Supplementation of polar lipids-enriched milk fat globule membrane in high-fat diet-fed rats during pregnancy and lactation promotes brown/beige adipocyte development and prevents obesity in male offspring

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Abstract
Promoting brown adipose tissue (BAT) function or browning of white adipose tissue (WAT) provides a defense against obesity. The aim of the study was to investigate whether maternal polar lipids-enriched milk fat globule membrane (MFGM-PL) supplementation to high-fat diet (HFD) rats during pregnancy and lactation could promote brown/beige adipogenesis and protect against HFD-induced adiposity in offspring. Female SD rats were fed a HFD for 8 weeks to induce obesity and, then, a HFD during pregnancy and lactation with or without MFGM-PL. Male offspring were weaned at postnatal Day 21 and then fed a HFD for 9 weeks. MFGM-PL treatment to HFD dams decreased the body weight gain and WAT mass as well as lowered the serum levels of insulin and triglycerides in male offspring at weaning. MFGM-PL+HFD offspring showed promoted thermogenic function in BAT and inguinal WAT through the upregulation of UCP1 and other thermogenic genes. In adulthood, maternal MFGM-PL supplementation reduced adiposity and increased oxygen consumption, respiratory exchange ratio, and heat production in male offspring. The enhancement of energy expenditure was correlated with elevated BAT activity and inguinal WAT thermogenic program. In conclusion, maternal MFGM-PL treatment activated thermogenesis in offspring, which exerted long-term beneficial effects against HFD-induced obesity in later life.

KEYWORDS
beige adipogenesis, maternal obesity, polar lipids-enriched milk fat globule membrane, thermogenesis, uncoupling protein 1

Abbreviations: AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; CD137, cluster of differentiation 137; Cidea, cell death-inducing DFFA-like effector A; CON, control diet; Cyto C, cytochrome c; FABP4, fatty acid-binding protein 4; H&E, Haemotoxylin and Eosin; HFD, high-fat diet; Hoxc9, homeobox C9; MFGM, milk fat globule membrane; MFGM-PL, polar lipids-enriched milk fat globule membrane; mtDNA, mitochondrial DNA; PGC-1α, peroxisomal proliferator-activated receptor γ coactivator-1α; RER, respiratory exchange ratio; PPARγ, peroxisomal proliferator-activated receptor γ; PRDM16, PR domain-containing 16; Tbx15, T-box 15; Tfam, mitochondrial transcription factor A; TMEM26, transmembrane protein 26; UCP1, uncoupling protein 1.
Obesity represents a major public health problem in countries around the world. Maternal under- or over-nutrition during gestation and lactation period leads to developmental alterations and defects in organ function and metabolism and has long-term effects on the health status of offspring in adulthood, a phenomenon referred to as “metabolic programming”. Increasing evidence indicates that maternal obesity or high-fat diet (HFD) consumption increases the risk of insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, cardiovascular disorders, and steatohepatitis in offspring later in life. Therefore, maternal nutrition is of great significance to the health outcome of the offspring.

In obesity-caused early life insults, adipose tissue dysfunction is an important factor in the metabolic alterations in humans and rodents. There were two main types of adipose tissues: white adipose tissue (WAT), which stores excess energy as triglycerides, and brown adipose tissue (BAT), which dissipates energy in the form of heat by the process of adaptive thermogenesis. The function of BAT is mainly dependent on the activation of lipolysis and fatty acid oxidation through uncoupling protein 1 (UCP1), which uncouples mitochondrial respiration from ATP synthesis. Moreover, brown-like adipocytes, also called “beige” adipocytes, have been discovered in WAT depots. Beige adipocytes showed multilocular lipid droplets and abundance of mitochondria enriched with UCP1, resembling the BAT phenotype upon physiological stimuli such as cold exposure, pharmacological treatment or beta-adrenergic stimulation. The growth of BAT in the fetus is a coordinated process that refers to the lipid accumulation and BAT-specific UCP1 synthesis. In the newborn, BAT is responsible for ensuring sufficient adaptation to the extraterine environment. Adipose tissue formation starts in mid-gestation and the mass of adipose increases during late gestation. Then, some of the BAT depots are replaced by WAT during postnatal life, which ensures sufficient lipid to provide energy for nonshivering thermogenesis. The BAT deposition reduction in early life may perpetuate throughout life, thus suppressing energy expenditure and causing obesity. Normal fat development profiles in the offspring are altered by changes in maternal diet, ultimately leading to long-term outcomes. HFD during pregnancy and lactation programs a deleterious response to the consumption of a HFD in offspring later in life including the adipocyte dysfunction. Maternal high-fat diet during lactation impaired the adaptive thermogenesis of BAT at weaning and lowered the expression of UCP1 and thermogenic activity of BAT in adulthood. Maternal obesity also aggravated the abnormal expansion and inflammatory infiltration and reduced the formation of beige adipocytes in WAT in the fetus and adulthoods. Therefore, improvement of maternal nutrition is an effective approach to facilitate the development of BAT and the browning of WAT against obesity in offspring.

Maternal administration of bioactive components from food can affect adipose tissue programming and regulate energy homeostasis in early life and adulthood, thus alleviating the adverse effects of maternal obesity on offspring health. Pregnant mice fed a high-fat diet supplemented with retinoids or resveratrol exhibited a reduction of adipocyte hypertrophy, an increase in energy expenditure and an increase in the formation of beige adipocytes in WAT, which have a positive effect on diet-induced obesity in offspring. Treatment of conjugated linoleic acid to HFD dams alleviated lipid deposition and improved insulin sensitivity in adult offspring caused by HFD. The milk fat globule membrane (MFGM) in breast milk is highly structured and contains many bioactive components including glycoproteins and polar lipids, which give MFGM many beneficial functions. In particular, the polar lipids in milk are located mainly (60% to 70%) in the MFGM and infant formula that contained MFGM or its polar lipid component promoted brain development, cognitive function, muscle development, immunity, and gut physiology in infants and young children. Active polar lipid components such as phosphatidylcholine and sphingomyelin in milk improved metabolic disorders by regulating lipogenesis-related transcription factors, reducing inflammation, and lipid accumulation in adipose tissue of obese mice. Our previous studies have shown that MFGM supplementation suppressed body weight gain induced by HFD and increased the protein expression of UCP1 in inguinal WAT of adult HFD mice. However, whether maternal polar lipids-enriched milk fat globule membrane (MFGM-PL) administration could exert beneficial effects on offspring remains unclear. Therefore, in this study, the effects of MFGM-PL administration to HFD dams during pregnancy and lactation on the BAT function and beige adipogenesis in early life were investigated. Besides, whether maternal MFGM-PL treatment could prevent obesity in adulthood offspring challenged with the HFD was also explored.

2 | MATERIAL AND METHODS

2.1 | Animal model and dietary intervention

All animal experiments were approved by the Animal Ethics Committee at China Agricultural University (the ethical review serial number is KY180026). Female SD rats (4-week-old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All animals used in this study were kept under controlled room temperature (23 ± 2°C) and light (12-12 hours light-dark cycle) with...
free access to food and water. After one-week acclimation, rats were randomly assigned to two dietary groups. One group received a control diet (10% energy from fat, D12450H; Research Diets, New Brunswick, NJ, USA) and another group received a HFD (60% energy from fat, D12492; Research Diets, New Brunswick, NJ, USA) for 8 weeks. Female rats were subsequently mated with a control male at a ratio of 2 females to 1 male. During pregnancy and lactation, the rats fed control diet were randomly divided into two groups and fed with control diet (CON group) or control diet with polar lipids-enriched milk fat globule membrane (MFGM-PL) at 400 mg/kg BW (CON + MFGM-PL group). The rats fed HFD were also divided into two groups and fed with HFD (HFD group; 45% energy from fat, D12451; Research Diets) or HFD with MFGM-PL at 400 mg/kg BW (HFD + MFGM-PL group). The MFGM-PL was prepared according to previous reports or used for UCP1 immunohistochemical analysis according to the standard protocol as previously described.29 The samples were observed under an Olympus IX 73 microscope (Olympus Corporation, Tokyo, Japan) and the images were captured at 200× magnification. Three images were captured per section and five sections were used for staining and quantitation. All adipocytes in each image were quantified using Image-Pro plus 6.0 software (Media Cybernetics, Inc, Rockville, MD, USA). For immunohistochemical staining, the UCP1-positive areas in five random fields in each section were determined by analyzing the staining intensity with Image-Pro plus 6.0 software and data were presented as a percentage of positive area.

2.5 | Quantitative real-time PCR

Total RNA from adipose tissues was isolated using TRIzol reagent (Life Technologies, CA, USA) according to the manufacturer's instructions and cDNA was generated using 5X All-In-One RT Master Mix (Applied Biosystems, MA, USA). For real-time quantitative PCR analysis, quantitative expression assays for genes were measured using SYBR master mix (Takara Bio, Tokyo, Japan) on Techne Quantica real-time PCR detection system (Techne, Staffordshire, UK) as described previously.30 The primer sequences are listed in Table 1.

2.6 | Western blot analysis

Proteins from adipose tissue were extracted after homogenization in a RIPA lysis buffer (Beyotime, Haimen, Jiangsu, China) containing protease and phosphatase inhibitors. The protein concentration was quantified using the BCA Protein Assay kit (Beyotime, Haimen, Jiangsu, China). The protein extracts were electrophoretically separated by SDS/PAGE on a 7.5%-12.5% running gel and then transferred to
polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Membranes were blocked and incubated with primary antibodies overnight at 4°C. The membranes were then incubated for 1 hour at room temperature with horseradish-peroxidase-conjugated secondary antibodies. All blots were washed and exposed using enhanced chemiluminescence (ECL) reagents (Millipore, Billerica, MA, USA). The obtained bands were scanned and analyzed using software ImageJ 1.47v (Wayne Rasband, Bethesda, MD, USA) by densitometry analysis and β-actin was used as the control.

2.7 Mitochondrial DNA copy number

Genomic DNA was extracted from BAT using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The content of mtDNA was assessed using real-time quantitative PCR by measuring the threshold cycle ratio of the mitochondrial gene (Cox2, cytochrome oxidase subunit II) to nuclear gene (β-globin).

2.8 Serum biochemistry determination

Rats were fasted overnight and then the blood glucose level was determined by Accu-CHEK Active glucometer (Roche Diagnostics, Mannheim, Germany). Serum insulin levels were determined by ELISA kits (R&D Systems, Minneapolis, MN, USA) and serum triglycerides levels were determined using the triglyceride colorimetric assay kit (Cayman, Ann Arbor, MI, USA) according to the manufacturers’ instructions.

2.9 Transmission electron microscopy

Tissue samples of BAT were fixed with 2.5% glutaraldehyde in a phosphate buffer (pH 7.4) for 4 hours at room temperature. Tissue samples were postfixed with 1% osmium tetroxide, dehydrated through an ethanol gradient and embedded in EPON812 resin for sectioning. Ultra-structural images of thin sections were observed using the transmission electron microscope (Hitachi S-3000N, Tokyo, Japan).

2.10 Statistical analyses

Data were analyzed and visualized using Prism, version 8 (GraphPad Software, San Diego, CA, USA) and are expressed as the mean ± SEM. One-way ANOVA followed by the post hoc Tukey’s test was used to explore differences between multiple groups. P < .05 was considered statistically significant. All statistical analyses were conducted with IBM SPSS (v.25.0; IBM, Armonk, NY, USA).

3 RESULTS

3.1 Maternal characteristics

To investigate the effects of MFGM-PL intervention at the end of lactation, the maternal phenotype, and metabolic parameters were measured. Before mating, the female rats fed high-fat diet gained more weight than those fed control diet after 8 weeks (Figure S1A), indicating that the high-fat diet had induced obesity. At the end of lactation, HFD dams had higher body weight (Figure S1B) and the ratio of WAT (inguinal WAT and perigonadal WAT) mass to body weight (Figure S1C) compared with dams in CON and CON + MFGM-PL group. MFGM-PL supplementation reversed HFD-induced body weight gain and fat accumulation. Besides, dams had no difference in energy intake (Figure S1D) and blood glucose level (Figure S1E) at the end of lactation. The levels of serum triglyceride (Figure S1F) and insulin concentrations (Figure S1G) were higher in the HFD dams than CON and CON + MFGM-PL dams, while MFGM-PL supplementation attenuated HFD-induced increase in levels of serum triglyceride and insulin.

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**TABLE 1** Primer sequences used for real-time quantitative PCR
3.2 Maternal MFGM-PL supplementation increased BAT metabolic activity in male HFD offspring of the rat at weaning

As shown in Figure 1A, HFD male offspring were significantly heavier than CON and CON + MFGM-PL offspring at postnatal Days 1, 10, and 21, whereas HFD + MFGM-PL offspring displayed lower body weight compared with HFD offspring. As shown in Figure 1B,C, HFD offspring displayed increased BAT mass (epididymal WAT, inguinal WAT, perirenal WAT, and mesenteric WAT). BAT mass of HFD + MFGM-PL offspring was significantly reduced compared with HFD offspring. Although there were no differences in the BAT index of the offspring (Figure 1B), histological analysis of BAT demonstrated that HFD offspring had larger lipid droplets, while lipid droplet size of HFD + MFGM-PL offspring resembled the CON and CON + MFGM-PL groups (Figure 1J). The level of blood glucose was not different among groups (Figure 1D). The serum triglyceride (Figure 1E) and insulin concentrations (Figure 1F) of HFD offspring exhibited a marked increase, which were reduced by maternal MFGM-PL treatment.

To investigate the effects of maternal MFGM-PL supplementation on the BAT metabolic activity of offspring at weaning, the thermogenic markers in BAT were measured by RT-PCR and western blot analysis. As shown in Figure 1G, the mRNA levels of thermogenic genes including Ucp1, Pgc-1a, Prdm16, Cidea, and Fgf21 were lower in HFD offspring, while HFD + MFGM-PL offspring presented an increased mRNA expression of Ucp1, Pgc-1a, Cidea, and Fgf21. The number of UCP1 positive adipocytes in BAT was lower in HFD offspring than CON and CON + MFGM-PL groups, while MFGM-PL treatment to HFD offspring increased UCP1 positive adipocytes in BAT (Figure 1J). Consistently, the protein levels of UCP1, PGC-1α, and PRDM16 were significantly decreased in BAT of HFD offspring, while expression levels of these proteins in BAT of HFD + MFGM-PL offspring were partly normalized to the levels of CON and CON + MFGM-PL groups (Figure 1H,I). At postnatal Day 1, MFGM-PL treatment to HFD dams also increased the protein levels of UCP1 and PGC-1α (Figure S2A,B). Besides, HFD offspring has a lower ratio of p-AMPKα/t-AMPKα in BAT, which was also recovered by maternal MFGM-PL treatment (Figure 1H,I). Transmission electron microscopy examination revealed that compared to the CON group, the brown adipocytes of HFD offspring had large and coalescing lipid droplets and contained enlarged mitochondria with irregular cristae structure (Figure 1K,L). After treatment with MFGM-PL, the brown adipocytes of HFD + MFGM-PL offspring filled with several small lipid droplets and contained numerous mitochondria packed with laminar cristae (Figure 1K,L). These results indicated that maternal MFGM-PL supplementation reduced body weight gain and increased BAT activity in HFD offspring during early life.

3.3 Maternal MFGM-PL supplementation induced brown fat-like changes in inguinal WAT of HFD offspring of the rat at weaning

Histological analysis of inguinal WAT showed that HFD feeding resulted in increased average adipocyte diameter compared to average adipocyte diameter in the CON and CON + MFGM-PL groups, whereas MFGM-PL administration reduced adipocyte enlargement induced by HFD (Figure 2A,B). By performing UCP1 immunohistochemistry, larger unilocular adipocytes with less UCP1 staining were detected in inguinal WAT of HFD offspring. HFD + MFGM-PL offspring possessed more UCP1-expressing multilocular adipocytes in inguinal WAT compared with HFD offspring (Figure 2A). In agreement, the relative mRNA (Figure 2C) and protein expression (Figure 2D) of UCP1 were also higher in HFD + MFGM-PL offspring compared with HFD offspring. The relative mRNA expressions of other thermogenic genes (Ucp1, Pgc-1a, Prdm16, and Cidea) and beige cell markers (Tmem26, Cd137, Hoxc9, and Tbx15) was decreased in the HFD offspring, while HFD + MFGM-PL offspring presented an increased expression of these mRNAs except Pgc-1a. Compared with CON and CON + MFGM-PL groups, HFD decreased the protein levels of UCP1, PGC-1α, PRDM16 as well as Cyto C, an important element of the mitochondrial respiratory chain, in inguinal WAT. MFGM-PL treatment to HFD dams increased these protein levels (Figure 2D,E). Similar results were observed in inguinal WAT of offspring at postnatal Day 10, accompanied by the increased protein levels of UCP1 and PGC-1α in the HFD + MFGM-PL group (Figure S2C,D). Besides, the p-AMPKα/t-AMPKα ratio was lower in the HFD offspring compared to CON and CON + MFGM-PL groups, which was also reversed by MFGM-PL treatment (Figure 2D,E). However, as shown in Figure 2D,E, the relative protein levels of PPARγ and FABP4, which are common markers of both brown and white adipocytes, displayed no differences among these four groups. These results indicated that maternal MFGM-PL treatment enhanced browning of inguinal WAT in HFD offspring during early life.

3.4 Maternal MFGM-PL supplementation did not induce brown-like adipocyte in epididymal WAT of HFD offspring of the rat at weaning

Although H&E analysis showed that MFGM-PL treatment to HFD dams reduced the average adipocyte diameter of epididymal WAT in offspring (Figure 3A,B), there was no significant difference among these groups in relation to the protein levels of UCP1 and PGC-1α (Figure 3C,D).
FIGURE 1  Maternal polar lipids-enriched milk fat globule membrane (MFGM-PL) supplementation increased BAT metabolic activity in male HFD offspring of rat at weaning. A, body weight. B and C, fat index (percentage of fat pad weight relative to the whole body weight) (B) and fat mass (C) of BAT, epididymal WAT, inguinal WAT, perirenal WAT, and mesenteric WAT. D-F, blood glucose (D) and serum triglyceride (E), and insulin concentrations (F) of the offspring at weaning. G, mRNA expression of thermogenic genes in BAT. H and I, western blot analysis for thermogenic markers and AMPKα. J, representative H&E staining and UCP1 staining for BAT sections. K and L, representative transmission electron microscopy images from BAT. Scale bars: 5 µm (K) and 1 µm (L). L, lipid droplets. Arrows point to mitochondria. *P < .05 vs CON group; #P < .05 vs HFD group. Data are expressed as the mean ± SEM (n = 6)
To determine whether the effects of MFGM-PL during early life could exert long-term benefits and alleviate HFD-induced obesity in adulthood, the body weight and energy expenditure were measured in adult offspring. As shown in Figure 4A, after 11 weeks of HFD challenge, the final body weight of HFD offspring was significantly increased relative to CON and CON + MFGM-PL offspring, while MFGM-PL treatment to HFD dams reduced body weight gain without affecting energy intake (Figure 4B).
Compared with CON and CON + MFGM-PL offspring, HFD offspring showed markedly lower VO$_2$ through a 12-h light/dark cycle, while MFGM-PL treatment to HFD dams increased VO$_2$ (Figure 4C,D). There was no significant difference in the VCO$_2$ among groups (Figure 4E,F). The respiratory exchange ratio (RER) reflects the relative contributions of carbohydrates and lipids to total energy expenditure. As a result, compared with HFD offspring, the RER was reduced significantly upon MFGM-PL treatment, demonstrating that more lipids were being oxidized (Figure 4G). Consistent with these results, the heat production of HFD offspring during the light and dark period was decreased compared with CON and CON + MFGM-PL groups, which was also recovered by MFGM-PL treatment (Figure 4H,I). These results indicated that maternal MFGM-PL treatment increased whole-body energy expenditure in adult HFD offspring.

### 3.6 | Maternal MFGM-PL supplementation improved BAT function in adult offspring of rat challenged with HFD

BAT plays an important role in energy expenditure and adaptive thermogenesis. To further examine the increased energy expenditure after MFGM-PL treatment, the BAT function was measured. As shown in Figure 5A,B, the relative protein levels of UCP1, PGC-1α, and PRDM16 were reduced in the HFD group, which was recovered by MFGM-PL supplementation. UCP1, PGC-1α, PRDM16, and other transcription factors regulate mitochondrial biosynthesis. As a consequence, MFGM-PL treatment to HFD dams increased the mitochondrial DNA (mtDNA) copy number (Figure 5C). H&E staining and UCP1 immunohistochemistry showed that compared with HFD offspring, HFD + MFGM-PL offspring had more UCP1-expressing brown adipocytes with reduced size of lipid droplets (Figure 5D).
Next, a cold tolerance test was performed to gauge adaptive thermogenesis in adult offspring. Under cold exposure, the dorsal interscapular surface temperature of HFD group dropped significantly, displaying impaired thermogenesis (Figure 5E,F). MFGM-PL treatment to HFD dams recovered the interscapular surface temperature (Figure 5E,F). In accordance with this, HFD offspring showed lower rectal temperature during 6 hours of exposure to cold, which was also
Maternal polar lipids-enriched milk fat globule membrane (MFGM-PL) supplementation improved BAT function in adult offspring of rat challenged with HFD. A and B, western blot analysis for thermogenic markers. C, mtDNA copy number of BAT. D, representative H&E staining and UCP1 staining of BAT. E and F, Representative infrared thermal images (E) and quantitation of dorsal interscapular surface temperature (F) at room temperature and under cold exposure (4°C for 6 h). G and H, rectal temperature at room temperature and under cold exposure (4°C for 6 h). *P < .05 vs CON group; #P < .05 vs HFD group. Data are expressed as the mean ± SEM (n = 5-6).
FIGURE 6 Maternal polar lipids-enriched milk fat globule membrane (MFGM-PL) supplementation enhanced inguinal WAT (inguinal WAT) thermogenic program in adult offspring of rat challenged with HFD. A and B, fat index (percentage of fat pad weight relative to the whole body weight) (A) and fat mass (B) of epididymal WAT, inguinal WAT, and perirenal WAT. C, image of fat pads. D, representative H&E staining in sections of inguinal WAT. E, average adipocyte diameters of inguinal WAT (F and G) and epididymal WAT (H and I). *P < .05 vs CON group; #P < .05 vs HFD group. Data are expressed as the mean ± SEM (n = 6).
increased by maternal MFGM-PL treatment to a level comparable with CON and CON + MFGM-PL groups (Figure 5G,H). These results indicated that maternal MFGM-PL treatment was able to increase body adaptation to cold exposure by improving the BAT function to generate more heat.

3.7 Maternal MFGM-PL supplementation enhanced inguinal WAT thermogenic program in adult offspring of rat challenged with HFD

To further investigate the long-term effects of maternal MFGM-PL supplementation on the WAT of offspring, the browning of WAT in adult offspring was measured. As shown in Figure 6A-C, HFD increased the fat index and fat mass of WAT (epididymal WAT, inguinal WAT, and perirenal WAT) in adult offspring, while HFD + MFGM-PL offspring showed less accumulation of WAT mass. Consistent with these findings, the size of adipocytes was larger in inguinal WAT of HFD offspring compared to CON and CON + MFGM-PL groups, which was recovered by MFGM-PL treatment (Figure 6D,E). The relative protein levels of UCP1, PGC-1α, and PRDM16 in inguinal WAT were all decreased in HFD offspring, while HFD + MFGM-PL offspring showed an increased expression in these protein levels (Figure 6F,G). However, the changes in the protein levels of UCP1, PGC-1α, and PRDM16 were not observed in epididymal WAT of HFD + MFGM-PL offspring, which was in accordance with the results in offspring at weaning (Figure 6H,I). These results suggested that maternal MFGM-PL treatment improved beige adipocyte development in inguinal WAT of adult HFD offspring.

4 DISCUSSION

Epidemiological studies have demonstrated that the offspring of obese mothers are at increased risk of obesity and other features of metabolic syndrome. MFGM contains unique polar lipids and membrane-specific proteins and possesses many nutritional and physiological functions, especially in infant's growth and development. In this study, we demonstrated that MFGM-PL to HFD dams during pregnancy and lactation promoted BAT function and browning of inguinal WAT in male offspring, which further increased energy expenditure and alleviated obesity in adult offspring challenged with HFD.

In general, HFD with 60% energy from fat was often used to establish and evaluate the high-fat-induced obese rat model. However, the use of 45% HFD during pregnancy is necessary because 60% HFD leads to fetal growth restriction, while 45% HFD leads to macrosomia, similar to obesity during pregnancy in humans. Therefore, we changed 60 kcal% to 45 kcal% HFD before mating, consistent with previous reports. As a consequence of HFD exposure during pregnancy and lactation, male offspring had increased body weight and adiposity in later life. Birth weight is an important pregnancy outcome linked to metabolic health in later life. Overnutrition during lactation is one of the main reasons for early obesity in the offspring, since the breast milk of HFD dams has a higher concentration of protein, lipids, and carbohydrates. Increased adiposity is commonly associated with glucose intolerance and insulin insensitivity. In this study, maternal MFGM-PL supplementation reduced the increase in serum levels of insulin and triglyceride induced by HFD in dams and offspring, whereas no change in serum glucose was observed. This finding is similar to previous reports showing that maternal polyphenol treatment had no obvious influence on blood glucose in HFD-fed dams at the end of lactation. A test in overweight and obese subjects showed that there existed a positive association between plasma phospholipids (indicative of full-fat dairy consumption) and insulin sensitivity. Addition of MFGM to a high-fat meal mitigated insulin secretion in the postprandial state in obese subjects, which indicates the potential role of MFGM in improving insulin response.

BAT generates heat through UCP1, while excess WAT accumulation causes adipocyte hypertrophy. Thus, pharmacological or dietary interventions facilitating the recruitment of brown fat cells or browning of WAT might be a new strategy for obesity therapy. The development of brown/beige adipocytes is initiated by PRDM16, followed by mitochondrial biogenesis and expression of specific markers of brown/beige adipocytes, including UCP1 and PGC-1α. AMP-activated protein kinase (AMPK), a major player in energy homeostasis, promotes glucose uptake and fatty acid β-oxidation and regulates mitochondrial biogenesis. Overexpression of UCP1 in adipose tissue leads to an increase in the AMP/ATP ratio and AMPK activation. AMPK also can increase PGC-1α expression and activity to control energy expenditure. In our previous study, MFGM inhibited fat accumulation in epididymal WAT by increasing AMPK phosphorylation and UCP1 expression in inguinal WAT of HFD mice. Dietary MFGM combined with exercise increased lipid metabolism in skeletal muscle by increasing the mRNA expression of thermogenic markers including PGC-1α in vitro and in vivo. Consistent with these results, maternal MFGM-PL supplementation increased the protein and mRNA levels of UCP1, PGC-1α, PRDM16, and other thermogenic genes as well as upregulated the phosphorylation of AMPK in both inguinal WAT and BAT of HFD offspring. These findings demonstrated that MFGM-PL to HFD dams promoted brown/beige adipogenesis and thermogenic function in offspring, which exhibited long-term effects against HFD-induced obesity in adulthood. Besides, compared with epididymal WAT, the
subcutaneous fat contains larger amounts of progenitors with the capacity of de novo beige adipogenesis. Since beige adipocytes are primarily derived from progenitors, the lack of progenitors in epididymal WAT might explain the indistinctive effects of MFGM-PL on the browning of epididymal WAT in offspring.

BAT-mediated thermogenesis is an important component of the thermostatic regulation to maintain body temperature during environmental changes such as cold or diet. The heat production capacity of BAT is due to the extraordinary metabolic activity reflected by abundant mitochondria. The offspring mice of HFD dams have greater body weight related to the reduction in O$_2$ consumption without affecting caloric intake, suggesting that the adiposity was primarily due to the lower energy expenditure. Mice given MFGM combined with exercise displayed higher oxygen consumption. Similar to this report, in our study, MFGM-PL supplementation to HFD dams significantly increased energy expenditure and heat production in adult offspring as well as upregulating mtDNA copy number in BAT. We measured RER in both light (inactive) and dark (active) phases. The basal metabolic rate during the inactive phase was greater in the HFD + MFGM-PL group compared to the HFD group and the difference was further enhanced during the active phase, showing that the physical activity of rats contributed to the difference observed. HFD offspring might move less which could contribute to the lower metabolic rate independent of the thermogenesis of brown/beige adipose tissue. Previous reports showed that maternal HFD feeding impaired the BAT thermogenic capacity of offspring accompanied by a decrease in body temperature under cold stimulus. To further examine the differences in energy expenditure among offspring, a cold tolerance test was conducted to gauge adaptive thermogenesis which is a major form of energy expenditure. In our study, HFD offspring also showed lower dorsal interscapular surface temperature and rectal temperature under cold exposure, while MFGM-PL treatment markedly reversed the effects, indicating the potential role of MFGM-PL in improving the adaptive thermogenesis.

Supplementing infants with MFGM improved brain development and cognitive functions prevented infection, improved gut barrier integrity in challenging conditions, and modulated gut microbiota. However, there were few reports about the effects of MFGM on the metabolism of infants. The young mice supplemented with MFGM prevented body fat accumulation in adulthood. In our previous study, MFGM supplementation alleviated obesity, inhibited adipogenesis of epididymal WAT, and induced the browning of inguinal WAT in 16-week HFD-fed mice. Therefore, maternal MFGM-PL supplementation may improve the metabolic disorders in HFD dams, which then improved the WAT/BAT development of the offspring indirectly. Besides, MFGM contains a wide array of polar lipids, including phospholipids, glycolipids, and sphingolipids. The mechanisms behind the potential effects of MFGM on obesity are unclear but may involve polar lipids-induced alterations in gene expression related to glucose and lipid metabolism. Animals fed high-fat diets supplemented with milk sphingolipids or milk phospholipids improved lipid metabolism, attenuated adipose inflammation, reduced intestinal cholesterol absorption, and decreased serum lipids and blood glucose, which results in improvement of metabolic complications associated with obesity. The most abundant phospholipids present in the MFGM is phosphatidylcholine. Dietary phosphatidylcholine intake during gestation and lactation was directly correlated with breast-milk phosphatidylcholine concentrations with increased choline and phosphocholine concentrations in breast milk. When choline transported into the mammary epithelium, it is rapidly phosphorylated to form phosphocholine (the major water-soluble form of choline in milk) to prevent diffusion back into maternal blood. Choline is critically needed for the growth and development of infants and directly affects cholinergic neurotransmission, transmembrane signaling and lipid transport/metabolism. Phosphatidylcholine can be well tolerated by pregnant and lactating women and alter the breast-milk composition, which may generate beneficial effects on the health of offspring. Besides, in human placental endothelial cells, phospholipid transfer protein is expressed and mediated a placental transfer of phospholipids in the early stage of pregnancy. Dietary phospholipids are a source of long-chain polyunsaturated fatty acids (LCPUFA) including docosahexaenoic acid (DHA) and arachidonic acid (AA), which are selectively transferred across the placenta to the fetal circulation, and benefits the embryonic development of the central nervous system. LCPUFA-containing lysophospholipids, choline, serine, and myoinositol in circulation, can be transported through the blood-brain barrier to support optimal phospholipid synthesis in the rapidly developing brain. Compared to the formula-fed infants, infants consuming formula supplemented with MFGM had a higher circulating lysophospholipids profile with more choline, betaine, and ketone bodies and less circulating amino acids, which made these infants become more metabolically similar to breast-fed infants. Therefore, MFGM-PL might also exert its effects by the placental transmission of phospholipids from MFGM-PL or their metabolites. However, due to the complex structure of MFGM, this study was not designed to evaluate which specific components are directly responsible for the observed effects and the specific mechanism remains to be further explored.

In our study, almost all the results showed that there was no significant difference between CON offspring and CON + MFGM-PL offspring. MFGM supplementation to infant formula had no effects on infant growth, weight gain, body mass index, and few effects on metabolic parameters such as cholesterol serum level in healthy infants compared
with infant formula group.\textsuperscript{23,65} Besides, other reports also showed that maternal diet supplemented with bioactive compounds such as grape seed procyanidin or resveratrol had weak effects on the biometric parameters and plasma parameters associated with metabolic disturbance in offspring born to healthy dams but obviously ameliorated metabolic impact of maternal obesity in offspring, which were consistent with our results.\textsuperscript{20,66} In our study, the effects of MFGM-PL to HFD dams were focused on male offspring, which are more susceptible to adiposity changes associated with metabolic programming.\textsuperscript{67} The underlying mechanisms in regulating energy metabolism may differ between sexes. For example, estrogen signaling is associated with lipogenesis, adipogenesis, and thermogenesis; however, maternal high-fat diet differentially changed the estrogen receptor across the white and brown adipose tissue of male and female offspring at weaning.\textsuperscript{68} Therefore, the effects of maternal MFGM-PL supplementation on the BAT and WAT development of female offspring warrant further investigation.

In summary, our data showed that MFGM-PL supplementation to rats fed a HFD during pregnancy and lactation decreased the body weight gain and WAT mass, improved the development of BAT and increased the browning of inguinal WAT in male offspring during early life. Furthermore, the enhanced brown/beige adipogenesis mitigated HFD-induced obesity and increased energy expenditure in adult life, demonstrating that MFGM-PL to HFD dams had long-term beneficial effects on health and metabolic state in offspring.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
T. Li, X. Mao, M. Du, and F. Ren designed the experiments; T. Li and H. Gong collected and analyzed the data; T. Li, H. Gong, and Q. Yuan performed the experiments; T. Li, X. Mao, and M. Du wrote the manuscript.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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