Maternal High Fat Feeding Increases Placenta Lipoprotein Lipase Activity by Reducing Sirt1 Expression in Mice

Liping Qiao1, Zhuyu Guo1, Chris Bosco1, Stefano Guidotti1, Yunfeng Wang2, Mingyong Wang3, Mana Parast4, Jerome Schaack5, William W. Hay, Jr.6, Thomas R. Moore7 and Jianhua Shao1

1Department of Pediatrics, University of California San Diego, La Jolla, California 92093; 2Department of Pediatrics, China-Japan Friendship Hospital, Beijing 100029; 3, Xinxian Medical University, Xinxiang, 453003; 4Department of Pathology, University of California San Diego, La Jolla, California 92093; 5Department of Microbiology, University of Colorado School of Medicine, Aurora, Colorado 80045; 6Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado 80045; 7Department of Reproductive Medicine, University of California San Diego, La Jolla, California 92093;

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Address correspondence to:
Jianhua Shao, M.D., Ph.D.
Department of Pediatrics
University of California San Diego
9500 Gilman Drive, MC 0983
La Jolla, CA 92093
Tel: (858)822-4720
Fax: (858)822-1966
Email: jishao@ucsd.edu

Abbreviations: GFP: green fluorescent protein; Sirt1: silent mating type information regulation 2 homology 1; PPARγ: peroxisome proliferator-activated receptor gamma; C/EBPα: CCAAT-enhancer-binding protein alpha; FA: fatty acid; FFA: free fatty acid; TG: triglyceride; LPL: lipoprotein lipase; VLDLr: very low density lipoprotein receptor; FABP3: fatty acid binding protein 3; FABPpm: plasma membrane fatty acid-binding protein; CD36: cluster of differentiation 36, a member of the class B scavenger receptor family; FASN: fatty acid synthase; SREBP1c: sterol regulatory element-binding protein 1c, also known as sterol regulatory element binding transcription factor 1; WT: wild type; HF: high fat.
Abstract

The objective of this study is to investigate how maternal overnutrition and obesity regulates expression and activation of proteins that facilitate lipid transport in placentas. To create a maternal overnutrition and obesity model, a high fat (HF) diet was provided to primiparous C57BL/6 mice throughout gestation. Fetuses from HF-fed dams had significantly increased serum free fatty acid and body fat. Despite no significant difference in placental weight, lipoprotein lipase (LPL) protein levels and activity were remarkably elevated in placentas from HF-fed dams. Increased triglyceride content and mRNA levels of CD36, VLDLr, FABP3, FABPpm and GPAT2&3 were also found in placentas from HF-fed dams. Although both PPARγ and C/EBPα protein levels were significantly increased in placentas of the HF group, only PPARγ exhibited a stimulative effect on LPL expression in cultured JEG-3 human trophoblasts. Maternal HF feeding remarkably decreased Sirt1 expression in placentas. By using a Sirt1 activator and inhibitor and cultured trophoblasts, an inhibitory effect of Sirt1 on LPL expression was demonstrated. We also found that Sirt1 suppresses PPARγ expression in trophoblasts. Most importantly, inhibition of PPARγ abolished the Sirt1-mediated regulatory effect on LPL expression. Together, these results indicate that maternal overnutrition induces LPL expression in trophoblasts by reducing the inhibitory effect of Sirt1 on PPARγ.
Introduction

Obesity is a risk factor of type 2 diabetes and cardiovascular diseases. Studies have demonstrated that pre-pregnant maternal body mass index (BMI) and gestational weight gain are closely associated with birth weight (1-3). Importantly, high birth weight predicts higher BMI and obesity during both childhood and adulthood (4,5). Therefore, in addition to calorie-rich foods, sedentary lifestyle and genetic defects, undesirable intrauterine metabolic exposure might contribute significantly to the ongoing obesity epidemic.

At birth, infant body weight is mainly determined by lean and fat tissue mass. Human studies have demonstrated that maternal obesity and excess gestational weight gain increase infant body fat and birth weight (1,3,6). Although rodent neonates have very limited amounts of fat tissue mass compared with humans, maternal high fat (HF) feeding increases fat tissue mass in both fetuses and newborn mice (7,8). Fetal lipid deposition increases exponentially with gestational age (9,10). Some of the accumulated lipids arise from de novo lipogenesis, but the great bulk of fetal lipids is derived from the maternal circulation through placental fatty acid (FA) transport (10).

Triglyceride (TG)-enriched VLDL in maternal circulation is the main FA supplier for the fetus (9,10). TG cannot be transported through the placenta. Only non-esterified FA can be taken up by the microvillous membrane and transported to the fetus. Therefore, FA from TG should be released by lipases at the placenta. Several TG lipases, including LPL and endothelial lipase, have been identified in human and rodent placentas (10-12).

LPL gene expression increases dramatically during the last trimester of pregnancy, paralleling with increased placental FA transport (12-14). Placental LPL expression and activity positively correlate with fetal size and fetal adipose tissue mass (14). Importantly, recent studies
have demonstrated that maternal obesity or high fat feeding increases placenta LPL expression and activity (15,16). Therefore, increased placental LPL may enhance fetal FA supply and increase fetal fat accretion. However, the mechanisms of maternal overnutrition and/or obesity - increased placental LPL expression are largely unknown.

SIRT1 (silent mating type information regulation 2 homology 1) is an NAD-dependent protein deacetylase that regulates energy metabolism, aging and other cellular processes (17). Energy deficiency increases NAD level or NAD/NADH ratio, leading to SIRT1 activation (18). In contrast, sufficient nutrient supply provides substrates to generate ATP while NAD is converted to NADH, leading to SIRT1 deactivation. In addition, overnutrition and obesity reduce SIRT1 expression in various tissues (17,19). In mammals, by controlling expression and/or acetylation of some transcription factors and enzymes, SIRT1 stimulates hepatic gluconeogenesis and suppresses lipogenesis in adipocytes (17,20-22). SIRT1 is highly expressed in trophoblasts (23,24). However, its regulatory role in placenta nutrient transport has yet to be studied.

By analyzing gene expression profiles between placentas from high fat (HF) diet and chow-fed dams, our study revealed that maternal HF feeding increased placenta LPL expression and activity accompanied with elevated PPARγ but decreased Sirt1 expression. Using cultured trophoblasts and Sirt1 or PPARγ-specific agonist and antagonist, our study further demonstrated that Sirt1 suppresses LPL expression through inhibiting PPARγ. These results led us to propose that maternal overnutrition decreases Sirt1 expression and consequently increases PPARγ activity and LPL expression in placenta.

Materials and Methods

Materials. Rosiglitazone, GW9662, resveratrol and 6-chloro-2,3,4,9-tetrahydro-1H-
carbazole-1-carboxamide (EX-527) were from Sigma (St. Louis, MO). Anti-LPL antibody was from GeneTex (Irvine, CA). The LPL activity assay kit was from Cell Biolabs, Inc (San Diego, CA). Antibodies for PPARγ, FASN and β-actin proteins were from Cell Signaling, Inc (Danvers, MA). Anti-Sirt1, C/EBPα and SREBP1c antibodies were from Santa Cruz Biotechnology (Dallas, TX). Fetal bovine serum, penicillin-streptomycin, Dulbecco’s modified Eagles’ medium (DMEM) and F-12K medium were from Invitrogen (Carlsbad, CA). HF diet (60 kCal% from fat, 20 kCal% from protein, 20 kCal% from carbohydrate, energy density: 5.24 kCal/g, catalog number D12492) was from the Research Diets, Inc (New Brunswick, NJ). Regular chow (17 kCal% from fat, 25 kCal% from protein, 58 kCal% from carbohydrate, energy density: 3.1 kCal/g, catalog number 7912) was from Harlan Laboratories (Madison, WI).

**Animal models.** C57BL/6 mice were from the Jackson Laboratory (Bar Harbor, ME). Three-month-old primiparous female mice were used as dams. Pregnancy was determined by the presence of a vaginal plug, and was assigned the title E0.5. HF diets were immediately provided to the vaginal plug-positive mice. The control dams were fed regular chow. Placentas and fetuses were collected by Caesarean section at E15.5, E17.5 and E18.5. After removing placentas and fetuses, dam body composition was determined using EchoMRI, which uses a specialized NMR-MRI-based technology and with a coefficient of variation at 3.71% (fat) or 4.55% (lean). Experiments using mouse models were carried out under the Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval from the University of California San Diego Animal Care and Use Committee.

**Blood glucose, FFA, TG and insulin assay.** Blood samples were collected from dams and fetuses by submandibular vein puncture or beheading, respectively. Serum glucose levels were measured using glucose oxidase reagents from Sigma (Saint Louis, MO). FFA and TG
levels were determined using kits from Wako (Richmond, Virginia). Serum insulin concentrations were measured using a mouse Diabetes 8-Plex kit and Bio-Plex MAGPIX reader (BioRad, Hercules, CA).

**Placental TG and fetal body fat measurement.** Placental tissues were homogenized in ice-cold phosphate-buffered saline. Placental lipids were extracted according to Bligh and Dyer (25), solvents were evaporated under nitrogen bath, and samples were re-dissolved in 2% Triton X (FisherBiotech, New Jersey) in water. A kit (Wako, Richmond, Virginia, US) was used to determine triglyceride content. Fetal body fat was determined by lipid extraction (26). Briefly, the fetal carcasses were dried at 103 °C till constant weight. The drying was followed by fat extraction with petroleum ether (Alfa Aesar, Heysham, England) in a Soxhlet apparatus (Cole Parmer, USA) for at least 10 cycles. Fat mass was calculated by the difference in dry mass of the carcass before and after the extraction.

**Western blot and real-time PCR assays.** Protein samples were separated using NuPAGE gels (Invitrogen). Protein was blotted with the indicated antibodies (see details in figure legends). The bands from Western blots were quantified using Quantity One software (Bio-Rad). Total RNA was prepared from placentas with Trizol following the manufacturer’s protocol (Invitrogen). cDNA was synthesized using SuperScript III Reverse Transcriptase and oligo(dT)\textsubscript{12-18} primer (Invitrogen). Real-time PCR was performed using an mx3000p Real-Time PCR system (Stratagene) and SYBR Green dye (Molecular Probes, Eugene, OR) with an annealing temperature at 60 °C and gene-specific primers (Table 1). The levels of PCR product were calculated from standard curves established from each primer pair. Expression data were normalized to the amount of 18S rRNA.

**Cell culture.** Human BeWo and JEG-3 trophoblasts were cultured in F-12K or DMEM
with 10% fetal bovine serum. Confluent cells were transduced with adenoviral vectors encoding Sirt1 or PPARγ for 24 hours. Ad-GFP was used as control. The construction and purification of the viral vectors were previously described (19).

Statistical analysis. Data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the Student t-test or ANOVA, followed by Bonferroni post-tests using Prism software. Differences were considered significant at p<0.05.

Results

Maternal HF feeding during gestation increases fetal body fat, placenta TG content and fetal serum FFA.

Pre-pregnant maternal obesity and excessive gestational weight gain associate with high birth weight (1-3). To focus on the effect of maternal overnutrition on fetal fat accumulation and to eliminate the effects of pre-existing maternal obesity on fetal development, HF diets were provided to dams only during the gestational period. Although energy intake of HF-fed dams was only slightly higher than that of controls (Supplemental Fig. 1a&b), remarkably elevated fat tissue mass was observed in HF-fed dams from E15.5 to E18.5 (Fig. 1a and Supplemental Fig. 1c). Similar to previous rodent studies which reported an adverse effect of maternal HF feeding on fetal growth (27-30), our study also found a trend of decrease in both body weight (Fig. 1b, p=0.0521) and lean body mass (Supplemental Fig. 1d) of E18.5 fetuses of HF-fed dams. There was no change in body fat content of E18.5 fetuses of HF-fed dams (Fig. 1c, p=0.5437). However, adiposity of E18.5 fetuses from HF-fed dams was significantly higher than that of control fetuses (Fig. 1d). At E15.5 and E17.5, there were no differences in either fetal body weight or adiposity (Supplemental Fig. 1e&f). Together, these data indicate that gestational period HF feeding induces both maternal and fetal adiposity in mice. However, the effect on fetal
adiposity happens at the late of gestation. For the rest of the study, only E18.5 fetal samples were analyzed.

To characterize the impact of maternal HF feeding on fetal metabolism, we measured metabolic parameters of blood and placentas. As shown in Fig. 1e-g, fetal serum free FA (FFA) of HF-fed dams was significantly increased, but TG and glucose levels were similar between the two groups. In maternal circulation, serum glucose and FFA levels were comparable between HF-fed and chow-fed dams (Fig 1h&i). However, there were significant decreases in TG levels of HF-fed dams (Fig. 1j). HF-feeding significantly increased insulin levels in maternal blood and but not in fetal circulation (Fig. 1k&l). Apparently, maternal HF-feeding alters glucose and lipid metabolism in both maternal and fetal compartment. However, the impacts on the major metabolic markers are different between fetuses and dams. These differences suggest that the effects of maternal obesity on fetal metabolism are not simply through a passive trans-placental nutrient diffusion. Interestingly, there was a slight increase of placental weight in the HF-fed group (Fig. 1m). However, placental TG from HF dams was remarkably higher than in the controls (Fig. 1n). The elevated fetal serum FFA and placenta TG content support the hypothesis that maternal HF feeding increases placental FA transport.

**Maternal HF feeding increases placental LPL expression and activity.**

To study the effect of maternal HF feeding on placental lipid metabolism, we compared mRNA levels of the key genes in these processes. Our results showed that mRNA levels of genes for TG hydrolysis and FA transport, such as LPL, CD36, VLDLr, FABP3 and FABPpm, were remarkably increased in placentas from HF-fed dams (Fig. 2a). The results of immunoblotting (Fig. 2b) also revealed a significant increase in LPL and CD36 protein in placentas from HF-fed dams. Consistent with elevated mRNA and protein levels, significantly-increased LPL activities
were detected in the placentas of HF-fed dams (Fig. 2c). Together, these results indicate that maternal HF feeding increases expression of proteins that facilitate TG hydrolysis, FA uptake and transport in placentas.

Our study also found that expression of rate-limiting enzymes of triacylglycerol synthesis glycerol-3-phosphate acyltransferase 2 (GPAT2) and GPAT3 were significantly increased by maternal HF feeding (Fig. 2a). Together with increased placental TG levels (Fig. 1n), these data suggest that maternal HF feeding increases placental FA transport and TG synthesis that may lead to higher placental TG content. However, unaltered fatty acid synthase (FASN) expression (Fig. 2a) implies that placental de novo lipogenesis is unlikely underlies maternal obesity-increased placental TG and fetal FFA.

Maternal HF feeding increases PPARγ expression leading to stimulation of LPL expression in trophoblasts.

Elevated mRNA levels of LPL and other genes in placentas from HF-fed dams suggest that the up-regulation may be at the transcriptional level. We measured protein levels of three key lipogenic transcription factors: PPARγ, C/EBPα and SREBP1c. Fig. 3a shows that both PPARγ and C/EBPα were significantly increased in placentas of HF-fed dams, while no change was apparent in mature SREBP1c.

The regulatory effects of PPARγ on LPL gene expression have been reported in adipocytes (31). To verify the role of increased PPARγ in maternal HF feeding-increased LPL expression in the placenta, we treated JEG-3 trophoblasts with the PPARγ agonist rosiglitazone (ROSI). Significantly high levels of LPL protein and activity were found in ROSI-treated JEG-3 cells (Fig. 3b&c), indicating that PPARγ stimulates LPL expression in trophoblasts.
We next studied the effect of C/EBPα on LPL gene expression using a C/EBPα-encoding adenovirus vector to transduce JEG-3 cells. As shown in Fig. 3d, LPL protein levels were similar between cells transduced with Ad-C/EBPα and Ad-GFP. This result indicates that, despite the maternal HF feeding increase in C/EBPα in placentas, C/EBPα does not increase LPL expression in trophoblasts. Together, these results suggest that increased PPARγ mediates maternal HF feeding-enhanced LPL expression in the placenta.

**Sirt1 inhibits LPL gene expression in JEG-3 trophoblasts.**

Sirt1 is a protein deacetylase that plays an important role in regulating cellular metabolism. Sirt1 expression is enhanced by fasting or calorie restriction and inhibited by HF feeding (22,32). As expected, significantly reduced Sirt1 protein and mRNA levels were found in placentas of HF-fed dams relative to controls (Fig. 4a&b), which indicates that maternal HF feeding reduces Sirt1 expression in placentas. This observation led us to hypothesize that decreased Sirt1 may be involved in maternal HF feeding-altered placental lipid metabolism.

To look at the regulatory effect of SIRT1 on LPL expression, mouse embryo fibroblasts (MEFs) from Sirt1−/− and wild type (WT) mice were used as a cellular model. We compared LPL expression levels between WT and Sirt1−/− MEFs. Remarkably higher levels of LPL mRNA were detected in Sirt1−/− MEFs than in WT cells (Fig. 4c), which suggests that Sirt1 suppresses LPL expression.

To further study the effect of Sirt1 on LPL expression in trophoblasts, we treated JEG-3 cells with the Sirt1 activator resveratrol (RSV) or the inhibitor 6-chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide (Ex-527). As shown in Fig. 4d, significantly reduced LPL protein was found in RSV-treated cells, while robustly increased LPL protein was observed in EX-527-treated cells. Parallel to the changes in LPL protein expression, remarkably altered LPL activities
were detected in RSV- or EX-527-treated JEG-3 cells (Fig. 4e). Together, these studies demonstrate that Sirt1 inhibits LPL expression in trophoblasts.

**Inhibition of PPARγ abolishes Sirt1-knocking-down-induced upregulation of LPL in JEG-3 cells.**

Results from the above studies demonstrate opposite effects of PPARγ and Sirt1 on LPL gene expression in trophoblasts. Interestingly, increased PPARγ but decreased Sirt1 proteins were observed in placentas from HF-fed dams (Fig. 3a&4a). Previous studies demonstrated that Sirt1 inhibits PPARγ expression and activity in adipocytes (22,33,34). Sirt1 and PPARγ can reciprocally regulate each other (34,35). This raises a question about the relationship of PPARγ and Sirt1 in maternal HF feeding-enhanced placental LPL expression.

Treatment of Sirt1−/− MEFs with the PPARγ agonist ROSI significantly increased LPL mRNA (Fig. 5a). This result indicates that PPARγ upregulates LPL expression independent of Sirt1. In addition, overnight ROSI treatment did not change Sirt1 protein levels in JEG-3 trophoblasts (Fig. 5b). In contrast, Sirt1 activation led to reduction of PPARγ protein levels in these cells (Fig. 4d). Together, these results suggest that Sirt1 inhibits PPARγ expression in trophoblasts. Therefore, we hypothesized that decreased Sirt1 mediates HF-feeding-induced placenta LPL expression by enhancing PPARγ activity.

We used Sirt1 gene knock-down and the PPARγ inhibitor GW9662 to test this hypothesis. Knock-down of Sirt1 significantly increased LPL gene expression (Fig. 5c). However, this stimulative effect was abolished in PPARγ inhibitor GW9662-treated cells, which suggests that Sirt1 regulates LPL gene expression through PPARγ. These results also support our hypothesis that maternal HF feeding increases PPARγ-controlled LPL expression by reducing Sirt1 expression in the placenta.
Discussion

Human fetal fat deposition increases exponentially with gestational age and most of the fat accumulation occurring during the third trimester. Unlike humans, mice are altricial having a very limited amount of fat tissue at birth. Despite the differences in fetal fat deposition, most human and mouse studies have demonstrated a positive association of maternal adiposity with fetal fat accumulation. In this study, all dams had similar pre-pregnant body fat levels (data not shown) and HF diet was provided only during the gestational period. Although total energy intakes were similar, HF-fed dams gained significantly more body fat. These data indicate that HF feeding during gestation induces maternal obesity in mice. The results of significantly increased adiposity of E18.5 fetuses further demonstrate that HF feeding or maternal obesity increases fetal fat in mice. An interesting finding of this study is that maternal HF feeding of mice has no significant effect on placental weight, while some studies found that maternal BMI correlates with placenta weight which is predictive of neonatal body weight (36,37). The discordant changes in fetal body fat and placental mass from HF fed dams led us to postulate that enhanced nutrient transport activity, other than placenta tissue mass, plays an important role in HF feeding or maternal obesity-induced fetal fat accumulation. The remarkably increased fetal blood FFA and placental TG content from HF-fed dams support this notion.

In the placenta, LPL is mainly expressed in syntotrophoblasts and catabolize TG from maternal blood. LPL gene expression and activity increase dramatically during the last trimester of pregnancy, correlating with increased placental FA transport (11,12,38). In line with two recent studies (15,39), our study showed that maternal HF feeding increases placental LPL activity. In addition, expression of key genes for placenta lipid transport, such as CD36 and VLDLr, were concurrently elevated in placentas of HF-fed dams. These results prompt us to propose that
increased expression and activity of LPL and other proteins enhance maternal-fetal FA transport, which contributes to maternal overnutrition-induced fetal fat accumulation.

While placental LPL expression positively correlates with fetal fat accumulation and newborn body weight, very limited mechanistic information is available regarding how maternal obesity and/or HF feeding increase LPL expression and activity in placentas. Regulation of LPL gene expression has been extensively studied in adipose tissue, skeletal muscle and heart. PPARγ is a nuclear receptor well known for its key role in lipogenesis. Similar other types of cells (31), our study demonstrated that PPARγ increases LPL expression in trophoblasts. Most importantly, our results also showed that maternal HF feeding increases PPARγ protein levels in placentas, which suggests that increased PPARγ might mediate maternal HF feeding-induced LPL expression in placentas. However, although increased LPL activity was found in placentas of obese mothers, no increase in PPARγ mRNA or protein were found in a human study (15). In addition, increased PPARγ was found only in mid gestation placentas of diet-induced obese ewes (40). These discrepancies may be due to differences in species and induction of maternal obesity.

The regulatory effects of Sirt1 on metabolism were initially observed in aging studies of calorie-restricted rodents. Although the beneficial effects of calorie restriction (CR) and Sirt1 on aging are still uncertain in mammals, CR-induced Sirt1 expression and activation are well conserved in many species. Previous studies have reported that prolonged CR reduces LPL gene expression in several tissues (41,42). In addition, significantly elevated LPL expression was found in hepatocytes in which Sirt1 was genetically reduced (43). These observations imply a connection between Sirt1 and LPL expression. By using Sirt1 activator and inhibitor, our study demonstrates that Sirt1 inhibits LPL expression in mammalian cells, including trophoblasts. Sirt1 is a protein deacetylase that regulates gene expression mainly through controlling transcription
factor activity. Consistent with previous studies (22,35,44), we found that Sirt1 reduces PPARγ in trophoblasts. Most importantly, PPARγ inhibition abolished Sirt1 knock down-enhanced LPL expression. These data indicate that Sirt1 regulates LPL expression through PPARγ.

During pregnancy, there is a significant adjustment of maternal lipid metabolism. From the middle gestation to the third trimester maternal lipid metabolism switches from anabolic to catabolic. Increased lipolysis provides FAs and glycerol for maternal hepatic VLDL synthesis, which creates a hyper-triglyceridemia in maternal circulation (38). Similar to humans, our study showed that late pregnant mice have significantly higher blood TG concentrations (data not shown). To our surprise, HF feeding does not further increase, actually but reduced maternal blood TG levels in mice (Fig. 1j). Interestingly, similar to other rodent studies (27-30), we also found that HF feeding even slightly reduces fetal body weight. Human studies have demonstrated that maternal blood TG levels correlate with fetal growth (45-47). Therefore, we speculate that decreased maternal blood TG may underpin the decreased fetal growth in HF-fed rodents (27-30). Another possibility is low dietary protein supply in HF-fed mice. We found that the daily food intake of the HF group was ~30% less than controls, despite similar in total caloric intake (Supplemental Fig 1a&b). In addition, the HF diet contains 5% less of proteins compared with chow. A separate study is investigating the mechanisms through which HF feeding reduces maternal blood TG and fetal growth. The intriguing observation of reduced blood TG in HF-fed dams raises another question about the lipid source of increased placental TG and fetal fat. The current study does not provide data to directly answer this question. However, similar levels of FASN and SREBP1c in placentas between HF and control groups do not support any contribution of de novo lipogenesis to these processes. We postulate that despite maternal HF feeding significantly decreases TG levels in maternal circulation, gestation-increased blood pool
and decreased TG/FA uptake in other peripheral tissues might still provide sufficient TG for fetal supply. Significantly elevated LPL and other genes for FA transport in placentas of HF-fed dams increase the efficiency of TG hydrolysis and FA uptake in the placenta.

In summary, using a pregnant mouse model, we found that maternal HF feeding increases fetal fat accumulation. Elevated placental LPL activity and expression of genes that facilitate placental lipid transport suggests that enhanced placental lipid transport may play a key role in maternal overnutrition and obesity-induced fetal fat accumulation. By using cultured trophoblasts, our study further demonstrates that Sirt1 inhibits LPL gene expression, while maternal HF feeding reduces Sirt1 protein levels in placentas. Inhibition of PPARγ attenuated the stimulative effect of Sirt1-knock down of LPL expression. Therefore, we propose that maternal overnutrition and/or obesity reduce placental Sirt1 leading to increase PPARγ transactivity and LPL expression in trophoblasts.

**Author Contributions**

L.Q., C.B., Z.G., S.G. Y.W. and B.L. contributed research data. J.Sch. created adenovirus vectors and manuscript writing. M.P., W.W.H.Jr. and T.R.M, contributed to research design and discussion. J.Sh. designed the study and wrote the manuscript

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References


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**Figure Legend**
**Fig. 1. Maternal HF feeding during gestation period increased fetal body fat and placental TG content.** 10-12 week-old nulliparous C57BL/6 female mice were mated with chow-fed males. HF diet was provided to dams once the vaginal plug was detected. Fetuses, placentas and other tissue samples were collected at E18.5 at fed state through C-section. Increased body weight (BW) and body fat were assayed in HF-fed dams by EchoMRI scanning (a, n=6-8). Fetal body fat was measured using petroleum ether fat extraction. Although there were no remarkable differences in fetal body weight (b) and body fat (c), significantly elevated adiposity (d) was observed in fetuses from HF-fed dams (n=20-36). Compared with fetuses of chow-fed dams, remarkably increased serum FFA levels (e), but not TG (f) and glucose (g), were detected in fetuses of HF-fed dams (n=8). Comparing HF-fed and chow-fed dams, there were no significant differences in blood glucose (h) and FFA (i). However, significantly decreased serum TG and increased insulin levels were found in HF-fed dams (j&k). Interestingly, fetal serum insulin concentrations were comparable between these two groups (l). There was no difference in placental weight (m), but placental TG content was significantly higher in the HF group (n, n=6).

**Fig. 2. Maternal HF feeding increased placental LPL expression and activity and genes that facility lipid transport.** E18.5 placental samples were collected from chow- or HF-fed dams. Using real-time PCR, significantly increased mRNA of LPL and other key genes that facilitate lipid transport was detected in placentas from HF-fed dams (a, n=6). Western blotting further revealed higher protein levels of LPL and CD36 in placentas from HF-fed dams (b, n=7). Parallel with the increased gene expression, significantly elevated LPL activities were found in placentas of HF-dams (c, n=8).

**Fig. 3. HF feeding during pregnancy increased PPARγ expression in placentas and**
**PPARγ stimulated LPL expression in trophoblasts.** Significantly increased PPARγ and C/EBPα protein levels were revealed in placentas of HF-fed dams (a, n=7-8, *p*<0.05 vs. control). Overnight (13h) treatment with the PPARγ agonist rosiglitazone (ROSI, 10 µM) robustly increased LPL expression and activities in confluent JEG-3 trophoblasts (b&c, n=6). However, adenovirus vector-mediated C/EBPα over-expression (24h) did not alter LPL protein levels in JEG-3 trophoblasts (d, n=6).

**Fig. 4. Maternal HF feeding reduced expression of Sirt1, which suppresses LPL expression in trophoblasts.** Western blotting and real-time PCR demonstrated remarkably reduced Sirt1 protein (a) and mRNA levels in placental samples of E18.5 HF-fed dams (n=6). Compared with WT MEFs, significantly higher LPL mRNA levels were found in Sirt1−/− MEFs (c, n=6). Confluent JEG-3 trophoblasts were treated with SIRT1 activator RSV (10 µM) or inhibitor EX-527 (1 µM) overnight (13h). EX-527 treatment robustly increased LPL protein levels and activities (d&e). In contrast, significantly reduced LPL protein levels and activities were found in RSV-treated trophoblasts (d&e, n=6).

**Fig. 5. SIRT1 inhibits LPL expression by suppressing PPARγ expression in trophoblasts.** To verify the relationship between SIRT1 and PPARγ in regulating LPL expression, Sirt1−/− MEFs (a) and JEG-3 trophoblasts (b) were treated with PPARγ agonist ROSI (10 µM) for 13 hours. ROSI treatment robustly increased LPL mRNA in Sirt1−/− MEFs (a, n=6) but showed no effect on SIRT1 protein expression in trophoblasts (b, n=6). By using viral vector mediated siRNA overexpression, Sirt1 was significantly knocked down in JEG-3 trophoblasts (c). However, increased LPL expression due to Sirt1 knock down was abolished in PPARγ-inhibitor GW9662 (3 µM, 13-hour)-treated cells (c, n=6).
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Fig. 1a

Dam body composition (g)

- Con
- HF

Fig. 1b

Fetal weight (g)

- Con
- HF

Fig. 1c

Fetal fat (mg)

- Con
- HF

Fig. 1d

Fetal fat (% of body weight)

- Con
- HF

Fig. 1e

Fetal serum FFA (mEq/L)

- Con
- HF

Fig. 1f

Fetal serum TG (mg/dl)

- Con
- HF

Fig. 1g

Fetal serum glucose (mg/dl)

- Con
- HF

Fig. 1h

Maternal serum glucose (mg/dl)

- Con
- HF

Fig. 1i

Maternal serum FFA (mEq/L)

- Con
- HF
Fig. 1j: Maternal serum TG

Fig. 1k: Maternal serum insulin

Fig. 1l: Fetal serum insulin

Fig. 1m: Placenta weight

Fig. 1n: Placenta TG
Fig. 3a

**Protein levels (arbitrary units)**

**Con**

- PPARγ
- C/EBPα
- SREBP1c
- β-actin

**HF**

- PPARγ
- C/EBPα
- SREBP1c
- β-actin

Fig. 3b

**LPL protein levels (arbitrary units)**

**Con**

- LPL
- β-actin

**ROSI**

- LPL
- β-actin

* p<0.01

Fig. 3c

**LPL activity (Units/mg protein)**

**Con**

**ROSI**

* p<0.01

Fig. 3d

**LPL protein levels (arbitrary units)**

**Ad-GFP**

- LPL
- C/EBPα
- β-actin

**Ad-C/EBPα**

- LPL
- C/EBPα
- β-actin

NS
Fig. 4a

**Sirt1**

<table>
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**β-actin**

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**Sirt1 protein levels** (arbitrary units)

![Graph](image5.png)

* p<0.05

Fig. 4b

**Sirt1 mRNA levels** (arbitrary units)

![Graph](image6.png)

* p<0.05

Fig. 4c

**LPL mRNA levels** (arbitrary units)

![Graph](image7.png)

* p<0.001

Fig. 4d

**LPL**

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**PPARγ**

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**β-actin**

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**Protein levels** (arbitrary units)

![Graph](image17.png)

* p<0.05

Fig. 4e

**LPL activity** (Units/mg protein)

![Graph](image18.png)

* *
Fig. 5a: LPL mRNA levels (arbitrary units)

![Graph showing LPL mRNA levels with Con and ROSI conditions, p<0.01](image)

Fig. 5b: Sirt1 protein levels (arbitrary units)

![Graph showing Sirt1 protein levels with Con and ROSI conditions](image)

Fig. 5c: Table and Western blot analysis

<table>
<thead>
<tr>
<th>Condition</th>
<th>GW9662</th>
<th>DMSO&amp;Ad-GFP</th>
<th>Ad-Sirt1 siRNA</th>
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</thead>
<tbody>
<tr>
<td>Con</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ROSI</td>
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<td>-</td>
<td>+</td>
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</tbody>
</table>

Western blot analysis:
- **LPL** 72 kDa
- **Sirt1** 120 kDa
- **β-actin** 45 kDa

![Graph showing protein levels with Con, Ad-Sirt1 siRNA, Ad-Sirt1 siRNA+GW9662](image)
Legend for supplemental figure

Fig 1. Maternal HF feeding and body composition of fetuses and dams. 10-12 week-old nulliparous C57BL/6 female mice were mated with chow-fed males. HF diet was provided to dams during gestation. From E10-E18, the daily food intakes of HF-fed mice were significantly lower than that of controls (a). However, total energy intakes were comparable (b). HF-feeding increased body fat mass of dams at both E15.5 and E17.5 (c). Maternal HF feeding slightly reduced dry lean body mass of fetuses at E18.5 (d). However, no significant effects on fetal weight (e) and adiposity (f) were found at E15.5 and E17.5.