

Maternal obesity restricts fetal growth through placental energy failure and iron-associated oxidative stress in mice[†]

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Abstract

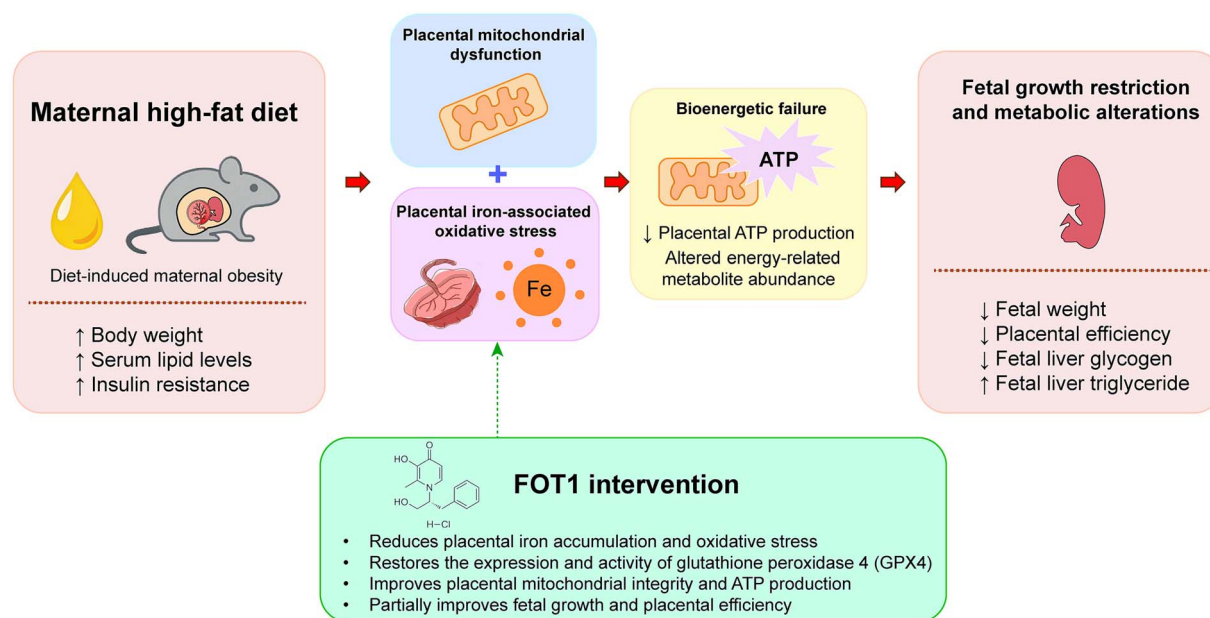
Maternal obesity compromises placental development and fetal growth, yet the mechanisms remain unknown. Here, we investigated the impact of high-fat diet (HFD) on placental energy homeostasis in mice. Female C57BL/6 mice were fed a control diet or an HFD, with a subset receiving FerroTerminator-1 (FOT1) to modulate metabolic dysfunction. At embryonic day 18.5, maternal metabolic status, fetal growth, placental morphology, energy metabolism, and oxidative stress were assessed. HFD-fed dams developed dyslipidemia and insulin resistance, and exhibited placental lipid accumulation and impaired labyrinth zone development, resulting in reduced fetal weight and altered fetal liver lipid and glycogen contents. The placenta showed mitochondrial damage, accompanied by reduced adenosine triphosphate (ATP) content, mitochondrial membrane potential, mitochondrial DNA copy number, and suppressed respiratory chain complex activities. Placental metabolomic profiling showed that HFD was associated with altered levels of metabolites in glycolysis and oxidative phosphorylation pathways. These alterations coincided with placental iron-associated oxidative stress, evidenced by elevated non-heme iron, lipid peroxidation products, upregulation of transferrin receptor and lysophosphatidylcholine acyltransferase 3, downregulation of ferritin heavy chain 1 and solute carrier family 40 member 1, a reduced glutathione to oxidized glutathione ratio, and impaired glutathione peroxidase 4 expression and activity. Meanwhile, FOT1 administration attenuated placental oxidative stress, partially restored ATP production and mitochondrial function, and improved fetal growth in HFD-fed mice. Collectively, maternal obesity was associated with placental iron-associated oxidative stress and bioenergetic impairment, which may contribute to reduced placental efficiency and fetal growth restriction.

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Graphical Abstract

Maternal high-fat diet links placental iron-associated oxidative stress to bioenergetic impairment and fetal growth restriction



Summary Sentence

Maternal obesity driven by a high-fat diet is associated with placental lipid overload and iron-associated oxidative stress, which compromise mitochondrial energy metabolism, placental efficiency, and fetal growth.

Key words: maternal obesity, placenta, energy metabolism, fetal growth restriction.

Introduction

Maternal obesity has emerged as a major global public health concern that compromises reproductive health and pregnancy outcomes [1, 2]. Chronic consumption of energy-dense, high-fat diet (HFD) disrupts maternal metabolism and reproductive physiology [3]. Epidemiological projections estimate that by 2030, more than 1.1 billion adults will be obese, including approximately 643 million women, highlighting the widespread prevalence of obesity among women of reproductive age [4]. During pregnancy, diet-induced metabolic disturbances alter the intrauterine milieu, contributing to placental dysfunction, fetal growth restriction (FGR), and an increased susceptibility to metabolic disorders in the offspring later in life [5, 6].

As the interface for maternal-fetal exchange and endocrine regulation, the placenta relies on energy homeostasis to sustain fetal development [7]. Placental energy homeostasis includes both energy production, which involves glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation, and energy utilization (nutrient transport, anabolic processes, and trophoblast development) [8]. Evidence has indicated that maternal obesity compromises placental energy homeostasis and is closely associated with adverse pregnancy outcomes, including FGR [9–11]. Specifically, metabolic stress induced by HFD intake disrupts this balance, leading to maternal metabolic syndrome, placental

mitochondrial dysfunction, reduced adenosine triphosphate (ATP) generation, and impaired nutrient transport capacity [10, 12, 13]. However, the mechanisms linking maternal metabolic disorder to placental energy failure remain unknown.

HFD-induced lipid overload enriches cellular membranes with polyunsaturated fatty acids, rendering them highly susceptible to iron-catalyzed lipid peroxidation [14]. HFD has been reported to disrupt systemic iron homeostasis, leading to excessive iron accumulation in multiple metabolic tissues and organs, including blood, adipose tissue, liver, kidney, and heart, thereby promoting Fenton chemistry and reactive oxygen species (ROS) generation [15–18]. Similarly, previous studies have found placental iron-associated oxidative stress in pregnancy complications such as preeclampsia and FGR [19–21]. Oxidative stress disrupts mitochondrial integrity and impairs oxidative phosphorylation, thereby exacerbating defects in cellular energy metabolism [22]. FerroTerminator-1 (FOT1, also known as CN128) has been used as an experimental modulator of genetic and HFD-induced metabolic disturbances through iron-chelating and lipid peroxidation suppression [15, 23].

In the present study, the effects of maternal obesity on placental lipid accumulation, energy homeostasis, iron-associated oxidative stress, and fetal growth were investigated in a mouse model. We further evaluated whether modulation of metabolic

stress could improve placental energy supply and fetal growth in maternal obesity.

Materials and methods

Ethics

This study was approved by the Institutional Animal Care and Use Committee of Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS2025-17).

Animal model and treatment

Eight-week-old female C57BL/6J mice were housed under controlled temperature (23°C) and humidity conditions with a 12-h light/dark cycle and free access to food and water. All animals were acclimatized to the same housing and feeding conditions for 1 week before the start of experimental procedures. Twenty-four female C57BL/6 mice were randomly assigned to receive either a control diet (CON, XTADG001, Xietong Shengwu Co. Ltd., China), a high-fat diet (HFD; 60% energy from fat, D12492, Research Diets, USA), or an HFD diet supplemented with FOT1 during pre-gestation (before mating for 28 days), and post-mating (18 days). FOT1 was dissolved in DMSO and diluted with saline for gavage, with a final DMSO concentration of 5% (v/v). The CON and HFD groups received the corresponding vehicle, whereas the FOT1 group received FOT1 (20 mg/kg/day) by gavage once every 2 days, beginning 28 days before mating and continuing throughout gestation until E18.5 d. The dosage of FOT1 was selected with reference to previous research [15]. Before mating, female mice were fed for 28 days. On day 28, they were housed with male mice overnight at a ratio of 2:1 per cage. The presence of a vaginal plug the following morning was considered indicative of successful mating and designated as embryonic day 0.5 (E0.5 d). Maternal body weight was recorded weekly during the 4-week pre-mating period. During pregnancy, weights were measured at E0.5 d, E1 wk, E2 wk, and E18.5 d.

Tissue collection

On E18.5 d, pregnant mice were euthanized by CO₂ inhalation after fasting for sample collection. Blood, maternal adipose tissue, liver, placentas, fetuses, and fetal livers were collected. Fetuses were weighed before fetal liver collection. Fetal liver samples used for biochemical assays were pooled within each litter and used as one litter-level biological replicate. Placental samples were allocated for hematoxylin and eosin (H&E), Oil Red O staining, transmission electron microscopy (TEM), biochemical, oxidative stress, mitochondrial, and molecular assays.

Serum, placental, and fetal liver biochemical analysis

Serum levels of triglycerides (TG, A110-1-1), total cholesterol (TC, A111-1-1), free fatty acids (FFA, A042-1-1), glucose (A154-1-1), and insulin (H203-1-2) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Glucose tolerance tests (GTTs) were performed after an overnight (12 h) fasting on E16.5 d. An injection of glucose solution was given at a dose of 2.0 g/kg body weight. Blood glucose was measured from the tail vein using a handheld glucometer at baseline (0 min) and at 15, 30, 60, and 120 min

after administration. Insulin tolerance tests (ITTs) were carried out following a 5-h fasting on E18.5 d. Mice received a single intraperitoneal injection of insulin (1.0 U/kg), and blood glucose was monitored at different time points. Placenta from each litter were randomly collected and homogenized to determine placental lipid levels (TG, TC, and FFA). Fetal liver TG content was measured using the same commercial TG assay kit described above. Glycogen content was measured using a commercial assay kit (BC0345, Solarbio, China) according to the manufacturer's instructions.

Histological analysis of adipose tissue, liver, and placenta

Subcutaneous fat, inguinal fat, liver, and placenta samples were excised and weighed. Subsequently, subcutaneous fat, liver, and placenta samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with H&E. For lipid assessment, a subset of fresh liver or placenta tissues were frozen at -80°C, cut into cryosections, and subjected to Oil Red O staining. Images were captured using a Leica microscope (DM3000, Germany). For maternal adipose and liver histological analyses, tissue samples from six dams per group were analyzed, and each dam was treated as one biological replicate. For placental H&E and Oil Red O analyses, placentas were selected from independent litters, and each placenta was treated as one biological replicate. Morphometric analyses, including lipid cell size, hepatic vacuole area, labyrinth zone proportion in the placenta, and Oil Red O regions, were quantified with ImageJ software (v1.53).

Litter performance

The placenta was carefully separated from the maternal decidua tissue and collected by cesarean section at E18.5 d. The total number of viable fetuses (litter size) was recorded for each dam. Fetal viability was assessed after uterine dissection based on gross morphology, as described previously [24]. Briefly, fetuses with intact morphology, normal gross appearance, and no signs of resorption or maceration were counted as viable. Resorption sites and fetuses showing degeneration were excluded from the viable litter size. The placenta was cleared of the umbilical cord and fetal membranes, then blotted dry using filter paper to ensure the removal of residual blood and fluids before analysis. The total fetal weight and total placental weight per litter were measured. The average fetal and placental weights were calculated by dividing the total weight by the litter size of the respective dam. Placental efficiency was determined as the ratio of average fetal weight to average placental weight.

Placental energy metabolism

Mitochondrial membrane potential of fresh placenta was assessed using a JC-1 assay kit according to the manufacturer's instructions (M8650, Solarbio, China). Briefly, fresh placental tissue was processed at low temperature, homogenized, and subjected to differential centrifugation to obtain mitochondrial fractions. The mitochondria were incubated with JC-1 working solution, and fluorescence intensity was measured using a fluorescence microplate reader. Mitochondrial membrane potential was calculated as the red/green fluorescence ratio of JC-1 aggregates to monomers. Placental ATP content was measured using an ATP content kit (BC0300, Solarbio,

China). The placental activities of respiratory chain complexes I–V were determined using the mitochondrial complex reductase activity assay kit from the same supplier (BC0510, BC3230, BC3240, BC0940, BC1440). Briefly, mitochondrial fractions were disrupted on ice to release mitochondrial proteins and then used for enzymatic measurements. Enzyme activities were calculated according to the manufacturers' instructions.

DNA isolation and mtDNA copy number assay

Placental total DNA was extracted using a genomic DNA extraction kit following manufacturer's instructions (Takara, Japan). Relative mitochondrial DNA (mtDNA) copy number was determined by quantitative polymerase chain reaction (PCR), and the mtDNA copy number was calculated as the ratio of mitochondrial DNA to nuclear DNA as previously described [25]. The sequences of the primers are shown in [Supplementary Table 1](#).

Iron-associated oxidative stress

Serum and placental non-heme iron levels were measured using a ferric/ferrous ion colorimetric assay kit (S1066S, Beyotime Biotechnology, China). Glutathione (GSH), oxidized glutathione (GSSG), malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE) contents were determined using commercial kits (A061-1-2, A003-1-2, H268-1-2, Nanjing Jiancheng Bioengineering Institute, China). Glutathione peroxidase 4 (GPX4) activity was measured by commercial kits (BC6260, Solarbio, China). Tissue samples were processed in accordance with the manufacturers' protocols to minimize oxidation during preparation.

Transmission electron microscopy

The TEM analysis was performed using placentas from three independent litters per group. Placental tissues were initially fixed in 2.5% glutaraldehyde for over 48 h, followed by post-fixation with 1% osmium tetroxide. Samples were then dehydrated at low temperature through a graded ethanol series, and subsequently treated with acetone for dehydration, embedding, and polymerization at room temperature. Ultrathin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and imaged using a JEM-1011 TEM (JEOL, USA). Digital images were acquired with a Hitachi camera system (HITACHI, Japan). Placental mitochondrial ultrastructure was examined by TEM. The degree of mitochondrial injury was assessed according to the Flameng scoring system [26]: 0, normal ultrastructure; 1, mild swelling, decreased matrix density, partial cristae separation; 2, reduced matrix density, transparent matrix, cristae separation without rupture; 3, severe swelling with cristae rupture and matrix coagulation; 4, loss of inner/outer membrane integrity, extensive vacuolization, severe swelling, and cristae rupture. Three different fields were captured from each placenta, and the mean value for each placenta was used as one biological replicate for statistical analysis.

Western blotting

Placental tissues were rinsed in ice-cold PBS and homogenized in protein lysate buffer containing protease inhibitors. Protein concentrations were determined using a protein assay kit (23225, Thermo Fisher, USA). Standardized amounts of protein were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred to

polyvinylidene difluoride (PVDF) membranes. Membranes were incubated with the following primary antibodies: transferrin receptor (TFRC, 32333-1-AP, Proteintech), acyl-CoA synthetase long-chain family member 4 (ACSL4, 22401-1-AP, Proteintech), solute carrier family 40 member 1 (SLC40A1, 26601-1-AP, Proteintech), ferritin heavy chain 1 (FTH1, 11682-1-AP, Proteintech), lysophosphatidylcholine acyltransferase 3 (LPCAT3, 67882-1-Ig, Proteintech), GPX4 (67763-1-Ig, Proteintech), and β -actin (66009-1-Ig, Proteintech). The optical density of the signals was quantified using ImageJ software.

Immunofluorescence analysis

Placental sections were deparaffinized, rehydrated, and subjected to antigen retrieval. After blocking with QuickBlock blocking buffer (Beyotime), sections were incubated overnight at 4°C with primary antibodies against TFRC, ACSL4, GPX4, and translocase of outer mitochondrial membrane 20 (TOMM20, 11802-1-AP, Proteintech). The sections were then incubated with fluorescent secondary antibodies for 1 h at 37°C. Nuclei were counterstained with DAPI for 5 min at room temperature. Images were acquired from the labyrinth zone, which was identified under low magnification based on placental morphology using a fluorescence microscope (Leica, Germany). Fluorescence intensity was quantified using ImageJ software.

Metabolomics analysis

For metabolite extraction, frozen placental tissue was transferred into centrifuge tubes containing grinding beads. Each sample was mixed with extraction solvent (methanol: water = 4:1, v/v) and homogenized using a cryogenic tissue grinder. The homogenates were then subjected to low-temperature ultrasonic extraction. The supernatant was used for analysis by UHPLC-Orbitrap Exploris (Thermo Fisher, USA). Chromatographic separation was performed on an HSS T3 column (100 mm × 2.1 mm, 1.8 μ m). The mobile phases consisted of solvent A (0.1% formic acid in water/acetonitrile, 95:5, v/v) and solvent B (0.1% formic acid in acetonitrile/isopropanol/water, 47.5:47.5:5, v/v/v). The flow rate was maintained at 0.40 mL/min, with the column temperature set at 40°C. Mass spectrometry was carried out in both positive and negative ionization modes, scanning within an m/z range of 70–1050. The ion spray voltages were 3500 V (positive) and –3000 V (negative), with sheath gas and auxiliary gas flow rates set at 50 and 13 arbitrary units, respectively. The ion source was operated at 20–40–60 V with stepped collision energy. Raw LC–MS data were processed using Progenesis QI software (Waters, USA) for peak alignment, normalization, and identification. Multivariate statistical analysis was performed using Partial Least Squares Discriminant Analysis (PLS-DA) to visualize group separation. Metabolites with VIP values >1.0 in Orthogonal PLS-DA and $P < 0.05$ in univariate testing were considered significantly different. These differential metabolites were subsequently subjected to pathway enrichment using the Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg/>).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 10). All datasets reflected mean $\pm$ standard error of the mean (SEM). Each dam/litter was used as the

experimental unit, and data are expressed as the mean value per litter for statistical analysis. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

Results

Maternal HFD induces obesity and metabolic dysfunction during gestation

As shown in [Figure 1A](#), twenty-four 8-week-old female C57BL/6 mice were assigned to a control diet (CON group, $n=8$), a high-fat diet (HFD group, $n=8$), or an HFD supplemented with the experimental metabolic modulator FOT1 (FOT1 group, $n=8$) during both pre-mating and pregnancy. Maternal obesity was induced by 4 weeks of HFD feeding before mating and maintained throughout gestation until E18.5 d. Compared with the CON-fed dams, HFD-fed dams had significantly elevated serum levels of FFA, TC, and TG on E18.5 d ($P < 0.05$, [Figure 1B–D](#)). Body weight had begun to differ significantly between groups since 1 week before gestation, with HFD mice showing higher body weight relative to the CON group ($P < 0.05$, [Figure 1E and F](#)). Consistently, maternal body weight at E0.5 d and E18.5 d was significantly higher in the HFD groups compared to the CON group ($P < 0.05$, [Figure 1G and H](#)). These results showed that HFD constructed a maternal obesity model.

On E18.5 d, fasting glucose and insulin levels were significantly increased in the HFD group ($P < 0.05$, [Figure 1I and J](#)). Moreover, HFD increased the area under the curve (AUC) during GTT and ITTs, indicative of impaired glucose tolerance and insulin resistance ($P < 0.01$, [Figure 1K–N](#)). HFD significantly increased the weight of subcutaneous and inguinal fat depots, whereas liver weight remained unchanged among groups ($P < 0.01$, [Figure 1O](#)). Histological analysis demonstrated that HFD increased the average area of lipid cells in the subcutaneous fat ($P < 0.01$, [Figure 1P and S](#)). H&E and Oil Red O staining showed that HFD increased the hepatic vacuolization and lipid droplet area ($P < 0.01$, [Figure 1Q, R, and T](#)).

Moreover, alleviation of metabolic disorders with FOT1 administration did not affect maternal body weight gain, but partially attenuated HFD-induced high serum TG, insulin resistance, and hepatic lipid accumulation ($P < 0.05$), supporting its use as a functional modulator in the HFD-induced metabolic stress.

Maternal obesity increases placental lipid accumulation, reduces placental efficiency, and impairs fetal growth and hepatic metabolism

There was no difference in litter size between groups ([Figure 2A](#)). Compared with CON, HFD significantly increased average placental diameter and placental weight, but decreased average fetal weight and placental efficiency ($P < 0.05$, [Figure 2B–E](#)). Biochemical analysis revealed that placental FFA and TG contents were markedly increased in the HFD group ($P < 0.01$, [Figure 2F–H](#)). Moreover, histological assessment showed HFD feeding decreased the relative labyrinth zone area of the placenta ($P < 0.01$, [Figure 2I and J](#)). Oil Red O staining further revealed extensive lipid droplets in the placenta of HFD-fed dams ($P < 0.01$, [Figure 2K and L](#)). HFD reduced fetal liver glycogen content ([Figure 2M](#), $P <$

0.05) and increased hepatic TG accumulation ([Figure 2N](#), $P < 0.05$).

Specifically, metabolic modulation by FOT1 significantly increased fetal weight, placental efficiency, and fetal liver glycogen toward control levels, reduced placental lipid accumulation, and preserved labyrinth zone structure ($P < 0.05$).

Maternal obesity exhibits placental iron accumulation and oxidative stress

The serum non-heme iron concentration did not differ among groups ([Figure 3A](#)). In contrast, placental non-heme iron content was significantly elevated in the HFD group compared with CON group ($P < 0.05$, [Figure 3B](#)), indicating tissue-specific iron accumulation under maternal metabolic stress. Attenuation of metabolic stress using FOT1 significantly attenuated this increase, restoring placental non-heme iron levels toward those observed in control dams. HFD reduced the GSH level and elevated the GSSG level, thereby decreasing the GSH/GSSG ratio, and FOT1 attenuated the ratio toward control levels ($P < 0.05$, [Figure 3C–E](#)). The activity of GPX4 was decreased in HFD group, and FOT1 increased the GPX4 activity ($P < 0.05$, [Figure 3F](#)). Placental 4-HNE and MDA levels were elevated in the HFD group, while FOT1 significantly reduced their production ($P < 0.01$, [Figure 3G and H](#)). At the protein level, HFD increased the expression of TFRC, a key regulator of cellular iron uptake, suggesting enhanced placental iron import under HFD conditions, and FOT1 significantly attenuated TFRC upregulation ($P < 0.05$, [Figure 3I and J](#)). Also, HFD reduced both FTH1 and SLC40A1 protein expression, and FOT1 only increased FTH1 expression ($P < 0.05$). Expression of LPCAT3, a lipid remodeling enzyme associated with polyunsaturated phospholipid enrichment, was also increased in the HFD group ($P < 0.05$). Conversely, antioxidant enzyme GPX4 was downregulated by HFD exposure but preserved in the FOT1 group ($P < 0.05$). Immunofluorescence staining further showed that HFD increased TFRC fluorescence intensity and reduced GPX4 intensity in the labyrinth zone ($P < 0.05$, [Figure 3K and L](#)), whereas ACSL4 fluorescence intensity was not significantly altered. FOT1 administration significantly normalized TFRC and GPX4 fluorescence intensity to levels comparable with the control group ($P < 0.05$).

Maternal obesity leads to structural damage in placental mitochondria and results in failure in ATP production

Although placental glucose levels were increased, ATP content was reduced in the HFD group compared with the CON group ($P < 0.05$, [Figure 4A and B](#)). In parallel, mitochondrial membrane potential was significantly decreased, indicating impaired mitochondrial bioenergetic capacity ($P < 0.05$, [Figure 4C](#)). Transmission electron microscope analysis revealed ultrastructural damage in placental mitochondria from HFD dams, including swelling, disrupted membranes, and loss of cristae ([Figure 4D](#)). Quantitative Flameng scoring further supported increased mitochondrial damage in the HFD group ($P < 0.05$, [Figure 4E](#)). Consistent with these structural defects, HFD significantly suppressed oxidative phosphorylation capacity, as evidenced by reduced activities of mitochondrial respiratory chain complexes I, III, and IV ($P < 0.05$, [Figure 4F–J](#)). Moreover, HFD reduced placental mtDNA copy number ($P < 0.01$, [Figure 4K](#)) and TOMM20

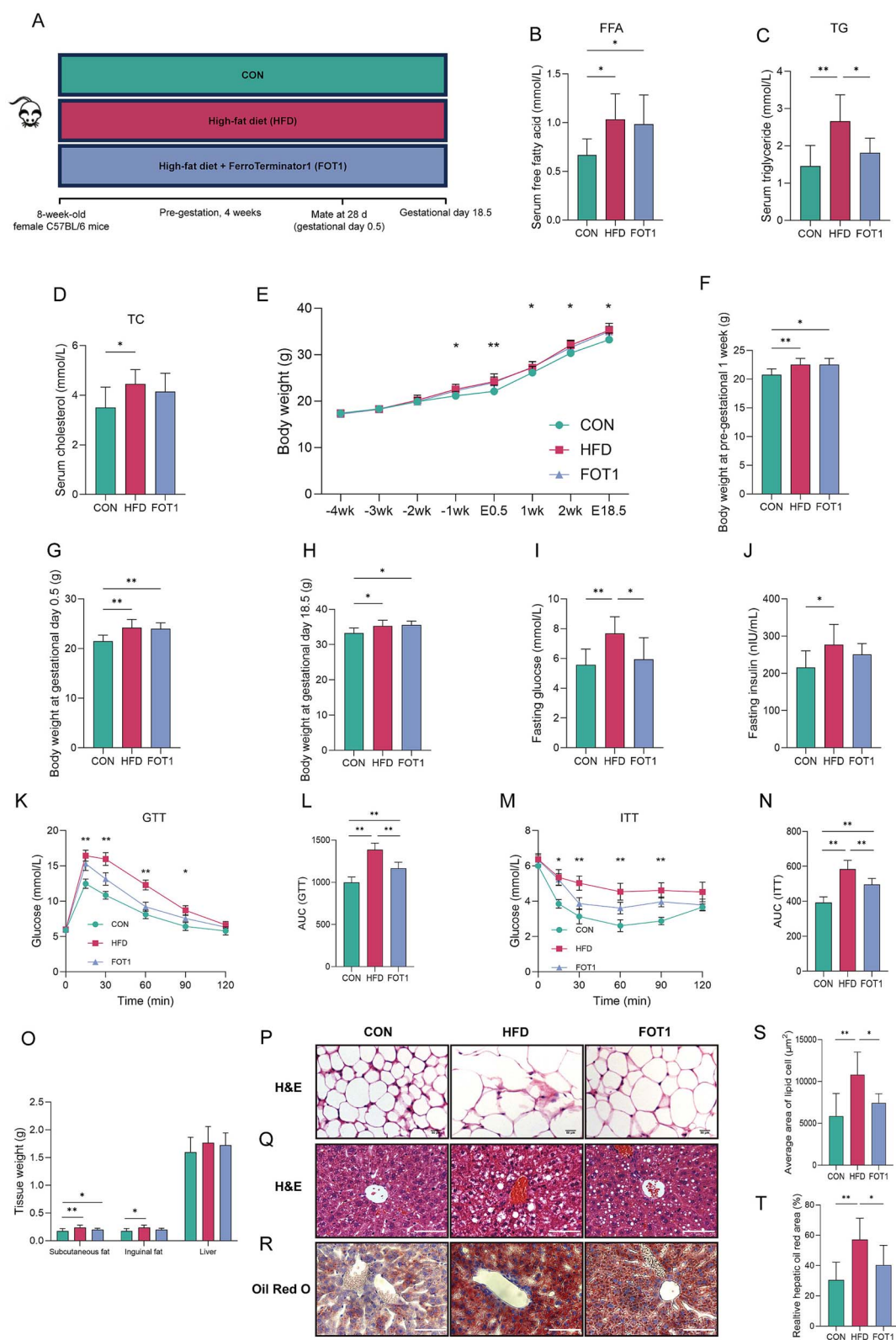


Figure 1. Maternal HFD leads to obesity and metabolic syndrome. (A) Schematic of experiment (n=8). (B) Serum FFA concentration (mmol/L). (C) Serum total cholesterol (TC) concentration (mmol/L). (D) Serum triglyceride (TG) concentration (mmol/L). (E) Maternal body weight changes during the pre-mating and gestational periods (g). (F) Maternal body weight at pre-gestational 1 week (g). (G) Maternal body weight at gestational day 0.5 (g). (H) Maternal body weight at gestational day 18.5 (g). (I) Serum fasting glucose concentration (mmol/L). (J) Serum fasting insulin concentration (nIU/mL). (K, L) GTT curve and AUC. (M, N) ITT curve and AUC. (O) Tissue weights of subcutaneous fat, inguinal fat, and liver (g). (P) Subcutaneous fat H&E staining (n=6 dams per group, scale bars = 50 μ m). (Q) Hepatic H&E staining (n=6 dams per group, scale bars = 75 μ m). (R) Hepatic Oil Red O staining (n=6 dams per group, scale bars = 75 μ m). (S) The quantification of lipid cell area (n=6 dams per group). (T) The quantification of hepatic Oil Red O area (n=6 dams per group). Data are presented as mean $\pm$ SEM; * means $P < 0.05$, ** means $P < 0.01$. n=8 dams/litters per group unless otherwise specified.

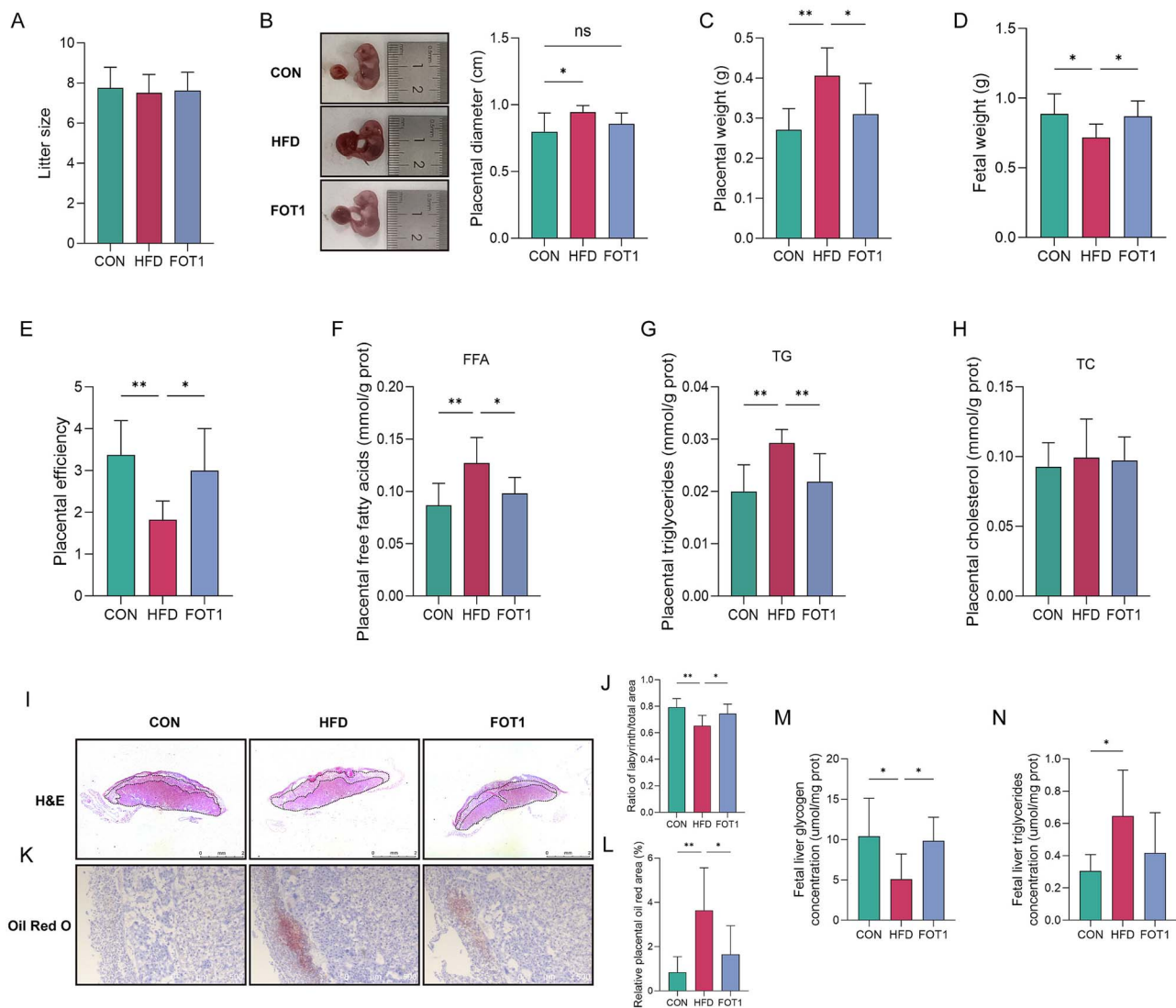


Figure 2. Maternal obesity restricts fetal growth, impairs placental efficiency and morphology, and induces placental lipid accumulation. (A) Litter size ($n = 8$ litters per group). (B) Placental diameter (cm, $n = 8$ placentas from independent litters per group). (C) Average of placental weight (g, $n = 8$ litters per group). (D) Average of fetal weight (g, $n = 8$ litters per group). (E) Placental efficiency, calculated as fetal weight divided by placental weight ($n = 8$ litters per group). (F) Placental FFA content (mmol/g protein). (G) Placental TG content (mmol/g protein). (H) Placental TC content (mmol/g protein). (I, J) Placental H&E staining and labyrinth/total area ratio ($n = 6$ placentas from independent litters per group). The dotted lines frame the regions of the junctional zone (upper) and labyrinth zone (lower). Scale bars = 2 mm. (K, L) Placental Oil Red O staining and quantification of relative placental Oil Red O area ($n = 6$ placentas from independent litters per group, scale bars = 500 μ m). (M) Fetal liver glycogen content (μ mol/mg protein). (N) Fetal liver TG content (μ mol/mg protein). Data are presented as mean $\pm$ SEM; * means $P < 0.05$, ** means $P < 0.01$. $n = 8$ dams/litters per group unless otherwise specified.

fluorescence intensity in the labyrinth zone ($P < 0.01$, Figure 4L and M).

Conversely, attenuation of metabolic stress significantly normalized the decreased ATP production, mitochondrial membrane potential, activities of complexes I and III, mtDNA copy number, and TOMM20 fluorescence intensity compared with the HFD group ($P < 0.05$).

Maternal obesity alters metabolites related to glycolysis, oxidative phosphorylation, and the pentose phosphate pathway

To characterize the impact of maternal HFD on placental energy metabolic metabolites, untargeted metabolomic profiling was performed. PLS-DA revealed distinct metabolic

patterns across groups ($P < 0.05$, Figure 5A). Compared with the CON group, the HFD group exhibited 94 upregulated and 148 downregulated metabolites ($P < 0.05$, Figure 5B). Relative to the HFD group, the FOT1 group resulted in 86 upregulated and 81 downregulated metabolites. A total of 34 significantly different expressed metabolites (DEMs) were consistently altered among the 3 groups ($P < 0.05$, Figure 5B). KEGG enrichment of these overlapping DEMs revealed significant enrichment in pathways such as efferocytosis, pentose phosphate pathway (PPP), and biosynthesis of cofactors ($P < 0.01$, Figure 5C). These DEMs and their associated KEGG pathways are provided in Supplementary File 1. Among 34 overlapped metabolites, 4 metabolites were enriched in KEGG pathway, as shown in Figure 5D. ATP and L-ornithine, which are involved

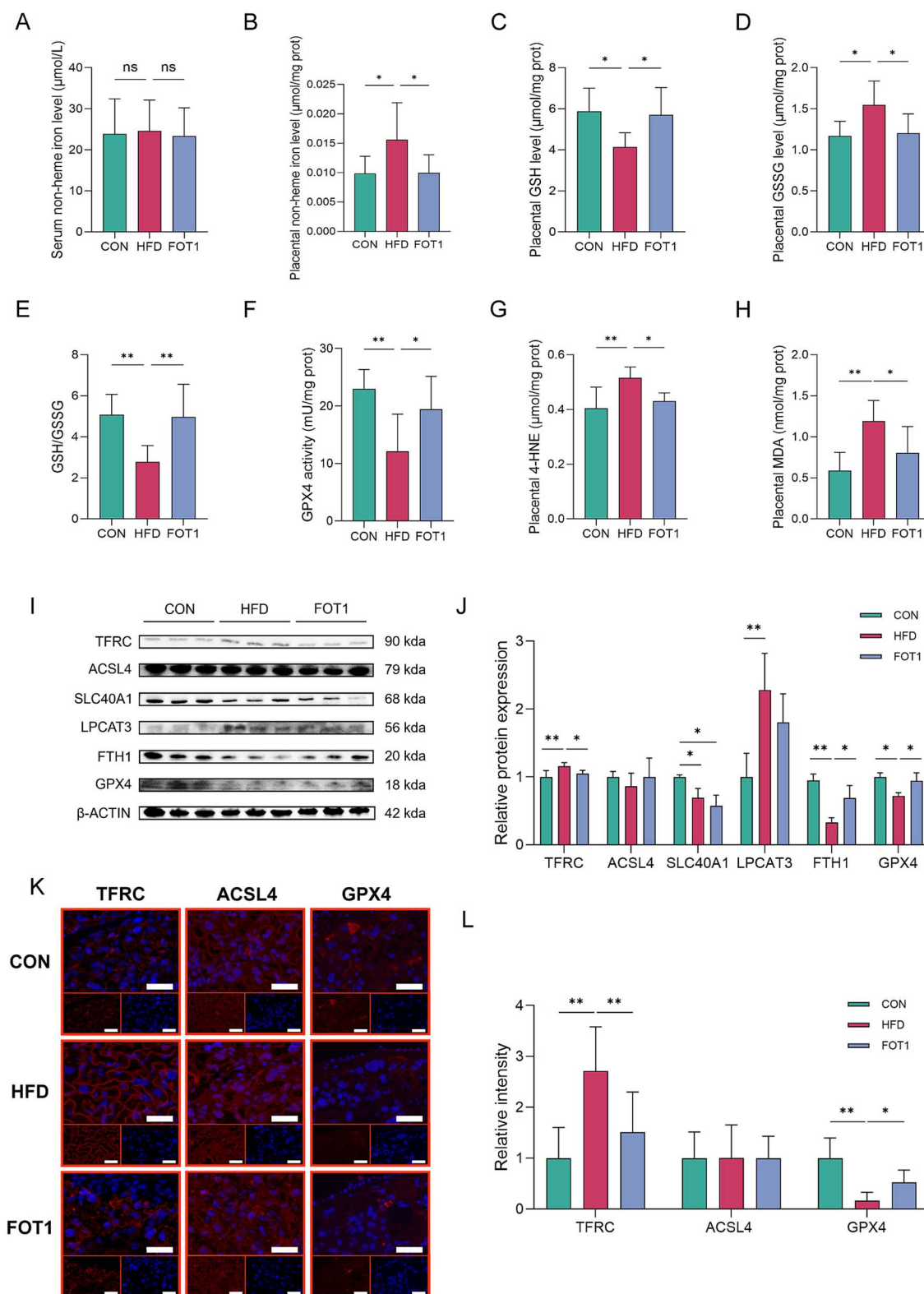


Figure 3. Maternal obesity induces placental iron accumulation and oxidative stress. (A) Serum non-heme iron content ($\mu\text{mol/L}$). (B) Placental non-heme iron content ($\mu\text{mol/mg protein}$). (C) Placental glutathione (GSH) content ($\mu\text{mol/mg protein}$). (D) Placental oxidized glutathione (GSSG) content ($\mu\text{mol/mg protein}$). (E) GSH/GSSG ratio. (F) Placental GPX4 activity (mU/mg protein). (G) Placental 4-Hydroxynonenal (4-HNE) content ($\mu\text{mol/mg protein}$). (H) Placental malondialdehyde (MDA) content (nmol/mg protein). (I, J) Immunoblots and quantification of transferrin receptor (TFRC), acyl-CoA synthetase long-chain family member 4 (ACSL4), solute carrier family 40 member 1 (SLC40A1), lysophosphatidylcholine acyltransferase 3 (LPCAT3), ferritin heavy chain 1 (FTH1), and GPX4 protein expression in the placenta. (K, L) Representative immunofluorescence images of TFRC, ACSL4, and GPX4 and quantification of relative fluorescence intensity in the labyrinth zone (main panel: merge of target protein and DAPI). Bottom-left inset: protein signal. Bottom-right inset: DAPI channel. $n = 6$ placentas from independent litters per group, scale bars = $50 \mu\text{m}$. Data are presented as mean $\pm$ SEM; *means $P < 0.05$, **means $P < 0.01$. $n = 8$ dams/litters per group unless otherwise specified.

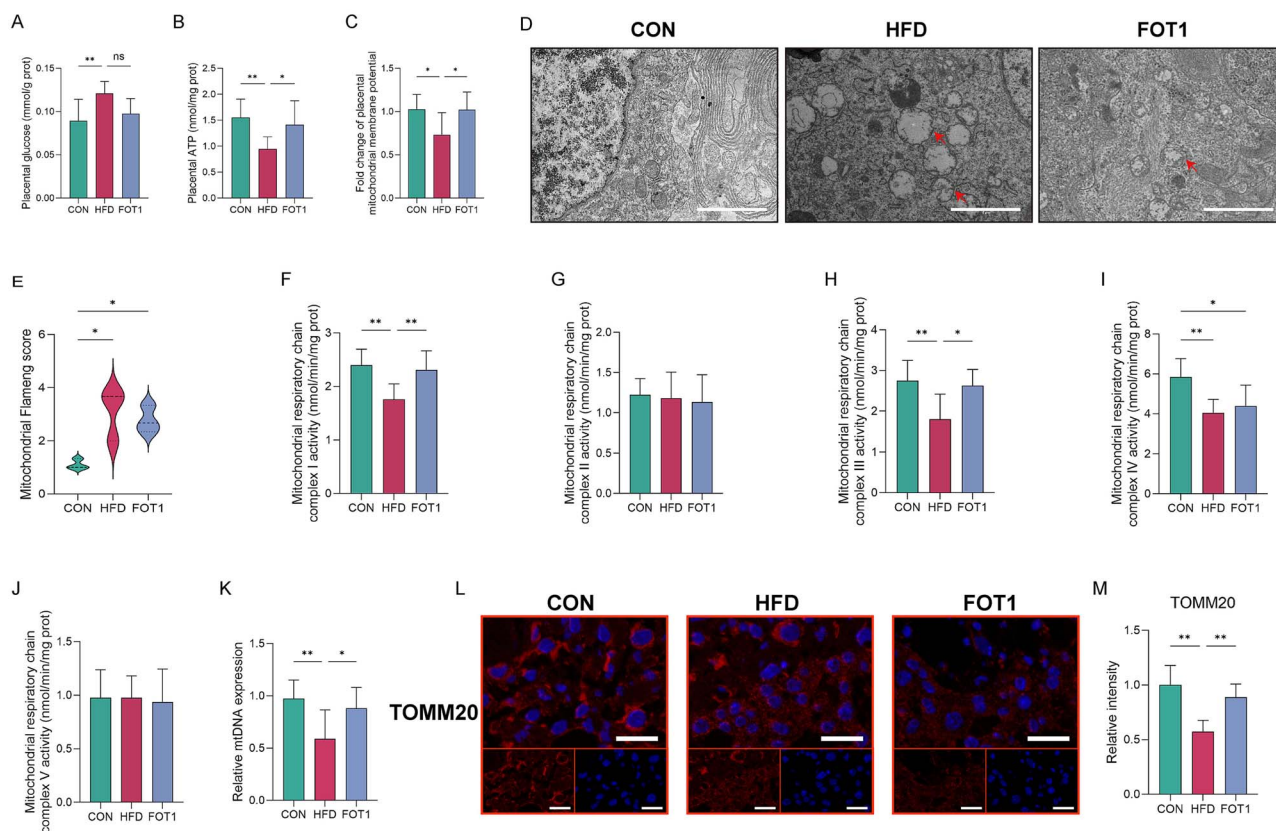


Figure 4. Maternal obesity impairs placental mitochondrial integrity and ATP production. (A) Placental glucose content (mmol/g protein). (B) Placental ATP content (nmol/mg protein). (C) Placental mitochondrial membrane potential. (D) Placental mitochondrial ultrastructure observed by transmission electron microscope ($n=3$ placentas from 3 independent litters per group. Three fields were analyzed per placenta, and the placenta-level mean was used as one biological replicate). (E) Placental mitochondrial damage score assessed by Flameng score. (F) Mitochondrial respiratory chain complex I activity (nmol/min/mg protein). (G) Complex II activity (nmol/min/mg protein). (H) Complex III activity (nmol/min/mg protein). (I) Complex IV activity (nmol/min/mg protein). (J) Complex V activity (nmol/min/mg protein). (K) Placental mitochondrial DNA (mtDNA) copy number. (L, M) Representative immunofluorescence images of translocase of outer mitochondrial membrane 20 (TOMM20) and quantification of fluorescence intensity in the labyrinth zone (main panel: merge of TOMM20 and DAPI). Bottom-left inset: protein signal. Bottom-right inset: DAPI channel. $n=6$ placentas from independent litters per group, scale bars = 50 μm). Data are presented as mean $\pm$ SEM; *means $P < 0.05$, **means $P < 0.01$. $n=8$ dams/litters per group unless otherwise specified.

in efferocytosis, were significantly reduced in the HFD group ($P < 0.05$, Figure 5E). Remarkably, FOT1 treatment modulated ATP levels comparable with CON. Given the marked mitochondrial dysfunction and ATP reduction observed in HFD, we next focused on energy metabolism, glucose metabolism, and corresponding DEMs in pairwise comparison.

KEGG enrichment analysis of DEMs between the HFD and CON groups revealed significant enrichment in glycolysis/gluconeogenesis, oxidative phosphorylation, and biosynthesis of cofactors pathways, and several metabolites were shared among these pathways ($P < 0.05$, Figure 5F). For instance, 3-phosphoglycerate was enriched in all these pathways ($P < 0.05$, Figure 5G). Adenosine 5'-diphosphate (ADP) was associated with oxidative phosphorylation, and D-ribulose 5-phosphate was enriched in the biosynthesis of cofactors ($P < 0.05$). Notably, the abundance of 3-phosphoglycerate, D-ribulose 5-phosphate, ADP, and spermine was significantly reduced in the HFD group compared with the CON group, indicating impaired placental energy metabolism under maternal obesity ($P < 0.05$, Figure 5).

To further assess whether metabolic modulation could alleviate HFD-induced energy impairment, DEMs between the HFD and FOT1 group were mapped to KEGG pathways.

Compared with the FOT1 group, two major energy-related pathways, glycolysis/gluconeogenesis and the PPP, were lower in the HFD group ($P < 0.05$, Figure 5H). FOT1 administration increased the abundance of glycolytic intermediates (3-phosphoglycerate and dihydroxyacetone phosphate) and pentose phosphate metabolites (D-ribulose 5-phosphate, 6-phosphogluconic acid, and 3-phosphoglycerate) ($P < 0.05$, Figure 5I). In conclusion, maternal obesity was associated with altered abundance of metabolites related to placental central carbon metabolism, including glycolysis/gluconeogenesis, oxidative phosphorylation, and the PPP. FOT1 partially shifted these pathway-related metabolite profiles toward control levels, supporting their association with placental bioenergetic impairment under maternal HFD exposure (Figure 5J).

Discussion

Maternal obesity is a recognized risk factor for FGR, but the mechanisms linking metabolic stress to impaired fetal growth remain incompletely understood. In the present study, we provide evidence that HFD induces placental lipid overload, placental energy insufficiency, and placental dysfunction, ultimately contributing to FGR. To further study the

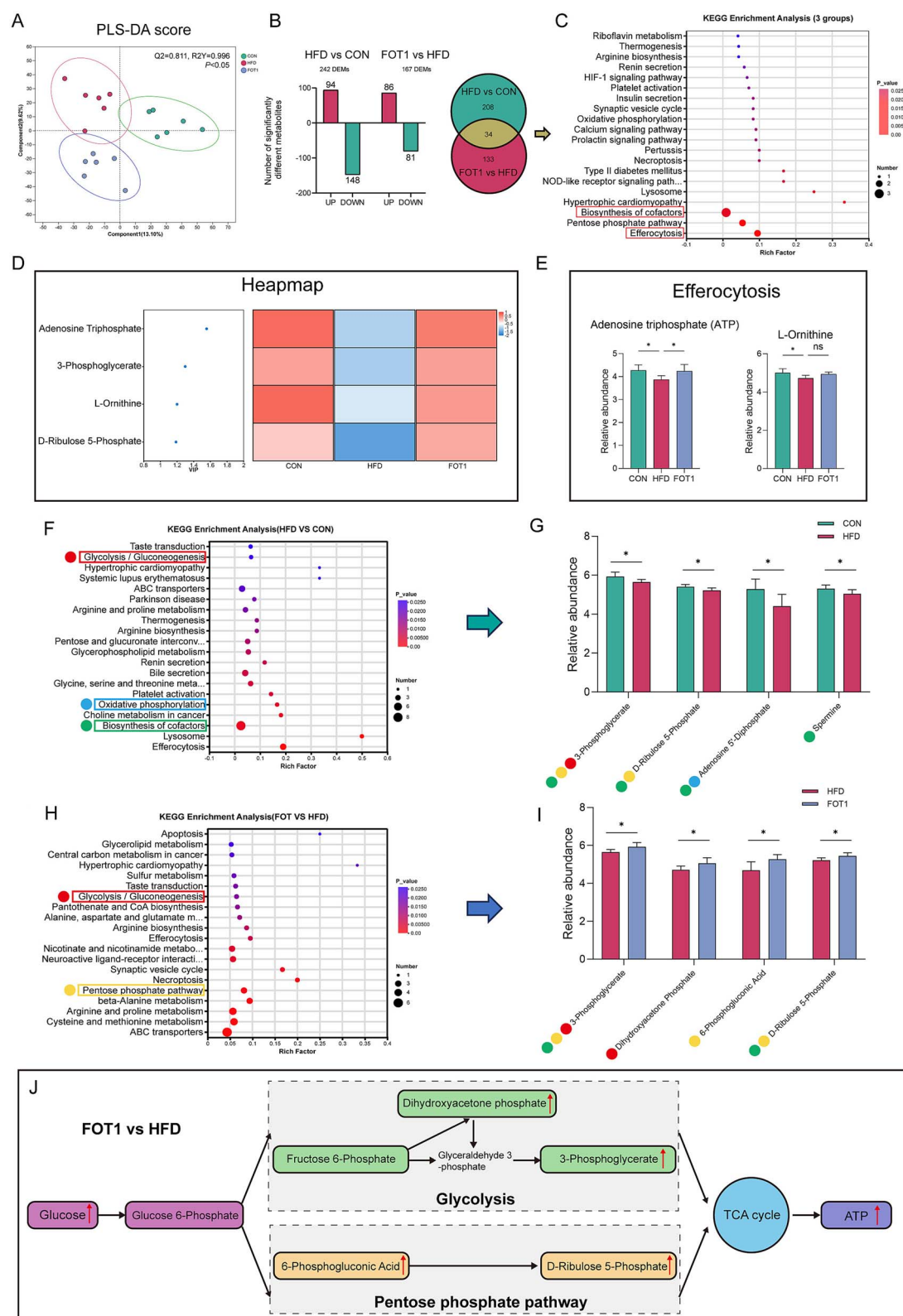


Figure 5. Maternal obesity alters placental metabolites related to glycolysis, oxidative phosphorylation, and the pentose phosphate pathway. (A) PLS-DA analysis of placental metabolomics of 3 groups ($n=6$). (B) Significantly DEMs across groups. (C) KEGG pathway enrichment of DEMs from three groups. (D) Heatmap of overlapped DEMs enriched in the KEGG pathway. Left: bubble plot showing metabolites with corresponding VIP values (x-axis). Right: heatmap of metabolite abundances, where columns represent groups and rows represent metabolites; color intensity indicates relative expression according to the scale bar. (E) DEMs from three groups enriched in the efferocytosis pathway. (F) KEGG pathway enrichment of DEMs from HFD and control (CON) groups. (G) Relative abundance of representative DEMs (HFD and CON groups) and their associated pathways. (H) KEGG pathway enrichment of DEMs from FOT1 and HFD groups. (I) Relative abundance of representative DEMs (FOT1 and HFD groups) and their associated pathways. (J) FOT1 increased placental glucose concentration, intermediates in glycolysis (3-phosphoglycerate, dihydroxyacetone phosphate), and the pentose phosphate pathway (6-phosphogluconic acid, D-ribulose 5-phosphate), which might increase ATP level.

involvement of iron-associated oxidative stress within this HFD-driven metabolic disorder, FOT1 was applied as an experimental metabolic modulator with iron-chelating and lipid peroxidation-suppressing properties.

The HFD administered for 4 weeks before mating and maintained throughout gestation was used to establish a maternal obesity model. Consistent with previous reports, HFD-fed dams developed a metabolic syndrome-like phenotype characterized by increased body weight, dyslipidemia, insulin resistance, increased subcutaneous fat weight, and hepatic lipid accumulation [5, 27]. Our findings showed that metabolic modulation by FOT1 partially alleviated maternal hyperglycemia and insulin resistance, although it did not prevent HFD-induced weight gain during gestation. These findings support its use as a functional modulator in HFD-induced metabolic stress. However, the absence of an only FOT1 group prevents us from distinguishing whether these protective effects of FOT1 are mediated by systemic maternal metabolic improvement, placenta-local actions, or both.

In the present study, maternal HFD exposure did not affect litter size but significantly reduced fetal weight and placental efficiency, which is similar to earlier reports [5, 27, 28]. These results suggested that HFD-induced metabolic disturbances compromised the intrauterine environment rather than ovulation or implantation. Conversely, another study reported decreased placental weight or increased placental efficiency in response to high-fat and high-sugar diets, which may result from differences in dietary fat content (60% vs. 30%) or in placental lipid accumulation across studies [21]. In our study, HFD resulted in an enlarged placental diameter accompanied by reduced efficiency, suggesting compensatory placental growth in response to functional insufficiency. Both clinical and experimental evidence indicate that such placental remodeling often reflects impaired nutrient transport, defective trophoblast differentiation, and abnormal vascular development, all of which contribute to FGR [21, 29, 30]. The placental labyrinth zone area, which mediates nutrient exchange and placental angiogenesis, was reduced in the HFD group, supporting impaired placental efficiency [31]. Reduced labyrinth zone area showed impaired placental development and was consistent with the reduced placental efficiency and fetal growth observed in HFD-fed dams. Interestingly, attenuation of metabolic stress by FOT1 modulated the labyrinth zone organization, indicating reduced structural disruption of the placenta under HFD exposure [32, 33]. Mechanistically, placental lipid overload has been recognized as a mediator of placental dysfunction in maternal obesity through lipid peroxidation, lipotoxic signaling, and oxidative stress [9, 34, 35]. Consistent with these findings, we observed increased placental TG and FFA content, accompanied by an increased lipid droplet area, as detected by Oil Red O staining. Metabolic modulation by FOT1 decreased placental lipid deposition, shifting placental lipid levels toward those observed in the control groups, and similar effects have been reported in other metabolic tissues under obesogenic conditions [15, 36, 37]. Fetal hepatic metabolism further extends the physiological relevance of placental bioenergetic impairment. Consistent with previous research, HFD increased fetal liver TG accumulation, which might be related to maternal dyslipidemia and altered placental lipid handling [38]. This alteration may represent an early prenatal metabolic signal associated with the long-term metabolic vulnerability of offspring exposed to maternal obesity [39]. In line with reduced fetal growth and placental efficiency, HFD reduced

fetal liver glycogen content, which reflects limited fetal glucose availability, placental glucose transport, and exchange capacity under maternal metabolic stress [40–42]. FOT1 attenuated the decrease in fetal hepatic glycogen, suggesting that improved placental bioenergetic status was associated with normalization of fetal metabolic indices. Future studies are needed to follow offspring's metabolism after birth and determine whether these prenatal hepatic changes persist into postnatal life.

Maternal obesity imposes metabolic stress on the placenta and challenges its capacity to maintain energy homeostasis [43]. Excessive lipid peroxidation and labile iron accumulation under HFD conditions may impair mitochondrial membranes, thereby reducing electron transport and ATP synthesis [22]. In this study, the increased glucose and decreased ATP levels suggest that glucose availability is not limiting, whereas mitochondrial oxidative capacity is compromised. Previous studies have reported reduced placental oxidative phosphorylation capacity under maternal HFD [44–46]. In HFD placentas, TEM provides supportive evidence for mitochondrial ultrastructural abnormalities, together with decreased mitochondrial membrane potential, reduced mtDNA copy number and TOMM20 intensity, and lower activities of respiratory chain complexes I, III, and IV. The mtDNA copy number is commonly used as an indicator of mitochondrial genome abundance or mitochondrial content, whereas TOMM20 marks the mitochondrial outer membrane [47]. As mtDNA encodes core subunits of complexes I, III, and IV, reduced mtDNA copy number may support the observed complex activity deficits induced by HFD [48]. FOT1 attenuated the decrease in mitochondrial function and abundance, suggesting that limiting iron-associated oxidative stress and lipid peroxidation may help preserve mitochondrial bioenergetic capacity under maternal HFD exposure. The lack of full recovery of complex IV activity suggests that additional regulatory mechanisms of mitochondrial damage may persist under HFD exposure [49]. Because mitochondrial dysfunction is regulated by upstream mechanisms, including mitochondrial biogenesis, dynamics, and mitophagy, these alterations in maternal obesity need to be explored in future studies [11, 47].

A key finding of this study is that placental lipid overload under HFD is accompanied by iron-associated oxidative stress. Previous studies have shown that HFD induces iron accumulation in multiple metabolic tissues and organs, including the blood, adipose tissue, liver, and kidney [15–18]. The western diet was associated with placental iron metabolism disorder and oxidative stress, which led to FGR [21]. In line with these observations, we found that HFD elevated placental non-heme iron levels and increased expression of TFRC, indicating enhanced cellular iron uptake. Excess labile iron can catalyze lipid peroxidation via Fenton chemistry, amplifying oxidative damage to cellular membranes [50]. TFRC mediates cellular iron uptake by endocytosing transferrin-bound Fe^{3+} and delivering Fe^{2+} to the labile iron pool, and its upregulation has been linked to iron-related oxidative stress, which is commonly observed in metabolic disorders [51–53]. SLC40A1 is the major iron-export protein, and FTH1 contributes to ferritin-mediated iron storage and detoxification of excess intracellular iron [54]. In our study, HFD increased TFRC expression but decreased SLC40A1 expression, suggesting enhanced iron uptake together with impaired iron export capacity in maternal-fetal exchange area. This pattern is consistent with placental non-heme iron accumulation and

may increase redox-active iron availability, thereby promoting lipid peroxidation and GPX4-related antioxidant failure. Changes in iron uptake, storage, and export proteins collectively support a state of intracellular iron retention and redox imbalance in the placenta under HFD [55]. Labyrinth zone is the main site for iron-related oxidative stress, and modulation of iron-associated oxidative stress preserves labyrinth zone function. In parallel, LPCAT3, a key enzyme promoting PUFA-containing phospholipid formation, was also upregulated by HFD, expanding the pool of oxidizable substrates and sensitizing placental membranes to lipid peroxidation, as evidenced by increased MDA and 4-HNE [56, 57]. In contrast, antioxidant defenses were compromised under HFD exposure, as reflected by decreased expression of GPX4, GPX activity, GSH, and a lower GSH/GSSG ratio, all of which weaken phospholipid hydroperoxide detoxification and accelerate oxidative stress [58, 59]. The reduced expression and activity of GPX4 reflect a broader failure of lipid peroxide detoxification and redox buffering capacity [60]. While indirect markers of oxidative stress were changed, direct quantification of mitochondrial ROS is needed to attribute oxidative damage to the mitochondrial compartment in future work. Moreover, HFD increased TFRC and reduced GPX4 fluorescence intensity in labyrinth zone, consistent with increased non-heme iron, 4-HNE, and MDA levels and the reduced GSH/GSSG ratio detected in whole-placenta assays. FOT1 attenuated the changes in TFRC and GPX4, suggesting that its protective effect may involve limiting iron-associated oxidative stress and preserving GPX4-related antioxidant defense and mitochondrial function [61]. However, these observations should be interpreted as compartment-level localization rather than definitive cell-type-specific evidence. Remarkably, FOT1 increased GSH/GSSG ratio and partially normalized TFRC and GPX4 expression, while reducing lipid peroxidation end products such as 4-HNE and MDA. These effects are consistent with the reported ability of FOT1 to chelate redox-active iron and suppress lipid peroxidation signaling [15]. Mechanistically, HFD-induced lipid overload was accompanied by iron-associated oxidative stress, which may contribute to mitochondrial membrane injury and reduced electron transport efficiency, though direct causal evidence requires further investigation. Metabolic modulation preserves mitochondrial structure and maintains energy-producing pathways, thereby limiting placental ATP depletion under HFD exposure [62]. However, as FOT1 also partially attenuated systemic insulin resistance and dyslipidemia, a contribution from improved systemic metabolic status cannot be fully excluded.

To further elucidate the metabolic alterations underlying HFD-induced energy insufficiency, placental LC-MS/MS metabolomic analysis was performed. KEGG enrichment analysis identified several pathways related to cellular metabolism, among which biosynthesis of cofactors, glycolysis, oxidative phosphorylation, and the pentose phosphate pathway were most directly linked to placental energy homeostasis, which is in line with a previous study [63]. Suppression of glycolytic intermediates such as 3-phosphoglycerate and dihydroxyacetone phosphate is likely to limit substrate-level phosphorylation and reduce pyruvate availability for mitochondrial oxidation, consistent with the constrained ATP production in maternal obesity [64]. Meanwhile, maternal HFD exposure was associated with a suppression of PPP metabolites, including D-ribulose-5-phosphate and 6-phosphogluconic acid, indicating a

reduced capacity for NADPH generation and a consequent impairment of glutathione-dependent antioxidant defenses [65, 66]. This loss of redox buffering capacity might sensitize the placenta to oxidative stress under placental lipid overload [67]. Consistently, the metabolic differences observed between the HFD and FOT1 groups suggest that iron-related oxidative stress plays a role in exacerbating HFD-induced damage. Attenuation of iron-dependent oxidative stress by FOT1 was accompanied by increased abundances of glycolytic and pentose phosphate pathway intermediates, suggesting that HFD-induced lipid overload constrains placental central carbon metabolism [68]. Notably, 3-phosphoglycerate serves as a precursor for serine biosynthesis, which fuels one-carbon metabolism and supports nucleotide synthesis, whose reduction compromises cellular antioxidant capacity in HFD, consistent with the reduced 4-HNE and MDA levels [69]. These results indicate that maternal HFD was associated with suppressed placental energy metabolite profiles and impaired antioxidant defense, and iron-associated oxidative stress might amplify placental mitochondrial dysfunction and energetic insufficiency.

Nevertheless, several limitations should be acknowledged. Although HFD may disrupt glycolysis to decrease ATP synthesis, metabolite abundance does not directly reflect metabolic flux, and isotope tracing approaches such as ^{13}C -glucose labeling are required to confirm changes [70]. The present study did not directly establish the specific causal role of iron-dependent oxidative stress in placental mitochondrial impairment. Future studies need to use pathway-specific rescue experiments and targeted manipulation of iron-regulatory proteins to define the causal relationship. Moreover, while FOT1 effectively modulated placental oxidative stress and mitochondrial function, it did not fully normalize maternal or placental metabolic dysfunction, indicating that additional lipid-independent mechanisms may contribute to HFD-induced placental insufficiency. Additionally, the absence of a CON + FOT1 group limits the ability to distinguish systemic from local placental effects of FOT1; this should be addressed in future studies.

Collectively, maternal obesity was associated with placental lipid overload, iron-associated oxidative stress, and bioenergetic impairment, which may contribute to FGR. Experimental attenuation of iron-associated oxidative stress using FOT1 partially rescued placental bioenergetic indices and fetal outcomes, supporting the involvement of this pathway in HFD-associated placental dysfunction.

Conclusion

In summary, maternal obesity induces placental iron-associated oxidative stress and bioenergetic impairment, which might contribute to placental insufficiency and FGR. FOT1 administration attenuated placental oxidative stress, partially restored placental bioenergetic function, and improved fetal metabolic indices. These findings identify placental iron-associated oxidative stress as a mechanistic link between maternal obesity and FGR, and highlight it as a potential target for future therapeutic strategies.

Author contributions

Ye qianli Wo (Writing—original draft), Jiabing Mao (Writing—original draft), Zeou Wei (Data curation), Ye Xu (Data curation), Xiaohong

Wang (Data curation), Dadan Liu (Data curation), Shusong Wu (Investigation), Jianhua He (Investigation), Martine Schroyen (Investigation), Bao Yi (Writing—review & editing), Liang Chen (Supervision; Funding acquisition), and Hongfu Zhang (Supervision; Funding acquisition)

Supplementary material

Supplementary material is available at *BIOLRE* online.

Conflict of Interest: The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

References

- Kalantari E, Tajvar M, Naderimagham S, Takian A. Maternal obesity management: a narrative literature review of health policies. *BMC Womens Health* 2024; 24:520. <https://doi.org/10.1186/s12905-024-03342-2>.
- Shirvanifar M, Ahlqvist VH, Lundberg M, Kosidou K, Herraiz-Adillo Á, Berglind D, Magnusson C, Henriksson P. Adverse pregnancy outcomes attributable to overweight and obesity across maternal birth regions: a Swedish population-based cohort study. *Lancet Public Health* 2024; 9:e776–e786. [https://doi.org/10.1016/S2468-2667\(24\)00188-9](https://doi.org/10.1016/S2468-2667(24)00188-9).
- Ruiz-González D, Cervero-Redondo I, Hernández-Martínez A, Baena-Raya A, Martínez-Forte S, Altmäe S, Fernández-Alonso AM, Soriano-Maldonado A. Comparative efficacy of exercise, diet and/or pharmacological interventions on BMI, ovulation, and hormonal profile in reproductive-aged women with overweight or obesity: a systematic review and network meta-analysis. *Hum Reprod Update* 2024; 30:472–487. <https://doi.org/10.1093/humupd/dmae008>.
- Collaborators GAB. Global, regional, and national prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: a forecasting study for the global burden of disease study 2021. *Lancet* 2025; 405:813–838. [https://doi.org/10.1016/S0140-6736\(25\)00355-1](https://doi.org/10.1016/S0140-6736(25)00355-1).
- Sanches APV, de Oliveira JL, Ferreira MS, Lima BS, Miyamoto JÉ, Simino LAP, Torsoni MA, Torsoni AS, Milanski M, Ignácio-Souza L. Obesity phenotype induced by high-fat diet leads to maternal-fetal constraint, placental inefficiency, and fetal growth restriction in mice. *J Nutr Biochem* 2022; 104:108977. <https://doi.org/10.1016/j.jnutbio.2022.108977>.
- Yang Z, Feng G, Gao X, Yan X, Li Y, Wang Y, Li S, Jiang Y, Zhao S, Zhao H, Chen ZJ. Maternal adiposity and perinatal and offspring outcomes: an umbrella review. *Nat Hum Behav* 2024; 8:2406–2422. <https://doi.org/10.1038/s41562-024-01994-6>.
- Díaz M, García C, Sebastiani G, de Zegher F, López-Bermejo A, Ibáñez L. Placental and cord blood methylation of genes involved in energy homeostasis: association with fetal growth and neonatal body composition. *Diabetes* 2017; 66:779–784. <https://doi.org/10.2337/db16-0776>.
- Armistead B, Johnson E, VanderKamp R, Kula-Eversole E, Kadam L, Drewlo S, Kohan-Ghadr HR. Placental regulation of energy homeostasis during human pregnancy. *Endocrinology* 2020; 161:bqaa076.
- He T, Chen Q, Yuan Z, Yang Y, Cao K, Luo J, Dong G, Peng X, Yang Z. Effects of maternal high-fat diet on fetal growth, placental nutrient transporters and circular RNA expression profiles. *Food Funct* 2023; 14:9391–9406. <https://doi.org/10.1039/D3FO02202A>.
- de Oliveira MP, da Silva LE, Fernandes BB, Steiner MR, Pistóia DG, Santos Cichella T, Jacinto LB, Spuldaro KM, Pinto Moehlecke Iser B, Rezin GT. The impact of obesity on mitochondrial dysfunction during pregnancy. *Mol Cell Endocrinol* 2025; 598:112463. <https://doi.org/10.1016/j.mce.2025.112463>.
- Kadam L, Chan K, Tawater E, Myatt L. Dysregulation of placental mitochondrial structure dynamics and clearance in maternal obesity and gestational diabetes. *Placenta* 2025; 171:140–149. <https://doi.org/10.1016/j.placenta.2025.09.019>.
- Aye ILMH, Aiken CE, Charnock-Jones DS, Smith GCS. Placental energy metabolism in health and disease—significance of development and implications for preeclampsia. *Am J Obstet Gynecol* 2022; 226:S928–S944. <https://doi.org/10.1016/j.ajog.2020.11.005>.
- Gallo LA, Barrett HL, Dekker NM. Review: placental transport and metabolism of energy substrates in maternal obesity and diabetes. *Placenta* 2017; 54:59–67. <https://doi.org/10.1016/j.placenta.2016.12.006>.
- Sui Y, Geng X, Wang Z, Zhang J, Yang Y, Meng Z. Targeting the regulation of iron homeostasis as a potential therapeutic strategy for nonalcoholic fatty liver disease. *Metabolism* 2024; 157:155953. <https://doi.org/10.1016/j.metabol.2024.155953>.
- Tao L, Yang X, Ge C, Zhang P, He W, Xu X, Li X, Chen W, Yu Y, Zhang H, Chen SD, Pan XY, et al. Integrative clinical and preclinical studies identify FerroTerminator1 as a potent therapeutic drug for MASH. *Cell Metab* 2024; 36:2190–2206.e2195. <https://doi.org/10.1016/j.cmet.2024.07.013>.
- Chen H, Zhang Y, Miao Y, Song H, Tang L, Liu W, Li W, Miao J, Li X. Vitamin D inhibits ferroptosis and mitigates the kidney injury of prediabetic mice by activating the klotho/p53 signaling pathway. *Apoptosis* 2024; 29:1780–1792. <https://doi.org/10.1007/s10495-024-01955-4>.
- Chen K, Qin Y-R, Liu S-Q, Chen R-L. Remission of iron overload in adipose tissue of obese mice by fatty acid-modified polyoxovanadates. *Rare Metals* 2025; 44:461–471. <https://doi.org/10.1007/s12598-024-02925-0>.
- Jiang S, Qiu S, Mu Y, Liu C, Han Y, Jiang J, Wang Y. Puerarin reduces susceptibility to ventricular arrhythmias and inhibits ferroptosis via Sirt1/Nrf2 signaling in high-fat-diet rats. *Free Radic Biol Med* 2025; 227:472–484. <https://doi.org/10.1016/j.freeradbiomed.2024.12.005>.
- Beharier O, Kajiwarra K, Sadovsky Y. Ferroptosis, trophoblast lipotoxic damage, and adverse pregnancy outcome. *Placenta* 2021; 108:32–38. <https://doi.org/10.1016/j.placenta.2021.03.007>.
- Park C, Alahari S, Ausman J, Liu R, Nguyen F, Sallais J, Post M, Caniggia I. Placental hypoxia-induced ferroptosis drives vascular damage in preeclampsia. *Cir Res* 2025; 136:361–378. <https://doi.org/10.1161/CIRCRESAHA.124.325119>.
- Zaugg J, Lopez-Tello J, Musial B, Vaughan OR, Fowden AL, Albrecht C, Sferruzzi-Perri AN. Obesogenic diet in pregnancy disrupts placental iron handling and ferroptosis and stress signalling in association with fetal growth alterations. *Cell Mol Life Sci* 2024; 81:151.
- Ahola S, Langer T. Ferroptosis in mitochondrial cardiomyopathy. *Trends Cell Biol* 2024; 34:150–160. <https://doi.org/10.1016/j.tcb.2023.06.002>.
- Chen W, Yuan X, Li Z, Lu Z, Kong S, Jiang H, Du H, Pan X, Nandi M, Kong X, Brown K, Liu Z, et al. CN128: a new orally active hydroxypyridinone iron chelator. *J Med Chem* 2020; 63:4215–4226. <https://doi.org/10.1021/acs.jmedchem.0c00137>.
- Dalton-O'Reilly J, Heazell AEP, Greenwood S, Dilworth M, Desforges M. Sex-specific alterations in placental mitochondria, oxidative damage and apoptosis in mice of advanced maternal age. *Reproduction* 2025; 170:e250160.
- Zhao M, Wang Y, Li L, Liu S, Wang C, Yuan Y, Yang G, Chen Y, Cheng J, Lu Y, Liu J. Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. *Theranostics* 2021; 11:1845–1863. <https://doi.org/10.7150/thno.50905>.
- Li L, Tan HP, Liu CY, Yu LT, Wei DN, Zhang ZC, Lu K, Zhao KS, Mägele M, Cai DZ, Gu ZT. Polydatin prevents the induction of secondary brain injury after traumatic brain injury by protecting

- neuronal mitochondria. *Neural Regen Res* 2019; 14:1573–1582. <https://doi.org/10.4103/1673-5374.255972>.
27. Huang S, Chen J, Cui Z, Ma K, Wu D, Luo J, Li F, Xiong W, Rao S, Xiang Q, Shi W, Song T, *et al.* Lachnospiraceae-derived butyrate mediates protection of high fermentable fiber against placental inflammation in gestational diabetes mellitus. *Sci Adv* 2023; 9:eadi7337.
28. Sasson IE, Vitins AP, Mainigi MA, Moley KH, Simmons RA. Pre-gestational vs gestational exposure to maternal obesity differentially programs the offspring in mice. *Diabetologia* 2015; 58: 615–624. <https://doi.org/10.1007/s00125-014-3466-7>.
29. Wallace JM, Agard JP, Horgan GW. A new customised placental weight standard redefines the relationship between maternal obesity and extremes of placental size and is more closely associated with pregnancy complications than an existing population standard. *J Dev Orig Health Dis* 2020; 11:350–359. <https://doi.org/10.1017/S2040174419000576>.
30. Badran M, Abuyassin B, Ayas N, Laher I. Intermittent hypoxia impairs uterine artery function in pregnant mice. *J Physiol* 2019; 597:2639–2650. <https://doi.org/10.1113/JP277775>.
31. Wu Y, Su K, Zhang Y, Liang L, Wang F, Chen S, Gao L, Zheng Q, Li C, Su Y, Mao Y, Zhu S, *et al.* A spatiotemporal transcriptomic atlas of mouse placentation. *Cell Discov* 2024; 10:110.
32. Zheng Y, Hu Q, Wu J. Adiponectin ameliorates placental injury in gestational diabetes mice by correcting fatty acid oxidation/peroxide imbalance-induced ferroptosis via restoration of CPT-1 activity. *Endocrine* 2022; 75:781–793. <https://doi.org/10.1007/s12020-021-02933-5>.
33. Hosni A, El-Twab SA, Abdul-Hamid M, Prinsen E, Abdelgawad H, Abdel-Moneim A, Beemster GTS. Cinnamaldehyde mitigates placental vascular dysfunction of gestational diabetes and protects from the associated fetal hypoxia by modulating placental angiogenesis, metabolic activity and oxidative stress. *Phar Res* 2021; 165:105426. <https://doi.org/10.1016/j.phrs.2021.105426>.
34. Zhou Y, Xu T, Cai A, Wu Y, Wei H, Jiang S, Peng J. Excessive backfat of sows at 109 d of gestation induces lipotoxic placental environment and is associated with declining reproductive performance1. *J Animal Sci* 2018; 96:250–257. <https://doi.org/10.1093/jas/skx041>.
35. Zhang L, Wang Z, Wu H, Gao Y, Zheng J, Zhang J. Maternal high-fat diet impairs placental fatty acid β -oxidation and metabolic homeostasis in the offspring. *Front Nutr* 2022; 9:849684. <https://doi.org/10.3389/fnut.2022.849684>.
36. Hong Y, Feng J, Dou Z, Sun X, Hu Y, Chen Z, Liu L, Xu H, Du M, Tang P, Li X, Zhang Y. Berberine as a novel ACSL4 inhibitor to suppress endothelial ferroptosis and atherosclerosis. *Biomed Pharmacother* 2024; 177:117081. <https://doi.org/10.1016/j.biopha.2024.117081>.
37. Bai T, Li M, Liu Y, Qiao Z, Wang Z. Inhibition of ferroptosis alleviates atherosclerosis through attenuating lipid peroxidation and endothelial dysfunction in mouse aortic endothelial cell. *Free Radic Biol Med* 2020; 160:92–102. <https://doi.org/10.1016/j.freeradbiomed.2020.07.026>.
38. Joselit Y, Nanobashvili K, Jack-Roberts C, Greenwald E, Malyshva OV, Caudill MA, Saxena A, Jiang X. Maternal betaine supplementation affects fetal growth and lipid metabolism of high-fat fed mice in a temporal-specific manner. *Nutr Diabetes* 2018; 8:41. <https://doi.org/10.1038/s41387-018-0035-z>.
39. Xue L, Wang Y, Sun J, Sun Y, Zhang K, Fan M, Qian H, Wang L, Li Y. Maternal brown rice diet alleviates adverse effects of maternal obesity on offspring metabolism via enhancing DNA demethylation of AdipoR2. *Food Biosci* 2024; 60:104392. <https://doi.org/10.1016/j.foodbio.2024.104392>.
40. Connor KL, Kibschull M, Matysiak-Zablocki E, Nguyen TT-TN, Matthews SG, Lye SJ, Bloise E. Maternal malnutrition impacts placental morphology and transporter expression: an origin for poor offspring growth. *J Nutr Biochem* 2020; 78:108329. <https://doi.org/10.1016/j.jnutbio.2019.108329>.
41. Yang X, Hu R, Zhang W, Shi M, Fan Z, He X, Fu C, Chen L, Zhang H, Yuan X, Wu M, Li Y, *et al.* Effect of fat deposition on placental function in Shaziling sows and modulation by resveratrol. *Animal Nutr* 2026; 24:368–377. <https://doi.org/10.1016/j.aninu.2025.08.009>.
42. Wang J, Wang N, Guo H, Niu X, Hu S, Pan X, Wang M, Zhang D, Song L, Sun B. Pre-pregnancy treatment of semaglutide improves high-fat-diet-induced placental and fetal hypothalamic alterations in a sex-specific manner in rats. *Biol Reprod* 2025; 113: 1463–1474. <https://doi.org/10.1093/biolre/iaof178>.
43. Zhang CXW, Candia AA, Sferuzzi-Perri AN. Placental inflammation, oxidative stress, and fetal outcomes in maternal obesity. *Trends Endocrin Met* 2024; 35:638–647. <https://doi.org/10.1016/j.tem.2024.02.002>.
44. Peng J, Peng J. PSXIII-3 maternal eicosapentaenoic acid feeding decreases placental and fetal lipid deposition and improves antioxidant capacity through a Sirtuin-1 independent. *J Animal Sci* 2019; 97:376–377. <https://doi.org/10.1093/jas/skz258.749>.
45. Yu Y, Wang L, Delguste F, Durand A, Guilbaud A, Rousselin C, Schmidt AM, Tessier F, Boulanger E, Nevriere R. Advanced glycation end products receptor RAGE controls myocardial dysfunction and oxidative stress in high-fat fed mice by sustaining mitochondrial dynamics and autophagy-lysosome pathway. *Free Radic Biol Med* 2017; 112:397–410. <https://doi.org/10.1016/j.freeradbiomed.2017.08.012>.
46. Sun Y, Ge X, Li X, He J, Wei X, Du J, Sun J, Li X, Xun Z, Liu W, Zhang H, Wang Z-Y, *et al.* High-fat diet promotes renal injury by inducing oxidative stress and mitochondrial dysfunction. *Cell Death Dis* 2020; 11:914.
47. He F, Liu G, Wu H, Elsabagh M, Huang Y, Wang J, Wang M, Zhang H. Maternal intestinal and placental mitochondrial dysfunction, autophagy, and ferroptosis involving intestinal microbiota by gut microbiota transplantation from sheep to mice. *Biol Reprod* 2026; 114:1030–1044. <https://doi.org/10.1093/biolre/iaof253>.
48. Bidne KL, Erickson KE, Powell TL, Jansson T. Mechanistic target of rapamycin signaling activity in the human placenta across gestation and in maternal obesity. *Biol Reprod* 2025; 112:540–549. <https://doi.org/10.1093/biolre/iaof007>.
49. Kadenbach B, Complex IV. The regulatory center of mitochondrial oxidative phosphorylation. *Mitochondrion* 2021; 58:296–302. <https://doi.org/10.1016/j.mito.2020.10.004>.
50. Dixon SJ, Olzmann JA. The cell biology of ferroptosis. *Nat Rev Mol Cell Biol* 2024; 25:424–442. <https://doi.org/10.1038/s41580-024-00703-5>.
51. Tao H, Dar HY, Tian C, Banerjee S, Glazer ES, Srinivasan S, Zhu L, Pacifici R, He P. Differences in hepatocellular iron metabolism underlie sexual dimorphism in hepatocyte ferroptosis. *Redox Biol* 2023; 67:102892. <https://doi.org/10.1016/j.redox.2023.102892>.
52. Wang H, Yu X, Liu D, Qiao Y, Huo J, Pan S, Zhou L, Wang R, Feng Q, Liu Z. VDR activation attenuates renal tubular epithelial cell ferroptosis by regulating Nrf2/HO-1 signaling pathway in diabetic nephropathy. *Adv Sci* 2024; 11:e2305563. <https://doi.org/10.1002/adv.202305563>.
53. Feng Q, Yang Y, Qiao Y, Zheng Y, Yu X, Liu F, Wang H, Zheng B, Pan S, Ren K, Liu D, Liu Z. Quercetin ameliorates diabetic kidney injury by inhibiting ferroptosis via activating Nrf2/HO-1 signaling pathway. *Am J Chin Med* 2023; 51:997–1018. <https://doi.org/10.1142/S0192415X23500465>.
54. Shi W, Wang J, Li Z, Xu S, Wang J, Zhang L, Yang H. Reprimo (RPRM) mediates neuronal ferroptosis via CREB-Nrf2/SCD1 pathways in radiation-induced brain injury. *Free Radic Biol Med* 2024; 213:343–358. <https://doi.org/10.1016/j.freeradbiomed.2024.01.021>.
55. Wang X, Wu Q, Zhong M, Chen Y, Wang Y, Li X, Zhao W, Ge C, Wang X, Yu Y, Yang S, Wang T, *et al.* Adipocyte-derived ferroptotic signaling mitigates obesity. *Cell Metab* 2025; 37:673–691.e677. <https://doi.org/10.1016/j.cmet.2024.11.010>.

56. Liu J, Kang R, Tang D. Signaling pathways and defense mechanisms of ferroptosis. *FEBS J* 2022; 289:7038–7050. <https://doi.org/10.1111/febs.16059>.
57. Wang L, Yu H, Wang D, Yin G, Chen S, Zhang X, Yu W, Meng D, Liu H, Jiang W, Zhang F. Diosgenin alleviates lipid accumulation in NAFLD through the pathways of ferroptosis defensive and executive system. *J Nutr Biochem* 2025; 140:109886. <https://doi.org/10.1016/j.jnutbio.2025.109886>.
58. Wu T, Teng Y, Song D, Yang Y, Shen H, Sun X, Chen R, Zhao L, Zhong X, Yan Q, Niu J, Ge J, *et al.* A strategy targeting ferroptosis for mitochondrial reprogramming and intervertebral disc degeneration therapy. *Theranostics* 2025; 15:9159–9178. <https://doi.org/10.7150/thno.117725>.
59. Huang C-D, Luo T, Zhang H, Cheng L-H, Peng S-H, Zhou X-W, Cao L, Chen F-Y, He J-W, Chen C, Zhang C-H, Zhang Y-A, *et al.* Ferroptosis as a potential therapeutic target for obesity-related metabolic diseases. *Front Pharmacol* 2025; 16: 1581632.
60. Yan J, Zeng Y, Guan Z, Li Z, Luo S, Niu J, Zhao J, Gong H, Huang T, Li Z, Deng A, Wen Q, *et al.* Inherent preference for polyunsaturated fatty acids instigates ferroptosis of Treg cells that aggravates high-fat-diet-related colitis. *Cell Rep* 2024; 43:114636. <https://doi.org/10.1016/j.celrep.2024.114636>.
61. Yang X, Chen Y, Guo J, Li J, Zhang P, Yang H, Rong K, Zhou T, Fu J, Zhao J. Polydopamine nanoparticles targeting ferroptosis mitigate intervertebral disc degeneration via reactive oxygen species depletion, iron ions chelation, and gpx4 ubiquitination suppression. *Adv Sci* 2023; 10:2207216.
62. Xiong M, Li L, Wen L, Zhao A. Decidual stromal cell-derived exosomes deliver miR-22-5p_R-1 to suppress trophoblast metabolic switching from mitochondrial respiration to glycolysis by targeting PDK4 in unexplained recurrent spontaneous abortion. *Placenta* 2024; 153:1–21. <https://doi.org/10.1016/j.placenta.2024.05.131>.
63. Kelly A, Chan J, Powell TL, Cox LA, Jansson T, Rosario FJ. Maternal obesity alters the placental transcriptome in a fetal sex-dependent manner. *Front Cell Dev Biol* 2023; 11:1178533.
64. Wu YQ, Zhang CS, Xiong J, Cai DQ, Wang CZ, Wang Y, Liu YH, Wang Y, Li Y, Wu J, Wu J, Lan B, *et al.* Low glucose metabolite 3-phosphoglycerate switches PHGDH from serine synthesis to p53 activation to control cell fate. *Cell Res* 2023; 33:835–850. <https://doi.org/10.1038/s41422-023-00874-4>.
65. Reyes JS, Cortés-Ríos J, Fuentes-Lemus E, Rodríguez-Fernández M, Davies MJ, López-Alarcón C. Competitive oxidation of key pentose phosphate pathway enzymes modulates the fate of intermediates and NADPH production. *Free Radic Biol Med* 2024; 222: 505–518. <https://doi.org/10.1016/j.freeradbiomed.2024.05.050>.
66. Tang Y, Zhang X, Chen X, Li J, Xie M, Wu Q, Zhang Y, Do C-w, Sun X. Targeted LC-MS profiling reveals dysregulated glycolytic flux and TCA cycle stalling in POAG plasma. *Sci Rep* 2025; 15:34212.
67. Liu N, Wu W, Wan X, Wang J, Huang J, Jiang Y, Sheng Y, Wu J, Liang Z, Qin Z, Wang Y. Regulation of FSP1 myristoylation by NADPH: a novel mechanism for ferroptosis inhibition. *Redox Biol* 2024; 73:103176. <https://doi.org/10.1016/j.redox.2024.103176>.
68. Tai Y, Zhang Z, Liu Z, Li X, Yang Z, Wang Z, An L, Ma Q, Su Y. D-ribose metabolic disorder and diabetes mellitus. *Mol Biol Rep* 2024; 51:220. <https://doi.org/10.1007/s11033-023-09076-y>.
69. Yagi H, Kasai T, Rioual E, Ikeya T, Kigawa T. Molecular mechanism of glycolytic flux control intrinsic to human phosphoglycerate kinase. *Proc Natl Acad Sci U S A* 2021; 118:e2112986118. <https://doi.org/10.1073/pnas.2112986118>.
70. Habashy A, Acree C, Kim K-Y, Zahraei A, Dufresne M, Phan S, Cutler M, Patterson E, Mulligan AG, Burkewitz K, Flynn CR, Lantier L, *et al.* Spatial patterns of hepatocyte glucose flux revealed by stable isotope tracing and multi-scale microscopy. *Nat Comm* 2025; 16:5850. <https://doi.org/10.1038/s41467-025-60994-w>.