Placenta 178 (2026) 30–38

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Maternal high-protein diet impairs fetal growth through placental dysfunction in mice

Ying Zhao [a,b,1], Long Cai [c,1], Liyuan He [a,d], Ge Gao [a], Martine Schroyen [b], Yu Pi [a], Wenjuan Sun [a], Dongxu Ming [a], Yanpin Li [a,*], Xilong Li [a,**]

[a] Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
[b] Animal Sciences, TERRA Teaching and Research Centre, Gembloux Agro-Bio Tech, University of Liège, Gembloux, 5030, Belgium
[c] Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, China
[d] College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang, 453003, China

ARTICLE INFO

Keywords:
High-protein diet
Placental function
Amino acid transport
Oxidative stress
Mitochondrial function
Fetal development

ABSTRACT

Introduction: Maternal dietary protein intake plays a vital role in pregnancy outcomes. However, the impact of popular high-protein diets, often accompanied by carbohydrate restriction, remains poorly understood. We investigated the effect of different levels of maternal protein during pregnancy on embryo implantation, placental development, and fetal growth in mice.

Methods: Pregnant C57BL/6 mice were fed isocaloric purified diets containing 10% (low protein, LP), 20% (medium protein, MP), or 40% (high protein, HP) protein. Implantation was assessed on day 6.5, and gestational outcomes were analyzed on day 18.5.

Results: Maternal protein did not affect implantation number or litter size. However, HP intake reduced fetal weight and placental efficiency. Morphologically, HP intake decreased the placental labyrinth zone/total area ratio compared to LP. It was further revealed that maternal HP intake reduced fetal serum glucose, increased fetal serum urea nitrogen, and activated hepatic gluconeogenic and amino acid catabolic pathways compared to MP. Mechanistically, HP intake induced placental oxidative stress, evidenced by enhanced malondialdehyde content, and decreased the activity of placental catalase, superoxide dismutase, and glutathione peroxidase. The expression of genes involved in antioxidant defense and amino acid transport was also suppressed in placenta. Moreover, maternal HP intake reduced ATP production, lowered mitochondrial DNA copy number, and down-regulated gene expression of electron transport chain components.

Discussion: Excessive maternal protein intake under isoenergetic conditions disrupts placental redox homeostasis and mitochondrial function, and impairs nutrient transfer. These alterations compromise placental efficiency and fetal growth, highlighting the potential risks associated with high-protein dietary patterns during pregnancy.

1. Introduction

Protein intake during gestation is especially crucial for maternal physiology, fetal development and long-term offspring health [1]. The adverse impacts of protein restriction have been well studied [2–6], while the consequences of high protein (HP) intake on placental function and fetal development remain less elucidated. Current evidence regarding the impact of HP intake on fetal development is contradictory, with some studies reporting beneficial outcomes such as reduced incidence of low birth weight (LBW), small for gestational age (SGA), and intrauterine growth restriction (IUGR) [7–9], while others have observed adverse effects including decreased ponderal index and lower birth weight [10,11]. To date, few studies have concurrently evaluated the impact of maternal low- and high-protein intake during pregnancy

* Corresponding author. Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Haidian District, Beijing, 100081, China.
** Corresponding author. Key Laboratory of Feed Biotechnology of Ministry of Agriculture and Rural Affairs, Institute of Feed Research, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Haidian District, Beijing, 100081, China.
E-mail addresses: liyanpin@caas.cn (Y. Li), lixilong@caas.cn (X. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.placenta.2026.03.016
Received 6 October 2025; Received in revised form 13 February 2026; Accepted 23 March 2026
Available online 24 March 2026
0143-4004/© 2026 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

on fetal development and maternal physiological outcomes.

Embryo implantation and placental development are critical events that determine pregnancy success, and their outcomes are highly sensitive to maternal nutritional status [12]. The placenta adapts dynamically to maternal nutritional status to optimize nutrient transport and fetal growth [13,14], yet such adaptations may not always be beneficial [15]. These adaptations rely on finely tuned amino acid (AA) transport systems and optimal mitochondrial function to meet the high energy demands of fetal growth. Any disruption in these systems, whether due to nutrient scarcity or overload, can compromise fetal development. Mitochondria are the primary generators of ATP and a major source of reactive oxygen species (ROS). An imbalance between ROS production and antioxidant defense is closely associated with placental dysfunction and adverse pregnancy outcomes such as gestational morbidities, poor fetal growth, or premature birth [16–18]. Pregnancy pathologies are related to abnormal mitochondrial structure and function [19]. Crucially, mitochondrial function is sensitive to metabolic status. While protein restricted diets are known to induce oxidative stress [20], whether and how maternal HP intake disrupts placental redox and mitochondrial homeostasis remains unclear.

In recent years, high-protein, low-carbohydrate diets have become increasingly popular [21,22]. However, the consequence of such nutritional patterns during pregnancy remains poorly understood. In isoenergetic dietary models, an increase in protein intake causes a proportional reduction in carbohydrates. Therefore, an HP diet creates a low-carbohydrate environment, whereas a low-protein (LP) diet results in high-carbohydrate intake. Consequently, this study aimed to evaluate the effects of maternal dietary protein levels and the associated change in carbohydrates on early embryo implantation and fetal growth in mice, with an emphasis on placental AA transporters, redox balance, and mitochondrial function.

2. Materials and methods

2.1. Animal treatments and sample collection

All animal procedures were approved by the Ethics Committee of the Institute of Feed Research of Chinese Academy of Agricultural Sciences (Protocol No. IFR-CAAS-20240820). Eight-week-old female C57BL/6 mice were purchased from SPF (Beijing, China) Biotechnology Co., Ltd. and housed under controlled conditions with free access to food and water at the Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences. After a one-week acclimation period, females were mated with male C57BL/6 mice overnight at a 2:1 ratio. Mated females (vaginal plug = gestational day 0.5, GD 0.5) were randomly assigned to one of the isocaloric diets containing 10% protein (LP), 20% protein (MP), 40% protein (HP). Carbohydrates were adjusted inversely to the protein content. The composition of these diets is shown in Supplementary Table 1. The MP group (standard protein and carbohydrate level) served as the control.

Two experiments were performed. In experiment 1, a total of 45 vaginal plug-positive females were randomly assigned to one of three groups (n = 15 per group). On GD 6.5, blood was collected via orbital bleeding. The number of embryo implantation sites in both uterine horns was recorded. The number of confirmed pregnancies per group was: LP = 12, MP = 13, HP = 11. In experiment 2, another 45 vaginal plug-positive mice were selected and they were sacrificed on GD 18.5. Feed intake was recorded daily throughout gestation. On GD 18.5, blood was collected. After uterus was exposed, the number of total and live fetuses, individual fetal and placental weights were recorded. Fetal blood was collected via decapitation of the fetuses immediately after removal from the uterus. Amniotic fluid, uterine tissue, placentas, maternal serum, and fetal serum were collected and stored at −80 °C. Placentas were fixed in 4% paraformaldehyde. The number of mice confirmed pregnant per group was: LP = 11, MP = 10, HP = 12. Index was calculated as: Weight gain (g) = final weight-initial weight. Placental efficiency = fetal weight/placental weight [23,24].

2.2. Concentrations of glucose and blood urea nitrogen (BUN) in serum and amniotic fluid

The concentrations of glucose and BUN in maternal serum, fetal serum, and amniotic fluid were determined using a commercial kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

2.3. Concentrations of amino acids in serum and amniotic fluid

Amino acid concentrations were analyzed using high-performance liquid chromatography (HPLC) as previously described [25]. Briefly, samples of maternal serum, amniotic fluid, and fetal serum, collected on GD 18.5, were deproteinized using 1.5M $HClO_4$, neutralized with 2M H_2CO_3, and centrifuged. Supernatants were mixed with 0.1M HCl and internal standard, then filtered and analyzed for 16 types of amino acids. For fetal blood and amniotic fluid, the available volume was sometimes insufficient, and six to seven samples per group were analyzed.

2.4. RNA extraction and RT-qPCR

Total RNA was extracted from homogenized tissues following a previous protocol [26]. RNA quality was assessed with the NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, USA), and 1000 ng of RNA was reverse-transcribed using the PrimeScript® RT Reagent Kit with gDNA Eraser (Takara, China). The RT-qPCR was conducted using TB Green Premix Ex Taq™ Kit (Takara, China) on a CFX96 Real-Time PCR System (Bio-Rad, USA), normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), and analyzed by the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Supplementary Table 2.

2.5. Protein extraction and Western blot

The placental protein was extracted and quantified by BCA assay (Beijing Huaxing Bochuang Gene Technology Co., Ltd.). Equal amounts (40 μg) were loaded onto 12% SDS-PAGE gels (Bio-Rad, USA). Proteins were separated by SDS-PAGE, transferred to PVDF membranes, blocked and incubated with primary antibodies. After washing, membranes were incubated with secondary antibodies and visualized using an Odyssey CLx infrared imaging system (LI-COR Biosciences, USA). Band intensities were quantified with ImageJ and normalized to actin beta (ACTB). Antibodies are listed in Supplementary Table 3.

2.6. Histological analysis

Fixed placentas were embedded and sectioned into 4 μm-thick slices. Hematoxylin and eosin (HE) staining, periodic acid-Schiff (PAS) staining, and immunohistochemistry (IHC) for CD31 were performed on 6 placentas per group randomly by Wuhan Servicebio Technology CO., LTD. Antibody information is provided in Supplementary Table 3. Placental tissue sections were scanned using a PANNORAMIC MIDI digital slide scanner (3DHISTECH Ltd., Budapest, Hungary). Image analysis of HE sections was conducted using CaseViewer 2.3 software. The areas of the whole placenta, decidua (DEC), labyrinth zone (LZ), and junctional zone (JZ) were quantified as previously described [27]. The proportion of PAS-positive glycogen area in JZ and the CD31-positive area in LZ were quantified using ImageJ software.

2.7. Oxidative stress and ATP content

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) level in the placenta were measured using kits from Nanjing Jiancheng Bioengineering

Institute (Nanjing, China). The concentration of ATP in the placenta was measured using the Enhanced ATP Assay Kit (Shanghai Beyotime Biotechnology Co., Ltd.).

2.8. mtDNA copy number

Mitochondrial DNA (mtDNA) copy number was quantified by qPCR, as previously described [28]. Total DNA was extracted from placental tissues using a commercial genomic DNA extraction kit (TIANamp genomic DNA Kit, China), and 10 ng of DNA was used in the qPCR reaction. The mtDNA copy number was calculated by normalizing the expression level of mitochondrially encoded cytochrome *c* oxidase II (*mt-Co2*) to nuclear ribosomal protein S18 (*Rps18*). Primer sequences for mice *mt-Co2 and Rps18* were obtained from a previous study and provided in Supplementary Table 2 [29,30].

2.9. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS Statistics 20 and visualized with GraphPad Prism 10.1.2. One-way analysis of variance (ANOVA) was used to determine differences between groups. Multiple comparisons of significant differences were performed using the Tukey test. For unequal variances (Levene's test, $P < 0.05$), Welch's ANOVA with Games-Howell correction was used. Different superscript letters indicate significant differences between groups. * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and $0.05 \leq P < 0.10$ indicates a trend toward significant difference.

3. Results

3.1. Effects of different maternal protein levels on feed intake, weight gain, and reproductive performance

Detailed maternal average daily feed intake (ADFI) data throughout gestation are presented in Supplementary Table 4. There were no significant differences in ADFI among groups during GD 0.5 - 6.5 or GD 6.5 - 13.5, while it was significantly decreased with the protein level increased during GD 13.5 - 18.5 ($P < 0.001$). However, total gestational ADFI did not differ between HP and MP. Despite the reduced feed intake in late gestation, maternal weight gain and uterus weight remained unaffected among groups (Table 1). Regarding the reproductive performance (Table 1), no significant differences were observed in implantation numbers in mice at GD 6.5, or total litter size, live litter size, total litter weight and average placenta weight at GD 18.5 ($P > 0.05$). However, average fetal weight was significantly lower in HP relative to MP, accompanied by reduced placental efficiency ($P < 0.05$).

3.2. Effects of different maternal protein levels on glucose and BUN concentrations in maternal serum, amniotic fluid and fetal serum at GD 18.5

As shown in Table 2, compared to MP, glucose concentrations in maternal serum and amniotic fluid were significantly elevated in LP ($P < 0.01$), whereas no significant difference was observed between MP and HP. Notably, fetal serum glucose was significantly reduced in HP ($P < 0.05$). The BUN concentration in maternal serum, amniotic fluid, and fetal serum exhibited a dose-dependent increase with dietary protein levels ($P < 0.05$), confirming the altered nitrogen metabolism.

3.3. Effects of different maternal protein levels on amino acid profiles in maternal serum, amniotic fluid and fetal serum at GD 18.5

Amino acid profiles are detailed in Table 2. In maternal serum, the level of valine (Val) was significantly higher in HP than MP ($P < 0.05$). No other amino acids were significantly changed in HP or LP compared to MP. However, the levels of branched chain amino acids (BCAA, including valine, leucine, and isoleucine), and histidine (His), threonine (Thr), tyrosine (Tyr), and methionine (Met) were elevated in HP relative to LP ($P < 0.05$). In amniotic fluid, we found that there was no significant difference between LP and MP or between HP and MP, except that lysine (Lys) was significantly higher in LP than in MP, and arginine (Arg) was significantly lower in HP than in LP ($P < 0.05$). However, the HP showed higher levels of Val and Ile but significantly lower level of non-essential AA (NEAA), including Asp, glutamate (Glu), serine (Ser), glycine (Gly), Arg, and alanine (Ala) compared to LP ($P < 0.05$). Specifically, Ala tended to be higher in LP than in MP ($0.05 \leq P < 0.10$), and was significantly higher than in HP ($P < 0.05$). In fetal serum, many AA levels showed no significant difference among the groups. The LP exhibited elevated Ala and Lys but decreased Trp compared to MP ($P < 0.05$). Met was substantially higher in HP compared to LP ($P < 0.05$), and showed a trend to be higher than MP ($0.05 \leq P < 0.10$).

Table 1
Effect of different protein levels on implantation and reproductive performance in pregnant mice.

Items	Treatment groups[a]			SEM	*P*-value
	LP	MP	HP		
GD 6.5[b]					
Number of embryo implantation sites	9.25	9.00	9.10	0.38	0.793
Left number of implanted embryos	4.83	5.00	4.54	0.50	0.658
Right number of implanted embryos	4.41	3.62	4.18	0.62	0.405
GD 18.5[c]					
Total litter size	8.64	8.90	9.25	0.37	0.481
Live litter size	8.27	8.40	8.92	0.39	0.453
Weight gain during pregnancy (g)	13.54	14.75	13.90	0.47	0.219
Uterus weight (g)	11.52	11.83	11.76	0.45	0.874
Total litter weight (g)	9.03	9.43	9.33	0.37	0.752
Average fetal weight (g)	1.09^{ab}	1.13^{a}	1.05^{b}	0.02	0.013
Average placenta weight (g)	0.10	0.10	0.10	0.00	0.377
Placental efficiency	11.50^{ab}	12.06^{a}	10.55^{b}	0.30	0.005

[a,b] indicates significant differences between means ($P < 0.05$). Within the same row, values sharing the same letter are not significantly different.
[a] LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%).
[b] At GD 6.5, the replicates were 12, 13, and 11 in LP, MP, and HP groups, respectively.
[c] At GD 18.5, the replicates were 11, 10, and 12 in LP, MP, and HP groups, respectively.

3.4. Effects of different maternal protein levels on placenta morphology and nutrient transporters in placentas of mice at GD 18.5

Since the placenta acts as the primary interface for nutrient transfer, we investigated whether the placenta morphology and nutrient transporter function was compromised in the HP group. Fig. 1A shows the HE staining results, which illustrate the placental morphology, which consists of the maternal DEC, JZ, and LZ. According to Fig. 1D, no significant differences were observed in the decidua/total area ratio among groups. There was no significant difference in the JZ/total area ratio or LZ/total area ratio between MP and HP or MP and LP. However, the JZ/total area ratio was significantly increased and the LZ/total area ratio was decreased in HP compared to LP ($P < 0.05$). Glycogen storage (PAS staining) and vascular coverage (CD31) did not differ significantly among groups (Fig. 1B, C, E, F). We further analyzed placental nutrient transporter genes (Fig. 1G). Compared to MP, L-type amino acid transporter 3 (*Lat3*), L-type amino acid transporter 4 (*Lat4*), cationic amino acid transporter 1 (*Cat1*), and glucose transporter 1 (*Glut1*) were significantly downregulated in HP ($P < 0.05$), and L-type amino acid transporter 2 (*Lat2*) and hexokinase 1 (*Hk1*) showed a downward trend

Table 2
Concentrations of glucose (mmol/L), BUN (mmol/L), and amino acids (μmol/mL) in maternal serum, amniotic fluid and fetal serum of mice fed different protein levels on GD 18.5.

Items	Maternal serum					Amniotic fluid					Fetal serum				
	LP	MP	HP	SEM	*P*-value	LP	MP	HP	SEM	*P*-value	LP	MP	HP	SEM	*P*-value
GLC	6.3^{a}	4.7^{b}	4.2^{b}	0.4	0.001	2.0^{a}	1.5^{b}	1.5^{b}	0.1	0.007	3.8^{ab}	3.9^{a}	2.7^{b}	0.4	0.031
BUN	2.7^{c}	4.2^{b}	5.2^{a}	0.2	0.000	3.2^{c}	5.6^{b}	7.2^{a}	0.3	0.000	4.5^{c}	7.9^{b}	9.8^{a}	0.3	0.000
Asp	13.2^{y}	13.9	20.1^{x}	2.1	0.052	20.1^{a}	17.3^{ab}	15.0^{b}	1.1	0.021	46.8	36.8	41.8	3.2	0.121
Glu	50.8	55.4	64.7	8.1	0.534	114.1^{a}	100.5^{ab}	72.3^{b}	10.9	0.049	234.4	216.2	212.4	13.2	0.498
Ser	103.2	103.3	113.3	10.3	0.747	171.6^{a}	156.6^{ab}	139.6^{b}	8.2	0.053	115.6	92.5	93.9	7.6	0.088
His	30.9^{b}	40.6^{ab}	49.2^{a}	4.3	0.024	84.2	97.6	82.0	4.9	0.104	85.5	96.4	99.5	5.1	0.166
Gly	65.0	71.6	71.4	5.2	0.577	169.2^{a}	157.7^{ab}	138.7^{b}	7.7	0.044	159.8	132.9	138.0	15.0	0.447
Thr	127.9^{b}	186.7^{ab}	244.2^{a}	17.8	0.001	246.9	294.5	267.0	15.7	0.166	157.1	160.1	198.0	17.1	0.251
Arg	7.1	4.9	4.4	1.5	0.467	87.5^{a}	84.7^{a}	60.4^{b}	6.00	0.016	35.4	37.8	23.6	9.3	0.573
Ala	353.7	294.1	353.9	39.8	0.517	$564.2^{a,x}$	$485.4^{ab,y}$	419.9^{b}	23.4	0.003	522.9^{a}	370.3^{b}	395.9^{b}	23.0	0.001
Tyr	34.9^{b}	45.5^{ab}	55.5^{a}	5.2	0.032	186.2	202.8	222.5	14.8	0.257	102.4	118.4	131.4	8.7	0.143
Val	83.2^{b}	107.5^{b}	159.3^{a}	13.3	0.003	250.2^{b}	275.3^{ab}	295.8^{a}	10.6	0.059	177.1	179.7	187.2	7.9	0.733
Met	37.0^{b}	44.4^{ab}	58.7^{a}	4.2	0.041	128.5	125.7	118.5	5.9	0.483	$75.7^{b,xy}$	$77.3^{ab,y}$	$89.7^{a,x}$	3.3	0.030
Trp	43.0^{y}	54.9^{x}	56.1^{x}	3.7	0.041	53.5	51.2	53.2	4.2	0.930	36.6^{b}	48.6^{a}	36.9^{b}	2.6	0.006
Ile	33.8^{b}	43.7^{ab}	55.5^{a}	4.7	0.014	87.7^{b}	94.7^{ab}	109.1^{a}	4.7	0.034	57.7	58.0	65.4	2.9	0.225
Phe	32.0^{y}	41.9^{x}	54.6^{x}	4.8	0.023	116.1	114.9	123.2	6.3	0.626	69.8	76.3	79.0	5.0	0.504
Lys	360.8	392.3	355.2	25.3	0.552	799.5^{a}	688.2^{b}	633.7^{b}	28.7	0.003	669.3^{a}	582.1^{b}	560.8^{b}	21.7	0.014
Leu	56.3^{b}	74.6^{ab}	92.4^{a}	8.4	0.024	220.3	242.1	260.4	12.8	0.138	109.3	102.8	113.7	6.7	0.567

[1] LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%).
[2] Replicates were n = 10 for maternal serum, n = 6 for amniotic fluid, and n = 7 for fetal serum.
^{a,b} indicates significant differences between means ($P < 0.05$); ^{x,y} indicates a trend toward significance ($P < 0.10$). Within the same row, values sharing the same letter are not significantly different.
GLC, Glucose; BUN, Blood urea nitrogen; Asp, Aspartate; Glu, Glutamate; Ser, Serine; His, Histidine; Gly, Glycine; Thr, Threonine; Arg, Arginine; Ala, Alanine; Tyr, Tyrosine; Val, Valine; Met, Methionine; Trp, Tryptophan; Ile, Isoleucine; Phe, Phenylalanine; Lys, Lysine; Leu, Leucine.

($0.05 \leq P < 0.10$). Conversely, CD98 heavy chain (*4F2hc*) and y^+ L amino acid transporter 1 (y^+ *Lat1*) were upregulated in LP.

3.5. Effects of different maternal protein levels on placental oxidative stress status of mice at GD 18.5

To explore the mechanisms underlying the observed morphological and transport damage, we assessed the placental oxidative stress status. From Fig. 2A–D, in the HP group, the lipid peroxidation marker MDA was significantly elevated, while the key antioxidant enzyme activities, including SOD, CAT, and GPx, were significantly decreased compared to MP ($P < 0.05$). Consistently, mRNA levels of antioxidant genes such as glutathione peroxidase 4 (*Gpx4*), NAD(P)H dehydrogenase quinone 1 (*Nqo1*), superoxide dismutase 1 (*Sod1*) and catalase (*Cat*) were significantly decreased in HP (Fig. 2E). In contrast, MDA levels in LP were significantly lower than in MP ($P < 0.05$), with a decreasing trend of SOD observed in the LP group ($0.05 \leq P < 0.10$). Regarding the NFE2L2 pathway (Fig. 2F–I), the protein expression of nuclear factor erythroid derived 2 like 2 (NFE2L2), the master transcriptional regulator of antioxidant defense, was significantly upregulated in LP compared to MP ($P < 0.05$), while kelch-like ECH-associated protein 1 (KEAP1), a negative regulator of NFE2L2, was significantly decreased ($P < 0.05$). Additionally, the pro-apoptotic protein, BCL2-associated X protein (BAX) was also significantly decreased in LP ($P < 0.05$).

3.6. Effects of different maternal protein levels on mitochondrial function in placenta of mice at GD 18.5

Given the elevated oxidative stress in HP, we further examined mitochondrial function (Fig. 3). According to the protein results of mitofusin 2 (MFN2) which mediates mitochondrial fusion, and dynamin 1-like (DNM1L) which drives mitochondrial fission (Fig. 3A–B), MFN2 levels were significantly increased while DNM1L levels were significantly decreased in the LP group ($P < 0.05$), suggesting the enhanced mitochondrial fusion in LP. From Fig. 3C, ATP level had no significant difference in HP compared to MP, while it was significantly lower than in LP ($P < 0.05$). Furthermore, the relative mtDNA copy number of *mt-Co2* (Fig. 3D) was significantly reduced in HP compared to both MP and LP ($P < 0.05$). Gene expression analysis (Fig. 3E–F) showed that mitochondrial biogenesis and dynamic genes such as *Mfn2*, mitochondrial fission 1 (*Fis1*), peroxisome proliferative activated receptor gamma coactivator 1 alpha (*Ppargc1a*), *and* transcription factor A mitochondrial (*Tfam*), as well as mitochondrial electron transport genes such as NADH: ubiquinone oxidoreductase core subunit S8 (*Ndufs8*), succinate dehydrogenase complex subunit B (*Sdhb*), and cytochrome *c* oxidase subunit 4I1 (*Cox4i1*) were significantly downregulated in HP compared to MP ($P < 0.05$).

3.7. Effects of different maternal protein levels on fetal glucose and amino acid metabolism at GD 18.5

Finally, we evaluated fetal metabolic adaptations in the liver (Fig. 4). In the HP group, genes related to glucose metabolism, including glucose transporter 1 (*Glut1*), phosphoenolpyruvate carboxykinase 1 (*Pck1*), and glycogen phosphorylase L (*Pygl*) were significantly upregulated compared to MP ($P < 0.05$), with glucose-6-phosphatase catalytic subunit (*G6pc*) showing an increasing trend ($0.05 \leq P < 0.10$). Fig. 4B showed that amino acid transporters *Lat1* and *Lat2*, and catabolic enzymes, such as branched-chain amino acid transaminase 2 (*Bcat2*), glutamic-pyruvic transaminase 1 (*Gpt1*), and glutamic-oxaloacetic transaminase 1 (*Got1*), were significantly increased in HP ($P < 0.05$).

4. Discussion

This study provides a comprehensive investigation into the effects of varying maternal dietary protein levels on pregnancy outcomes. Neither low nor high protein diets significantly altered the number of implanted embryos at GD 6.5 or litter size at GD 18.5. These findings are consistent with previous reviews indicating that maternal low- or high-protein diets during pregnancy have minimal impact on litter size in rats [31]. However, maternal HP intake was associated with reduced average fetal weight and decreased placental efficiency. Placental efficiency, a key indicator of placental function [23,24], was notably reduced in HP, implying that each unit of placental tissue supports less fetal growth. Notably, although the HP group exhibited reduced ADFI during late gestation, no significant difference was observed between HP and MP

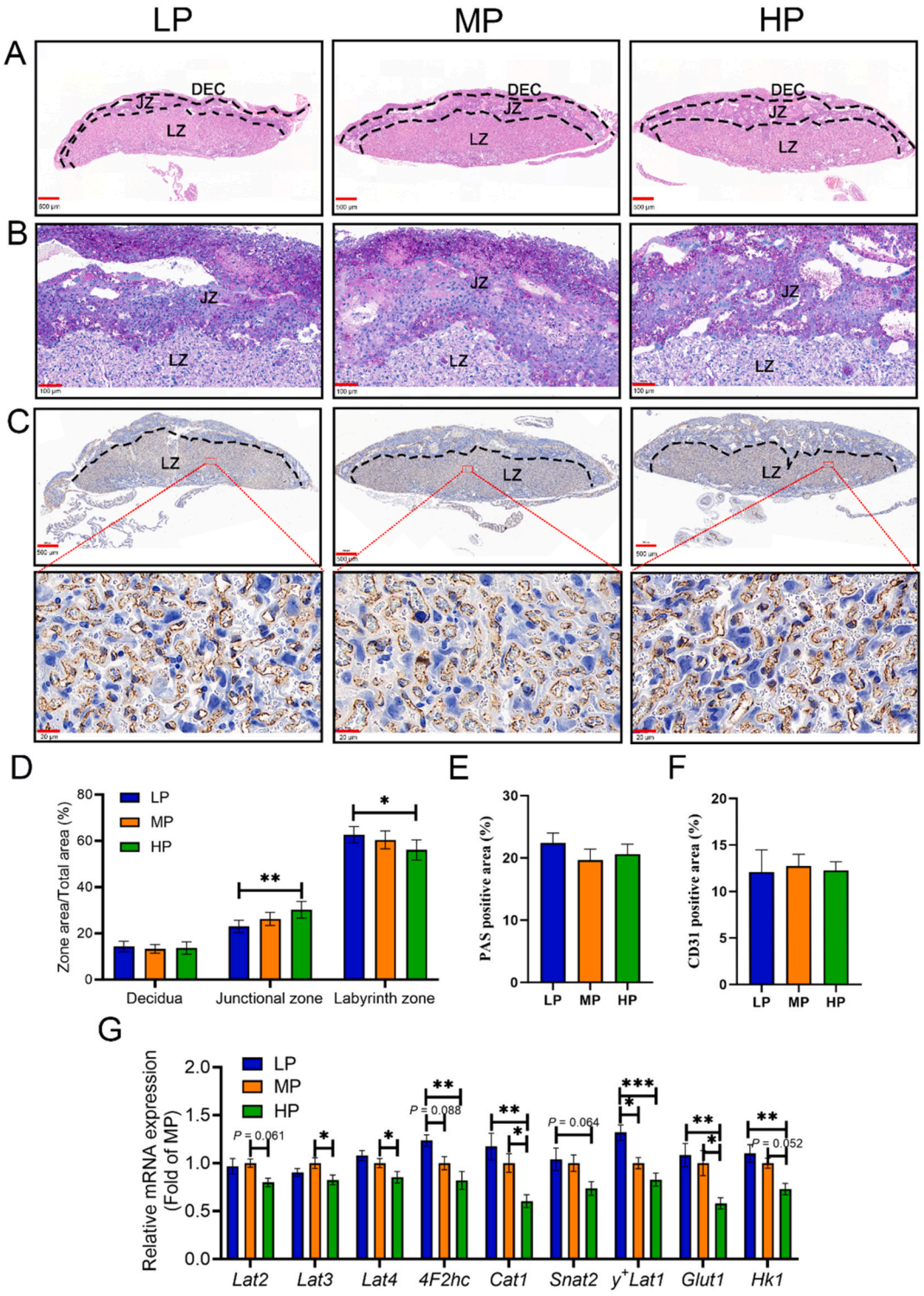

Fig. 1. Effects of different protein levels on the placenta structure, glycogen storage, vascularization, and nutrient transport of mice on GD 18.5. (A) Representative images of HE stained placental cross-sections of LP, MP, and HP groups, scale bar = 500 μm. (B) Representative images of PAS staining showing glycogen, scale bar = 100 μm. (C) Representative images of IHC staining for CD31 in the labyrinth zone, scale bar = 500 μm and 20 μm. (D) Quantitative analysis of the proportional areas of decidua, junctional zone, and labyrinth zone to total placental sectional area (n = 6). (E) Quantitative analysis of PAS-positive area percentage (n = 6). (F) Quantification of CD31-positive area percentage (n = 6). (G) The mRNA expression of amino acid transporter, glucose transporter/metabolism related genes in placenta (n = 10). LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%). Data are means ± SEM, $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

DEC, Decidua; JZ, Junctional zone; LZ, Labyrinth zone; Lat2, L-type amino acid transporter 2; Lat3, L-type amino acid transporter 3; Lat4, L-type amino acid transporter 4; 4F2hc, 4F2 cell-surface antigen heavy chain; Cat1, Cationic amino acid transporter 1; Snat2, Sodium-coupled neutral amino acid transporter 2; y^{+}Lat1, y^{+}L amino acid transporter 1; Glut1, Glucose transporter 1; Hk1, Hexokinase 1.

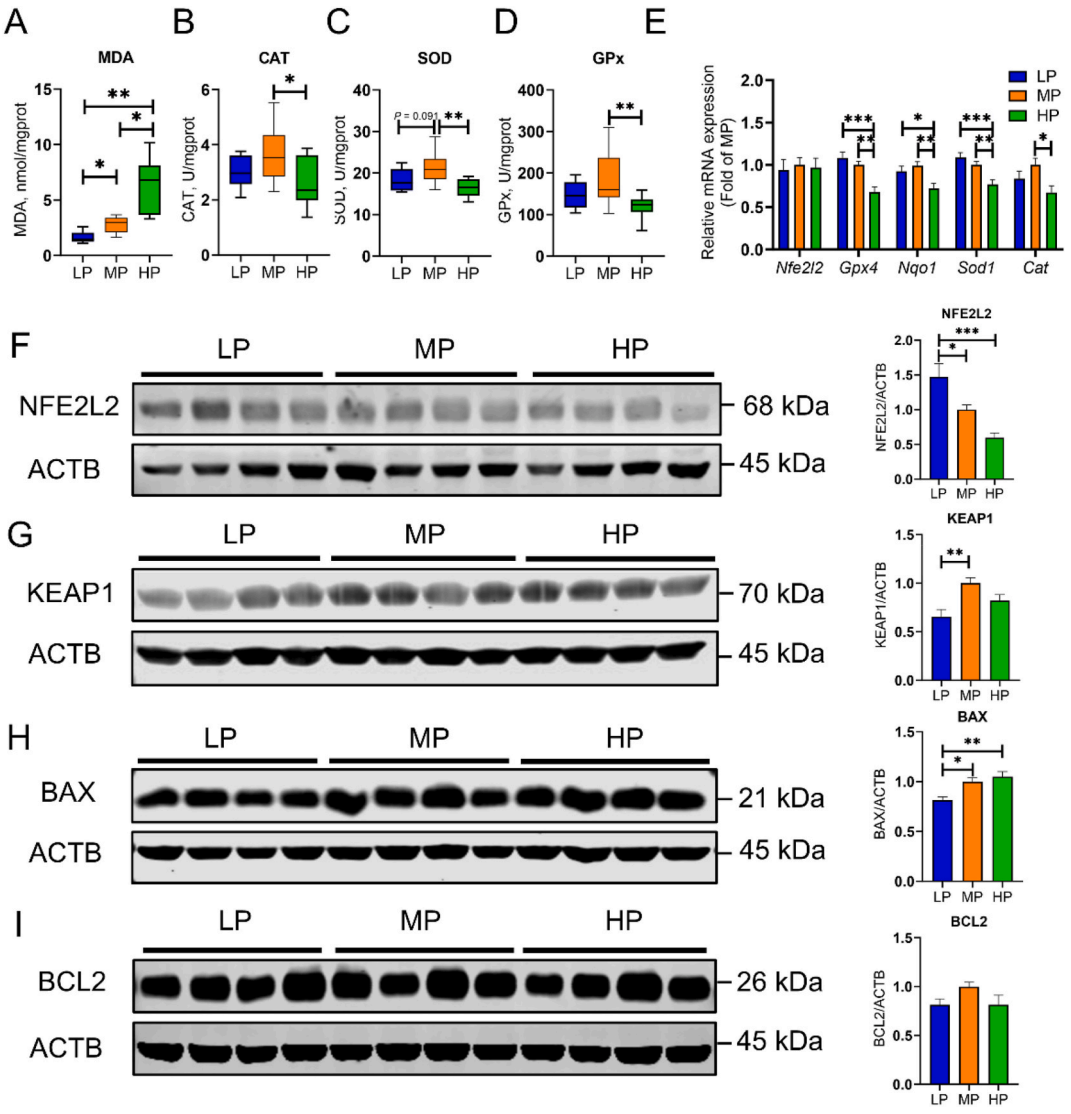

Fig. 2. Placental oxidative stress status in mice fed with different protein level at GD 18.5. (A-D) The MDA level and antioxidant enzyme levels in placenta of mice at GD 18.5 (n = 10). (E) The relative mRNA expression of oxidative stress related genes in placenta (n = 10). (F-I) The expression of oxidative stress and apoptosis related proteins (n = 8). LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%). Data are presented relative to the MP group. Data are means ± SEM. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

MDA, Malondialdehyde; CAT, Catalase; SOD, Superoxide dismutase; GPx, Glutathione peroxidase; NFE2L2, Nuclear factor erythroid derived 2 like 2; NQO1, NAD(P)H dehydrogenase quinone 1; KEAP1, Kelch-like ECH-associated protein 1; BAX, BCL2-associated X protein; BCL2, B cell leukemia/lymphoma 2.

during whole gestation. Furthermore, maternal weight gain remained unaffected. This indicated that the impaired fetal growth was driven by the nutrient rather than insufficient caloric intake.

A critical finding of this study is the fetal metabolic adaptation induced by the HP diet. Although the diets were formulated to differ primarily in protein, the HP diet is relatively low in carbohydrates. Notably, the fetal serum glucose level was significantly decreased. Since glucose is the main energy source for fetus and is derived mainly from maternal circulation [32], the HP fetuses undergo extensive metabolic changes in response to this environment. The significant upregulation of hepatic glucose transporter *Glut1* and key gluconeogenic enzymes, including *Pck1* and *G6pc* in the HP fetus, indicates an active shift to endogenous glucose production. Crucially, this process is fueled by amino acid catabolism, evidenced by the upregulation of hepatic catabolic enzymes *Gpt1*, *Got1*, *Bcat2* and the markedly elevated BUN levels in fetal serum and amniotic fluid. Collectively, these findings demonstrate that the high fetal amino acids are diverted towards oxidative catabolism to compensate for the carbohydrate deficit, directly contributing to the growth restriction.

To further elucidate the dynamics of nutrient supply, we analyzed AA profiles. As expected, maternal serum in the HP group showed elevated levels of most essential AAs and some non-essential AAs (NEAAs), reflecting the direct dietary input. This is consistent with established evidence that maternal circulating AA concentrations are highly sensitive to dietary protein intake [33]. However, the AA profile in fetal serum did not linearly correlate with the maternal profile. In our study, although we observed a coordinated downregulation of key amino acid transporters, including *Snat2* (e.g., for alanine), *Cat1* (e.g., for lysine), and *Lat2*/Lat3/*Lat4* (e.g., for BCAAs) in the HP placenta, fetal AA levels did not decrease. Placental amino acid transport is a complex process, and the net transfer rate is determined by multiple factors, including transporter activity, placental surface area, blood flow, maternal amino acid concentrations, and placental and fetal metabolism [34]. We attribute this maintenance to increased maternal substrate availability. Furthermore, as the HP fetuses suffer from carbohydrate restriction, the utilization of amino acids for protein accretion is impaired, contributing to their accumulation in the fetal circulation. Amniotic fluid composition reflects both fetal metabolic status and

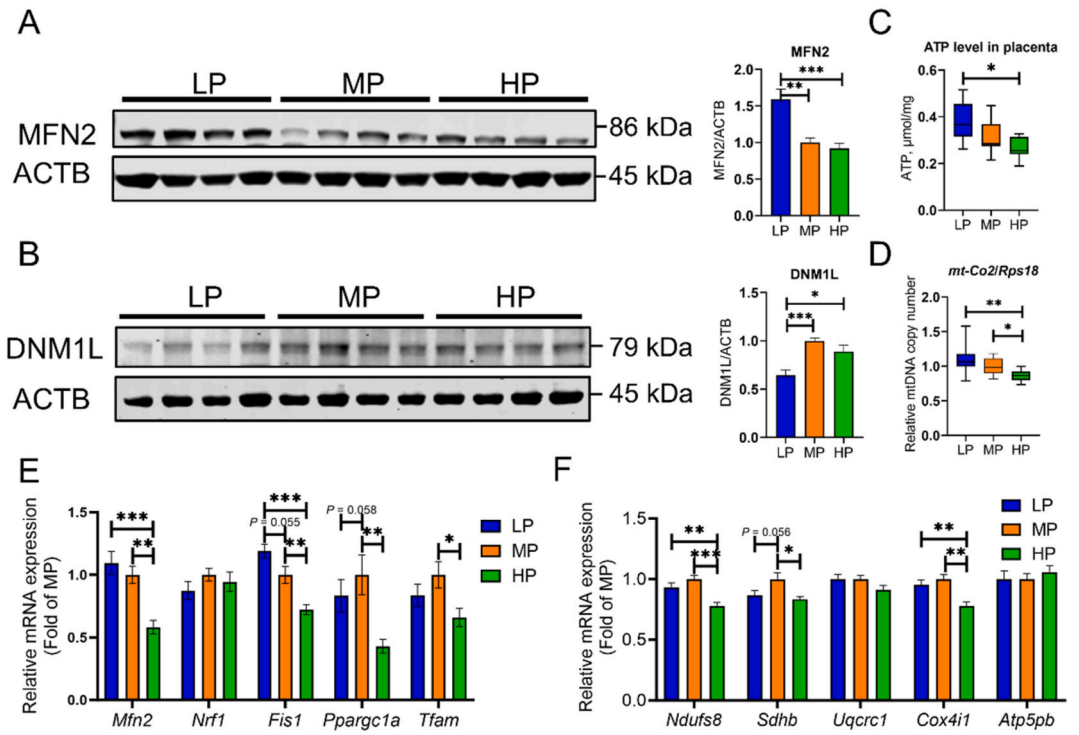

Fig. 3. Mitochondrial function of placenta in mice fed with different protein level at GD 18.5. (A-B) The expression of mitochondrial fusion and fission proteins (n = 8). (C) The ATP content in placenta (n = 8). (D) The mtDNA copy number in placenta (n = 10). (E-F) The mRNA expression of mitochondrial function related genes in placenta (n = 10). LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%). Data are presented relative to the MP group. Data are means ± SEM. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

MFN2, Mitofusin 2; DNM1L, Dynamin 1-like; mt-Co2, Mitochondrially encoded cytochrome *c* oxidase II; Rps18, Ribosomal protein S18; Nrf1, Nuclear respiratory factor 1; Fis1, Mitochondrial fission 1; Ppargc1a, Peroxisome proliferative activated receptor gamma coactivator 1 alpha; Tfam, Transcription factor A mitochondrial; Ndufs8, NADH: ubiquinone oxidoreductase core subunit S8; Sdhb, Succinate dehydrogenase complex subunit B; Uqcrc1, Ubiquinol-cytochrome c reductase core protein 1; Cox4i1, Cytochrome *c* oxidase subunit 4I1; Atp5pb, ATP synthase peripheral stalk-membrane subunit b.

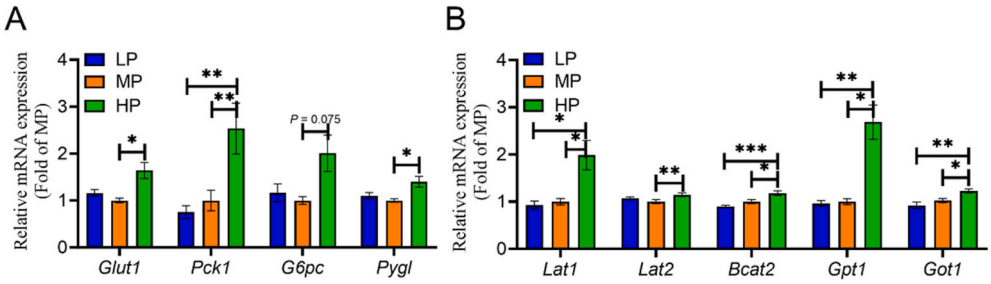

Fig. 4. Fetal hepatic amino acid and glucose metabolism related genes expression at GD 18.5. (A) Relative mRNA expression of genes related to glucose uptake and gluconeogenic genes in fetal liver (n = 10). (B) Relative mRNA expression of genes related to amino acid transport and catabolism in fetal liver (n = 10). LP, low protein diet (10%); MP, middle protein diet (20%); HP, high protein diet (40%). Data are presented relative to the MP group. Data are means ± SEM. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

Glut1, Glucose transporter 1; Pck1, Phosphoenolpyruvate carboxykinase 1; G6pc, Glucose-6-phosphatase catalytic subunit; Pygl, Glycogen phosphorylase L; Lat1, L-type amino acid transporter 1; Lat2, L-type amino acid transporter 2; Bcat2, Branched-chain amino acid transaminase 2; Gpt1, Glutamic-pyruvic transaminase 1; Got1, Glutamic-oxaloacetic transaminase 1.

placental transport activity and it represents the net result of bidirectional exchange between the mother and fetus [35,36]. We propose that HP fetuses use non-essential amino acids, such as Ala, Gly, and Glu, to fuel hepatic gluconeogenesis, leading to their reduced concentrations in the amniotic fluid. Collectively, the adverse effect of HP intake on fetal growth is driven not only by placental transport regulation, but also by the fetal utilization efficiency driven by the low carbohydrate environment.

The placenta serves as the interface for nutrients and oxygen exchange. To determine whether the HP-induced growth restriction stemmed from structural defects or functional impairment, we performed a detailed histological analysis. The most significant morphology alteration was the altered zone distribution. Specifically, since the LZ is the primary site for maternal-fetal exchange [30], this structural reduction implies a diminished functional surface area for nutrient transfer, directly correlating with the reduced placental efficiency. JZ is the major endocrine layer of the placenta, plays a crucial role in maternal adaptation by regulating blood flow [37], and in late gestation, it also contributes to glycogen storage for the fetus [38]. Given the low-carbohydrate content in the HP diet and the observed fetal hypoglycemia, we employed PAS staining to investigate whether placental glycogen reserves were depleted by the fetuses. Interestingly,

despite the structural expansion of the JZ, the glycogen density (PAS--positive area) remained comparable to the MP group. This may represent a compensatory adaptation to metabolic stress. Crucially, the lack of difference in vascular coverage (CD31) suggests that the placental insufficiency is not due to angiogenic failure, but rather due to compromised transport efficiency.

Oxidative stress has been shown to disrupt placental structure and is associated with adverse pregnancy outcomes [39,40]. In the HP group, the placental structural and functional impairments are accompanied by a marked disruption of placental redox homeostasis, evidenced by elevated lipid peroxidation levels and decreased activities of antioxidant enzymes which form the primary defense mechanism against oxidative stress [41]. NFE2L2 is a critical regulator of antioxidant response [42], and the inhibited NFE2L2 pathway in HP also supported the oxidative damage. Conversely, the LP diet appeared to activate antioxidant defenses, with lower MDA levels, higher NFE2L2 protein expression, lower KEAP1 protein expression, and lower levels of the pro-apoptotic protein BAX. Since KEAP1 acts as a negative regulator that promotes NFE2L2 degradation [42], the higher expression of NFE2L2 and lower expression of KEAP1 in LP placentas indicate an adaptive activation of NFE2L2 pathway. Collectively, the results suggest that mild protein restriction may elicit adaptive redox regulation. In contrast, the HP diet appears to exceed the defensive capacity, leading to oxidative damage.

The placenta is a highly metabolically active organ rich in mitochondria, making it particularly vulnerable to oxidative damage. Mitochondria serve as the primary source of placental ATP and dynamically respond to environmental and nutritional cues. In the HP group, we observed clear signs of mitochondrial dysfunction, including reduced ATP production, decreased mtDNA copy number, and the downregulation of genes related to the electron transport chain (ETC) and mitochondrial biogenesis. The resulting energy deficit directly limits the placenta's ability to transport nutrients, which are highly energy-dependent. This aligns with previous findings linking placental mtDNA content to fetal weight [43]. Furthermore, the impaired ETC tends to generate excess ROS, which aggravate oxidative damage and further compromise mitochondrial function [44]. MFN2 promotes mitochondrial fusion, which is often associated with improved efficiency and stress resistance [45]. The LP diet promoted mitochondrial fusion (increased MFN2, decreased DNM1L), suggesting the formation of a stable network resistant to damage. Crucially, we propose that the mitochondrial failure in the HP group is exacerbated by limited substrate availability. Glucose is the primary fuel for placental growth and ATP generation. Since the HP diet is inherently low in carbohydrates, the relative glucose scarcity creates a metabolic challenge, especially during late gestation when fetal demand peaks and the ADFI decreases. Collectively, our findings support that maternal HP intake induces placental oxidative stress and impairs placental mitochondrial function, which in turn impairs amino acid transport capacity.

5. Conclusion

In conclusion, although both protein deficiency and excess can disrupt physiological balance, our findings highlight that the excessive protein intake, coupled with lower carbohydrate, was associated with changed placental morphology, downregulation of placental AA transporters, heightened oxidative stress, and mitochondrial dysfunction. Furthermore, the fetus exhibited enhanced gluconeogenesis and amino acid catabolism, leading to growth restriction. This indicates that maternal supply can affect placental adaptation and fetal utilization, potentially contributing to reduced fetal weight. Given that current recommendations for protein intake during pregnancy remain inconsistent, these findings have significant implications for establishing appropriate protein recommendations during pregnancy and supporting healthy fetal growth.

Funding sources

This research was supported by the National Natural Science Foundation of China (Grant number 32272908). The author Ying Zhao is grateful for the financial support provided by the China Scholarship Council program (Grant number 202403250107).

CRediT authorship contribution statement

Ying Zhao: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Long Cai:** Data curation, Investigation, Visualization, Writing – review & editing. **Liyuan He:** Data curation, Investigation, Writing – review & editing. **Ge Gao:** Investigation, Writing – review & editing. **Martine Schroyen:** Writing – review & editing. **Yu Pi:** Writing – review & editing. **Wenjuan Sun:** Writing – review & editing. **Dongxu Ming:** Writing – review & editing. **Yanpin Li:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Xilong Li:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

Thanks to Xiaowen Li for assistance with animal feeding and to my colleagues for helping with sample collection.

List of abbreviations

ADFI: Average daily feed intake; Ala: Alanine; Arg: Arginine; Asp: Aspartate; BAX: BCL2-associated X protein; Bcat2: Branched-chain amino acid transaminase 2; BCL2: B cell leukemia/lymphoma 2; BUN: Blood urea nitrogen; CAT: Catalase; Cat1: Cationic amino acid transporter 1; COX4I1: Cytochrome *c* oxidase subunit 4I1; DEC: Decidua; DNM1L: Dynamin 1-like; FIS1: Mitochondrial fission 1; 4F2hc: 4F2 cell-surface antigen heavy chain; G6pc: Glucose-6-phosphatase catalytic subunit; GLC: Glucose; Glu: Glutamate; Glut1: Glucose transporter 1; Gly: Glycine; GPX: Glutathione peroxidase; Gpt1: Glutamic-pyruvic transaminase 1; Got1: Glutamic-oxaloacetic transaminase 1; HP: High protein; His: Histidine; Hk1: Hexokinase 1; IUGR: Intrauterine growth restriction; Ile: Isoleucine; JZ: Junctional zone; KEAP1: Kelch-like ECH-associated protein 1; Lat1: L-type amino acid transporter 1; Lat2: L-type amino acid transporter 2; Lat3: L-type amino acid transporter 3; Lat4: L-type amino acid transporter 4; LBW: Low birth weight; Leu: Leucine; Lys: Lysine; LZ: Labyrinth zone; MDA: Malondialdehyde; mt-Co2: Mitochondrially encoded cytochrome *c* oxidase II; Met: Methionine; mtDNA: Mitochondrial DNA; MFN2: Mitofusin 2; NQO1: NAD(P)H dehydrogenase quinone 1; NDUFS8: NADH: ubiquinone oxidoreductase core subunit S8; NEAA: Non-essential AAs; NFE2L2: Nuclear factor erythroid derived 2 like 2;Pck1: Phosphoenolpyruvate carboxykinase 1; Phe: Phenylalanine; PPARGC1A: Peroxisome proliferative activated receptor gamma coactivator 1 alpha; Pygl: Glycogen phosphorylase L; ROS: Reactive oxygen species; RPS18: Ribosomal protein S18; Ser: Serine; SDHB: Succinate dehydrogenase complex subunit B; SGA: Small for gestational age; Snat2: Sodium-coupled neutral amino acid transporter 2; SOD: Superoxide dismutase; TFAM: Transcription factor A mitochondrial; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine; y^+Lat1: y^+L amino acid transporter 1.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2026.03.016.

References

[1] R. Elango, R.O. Ball, Protein and amino acid requirements during pregnancy, Adv. Nutr. 7 (4) (2016) 839S–844S, https://doi.org/10.3945/an.115.011817.

[2] S.C. Langley-Evans, S.J. Welham, R.C. Sherman, A.A. Jackson, Weanling rats exposed to maternal low-protein diets during discrete periods of gestation exhibit differing severity of hypertension, Clin. Sci. 91 (5) (1996) 607–615, https://doi.org/10.1042/cs0910607.

[3] D.S. Fernandez-Twinn, S. Ekizoglou, A. Wayman, C.J. Petry, S.E. Ozanne, Maternal low-protein diet programs cardiac β-adrenergic response and signaling in 3-mo-old male offspring, Am. J. Physiol. Regul. Integr. Comp. Physiol. 291 (2) (2006) R429–R436, https://doi.org/10.1152/ajpregu.00608.2005.

[4] C.J. Petry, M.W. Dorling, D.B. Pawlak, S.E. Ozanne, C.N. Hales, Diabetes in old male offspring of rat dams fed a reduced protein diet, Int. J. Exp. Diabetes Res. 2 (2) (2001) 139–143, https://doi.org/10.1155/edr.2001.139.

[5] V. Zohdi, K. Lim, J.T. Pearson, M.J. Black, Developmental programming of cardiovascular disease following intrauterine growth restriction: findings utilising a rat model of maternal protein restriction, Nutrients 7 (1) (2014) 119–152, https://doi.org/10.3390/nu7010119.

[6] C. Guzman, R. Cabrera, M. Cardenas, F. Larrea, P.W. Nathanielsz, E. Zambrano, Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny, J. Physiol. 572 (1) (2006) 97–108, https://doi.org/10.1113/jphysiol.2005.103903.

[7] J. Yang, Q. Chang, X. Tian, B. Zhang, L. Zeng, H. Yan, S. Dang, Y.-H. Li, Dietary protein intake during pregnancy and birth weight among Chinese pregnant women with low intake of protein, Nutr. Metab. (Lond.) 19 (1) (2022) 43, https://doi.org/10.1186/s12986-022-00678-0.

[8] H. Zhang, A.M. Senior, C. Saner, N.A. Koemel, S.J. Simpson, D. Raubenheimer, B. L. Heitmann, Maternal protein intake during pregnancy and obesity risk in mothers and offspring: a prospective cohort study, Am. J. Clin. Nutr. (2025), https://doi.org/10.1016/j.ajcnut.2025.04.011.

[9] D. Mikolainis, Impact of the higgins nutrition intervention program on birth weight: a within-mother analysis, J. Am. Diet. Assoc. 89 (8) (1989) 1097–1103.

[10] K. Andreasyan, A.-L. Ponsonby, T. Dwyer, R. Morley, M. Riley, K. Dear, J. Cochrane, Higher maternal dietary protein intake in late pregnancy is associated with a lower infant ponderal index at birth, Eur. J. Clin. Nutr. 61 (4) (2007) 498–508, https://doi.org/10.1038/sj.ejcn.1602552.

[11] C. Rehfeldt, L. Lefaucheur, J. Block, B. Stabenow, R. Pfuhl, W. Otten, C.C. Metges, C. Kalbe, Limited and excess protein intake of pregnant gilts differently affects body composition and cellularity of skeletal muscle and subcutaneous adipose tissue of newborn and weanling piglets, Eur. J. Nutr. 51 (2) (2012) 151–165, https://doi.org/10.1007/s00394-011-0201-8.

[12] J.C. Cross, L. Mickelson, Nutritional influences on implantation and placental development, Nutr. Rev. 64 (suppl_2) (2006) S12–S18, https://doi.org/10.1301/nr.may.s12-s18.

[13] I. Cetin, G. Alvino, Intrauterine growth restriction: implications for placental metabolism and transport. A review, Placenta 30 (2009) 77–82, https://doi.org/10.1016/j.placenta.2008.12.006.

[14] A.L. Fowden, A. Sferruzzi-Perri, P. Coan, M. Constancia, G. Burton, Placental efficiency and adaptation: endocrine regulation, J. Physiol. 587 (14) (2009) 3459–3472, https://doi.org/10.1113/jphysiol.2009.173013.

[15] P.D. Gluckman, M.A. Hanson, C. Cooper, K.L. Thornburg, Effect of in utero and early-life conditions on adult health and disease, N. Engl. J. Med. 359 (1) (2008) 61–73, https://doi.org/10.1056/NEJMra0708473.

[16] O. Holland, M.D. Nitert, L.A. Gallo, M. Vejzovic, J.J. Fisher, A.V. Perkins, Placental mitochondrial function and structure in gestational disorders, Placenta 54 (2017) 2–9, https://doi.org/10.1016/j.placenta.2016.12.012.

[17] Z.C. Luo, W.D. Fraser, P. Julien, C.L. Deal, F. Audibert, G.N. Smith, X. Xiong, M. Walker, Tracing the origins of "fetal origins" of adult diseases: programming by oxidative stress? Med. Hypotheses 66 (1) (2006) 38–44, https://doi.org/10.1016/j.mehy.2005.08.020.

[18] S. Gupta, A. Agarwal, R.K. Sharma, The role of placental oxidative stress and lipid peroxidation in preeclampsia, Obstet. Gynecol. Surv. 60 (12) (2005) 807–816, https://doi.org/10.1097/01.ogx.0000193879.79268.59.

[19] T.-H. Hung, J.N. Skepper, D.S. Charnock-Jones, G.J. Burton, Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia, Circ. Res. 90 (12) (2002) 1274–1281, https://doi.org/10.1161/01.res.0000024411.22110.aa.

[20] J.O. Lo, M.C. Schabel, J. Gaffney, K.S. Lewandowski, C.D. Kroenke, C. T. Roberts Jr., B.P. Scottoline, A.E. Frias, E.L. Sullivan, V.H. Roberts, Impaired placental hemodynamics and function in a non-human primate model of gestational protein restriction, Sci. Rep. 13 (1) (2023) 841, https://doi.org/10.1038/s41598-023-28051-y.

[21] A. Trichopoulou, T. Psaltopoulou, P. Orfanos, C. Hsieh, D. Trichopoulos, Low-carbohydrate–high-protein diet and long-term survival in a general population cohort, Eur. J. Clin. Nutr. 61 (5) (2007) 575–581, https://doi.org/10.1038/sj.ejcn.1602557.

[22] P. Clifton, D. Condo, J. Keogh, Long term weight maintenance after advice to consume low carbohydrate, higher protein diets–a systematic review and meta analysis, Nutr. Metabol. Cardiovasc. Dis. 24 (3) (2014) 224–235, https://doi.org/10.1016/j.numecd.2013.11.006.

[23] E. Mellott, D. Moronge, K. Backer, G. Cooper, S. Ogbi, J. Maher, J. Faulkner, Mid-late gestation eplerenone treatment improves maternal cardiovascular, but not fetal, outcomes in a mouse model of leptin-induced Preeclampsia, Physiology 40 (S1) (2025) 252, https://doi.org/10.1152/physiol.2025.40.S1.0252.

[24] C.E. Hayward, S. Lean, C.P. Sibley, R.L. Jones, M. Wareing, S.L. Greenwood, M. R. Dilworth, Placental adaptation: what can we learn from birthweight: placental weight ratio? Front. Physiol. 7 (2016) 28, https://doi.org/10.3389/fphys.2016.00028.

[25] Z. Dai, Z. Wu, S. Jia, G. Wu, Analysis of amino acid composition in proteins of animal tissues and foods as pre-column o-phthaldialdehyde derivatives by HPLC with fluorescence detection, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 964 (2014) 116–127, https://doi.org/10.1016/j.jchromb.2014.03.025.

[26] J. Wu, M. Zhang, H. Zhang, M. Sheng, J. Sun, F. Wu, H. Gao, L. Chen, Z. Li, Q. Tian, PMN-MDSC: a culprit behind immunosenescence and increased susceptibility to Clostridioides difficile infection during aging, Engineering 42 (2024) 59–73, https://doi.org/10.1016/j.eng.2024.06.014.

[27] M.-y. He, G. Wang, S.-s. Han, Y. Jin, H. Li, X. Wu, Z.-l. Ma, X. Cheng, X. Tang, X. Yang, Nrf2 signalling and autophagy are involved in diabetes mellitus-induced defects in the development of mouse placenta, Open Biol. 6 (7) (2016) 160064, https://doi.org/10.1098/rsob.160064.

[28] J.S. Eaton, Z.P. Lin, A.C. Sartorelli, N.D. Bonawitz, G.S. Shadel, Ataxia-telangiectasia mutated kinase regulates ribonucleotide reductase and mitochondrial homeostasis, J. Clin. Investig. 117 (9) (2007) 2723–2734, https://doi.org/10.1172/JCI31604.

[29] M. Zhao, S. Liu, C. Wang, Y. Wang, M. Wan, F. Liu, M. Gong, Y. Yuan, Y. Chen, J. Cheng, Mesenchymal stem cell-derived extracellular vesicles attenuate mitochondrial damage and inflammation by stabilizing mitochondrial DNA, ACS Nano 15 (1) (2020) 1519–1538, https://doi.org/10.1021/acsnano.1c09895.

[30] E.D. Watson, J.C. Cross, Development of structures and transport functions in the mouse placenta, Physiology 20 (3) (2005) 180–193, https://doi.org/10.1152/physiol.00001.2005.

[31] P.K. Ajuogu, M. Wolden, J.R. McFarlane, R.A. Hart, D.J. Carlson, T. Van der Touw, N.A. Smart, Effect of low-and high-protein maternal diets during gestation on reproductive outcomes in the rat: a systematic review and meta-analysis, J. Anim. Sci. 98 (1) (2020), https://doi.org/10.1093/jas/skz380 skz380.

[32] K.E. Brett, Z.M. Ferraro, J. Yockell-Lelievre, A. Gruslin, K.B. Adamo, Maternal–fetal nutrient transport in pregnancy pathologies: the role of the placenta, Int. J. Mol. Sci. 15 (9) (2014) 16153–16185, https://doi.org/10.3390/ijms150916153.

[33] K.K.S. Bhasin, A. van Nas, L.J. Martin, R.C. Davis, S.U. Devaskar, A.J. Lusis, Maternal low-protein diet or hypercholesterolemia reduces circulating essential amino acids and leads to intrauterine growth restriction, Diabetes 58 (3) (2009) 559–566, https://doi.org/10.2337/db07-1530.

[34] J.K. Cleal, E.M. Lofthouse, B.G. Sengers, R.M. Lewis, A systems perspective on placental amino acid transport, J Physiol. 596 (23) (2018) 5511–5522, https://doi.org/10.1113/JP274883. Epub 2018 Sep. 7.

[35] M.A. Underwood, W.M. Gilbert, M.P. Sherman, Amniotic fluid: not just fetal urine anymore, J. Perinatol. 25 (5) (2005) 341–348, https://doi.org/10.1038/sj.jp.7211290.

[36] W. Schmidt, The amniotic fluid compartment: the fetal habitat, Adv. Anat. Embryol. Cell Biol. 127 (1992) 1–100, https://doi.org/10.1007/978-3-642-77300-6.

[37] J. Cross, How to make a placenta: mechanisms of trophoblast cell differentiation in mice–a review, Placenta 26 (2005) S3–S9, https://doi.org/10.1016/j.placenta.2005.01.015.

[38] P. Coan, N. Conroy, G. Burton, A. Ferguson-Smith, Origin and characteristics of glycogen cells in the developing murine placenta, Dev. Dyn. 235 (12) (2006) 3280–3294, https://doi.org/10.1002/dvdy.20981.

[39] E.H. Joo, Y.R. Kim, N. Kim, J.E. Jung, S.H. Han, H.Y. Cho, Effect of endogenic and exogenic oxidative stress triggers on adverse pregnancy outcomes: preeclampsia, fetal growth restriction, gestational diabetes mellitus and preterm birth, Int. J. Mol. Sci. 22 (18) (2021) 10122, https://doi.org/10.3390/ijms221810122.

[40] L. Myatt, X. Cui, Oxidative stress in the placenta, Histochem. Cell. Biol. 122 (2004) 369–382, https://doi.org/10.1007/s00418-004-0677-x.

[41] O. Ighodaro, O. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, Alex. J. Med. 54 (4) (2018) 287–293, https://doi.org/10.1016/j.ajme.2017.09.001.

[42] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (20) (2009) 13291–13295, https://doi.org/10.1074/jbc.R900010200.

[43] D. Poidatz, E. Dos Santos, F. Duval, H. Moindjie, V. Serazin, F. Vialard, P. De Mazancourt, M.-N. Dieudonné, Involvement of estrogen-related receptor-γ and mitochondrial content in intrauterine growth restriction and preeclampsia, Fertil. Steril. 104 (2) (2015) 483–490, https://doi.org/10.1016/j.fertnstert.2015.05.005.

[44] A. Musatov, N.C. Robinson, Susceptibility of mitochondrial electron-transport complexes to oxidative damage. Focus on cytochrome c oxidase, Free Radic. Res. 46 (11) (2012) 1313–1326, https://doi.org/10.3109/10715762.2012.717273.

[45] M. Adebayo, S. Singh, A.P. Singh, S. Dasgupta, Mitochondrial fusion and fission: the fine-tune balance for cellular homeostasis, FASEB J. 35 (6) (2021) e21620, https://doi.org/10.1096/fj.202100067R.