Adaptive responses by mouse fetus to a maternal HLE diet by downregulating SREBP1: a microarray- and bio-analytic-based study

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Abstract Maternal diet has long been recognized as a significant factor affecting offspring development and health, but the target genes affected by a maternal high-lipid diet are currently unknown. In this study, the gene expression profile of neonatal mouse liver was analyzed using gene chips to identify genes with significant up- or downregulated expression levels due to maternal high-fat diet during gestation. Real-time PCR and Western blotting were used to measure key genes selected using microarray. Serum lipid, glucose, and insulin levels in adult offspring from dams fed with chow or a high-lipid diet were measured using commercial kits. Results indicate that the expression of genes involved in cholesterol and fatty acid synthesis were significantly inhibited, while the expression of genes involved in glycolysis were significantly decreased by maternal high-lipid diet during gestation. SREBP1 might be the key gene regulating genes involved in fatty acid, glucose, and cholesterol metabolism in response to a maternal high-fat diet. Yu, H-L., H-T. Miao, L-F. Gao, L. Li, Y-D. Xi, S-P. Nie, and R. Xiao. Adaptive responses by mouse fetus to a maternal HLE diet by downregulating SREBP1: a microarray- and bio-analytic-based study. J. Lipid Res. 2013. 54: 3269–3280.

Supplementary key words maternal diet • dietary cholesterol • high-energy high-lipid diet • gestational diet

Maternal nutrient intake during gestation has long been recognized as a significant factor affecting the incidence of features of the adult metabolic syndrome in offspring. Indeed, human studies demonstrated that maternal over-nutrition during gestation predisposes offspring to a much higher prevalence of obesity, hypertension, glucose intolerance, insulin resistance, and vascular dysfunction (1–3). Accumulating evidence from animal studies also indicates that over-nutrition during gestation induces the same health problems as the ones observed in epidemiological studies (4, 5). So, maternal high-fat diet is an important predisposing factor to the onset and development of obesity, insulin resistance, and cardiovascular diseases in offspring (6). Indeed, a high-fat diet during gestation has been shown to lead to a lack of increase in insulin release and ATP content in response to glucose stimulation in islets from 3-month-old male and female offspring (5).

However, the mechanisms linking high-fat nutrition in early life with later health problems remain unclear. Preliminary work has been performed on several candidate mechanisms, including: (1) white adipose tissue glucocorticoid sensitivity (7); (2) gene modification status, such as DNA methylation (epigenetic mechanism) (4); (3) oxidative stress in dams, which might be transferred to the pups during gestation, later promoting disease development in the offspring (8); and (4) a maternal high-fat diet by itself, which triggers lipotoxicity in the fetal livers and later affects the offspring’s health status (9). Indeed, the metabolic alterations usually observed during pregnancy combined with increased dietary fat intake, increase the triglycerides available to be hydrolyzed for transfer to the offspring, inducing an adaptive toxic response in the offspring’s liver; this lipotoxicity leads to macrophage infiltration into the liver, and to increased cytokine secretion. This inflammatory state activates the pathways involved in oxidative stress and in gluconeogenesis (9). These conditions may predispose the offspring to alterations in their lipid metabolism in adult life.

An understanding of how maternal nutrients influence offspring’s development of adult diseases may be gained using proteomic methodologies and microarray analyses.

Abbreviations: CD, chow diet; Ct, cycle threshold; ER, endoplasmic reticulum; FC, free cholesterol; FDR, false discovery rate; GO, gene ontology; HDL-C, high density lipoprotein cholesterol; HLE, high-lipid high-energy; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol.

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4 The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one table.
Indeed, Novak et al. (10) identified proteins regulated by maternal dietary fatty acid composition in neonatal rat liver and concluded that early fatty acid nutrition impacts hepatic metabolic pathways involved in gluconeogenesis, redox balance, and nitric oxide signaling.

Thus, considering the number of different factors seemingly associated with effects on pups after a high-fat diet during gestation, we hypothesized that a number of interrelated pathways or key molecular signals are involved in this process. Consequently, the aim of the present study was to obtain a comprehensive and unbiased analysis of adaptive responses in hepatic gene expression patterns of prenatal mouse liver to maternal high-fat intake. To do so, we carried out microarray analyses of changes in C57BL/6J neonatal mouse liver and concluded that early fatty acid nutrition impacts hepatic metabolic pathways involved in gluconeogenesis, redox balance, and nitric oxide signaling.

### MATERIALS AND METHODS

#### Animals and diets

The Beijing Administrative Committee for Laboratory Animals and the Ethical Committee for Animal Care and Use of the Capital Medical University reviewed and approved all animal experiments carried on in the present study.

**Experiment 1.** Male and female C57BL/6j mice (specific pathogen-free, 9 weeks old) were housed in groups of 4 mice per cage at 22°C, with a 12 h light/dark cycle, and were given free access to food and water. After 1 week, 15 male and 15 female mice were transferred to one cage and fed the control diet [chow diet (CD)] or a high-lipid high-energy (HLE) diet (composed of 84% CD, 15.8% lard fat, and 0.2% cholesterol). These diets were used in one of our previous studies (11). Two days later, pregnant female mice were individually housed in cages. Total food intake and body weight gain were recorded twice a week. After birth, the neonatal livers were sampled. Biochemical measurements, quantitative PCR, Western blot, and micro-array analyses were performed. After a 12 h fast, blood was sampled from the eye of the dams.

**Experiment 2.** Male and female mice (refer to experiment 1) were housed in one cage for 2 days. Pregnant mice were housed individually, and fed the chow or HLE diet. After giving birth, dams were fed the same diet. After 21 days, the weaning offspring were fed the CD until they were 10 weeks old. Blood (nonfasting) was sampled, and serum cholesterol, insulin, and glucose levels were measured.

#### Blood biochemical analysis

Eye vein blood samples were collected. After centrifugation (15 min at 3,500 rpm), the serum was obtained. Serum insulin levels were measured using an ELISA kit (Beijing North Institute of Biological Technology, China). Total serum cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and free fatty acid (FFA) levels were measured using an autoanalyzer (New Alkaline S, Japan).

#### cDNA microarray analysis

Total RNA was isolated from six neonatal mouse livers from the two groups using Trizol reagent (Invitrogen, Carlsbad, CA) and purified according to the manufacturer’s instructions (Qiagen, Valencia, CA). RNA quality was assessed by electrophoresis on a 1.5% denaturing agarose gel containing formaldehyde. RNA concentrations were measured using a Smart Spec Plus (BioRad, Hercules, CA). Purified mRNA (2 μg) was used to synthesize the first strand of cDNA using SuperScript II (Invitrogen). cDNA was purified using an RNaseasy Mini kit (Qiagen), labeled with Cy3, and hybridized at 65°C for 17 h onto an Agilent whole mouse genome microarray (Affymetrix). This chip contains 764,885 probes representing 28,869 genes, each of which is represented on the array by approximately 26 probes spread across the full length of the gene. For each sample, three biological replicates were performed. All arrays were washed and scanned using an Agilent DNA microarray scanner (Agilent Technologies). Hybridization signals were acquired and normalized using Agilent’s feature extraction software (v.9.5).

#### Data analysis

We used the random variance model t-test to filter the differentially expressed genes for the control and experimental groups.
because the random variance model t-test can effectively increase the degree of freedom for small sample sizes. After a significant analysis and the false discovery rate (FDR) analysis, P values and FDRs of less than 0.05 were considered statistically significant (12–14). Hierarchical clustering analysis (Cluster 3.0) and TreeView analysis (Stanford University) were performed to generate a dendrogram for each cluster of genes based on their expression profiling similarities. Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, 49 significantly changed pathways were identified. Based on these significantly changed pathways, a pathway network was created (15). Afterward, based on the gene ontology (GO) (16, 17) and pathway analyses (18, 19), the metabolism-related GenRelNet (20, 21) was established. By subtracting the degrees of homologous genes between these two networks, genes with the highest degrees were identified as the regulated gene list of tests performed times the degree of freedom for small sample sizes. After a significant analysis and the false discovery rate (FDR) analysis, P values and FDRs of less than 0.05 were considered statistically significant.

### Quantitative real-time RT-PCR

Real-time PCR was performed as described by Liu et al. (22). Total RNA was isolated from neonatal mouse livers using the TRIzol reagent (Invitrogen), according to the manufacturer’s protocol. Aliquots (2 μg) of total RNA were converted into first-strand cDNA using the reverse transcription kit (A3500; Promega) according to the manufacturer’s guidelines. Quantitative real-time PCR was performed on an ABI 7500 device (Applied Biosystems) using the A6001 real-time PCR system (Promega). β-Actin, an endogenous housekeeping gene, was used to normalize the results. Real-time PCR reactions were performed in triplicate, in 96-well plates, using the following thermo-cycling conditions: 95°C for 10 min, 42 cycles of 15 s at 95°C, and 60°C for 1 min. The primers for quantitative real-time PCR are listed in Table 1. The point at which the PCR product was first detected above a fixed threshold [the cycle threshold (Ct)] was determined for each sample. Changes in the expression of target genes were calculated using the 2^(-ΔΔCt) method, where ΔΔCt = (Ct_target - Ct_β-actin)_sample - (Ct_target - Ct_β-actin)_control, taking the mean of Ct in the CD group as the control.

### Western blotting

For the Western blot analysis, livers from neonatal mice were homogenized on ice in radio-immunoprecipitation assay buffer (Sigma) containing phenylmethanesulfonyl fluoride (PMSF) as a proteinase inhibitor. Homogenates were centrifuged for 10 min at 12,000 rpm at 4°C, and the supernatants were collected. The total protein content was determined using the bicinchoninic acid method. Then, 50 μg of protein extracts from each liver tissue were resuspended in a sodium dodecyl sulfate (SDS)-containing buffer, maintained in boiling water for 5 min, and separated by SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to nitrocellulose membranes, and SREBP1 was detected using an anti-SREBP1 antibody (ab3259, 1:1,000). An anti-β-actin antibody (1:1,000) was used as control. Signals were revealed using an ECL chemiluminescence kit (FIVEphoton Biochemicals), and the dilution ratio of IgG was 1:5,000. ImageJ was used to analyze the density of the bands.

### Liver lipid extraction

Liver total lipids were determined according to the method of Folch et al. (23). Minced liver tissues were placed in a glass homogenizer with 5 ml of CHCl₃:methanol (2:1). The homogenate was removed and placed into a tube. The tube was centrifuged (2,000 rpm for 15 min at room temperature), and the extract was collected in a clean tube. Tissues were rinsed with 0.5 ml CHCl₃:methanol (2:1), centrifuged in the tube, and the extracts were pooled. The lipid extract was dried under a N₂ flow. After CHCl₃:methanol (2:1) and H₂SO₄ treatment and centrifugation at 2,000 rpm for 10 min at room temperature, the bottom phase was removed and placed in a clean tube that had been weighed (weight1) and dried under N₂ flow. The tube was then weighed again (weight2). The difference between weight1 and weight2 was recorded as the total lipid weight.

### Statistical analysis

Results are expressed as mean ± SD. Significant differences were assessed using the Student’s t-test (two-tailed). A P value <0.05 was considered statistically significant.

### Table 3. Serum insulin, lipid, and cholesterol level in adult male offspring

| Group    | TC (mmol/l) | LDL-C (mmol/l) | HDL-C (mmol/l) | FFA (mmol/l) | Insulin (UIu/ml) | Glucose (mg/dl) |
|----------|-------------|----------------|----------------|--------------|-----------------|----------------|
| CD       | 2.13 ± 0.34 | 0.14 ± 0.06    | 1.57 ± 0.29    | 3.22 ± 0.21  | 3.30 ± 2.46     | 10.03 ± 0.71   |
| HLE diet | 2.57 ± 0.31 | 0.25 ± 0.05    | 1.87 ± 0.38    | 3.16 ± 0.23  | 2.79 ± 1.00     | 10.00 ± 1.45   |

Data are presented as mean ± SD. Serum of weaned mice was not sampled in the fasting state.

^Significant mean difference as compared with CD group (P < 0.05).
RESULTS

Maternal HLE diet during gestation significantly increased serum cholesterol levels of dams

Fasting serum TC, LDL-C, HDL-C, FFA, and insulin levels were significantly greater in the HLE group than in the CD group (Table 2). There were no significant differences in maternal body weight and food intake during gestation between the two groups (Fig. 1).

Serum lipid and insulin levels in adult male offspring were affected by maternal HLE diet during gestation and lactation

Maternal serum lipids and insulin levels were changed by the HLE diet during gestation (24). The effects of a maternal HLE diet on offspring have been previously reported. We next measured the adult serum lipids and insulin levels. Adult offspring from dams fed a HLE diet during gestation and lactation had significantly higher serum TC levels and lower nonfasting serum insulin levels. Serum glucose (non-fasting) levels were not significantly changed (Table 3).

Liver free cholesterol content in male adult offspring was affected by maternal HLE diet during gestation

Adult mouse liver TC and total lipid contents were not significantly higher in the HLE diet group. However, free cholesterol (FC) content and the ratio of FC to TC were significantly higher in the HLE group (Table 4).

mRNA expression profiles of neonatal mouse liver were changed by the maternal diet during gestation

Using the whole transcript expression arrays platform, we first assessed the whole mRNA expression profiles in neonatal mouse livers whose dams were fed with the chow or HLE diets. The expression profiles of 1,027 mRNAs were regulated by maternal HLE diet during gestation. Among these, 144 mRNAs were identified as being up- or downregulated more than 2-fold in the HLE group compared with the CD group. These 144 genes were sufficient to separate samples into biologically interpretable groups. Eight mRNAs among those filtered were validated as significantly different between the two groups (P < 0.05). As illustrated in Fig. 2A–D, the levels of fatty acids and cholesterol synthesis genes were significantly inhibited.

Microarray-based GO and pathway analysis revealed the functional and signal change related to the SREBP1 gene

Using a significantly changed threshold of GOs, the P value and FDR were <0.001 and <0.05, respectively. The highly significant GOs downregulated by maternal HLE diet included brown fat cell differentiation, response to drug, response to glucocorticoid stimulus, transport, cholesterol biosynthetic processes, transcription, and cellular response to insulin stimulus. SREBP1-related functions included aging, cellular response to starvation, positive regulation of gene-specific transcription, cholesterol metabolic processes, response to retinoic acid, negative regulation of insulin secretion, and regulation of insulin secretion (Fig. 3).

A pathway analysis was used to identify the significant pathways based on the KEGG database. Fisher’s exact tests and χ² tests were used to determine significant pathways. The threshold of significance was defined by a P value and FDR of <0.05. The pathways significantly downregulated (Table 5) by maternal HLE diet included steroid biosynthesis, insulin signaling pathway, galactose metabolism, and glycolysis/gluconeogenesis. In contrast, the upregulated (Table 6) pathways included peroxisome proliferator-activated receptor signaling pathway, citrate cycle (TCA cycle), and the biosynthesis of unsaturated fatty acids. Pathways related to SREBP1 included the insulin signaling pathway.

These pathways interacted with each other, as revealed by the pathway net analysis based on the KEGG database. The analysis indicated that the insulin signaling pathway was an upstream pathway, in which changes resulted in changes in other pathways (Fig. 4).

Microarray-based signal network, signal-flow, and TFactS analysis revealed the key role of SREBP1

Based on the KEGG database, the signal network analysis indicated that relationships between the differentially expressed genes and the upstream genes would be found. From the Signet map, we observed that SREBP1 was the upstream gene that was regulated by maternal HLE diet during gestation (Fig. 5).

Signal-flow analysis revealed the important genes involved in cholesterol and fatty acid synthesis; these genes included SREBP1, HmgcoR, FDPS, and others (Fig. 6, supplementary Table 1).

Based on the differentially expressed genes, we included up- and downregulated genes according to the guidelines on the website. The TFactS analysis predicted that SREBP1, Ppara, Sreb2p, SP1, Hnf4a, RBPj, Foxo1, Myc, CREBBP, HIF1A, CEBPA, and USF1 were inhibited in neonatal mouse livers delivered by dams fed a HLE diet during gestation (Fig. 7).

Expression of SREBP1 and related genes were inhibited by maternal HLE diet during gestation

The protein content of SREBP1 was significantly decreased in neonatal mouse livers by maternal HLE diet during gestation. Insig1 and SREBP1 mRNA expression were also significantly inhibited by maternal HLE diet. SREBP1-regulated genes, Acaca, Gck, and Pklr, were significantly inhibited by maternal HLE diet (Fig. 8).

Expression of FASN, HMG-CoA reductase, and SREBP1 genes were inhibited by maternal HLE diet in adult offspring

FASN and SREBP1 gene expression were significantly decreased in adult offspring from dams fed a HLE diet

| Group | Neonate | Weaning | Male adult |
|-------|---------|---------|------------|
| CD    | 1.92 ± 0.45 | 2.33 ± 0.19 | 1.83 ± 0.15 |
| HLE diet | 2.61 ± 0.94* | 2.59 ± 0.30* | 1.87 ± 0.38 |

Data are presented as %, mean ± SD.

*Significant mean difference as compared with CD group (P<0.05)
Fig. 2. A: Hierarchical clustering of 144 transcripts (changes by more than 2-fold) altered by a maternal HLE diet in neonatal offspring mouse livers. Gene expression was assessed in neonatal offspring livers using mouse genome microarrays (Affymetrix 1.0) (n = 3 microarrays per group). Fatty acid synthesis genes and cholesterol synthesis genes were identified as significantly changed. B, D: Validation of microarray data using real-time PCR. Triplicate assays were performed for each RNA sample, and the relative amount of each mRNA was normalized to β-actin. Statistically significant differences between the chow and HLE diet groups are indicated by *P < 0.05. C: Enlarged photo of the marked area.
Through developmental programming, a maternal high-lipid diet during gestation can predispose the offspring to...
glucose intolerance and to increased adiposity in adulthood (25, 26). In the present study, results indicated that a high-lipid diet during gestation induced serum cholesterol, FFA, and insulin levels (fasting) to significantly increased levels in dams. Thus, total lipid contents in neonatal and weaning mouse livers were significantly increased, and serum cholesterol levels were also significantly increased, while serum nonfasting insulin levels were significantly decreased in adult offspring. Using microarray and bio-analytic methods, we showed that among the differentially expressed genes in livers of neonates born from dams fed with a HLE diet during gestation, SREBP1 was identified as the targeted gene for adaptation to maternal diet.

Adult offspring born from obese (high-fat diet-induced) dams have shown insulin resistance (27), sensitivity to weight gain, mitochondrial dysfunction (28), and increased endogenous cholesterol synthesis (29). Our previous study indicated that adult offspring brain polyunsaturated fatty acid content correlated with maternal serum LDL-C levels (11). In this present study, in the absence of maternal obesity induced by HLE diet, the adult male offspring also showed higher serum cholesterol and lower nonfasting serum insulin levels. These results indicated that maternal HLE diet during gestation itself might program the development without obesity. Other researchers also observed the absence of obesity during gestation in rodents fed with HLE diet (30). Because chronic high-fat diet consumption may result in a greater lipid transfer to the fetus regardless of maternal obesity (9), the total lipid content in neonate and weaning mouse livers was significantly increased in the HLE diet group in our study. However, the liver total lipid content of adult mice in the HLE diet group was not significantly different from the CD group. This might indicate that using CD after weaning of HLE mice might help to alleviate the liver fat content. However, the weaned mice were not sampled in the

| Path Identification | Path Name                                      | P       | FDR     |
|---------------------|------------------------------------------------|---------|---------|
| 1100                | Metabolic pathways                            | 4.39E-43| 2.19E-41|
| 850                 | Retinol metabolism                            | 3.05E-11| 7.62E-10|
| 71                  | Fatty acid metabolism                          | 6.51E-11| 1.08E-09|
| 260                 | Glycine, serine, and threonine metabolism      | 7.70E-10| 7.97E-09|
| 3320                | PPAR signaling pathway                         | 8.40E-10| 8.38E-09|
| 410                 | β-Alanine metabolism                           | 1.50E-09| 1.24E-08|
| 340                 | Histidine metabolism                           | 4.12E-09| 2.72E-08|
| 983                 | Drug metabolism, other enzymes                 | 4.86E-09| 3.03E-08|
| 982                 | Drug metabolism, cytochrome P450               | 1.36E-08| 7.17E-08|
| 590                 | Arachidonic acid metabolism                    | 1.70E-08| 8.49E-08|
| 310                 | Lysine degradation                             | 1.70E-07| 7.71E-07|
| 280                 | Valine, leucine, and isoleucine degradation    | 6.10E-07| 2.23E-06|
| 20                  | Citrate cycle (TCA cycle)                      | 6.67E-07| 2.38E-06|
| 640                 | Propanoate metabolism                          | 6.67E-07| 2.38E-06|
| 980                 | Metabolism of xenobiotics by cytochrome P450   | 9.73E-07| 3.23E-06|
| 380                 | Tryptophan metabolism                          | 3.24E-06| 9.98E-06|
| 620                 | Pyruvate metabolism                            | 5.03E-06| 1.46E-05|
| 591                 | Linoleic acid metabolism                       | 6.62E-06| 1.83E-05|
| 561                 | Glycerolipid metabolism                        | 1.57E-05| 3.95E-05|
| 250                 | Alanine, aspartate, and glutamate metabolism   | 2.00E-05| 4.48E-05|
| 330                 | Arginine and proline metabolism                | 2.18E-05| 5.18E-05|
| 5012                | Parkinson’s disease                             | 0.000102076| 2.13E-04|
| 53                  | Ascorbate and aldarate metabolism              | 0.000104286| 2.17E-04|
| 910                 | Nitrogen metabolism                            | 0.000104286| 2.17E-04|
| 5016                | Huntington’s disease                           | 0.00015968| 3.08E-04|
| 300                 | Lysine biosynthesis                            | 0.000164965| 3.16E-04|
| 4142                | Lysosome                                       | 0.000362158| 6.39E-04|
| 190                 | Oxidative phosphorylation                      | 0.000373589| 6.57E-04|
| 4080                | Neuroactive ligand-receptor interaction        | 0.00042894| 7.38E-04|
| 129                 | Primary bile acid biosynthesis                 | 0.000626696| 1.01E-03|
| 770                 | Pantethene and CoA biosynthesis                | 0.000780438| 1.20E-03|
| 650                 | Butanoyl metabolism                            | 0.000863415| 1.30E-03|
| 860                 | Porphyrin and chlorophyll metabolism           | 0.002274949| 3.15E-03|
| 230                 | Purine metabolism                              | 0.002319934| 3.29E-03|
| 450                 | Selenoamino acid metabolism                    | 0.002538206| 3.94E-03|
| 4270                | Vascular smooth muscle contraction             | 0.002590194| 3.95E-03|
| 760                 | Nicotinate and nicotinamide metabolism         | 0.002961019| 3.95E-03|
| 110                 | Ubiquinone and other terpenoid-quione biosynthesis| 0.003391081| 4.38E-03|
| 1040                | Biosynthesis of unsaturated fatty acids         | 0.003715258| 4.75E-03|
| 920                 | Sulfur metabolism                              | 0.007073207| 8.51E-03|
| 270                 | Cysteine and methionine metabolism             | 0.009228152| 1.07E-02|
| 564                 | Glycerophospholipid metabolism                 | 0.009870128| 1.13E-02|
| 4610                | Complement and coagulation cascades            | 0.009870128| 1.13E-02|
| 10                  | Glycolysis/gluconeogenesis                     | 0.010582907| 1.20E-02|
| 4710                | Circadian rhythm, mammal                       | 0.01396364| 1.55E-02|
| 240                 | Pyrimidine metabolism                          | 0.030431068| 3.18E-02|
| 40                  | Pentose and glucuronate interconversions       | 0.046418741| 4.60E-02|
| 4950                | Maturity onset diabetes of the young           | 0.046418741| 4.60E-02|
Fig. 4. Pathway network created from significant pathways regulated by maternal HLE diet. Red circles represent the upregulated pathways and blue circles represent the downregulated pathways. Yellow circles indicate that there were up- and downregulated genes related to the pathways.

Fig. 5. Signal network analysis based on the KEGG database. Red circles represent the upregulated genes and blue circles represent the downregulated genes. The larger networks indicate a greater ability to regulate other genes’ expression.

fasting state, which could lead to variations in the lipid and insulin levels observed. Further experiments are needed on this point.

After identifying a set of differentially expressed genes of the neonatal livers of mice born from dams fed with chow or HLE diet, gene functions were analyzed. GO analysis
SREBP1 targeted gene expression changed by gestational diet

GO analysis revealed that fatty acid biosynthesis (GO:0006633) was not significantly inhibited, but that fatty acid biosynthesis-related processes, such as the acetyl-CoA biosynthetic process (GO:0006085), were downregulated. Inhibition of these related processes will limit the supply of precursors, and will therefore inhibit fatty acid synthesis, which was observed after further analyses. Pathway net analysis indicated that fatty acid biosynthesis was a crucial downstream pathway, while insulin signaling pathway was the crucial upstream pathway.

Signal net analysis helped to determine the relationship between the differentially expressed and upstream genes.

Fig. 6. The interaction network between the various genes generated by the microarray experiments. The upper figure illustrates genes from the CD group, and the lower figure displays genes from the HLE diet group. The red dots indicate upregulated genes and the blue dots indicate downregulated genes. Straight lines indicate interaction relationships between the genes. The solid line indicates a direct interaction, and the dashed line indicates an indirect interaction.

revealed a particularly weakened function among genes responsible for cholesterol biosynthesis and response to glucose stimulation. Genes were subsequently organized into hierarchical categories based on the pertinent biological processes. Cholesterol biosynthesis- and transformation-related GO categories were identified as the fifth and third most significantly changed ones, as confirmed by the downregulation of cholesterol synthesis genes, such as HMG-CoA reductase, LSS, and FDPS. Further pathway analyses identified that pyruvate metabolism, terpenoid backbone biosynthesis, and steroid biosynthesis were the third, fourth, and fifth most significantly downregulated pathways.
TFactS is designed to predict which transcription factors are regulated, inhibited, or activated in a biological system based on lists of up- and downregulated genes generated by microarray experiments. In our study, SREBP1 was found to be the upstream gene and TFactS analysis predicted that it was one of the regulated transcription factors. In signal-flow analysis, SREBP1 and its target gene FDPS were among the important genes significantly affected by HLE maternal diet.

SREBPs activate the expression of more than 30 genes involved in the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids, as well as the NADPH cofactor required to synthesize these molecules (31). In mouse liver, transcription of SREBP-1c is regulated by liver X receptors (LXRs), insulin, and glucagon (31). The posttranscriptional regulation of SREBPs includes the sterol-mediated suppression of the movement of the SCAP/SREBP complex from the endoplasmic reticulum (ER) to the Golgi apparatus (31). SREBPs can be upregulated upon ER stress (32). Most recently, researchers observed that ER stress in the liver would accumulate through generational effects in mice from dams fed with high-fat diets (33). In our study, we observed a decreased SREBP1 expression in neonates from dams fed with HLE diet during gestation, and this decrease persisted in adults. However, the exact source of this decrease is unknown: it might result from changes in serum lipids, insulin, or ER stress. Based on these, we may hypothesize that the neonates' liver lipid content was received from the dams via placental circulation, therefore decreasing SREBP in the liver, as well as FASN and HMG-CoA reductase. However, this hypothesis and its implications need to be tested. Nevertheless, our results show that mice from HLE-fed dams have higher liver lipid content at birth, which gradually decreases when weaned on CD, to be finally similar to mice from chow-fed dams. Interestingly, even if the lipid content of the liver is near normal in adults, their FASN, HMG-CoA reductase, and SREPB1 levels remain decreased, suggesting a dysregulation persisting in adult life. Further studies are needed on this point, as well as on the effects of maternal HLE diet on adipose tissues.

Compared with normal chow-fed mice, HLE-fed mice had lower SREBP1 expression during the fetal and adult periods, indicating an inhibitory adaptive response in SREBP1 expression in these mice. Indeed, in addition to the increased lipolysis of adipose tissue observed in mammal pregnancy, the liver of these mice had to adapt to the high levels of lipids they were fed to cope with the potential lipotoxicity (9). In addition, serum triglyceride levels were higher in HLE-fed mice, also showing that they adapted to their diet. The combination of increased lipolysis and increased dietary fats leads to more triglycerides available to be delivered to the offspring. Some adaptive mechanisms, such as reduced triglyceride secretion and reduced β-oxidation might predispose the offspring’s livers to lipotoxicity in the adult life (9). This might be the reason why their offspring more easily exhibited lipid metabolism disorders after being fed the HLE diet. However, the exact mechanisms still need to be clarified.

In conclusion, using microarray and bio-analysis methods, we showed that the Srebp1 gene might be a key gene in the processes regulated by maternal HLE diet involved in the offspring’s lipid metabolism. The authors would like to acknowledge the technical assistance of the Genminix Company (Shanghai, China).
Fig. 8. SREBP1 expression measured by quantitative PCR (A) and Western blotting (B). Expression levels of SREBP1 target genes were significantly decreased (C). Acaca, acetyl-CoA carboxylase α; gck, glucokinase; pklr, pyruvate kinase liver and red blood cell; insig1, insulin-induced gene 1. Statistically significant differences between the chow and HLE diet groups are indicated by *P < 0.05.
Fig. 9. FASN, HMG-CoA reductase, and SREBP1 gene expression in adult offspring mouse livers from chow and HLE diet groups. Assays were performed in triplicate and the relative amount of each mRNA was normalized to β-actin. *P < 0.05 versus CD mice.

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