of the recorded CYP3A2 expression levels were extremely low on days 56 and 84. CYP3A2 expression was significantly higher in the LH group than that in the

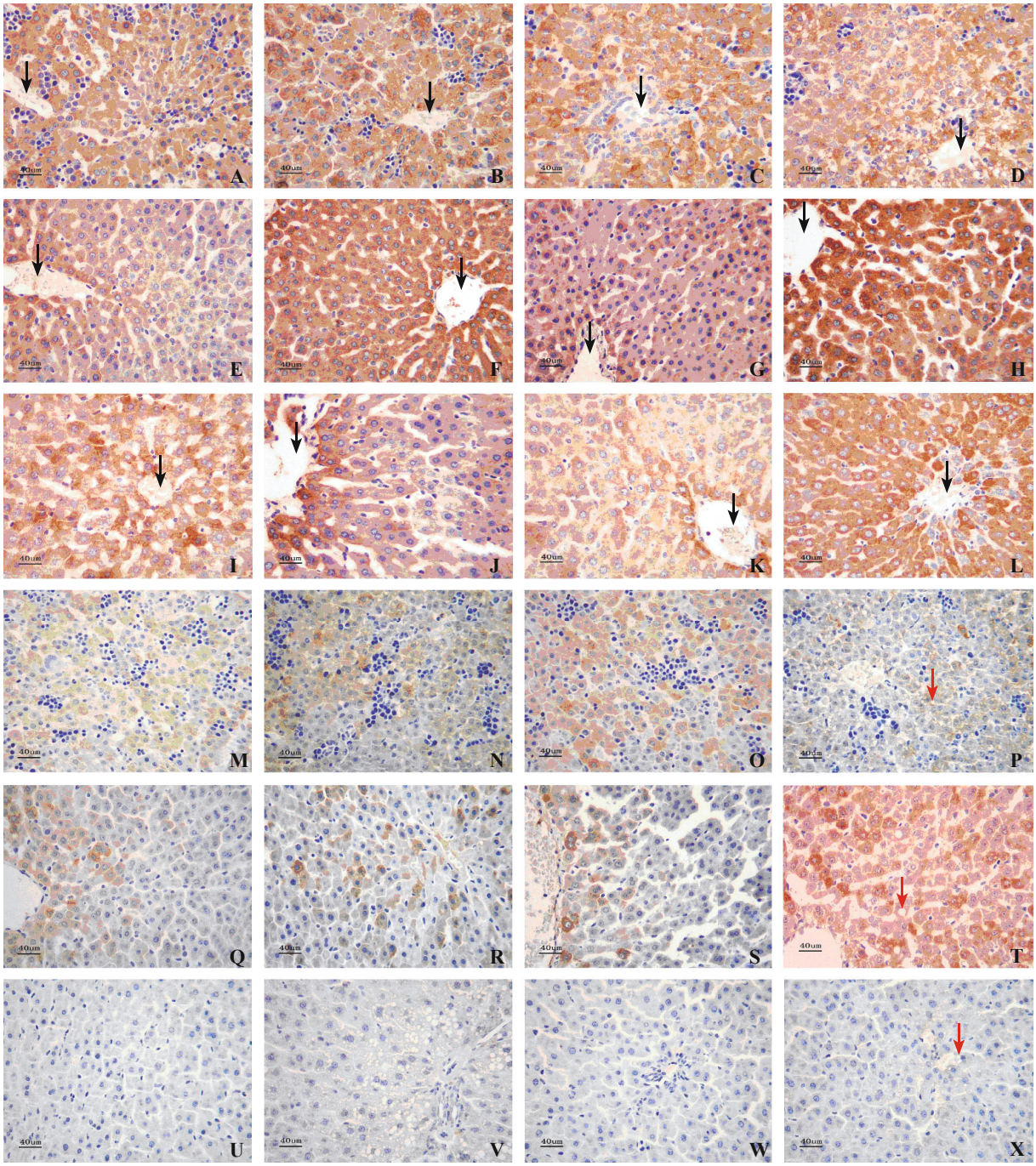

Fig. 2. Photomicrographs of hepatic sections from developing rats in the different birth weight and diet groups that were immunostained for CYP3A1 (**A-L**) and CYP3A2 (**M-X**) (n=6/group). Day 3 (**A-D** and **M-P**), day 28 (**E-H** and **Q-T**) and day 84 (**I-L** and **U-X**) in the NN group (the first column), NH group (the second column), LN group (the third column) and LH group (the fourth column). Protein staining is brown, and the hematoxylin counterstain is blue (original magnification × 200). Blue arrow: the central vein; red arrow: the typical fat vacuoles.

NN group at days 21 and 28 (P<0.05) (Supplementary Table 1). CYP3A2 mRNA expression in the LH group was similar with those of the NH and LN groups.

Immunohistology results

CYP3A1 localization

CYP3A1 immunolabeling was observed in the cytoplasm of hepatocytes (Fig. 2). There was no zonally restricted CYP3A1 expression pattern from days 3 to 21 in any of the groups, and expression was diffuse in the hepatocytes. More fat vacuoles were observed in the LH group than those in the NN, NH and LN groups (Fig. 2). Additionally, more than 30% of the hepatic cells contained fatty deposits in the LH rats. The staining intensity was higher in the hepatocytes with fat vacuoles around the central vein. After day 28 in the LN and NN groups, a higher staining intensity of hepatocytes was observed around the central vein, and the staining intensity was gradually reduced from the perivenous to periportal areas. However, on day 28, no obvious zonal expression was observed in the LH group, while there was a weak zonal expression pattern in the NH group. The zonal expression patterns in the NN and LN groups became increasingly apparent in an age-dependent manner. The NH group showed obvious zonal expression on day 84, whereas the CYP3A1-positive hepatocytes in the LH group remained diffusely distributed among the hepatocytes (Fig. 2).

Protein expression scores based on CYP3A1 immunohistology

The expression scores (Supplementary Table 2) of the LH group on days 7, 28, 56 and 84 were significantly higher than those of the LN group (Supplementary Table 3). Moreover, on day 84, the expression score of the LH group was significantly higher than those of all the other three groups (NN, NH, LN).

The localization pattern of CYP3A2 is similar to that of CYP3A1

Immunolabeling was evident in the cytoplasm of the hepatocytes. No zonally restricted expression of CYP3A2 was observed from days 3 to 21 in any group. CYP3A2 was diffusely expressed throughout the hepatocytes. In the NN and LN groups, an obvious zonally restricted expression pattern was detected after day 28. The staining intensity was stronger in the hepatocytes around the central vein, and the staining intensity became gradually weaker from the perivenous to periportal areas. However, no obvious zonal expression pattern was observed in the LH and NH groups, and CYP3A2 was expressed diffusely throughout the hepatocytes. The staining intensity was stronger in the hepatocytes with more fat vacuoles around the central vein. No CYP3A2-positive hepatocytes were observed at days 56 and 84 (Fig. 2).

Protein expression scores based on CYP3A2 immunohistology

CYP3A2 expression was significantly higher in the LH group than that in the NN group on day 28 (P=0.030). In contrast, on day 21, CYP3A2 expression was significantly lower in the LH group than that in the NN group (P=0.011) (Supplementary Table 3).

Cytochrome P450 isoform 3A activity in rat livers determined using DFB as a substrate

DFH production varied with age. The DFH production measured in the rat liver microsomes is shown in Fig. 3. The observed activity varied with age. The mean activity on day 21 was the highest among the groups. The LH group showed significant differences in the DFH production between days 3 and 84 as well as days 21 and 84.

Differences in CYP3A activity between the four groups

The mean CYP3A activity in the LH group was significantly higher than that in the NN group on days 3, 7, 14 and 28. Compared with the LN group, the LH group had significantly higher mean activity on days 7, 14, 28 and 56 (Fig. 3).

Discussion

This study suggests that an HFHE diet has a significant impact on CYP3A mRNA expression and activity. The developmental expression of P450 enzymes is

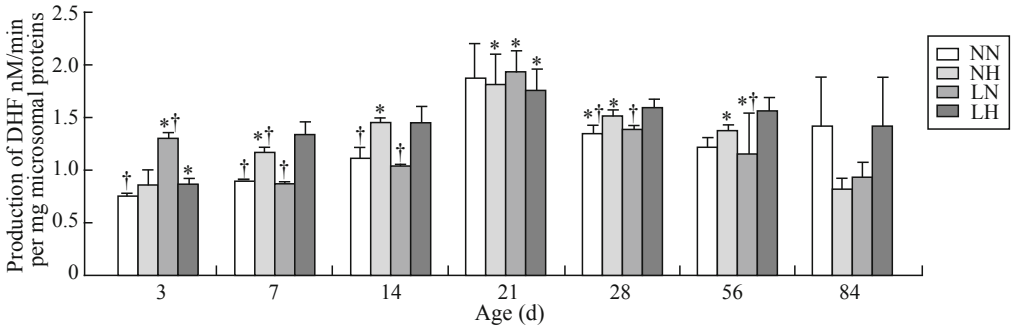

Fig. 3. DFH formation after DFB incubation in rat liver microsomes, which acts as a fluorescent CYP3A marker. NN: normal-birth-weight, normal diet group; NH: normal-birth-weight, highlipid diet group; LN: low-birth-weight, normal diet group; LH: low-birth-weight, high-lipid diet group. Error bars indicate the standard deviation (n=3-4). *: P<0.05, *vs.* the day 84 group with the same diet; †: P<0.05, *vs.* the LH group in the same age.

a key factor determining the pharmacokinetic status of developing individuals postnatally.[17] Detailed information concerning developmental changes in the major drug-metabolizing P450 enzymes is limited. The ontogeny of these drug-metabolizing enzymes under different conditions of maternal and postnatal nutrition is even less well characterized. In this study, we simulated two different maternal nutrition statuses and intervened with an HFHE diet, then observed the developmental changes in hepatic CYP3A1 and CYP3A2 expression and activity in female rats. To the best of our knowledge, this is the first report detailing the developmental changes in hepatic CYP3A1 and CYP3A2 under low-birth-weight and high-lipid, high-energy diet conditions.

The liver is the major drug metabolizing organ in the body. Therefore, any relative decrease or increase in its size may have functional implications for drug metabolism and elimination in children.[18] Our analyses revealed that the average body and hepatic weights were higher in the LH group than that in the LN group after day 7. Furthermore, the mean hepatic index (liver weight per unit of body weight) was higher in the LH group than the other three groups (NN, NH, LN) after day 28, whereas significant differences between the LH group and the NN and LN groups were only observed on day 56. Increased numbers of fat vacuoles were found in histological slices from the LH group compared with the NN, NH and LN groups. These results were similar with those of previous studies which suggested that growth-retarded neonatal rats accumulated more body fat and that the development of obesity in adulthood was favored when they were postnatally over-nourished.[3,4,19,20] A recent human study indicated that a high body fat percentage, which commonly precedes type 2 diabetes, could be achieved through rapid accumulation of fat in childhood following a low body weight in infancy.[21] Body composition affects the distribution of a number of drugs, and the fat mass of LBW children and juvenile may have to be adjusted for when determining appropriate doses of lipophilic compounds.[22]

Changes in fetal nutrition may result in developmental adaptations that permanently alter the structure, physiology, and metabolism of offspring, thereby predisposing individuals to metabolic, endocrine, and cardiovascular diseases in adult life. Obesity may change the pharmacokinetic and pharmacodynamic properties of several therapeutic drugs.[23] The present study demonstrated that a high-lipid, high-energy diet had a significant impact on *CYP3A* mRNA expression in LBW rats. *CYP3A1* mRNA expression was significantly higher in the LH group on days 56 and 84 than that in the NN group, which corresponded to the protein expression trend observed in hepatocytes. In general, metabolizing enzymes, including CYP3A enzymes, protect the body against potentially harmful environmental insults. When present in the body, many xenobiotics may induce signal transduction events, either specifically or non-specifically, leading to various cellular, physiological and pharmacological responses, including homeostasis, proliferation, and differentiation.[24] An HFHE diet may trigger a cellular stress response, leading to increased *CYP3A* gene expression, which would ultimately enhance the elimination and clearance of xenobiotics and/or "cellular stresses", including harmful reactive intermediates, such as reactive oxygen species (ROS), to expedite "stress" removal. We proposed that the higher CYP3A expression in the LH group represents a homeostatic response of the body to protect the organism against environmental insults.[24] Moreover, an HFHE diet may amplify the LBW phenotype observed under standard diet conditions.

Our analyses revealed that the timing of peak CYP3A1 expression varied between the different diet and birth weight groups. The expression level in the LH group reached a peak on day 56, which was later than in the other groups. A limited previous study suggested that obesity may influence the maturation process itself, and the starting point of weight gain may also influence the maturation process. Hence, these impacts represent additional factors determining variability in drug metabolism and elimination among obese children.[25] It has also been suggested that the effects of an HFHE diet in NBW and LBW offspring may be different.

Our experiments revealed that an HFHE diet had an obvious impact on the zonal expression patterns of CYP3A1 and CYP3A2. After day 28 in the NN and LN groups, CYP3A1 exhibited a zonal expression pattern, with increased expression being detected in the perivenous liver region. No obvious zonal CYP3A1 expression pattern was observed in the LH group, while a minor zonal expression pattern was observed in the NH group. Zonal expression of CYP3A1 became obvious in the NN and LN groups during development, and the NH group showed a clear zonal expression pattern on day 84. However, no zonal expression pattern was observed in the LH group until day 84. A previous study suggested that during maturation, hepatocytes acquired various phenotypes in the periportal and perivenous regions to respond differentially to endogenous and exogenous signals to control P450 expression.[26] Growth and thyroid hormones may regulate the expression of *CYP3A* genes in a zone-specific manner by suppressing their transcription in the periportal (upstream) region of the liver.[27] Results from previous studies suggest that early postnatal overfeeding in small litters may lead to developmental neuroendocrine malprogramming, resulting in

metabolic syndrome-like alterations throughout life. Early postnatally overfed rats from small litters display hyperinsulinemia as well as increased intrahypothalamic insulin concentrations during early postnatal life. The association between elevated insulin concentrations during early development and acquired alterations in hypothalamic regulatory areas may indicate disturbed hormone-dependent self-organization and programming of central circuits, regulating food intake, body weight and metabolism.[28,29] Elucidation of any other alterations related to zonal expression patterns in the developmental LH group will require further research.

Our findings indicated that exogenous signals from an HFHE diet may induce CYP3A expression in the periportal region of the liver. Our results further revealed that the staining intensity of CYP3A1 and CYP3A2 was stronger in hepatocytes with more fat vacuoles around the central vein. Previous research suggested that a prenatal low-protein intake and a postnatal high-fat intake resulted in adipose tissue catch-up growth through alterations in the expression of the Igf2 gene and DNA methylation within adipocytes.[30] Whether DNA methylation induced CYP3A expression in the LH group in the present work is unclear and should be the subject of future research.

The developmental changes in CYP3A activity measured via DFH production revealed that the mean activity on day 21 was the highest among the LH, LN, NH and NN groups. This result was similar to a previous study that measured testosterone hydroxylase activity by monitoring the 6β-hydroxylase activity of testosterone in non-treated Sprague-Dawley rats.[31] Our findings revealed that low birth weight had a minor impact on developmental CYP3A activity, with significant differences being observed compared with the NN group only on day 3. However, the HFHE diet had a great impact on LBW rats. The mean activity in the LH group was significantly higher than that in the NN group on days 3, 7, 14 and 28. Furthermore, significantly higher mean activity was observed in the LH group compared with the LN group on days 7, 14, 28 and 56. Previous studies have demonstrated that hepatic CYP3A activity is reduced in adult mice subjected to a high-fat diet intervention compared with mice on a low-fat diet. This reduction in drug-metabolizing enzymes can affect the concentration and efficacy of drugs that are substrates of the enzymes. The sleep time induced by midazolam (a Cyp3a substrate) was shown to be prolonged in mice on a high-fat diet, while zoxazolamine (a Cyp1a2 and Cyp2e1 substrate)-induced sleep time was unaltered.[32] A significantly lower clearance of CYP3A substrates was previously demonstrated in obese patients in 7 of 13 studies; however, 4 studies did not detect significantly lower absolute clearance values.[25] Combined with variables associated with development and low birth weight, the effects of an HFHE diet or obesity on CYP3A may be more complicated. The pharmacokinetics of CYP3A-metabolized drugs have not been studied in obese children or adolescents. As CYP3A plays a critical role in lipid homeostasis (cholesterol, vitamin D, oxysterol and bile acid metabolism) and in the detoxification of endogenous compounds (e.g., bile acids) and xenobiotic (drug) metabolism,[33] there is a rationale for monitoring the effectiveness of drug therapy in low-birth-weight, obese individuals.

In conclusion, the present study indicated that an HFHE diet could result in alterations of CYP3A expression in a developmental LBW female rat model. We did not include the quantification of fatty depots of hepatic cells. This is the limitation of this study. Within the limits of extrapolation from our rat models, our findings suggest that the respond to drugs of LBW, obese patients may need further research.

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Ethical approval: This protocol of study was approved by Ethics Committee of Children's Hospital Zhejiang University School of Medicine.

Competing interest: There is no conflict of interest associated with this work.

Contributors: Ni SQ is the first author of this article who was responsible for research design, experiment conduction, data analysis, manuscript writing and revision. Lou Y, Wang XM, Shen Z assisted with the research design and experiment conduction. All authors contributed to the intellectual content and approved the final version. Zeng S is the guarantor.

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