Maternal high-fat diet during pregnancy and lactation affects hepatic lipid metabolism in early life of offspring rat

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We investigated whether maternal over-nutrition during pregnancy and lactation affects the offspring's lipid metabolism at weaning by assessing liver lipid metabolic gene expressions and analysing its mechanisms on the development of metabolic abnormalities. Female Sprague–Dawley rats were fed with standard chow diet (CON) or high-fat diet (HFD) for 8 weeks, and then continued feeding during gestation and lactation. The offspring whose dams were fed with HFD had a lower birth weight but an increased body weight with impaired glucose tolerance, higher serum cholesterol, and hepatic steatosis at weaning. Microarray analyses showed that there were 120 genes differently expressed between the two groups. We further verified the results by qRT-PCR. Significant increase of the lipogenesis (*Me1*, *Scd1*) gene expression was found in HFD ($P<0.05$), and up-regulated expression of genes (*PPAR-α*, *Cpt1α*, *Ehhadh*) involved in β-oxidation was also observed ($P<0.05$), but the *Acsl3* gene was down-regulated ($P<0.05$). Maternal over-nutrition could not only primarily induce lipogenesis, but also promote lipolysis through an oxidation pathway as compensation, eventually leading to an increased body weight, impaired glucose tolerance, elevated serum cholesterol and hepatic steatosis at weaning. This finding may provide some evidence for a healthy maternal diet in order to reduce the risk of metabolic diseases in the early life of the offspring.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases that involves simple fat infiltration into hepatocytes (steatosis), fatty infiltration plus inflammation, non-alcoholic steatohepatitis (NASH), fibrosis and eventually cirrhosis. With increase in the prevalence of obesity and type 2 diabetes, NAFLD has become a common metabolic disease worldwide (Chitturi *et al*. 2011; Cao and Fan 2011). High-fat intake and overeating are thought to be the main causes of NAFLD.

The theory for Developmental Origins of Health and Disease (DOHaD) proposed that exposure to detrimental conditions *in utero* was critical in the risk of developing metabolic diseases in the early life of the offspring (Barker 1990; Uauy *et al*. 2011). Recent epidemiological and experimental studies have substantially demonstrated that an alteration of maternal nutrition could significantly

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impact the predisposition to developing metabolic abnormalities in adulthood (Beck *et al*. 2012; Stergiou *et al*. 2012). It was largely accepted that under-nutrition during gestation induced intrauterine growth restriction (IUGR) and increased the risk for metabolic disease in the offspring (Sohi *et al*. 2011; Plagemann *et al*. 2012). Is maternal over-nutrition related to the metabolic diseases in their offspring? With the recent progressive change of lifestyle and diet, and the increase of metabolic abnormalities with obesity during pregnancy, several studies suggest that maternal obesity might lead to insulin resistance, dyslipidemia and NAFLD in the offspring (Zhang *et al*. 2009; Dudley *et al*. 2011; Strakovsky *et al*. 2011; Takasaki *et al*. 2012). A previous study found that diet-induced obesity in dams during premating, gestation and lactation produced offspring with noticeable body mass gains, higher serum and hepatic triglycerides, higher large adipocytes, hepatic steatosis, and higher lipogenic genes expressions at week 15, suggesting that maternal over-nutrition diet might play an important role in developing NAFLD in the young offspring (Magliano *et al*. 2013).

High-fat food consumption by maternal dams during restricted periods, such as gestation and lactation, might be unfavorable to the development of their offspring. The dams and offspring were fed with the same diets in most of previous studies utilizing animal models. Results from those studies might be affected by the diet of offspring at post-weaning (Magliano *et al*. 2013; Pruis *et al*. 2014). The role of maternal HFD during pregnancy and lactation on the development of NAFLD in immediate life of the offspring is clearly limited. In this study, we aimed to assess lipid metabolism and lipid metabolism-related genes expressions on rat offsprings whose dams were fed with HFD during pregnancy and lactation, and to explore the mechanism on metabolic abnormalities in the offspring.

2. Materials and methods

2.1 *Animals and diets*

Female Sprague–Dawley rats (age 3 weeks) were housed under controlled conditions of light (0600–1800 h) and temperature (22±2°C). Rats were given ad libitum access to the standard chow diet (CON, 12.9% fat and 3.5 kcal/g) or a high-fat diet (HFD, 45.0% fat and 3.12 kcal/g) for 8 weeks. Thus, the dams were allocated to two groups: F0-CON (n=6) and F0-HFD (n=6).

After 8 weeks of feeding, females were mated with similar-age male SD rats. Female rats were continued on their respective diets during the entire period of pregnancy and lactation. The offspring were all weaned at postnatal day (PND) 21 and allocated into F1-CON group (n=10) and F1-HFD group (n=10).

2.2 *Glucose tolerance test (GTT)*

Intraperitoneal glucose tolerance tests (IPGTTs) were performed after an overnight fasting in dams at 11 weeks premate and offspring at weaning (Dunn and Bale 2011). Glucose measurements were analysed using One Touch Ultra (Johnson & Johnson, New Brunswick, NJ) system from tail blood before injection (G_0) and at 30 (G_{30}), 60 (G_{60}), and 120 (G_{120}) min after injection. The area under the curve (AUC) of IPGTTs was calculated by the trapezoid formula:

$$\mathrm{AUC} = (G_0 + G_{120})\Big/2 + G_{30} + G_{60}.$$

2.3 *Lipid metabolism*

Serum was obtained by centrifugation of blood samples at 3000 rpm/min. Liver tissues were resected and snap frozen in liquid nitrogen and stored at −80°C for further analysis. Serum triglyceride (TG) and total cholesterol (TC) levels are determined enzymatically using triglyceride quantification kit (Applygen, Beijing) and cholesterol quantification kit (Applygen, Beijing), respectively. Liver lipids were extracted from ~50 mg liver samples for triglyceride assay by kits (Applygen, Beijing) according to manufacturer's instructions.

2.4 *Liver histology*

The liver tissues were fixed with 10% paraformaldehyde solution. The paraffin-embedded tissue sections (3 μm) were stained with hematoxylin and eosin. The samples embedded in optimal cutting temperature compound were stained with freshly prepared Oil-Red-O and the nuclei of liver cells were counterstained in Mayer's hematoxylin in order to verify lipid content. Those stained liver sections were reviewed by the microscope (Leica DMI3000B, Germany).

2.5 *Gene array analysis*

Total RNA from liver tissues was extracted using TRIzol reagent (Life technologies, US) according to the manufacturer's instructions. Each group contained three biological replicates, and a total of six gene arrays were analysed. The samples were processed following Affymetrix recommendations, and the cDNA was hybridized using a GeneChip® Hybridization, Wash and Stain Kit (Affymetrix, Santa Clara, USA). Slides were scanned by GeneChip® Scanner 3000 (Affymetrix, Santa Clara, USA) and the data was read by Command Console Software 3.1 with default settings. Qualified data were normalized by MAS 5.0 algorithm, Gene Spring Software 11.0 (Agilent Technologies, Santa Clara, USA). Differentially expressed genes were hierarchically clustered using Pearson correlation analysis. The genes

were selected as significant using a combined criterion for true positive expression differences of a fold change >2.0 and $P<0.05$.

2.6 *Quantitative RT-PCR assay*

To validate the array results, nine genes from the gene list were selected for quantitative real time-PCR (qRT-PCR) analysis. Prior to PCR, the RNA was reverse-transcribed by 1 μg of total RNA from each sample using the Power cDNA Synthesis kit (Takara, Japan). The cDNA (2 μL) was amplified with Takara SYBR® Premix Ex TaqTM (Tli RNaseH Plus, Japan) in a final volume of 20 μL. The sequences of primers used are listed in table 1: PPAR-α (peroxisome proliferator activator receptor alpha), PPAR-γ (peroxisome proliferator activator receptor gamma), Acsl3 (acyl-CoA synthetase long-chain family member 3), Cpt1α (carnitine palmitoyltransferase 1a), Ehhadh (enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase), Scd1 (stearoyl-Coenzyme A desaturase 1), Me1 (malic enzyme1), Srebf-1 (sterol regulatory element binding transcription factor 1), FAS (fatty acid synthase), Acaca (acetyl-CoA carboxylase alpha). The reaction conditions consisted of an initial activation step (30 s at 95°C) and a cycling step (denaturation for 5 s at 95°C and annealing for 34 s at 60°C for 40 cycles). The production was accurately measured by Applied Biosystems 7500 Real-Time PCR Detection System (ABI, USA). The signal of the housekeeping gene 18s was used for normalization, and the relative expression levels were quantified by the $2^{-\Delta\Delta Ct}$ method, with the relative fold changes normalized to the control values.

2.7 *Statistical analysis*

All the data expressed as mean ± standard deviation were analysed with SPSS (SPSS v.19.0, IBM Inc., Chicago, IL). Statistical analyses were performed with independent Student's *t*-test after Pearson's normality test. $P<0.05$ was considered statistically significant.

3. Results

3.1 *Glucose metabolism in dams before mating*

The dams in F0-HFD group before mating showed a higher fasting serum glucose (5.83±0.64 vs. 4.40±0.56, $P=0.043$) and AUC of IPGTT (33.32±1.50 vs. 26.00±2.66, $P=0.014$), as compared to F0-CON, suggesting the dams in F0-HFD group had glucose intolerance before mating (table 2).

3.2 *Growth in offspring*

The effect of the maternal HFD was observed during pregnancy and lactation on offspring growth in their early life; body weight was recorded at PND1, PND7 and PND21 (at weaning). The results showed that the birth weight of the

Table 1. Primer sequences used for mRNA quantification by qRT-PCR

	Primer sequence	Product length
18s	Forward: 5′-AAGTTTCAGCACATCCTGCGAGTA-3′ Reverse: 5′-TTGGTGAGGTCAATGTCTGCTTTC-3′	140 bp
PPAR-α	Forward: 5′-CCATACAGGAGAGCAGGGATT-3′ Reverse: 5′-CCACCATTTCAGTAGCAGGA-3′	146 bp
Acsl3	Forward: 5′-TAACGGAACTTGGGAAGAGC-3′ Reverse: 5′-AAGGCATCAGTCACCAGACC-3′	171 bp
Cpt1α	Forward: 5′-ATCCACCATTCCACTCTGCT-3′ Reverse: 5′-TGTGCCTGCTGTCCTTGATA-3′	107 bp
Ehhadh	Forward: 5′-TGTGGATTTGGTGGTTGAAG-3′ Reverse: 5′-CTGTGGAAGAAGCAATGTCG-3′	146 bp
Scd1	Forward: 5′-TGTTCGTCAGCACCTTCTTG-3′ Reverse: 5′-AGTTGATGTGCCAGCGGTA-3′	219 bp
Srebf-1	Forward: 5′-GCGCTACCGTTCCTCTATCA-3′ Reverse: 5′-GGATGTAGTCGATGGCCTTG-3′	113 bp
FAS	Forward: 5′-GGAACTGCTTTCTCTTTCTGC-3′ Reverse: 5′-AACGCTCCTCTTCAACTCCA-3′	136 bp
Acaca	Forward: 5′-ATTCTGGCGGATCAGTATGG-3′ Reverse: 5′-AGCAATAGCAGCAGGAGCTT-3′	105 bp
PPAR-γ	Forward:5′-CTGACCCAATGGTTGCTGATTAC-3′ Reverse:5′-CCTGTTGTAGAGTTGGGTTTTTTCA-3′	119 bp

Table 2. IPGTTs in dams before mating

	F0-HFD	F0-CON	*P* Value
N	6	6	
IPGTTs			
Glucose G_0 (mmol/L)	5.83±0.64	4.40±0.56	0.043*
Glucose G_{30} (mmol/L)	16.07±1.04	12.03±2.01	0.037*
Glucose G_{60} (mmol/L)	10.10±0.52	8.40±0.62	0.022*
Glucose G_{120} (mmol/L)	8.47±0.21	6.73±0.55	0.007*
AUC	33.32±1.50	26.00±2.66	0.014*

Blood Glucose G_0, G_{30}, G_{60}, G_{120} indicates the blood glucose level at 0, 30, 60, 120 min during the IPGTTs.

* The significance between F0-HFD group and F0-CON group ($P<0.05$).

offspring in F1-HFD was lower than that in F1-CON (6.43 ±0.34 vs. 6.66±0.43; $P=0.027$), whereas the body weight in F1-HFD was increased much as compared to that in F1-CON at PND7 and PND21 ($P=0.0001$ and $P=0.0001$, respectively), suggesting that the offspring in F1-HFD had a catch-up growth after birth (table 3).

Table 3. Serum and liver lipid and glucide level in offspring

	F1-HFD	F1-CON	*P* Value
N	10	10	
Weight (g)			
PND 1	6.43±0.34	6.66±0.43	0.027*
PND 7	17.83±1.40	15.44±2.51	0.000*
PND 21	56.57±4.35	43.35±4.51	0.000*
Serum at PND 21			
Glucose G_0 (mmol/L)	6.57±1.21	5.15±0.81	0.011*
Glucose G_{30} (mmol/L)	19.28±2.55	15.63±2.48	0.004*
Glucose G_{60} (mmol/L)	14.81±2.30	10.61±1.31	0.000*
Glucose G_{120} (mmol/L)	8.64±1.47	6.43±0.84	0.000*
AUC	41.70±5.42	32.04±3.39	0.0001*
TC (mmol/L)	2.32±0.47	1.76±0.10	0.025*
TG (mmol/L)	1.24±0.56	0.40±0.30	0.031*
Liver			
TG at PND 7 (mmol/L)	159.73±46.50	41.01±30.78	0.008*
TG at PND 21(mmol/L)	139.05±68.97	27.87±7.64	0.010*

Blood Glucose G_0, G_{30}, G_{60}, G_{120} indicates the blood glucose level at 0, 30, 60, 120 min during the IPGTTs.

*The significance between F1-HFD group and F1-CON group ($P<0.05$).

3.3 *Glucose and lipid metabolism in offspring*

Glucose and lipid metabolism abnormalities were found in this study, along with the body weight changes in the offspring earlier life. A significant increase of serum glucose was observed at 0, 30, 60, 120 min during the IPGTT in F1-HFD at PND21 compared with those in F1-CON ($P=0.011$, $P=0.004$, $P=0.0001$, $P=0.0001$, respectively). The AUC of IPGTT in F1-HFD at PND21 was significantly higher than that in F1-CON (41.70±5.42 vs. 32.04±3.39, $P=0.0001$) (table 3).

The serum TC and TG at PND21 in F1-HFD were considerably higher than that in the CON ($P=0.025$, 0.031, respectively). Meanwhile, elevated liver TG in F1-HFD at PND7, PND21 were noted as well ($P=0.008$, 0.010, respectively) (table 3). Additionally, we also analysed and compared the level of serum TC and TG in these two groups by two genders respectively. As a result, we found a similar tendency of influence on hepatic lipid metabolism in offspring rats of both genders.

3.4 *Liver histology in offspring*

Liver tissue histology demonstrated a large amount of lipid deposition in the offspring liver from the dams fed with HFD. Lipid accumulation was observed in liver at PND1, PND7 and PND21 in F1-HFD. Furthermore, vacuolar and microvesicular steatosis with a distinct microanatomy in the liver of F1-HFD at PND21 was found. The widespread deposition of lipid droplets in hepatocytes was visualized by Oil-Red-O staining in F1-HFD. There were more intensive fatty droplets shown in F1-HFD at PND21 than that at PND1 and PND7, indicating that severe NAFLD and NASH developed with the time (figure 1).

3.5 *mRNA for lipid metabolism-related gene expressions in liver of offspring*

Metabolism-related gene expressions in offspring liver whose dams were fed with fatty diet during pre-pregnancy, pregnancy and lactation showed a discrepancy in their modalities. There were 120 abnormal expressed genes of the offspring in the liver in F1-HFD, compared with F1-CON, including 76 up-regulated genes (63.3%) and 44 down-regulated genes (36.7%). All genes expressed differentially (see supplementary material available online). A list of affected genes is shown in the form of heat map, hierarchical clustering, and two clusters – cluster 1 and cluster 2 – were presented with up-regulated and down-regulated gene expressions in F1-HFD (figure 2A). Meanwhile, a list of pathway enrichment analysis is shown in the form of histogram (figure 2B). Through filtering of the genes that were affected by the maternal HFD, a group of subset genes was found to be related to 17 abnormal signal pathways involved in lipid metabolism, such as PPAR signalling pathway,

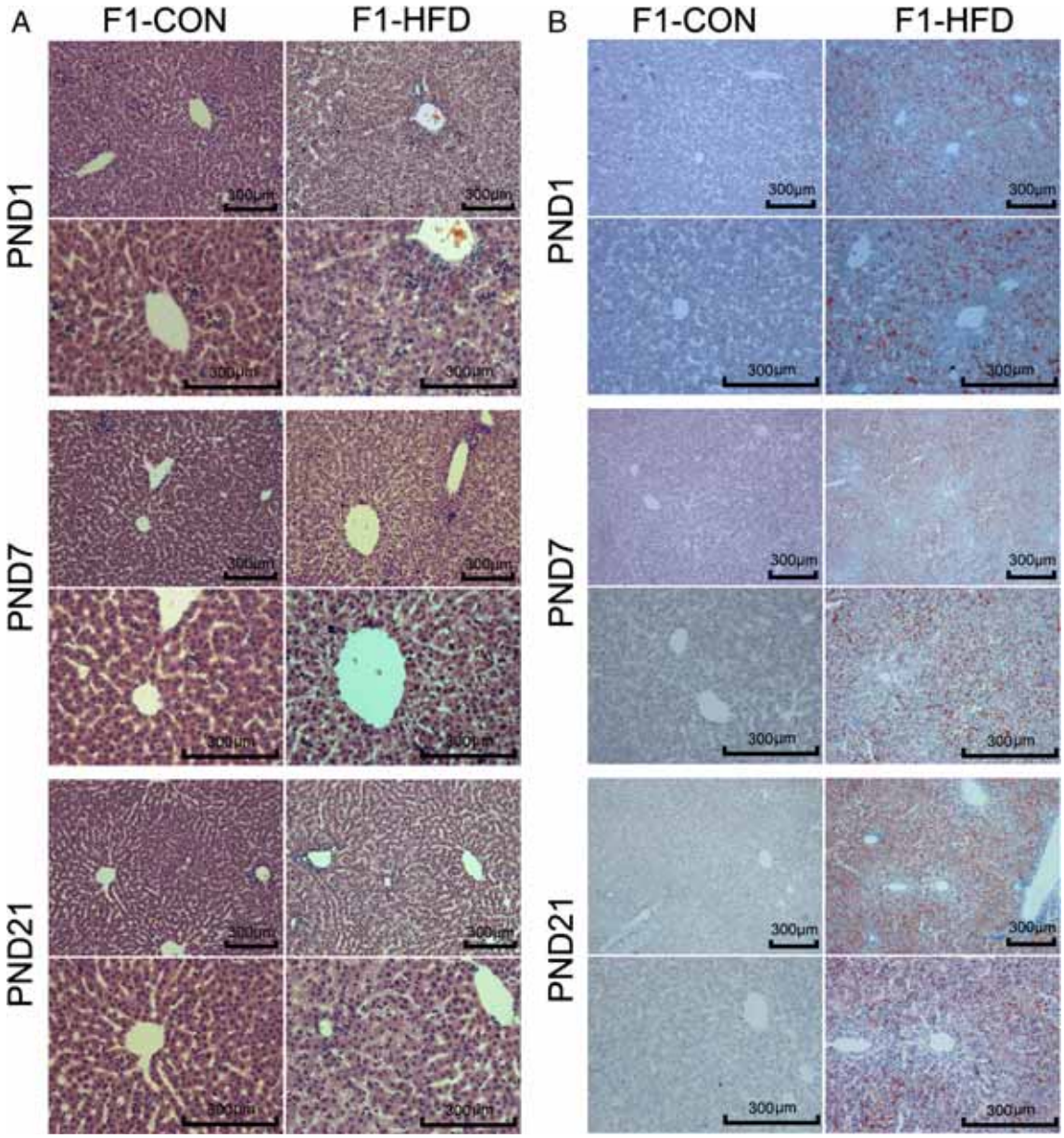

Figure 1. Histological analysis in the offspring livers from F1-CON and F1-HFD at PND1, PND7 and PND21. (**A**) HE staining, ×200, ×400. (**B**) Oil-Red-O staining, ×100, ×200).

unsaturated fatty acids biosynthesis, steroid synthesis, nitrogen metabolism, at cytokine signalling pathways, cell cycle, etc. Among these altered genes, *Scd1*, as one of the PPAR signalling pathway and in its role mainly to decompose saturated fatty acid (SFA) into monounsaturated fatty acid (MUFA), showed the most significant hierarchic in gene expressions in F1-HFD, much higher than that in F1-CON, about 25.77 times. Since the PPARs were the important transcription factors involved in glucose and fatty acid metabolism, PPAR signalling pathway was selected for main analysis. The results of gene array in F1-HFD showed that five genes were expressed differently in PPAR pathway, including *Me1*, *Scd1*, *Ehhadh*, *Acsl3* and *PPAR-α.* These abnormalities had been verified by microarray gene expression and qRT-PCR. A strong consistency was observed between the chip result and the qRT-PCR results in synthesis and lipid-related gene expressions of *Me1* and *Scd1*, which were significantly increased in F1-HFD ($P<0.05$). In addition, β-oxidation genes, *PPAR-α*, *Cpt1α* and *Ehhadh* were found with qRT-PCR in F1-HFD, associated with genes up-

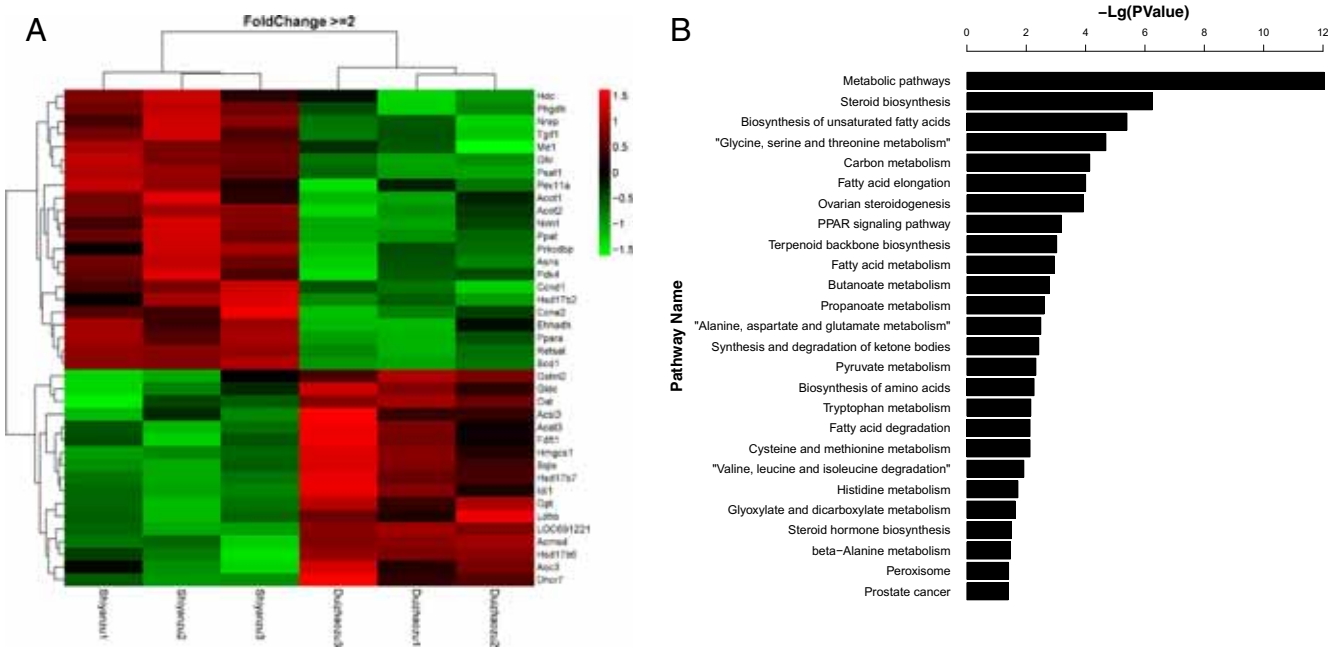

Figure 2. Gene array analysis for the offspring of dams fed with a high-fat diet or a standard chow diet at weaning. (**A**) Heatmap diagram: the differential expression of hepatic mRNAs in high-fat diet (n=3) versus standard chow diet (n=3) is illustrated. The tree is based on the $\log_2$ transformation of the normalized probe signal intensity using hierarchical clustering. The hierarchical clustering is based on 120 differentially expressed mRNAs; the expression levels in the high-fat diet (HFD) differentially presented an increased expression in red and a decreased expression in green. (**B**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis is shown in the offspring of maternal high-fat diet.

regulation (P<0.05). *Acsl3*, involved in the first step of the fatty acid catalysis, decreased in F1-HFD (P<0.05), consistent with the microarray result. However, *de novo* lipogenesis (DNL) key genes *Srebf-1*, *FAS*, *Acaca* and transcription factor *PPAR-γ*, involved in lipid biosynthesis, were expressed differently but were not statistically significantly (figure 3).

4. Discussion

Our study found that the offspring of the maternal dam with high-fat diet had a lower birth weight, and later at weaning increased body weight with impaired glucose tolerance, higher serum cholesterol, and hepatic steatosis. Along with the metabolic abnormalities, significant increased expressions of *Me1* and *Scd1*, both of which involved in lipogenesis, were observed in F1-HFD, and increased expression of three lipolysis genes (*PPAR-α, Cpt1α, Ehhadh*). Additionally, *Acsl3* was found to be down-regulated. Several previous studies have investigated the maternal over-nutrition as well. However, our studies had some differences with them. First, the composition of diet is different in our study compared with the one in the previous study (Gugusheff *et al.* 2015). We used a HFD with 45.0% fat, while Gugusheff used a diet including carbohydrate in form of dextrinised starch. Second, and more importantly, we mainly focused on of hepatic lipid metabolism in F1 offspring which were affected by maternal over-nutrition, while previous studies focused on gut taste receptor inflammatory gene expression (Reynolds *et al.* 2015) and mammary glands (Govindarajah *et al.* 2016).

Maternal nutrition modification during pregnancy and lactation was shown to influence health in the adult life of offspring, which could alter their responses to environmental challenges, and increase their susceptibility to disease (Zhang *et al.* 2009). It has been widely accepted that IUGR could lead to a small-for-gestational-age offspring, which present a lower birth weight followed by a rapid growth. In this study, although the dams were fed with high-fat diet in pre-pregnancy and lasting lactation period, their offspring also had a lower birth weight, and had rapidly increasing weight in later growth, similar to the IUGR offspring. Their maternal nutrition was totally different, but the offspring had similar outcome on birth weight. This growth phenomenon might be consistent with the theory of similarities in the

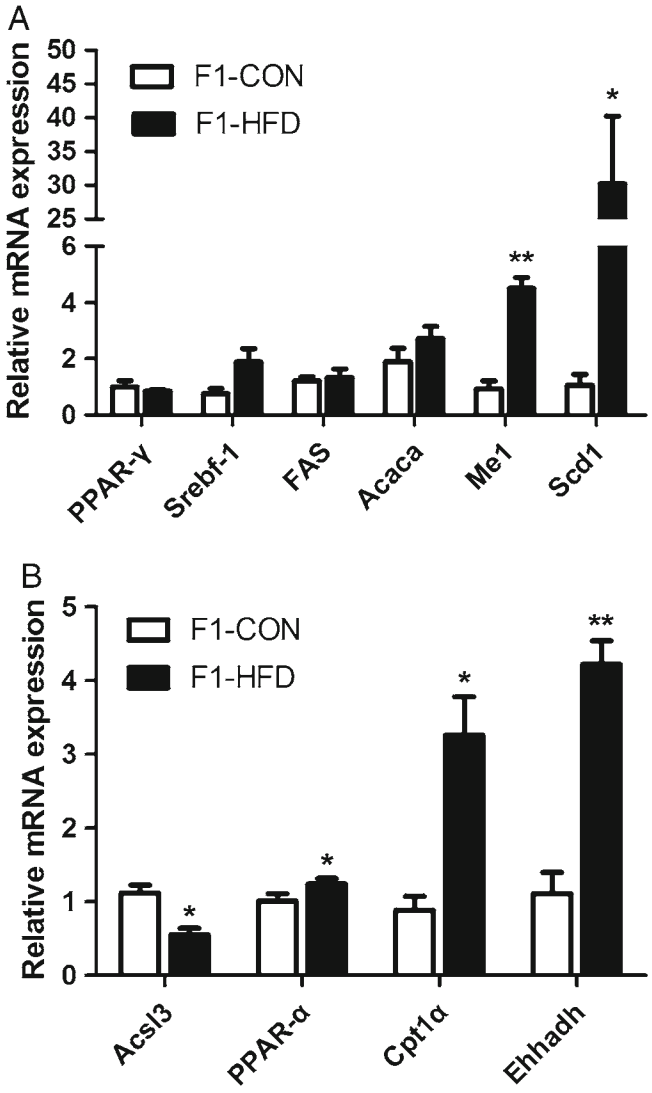

Figure 3. Hepatic lipid metabolic gene expression is involved in offspring of dams fed with a high-fat diet at weaning. (**A**) The mRNA genes expression related to lipogenesis. (**B**) The mRNA genes expression related to β-oxidation. *statistical significance between F1-HFD and F1-CON (P<0.05).

inequalities (Cunha Fda *et al.* 2015), proposing that the placentas suffered from the same results of exchange dysfunction and nutrition insufficiency whether with high-fat diet or with dietary restriction, which might explain the lower birth weight and catch-up growth with impaired glucose tolerance in our study.

Hepatic lipid disposition resulting from derangements in any lipids metabolism or processes could lead to the development of NAFLD. Many recent studies have demonstrated that diets could affect the metabolic gene expressions, including glucose and lipid metabolism pathway genes (Magliano *et al.* 2013; Fernandez Gianotti *et al.* 2013; Pruis *et al.* 2014). In this study, over 120 differently expressed genes were sorted out, which belonged to 17 signal pathways involved in lipid metabolism. Among the differently expressed genes from the investigation using the chip in F1-HFD, five belonged to the PPAR pathway, including *Me1*, *Scd1*, *Ehhadh*, *Acsl3* and *PPAR-α*. It is well known that the PPARs are important transcription factors involved in glucose and fatty acid metabolism. Our findings might be of significance in uncovering the association of maternal high-fat diet with metabolic syndrome in the early life of the offspring.

Hepatic adipogenesis gene expression in the offspring might be associated with disturbed lipid homeostasis. *PPAR-γ* is a master transcriptional regulator of adipogenesis and plays an important role in the process of lipid storage. The key transcriptional regulator, *Srebf-1*, in the main isoform of SREBPs family in rodent and human liver, with its target genes (*FAS* and *Acaca*), is supposed to be involved in DNL which promotes the development of fatty liver due to the lipids accumulation in hepatocytes (Silbernagel *et al.* 2012). A previous study found that maternal high-fat diet could lead to a reduction in hepatic *PPAR-γ* expression in offspring rats at weaning, which might be associated with disturbed lipid homeostasis (Yamaguchi *et al.* 2010). In this study, however, the transcription factor *PPAR-γ* and the DNL key genes *Srebf-1*, *FAS* and *Acaca*, which are involved in lipid biosynthesis, were expressed differently between F1-HFD and F1-CON, although not statistically significantly, impliying that maternal high-fat diet during pregnancy and lactation might not activate the DNL pathway in the early offspring life. Therefore, hepatocytes were protected from *de novo* synthesized saturated fatty acid-induced lipotoxicity (Silbernagel *et al.* 2012).

An elevated expression of *Me1* in offspring from dams with a high-fat diet might lead to the development of hepatic steatosis. *Me1* catalyses the reversible oxidative decarboxylation of malate to pyruvate, CO_2 and NADPH, and the latter contributes to fatty acid synthesis. It was reported that *Me1*-null mice showed a lower body and liver weight, reduced adipocyte in size and ameliorated hepatic steatosis (Al-Dwairi *et al.* 2012). *Scd1* gene was the most significant hierarchic in F1-HFD in this study, about 25.77 times higher than that in F1-CON. Among these differentially expressed genes, *Scd1* converts SFA to MUFA, which is the major substrate for the triglycerides formation (Ntambi and Miyazaki 2004). Interruption of triacylglycerol synthesis in the endoplasmic reticulum (ER) is the initiating event for SFA-induced lipotoxicity in liver cells (Mantzaris *et al.* 2011; Fernandez Gianotti *et al.* 2013). Investigation in mice indicated that the *Scd1* gene expression was significantly lower in livers of offspring from dams fed with high-fat, suggesting that diet-induced fatty liver was associated with down-regulation of hepatic *Scd1* transcript and

dedimerization of the protein. Interestingly, the current study showed that *Scd1* expression was significantly increased in the liver of the offspring from dams fed with a high-fat diet at weaning, which was inconsistent with previous studies (Cnop *et al.* 2012; Silbernagel *et al*. 2012). This increased *Scd1* expression could be associated with the adverse intrauterine environment of maternal high-fat diet. Under this intrauterine environment, the increased *Scd1* expression seems to be a protection. An increased *Scd1* expression may protect hepatocytes against endoplasmic reticulum stress, restore hepatic capacity to clear triglycerides, and thereby prevent from liver fat accumulation (Cnop *et al.* 2012; Silbernagel *et al*. 2012).

The multiple and altered genes expressions are correlated with lipid oxidation pathway in liver. The qRT-PCR results demonstrated that *PPAR-α* expression and its target genes *Cpt1α* and *Ehhadh* involved in β-oxidation were up-regulated in offspring from dams fed with a high-fat diet at weaning. *Acsl3* played a critical role in an initiation of fatty acids metabolism, and it was observed to be down-regulated in F1-HFD. All these findings were consistent with microarray analysis. Under this high maternal fat diet, *PPAR-α* activation might participate in modulation of *Cpt1α* and *Ehhadh* genes expressions, initiation of lipid β-oxidation, protection against steatosis (Tanaka *et al.* 2005; Houten *et al.* 2012). *Acsl3* suppression decreases lipid synthesis and triglycerides (TG) storage through cellular uptake reduction of fatty acids (Bu *et al.* 2009; Poppelreuther *et al.* 2012). The increased gene expression of *Cpt1α* and *PPAR-α* was accompanied with increased hepatic TG content at weaning as well. On the contrary, other studies (Zhang *et al.* 2005, 2009) found that the adult offspring, which weaned onto a standard chow diet after their dams fed with a high-fat diet during pregnancy and lactation, had the increased mRNA levels of key genes that regulated fatty acid oxidation, including *Cpt1α* and *PPAR-α*, but had reduced hepatic TG levels. The different hepatic TG levels might be due to maternal high-fat diet that affects the lipid homoeostasis of the early life of offspring, and this effect cannot be explained by the adaptive response of improved capacity to hepatic fatty acid oxidation, but is thought to be affected by the standard chow diet during post-weaning on F1 offspring.

Although the present study indicated that increased gene expressions were involved in fatty acids oxidation pathway in offspring from maternal high-fat diet at weaning, elevated hepatic triglycerides and diffused lipid droplets were still observed in early life of offspring. These consequences, obviously, cannot be balanced or even reversed by an increase of compensatory fatty acids oxidation.

In summary, maternal high-fat diet induced offspring obesity with impaired glucose tolerance, disordered lipid metabolism, which may relate to abnormal metabolic genes expressions, lead to an increase in fatty acid synthesis and also fatty acid β-oxidation in the liver of offspring. These findings provided evidence for guiding a healthy maternal diet by which a reduced risk of metabolic diseases in their early life of the offspring could be achieved.

Acknowledgements

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