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Original Article

Long-term exercise improves metabolism during pregnancy in high-fat fed mice

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Abstract: Background: Maternal obesity and abnormal glucose and lipid metabolism during pregnancy alter the intrauterine environment and these effects may persist in the offspring for a long time. Previous studies have reported that regular physical activity during pregnancy effectively improves maternal obesity and body metabolism. This study aimed to investigate whether exercising before and during pregnancy could affect energy metabolism during pregnancy. Methods: Female mice were subdivided into four groups based on standard chow diet and high-fat diet, (n = 10/group): SC, SC-Ex, HFD and HFD-Ex. On gestation days ~ 17-19, indirect calorimetry was performed to measure the energy metabolism and body composition analysis. Liver and serum tissues were taken to analyze the metabolism-related biochemical indicators. Results: During pregnancy, the weight between the four groups was gradually decreased, but exercise improved bodily composition. HFD elevated glucose, cholesterol, FFA, triglycerides, IL-6, insulin, leptin, and HOMA-IR. Exercise effectively increased energy metabolism and locomotor activity during pregnancy. In addition, exercise reduced liver Leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4), mitochondrial uncoupling protein 2 (UCP2) protein expression, and increased pyruvate dehydrogenase kinase (PDK4), peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α), peroxisome proliferator-activated receptor gamma (PPARγ) protein expression. Conclusions: These results suggest that long-term exercise (pre-pregnancy and pregnancy) can effectively improve energy metabolism. Exercise has no significant effect on weight during the third trimester, but effectively improved body composition.

Keywords: Pregnancy, energy metabolism, aerobic exercise, maternal obesity

Introduction

Obesity and sedentary lifestyle increases the risk of chronic diseases, especially during pregnancy. Intake of a high calorie diet, maternal excess obesity, and sedentary behavior increase the risk of gestational diabetes mellitus (GDM) [1, 2]. It is well-known that GDM patients or maternal obesity signify several adverse effects on offspring's health, subsequently increasing birth weight, and the odds of developing obesity or type 2 diabetes in the adulthood in the offspring [3-5]. Physical activity (PA) is defined as any movement of the body produced by skeletal muscle that results in energy expenditure [6]. Exercise is a subcategory of PA, in which a planned, structured, and repetitive bodily movements are performed to improve or maintain one or more components of physical fitness [6]. Exercise is one of the important aspects for maintaining a healthy lifestyle. Skeletal muscle increases the uptake of glucose during exercise. The pathway of glucose uptake by skeletal muscle is divided into insulin-dependent and non-insulin-dependent. During the resting state, glucose uptake is mainly achieved through insulin-dependent pathway, and the insulin-stimulated muscle glucose uptake pathway is impaired under insulin resistance conditions. However, the non-insulin-dependent glucose uptake caused by muscle contraction remains unaffected. Therefore, exercise plays an important physiological role in the regulation of glucose metabolism under insulin resistance [7]. Exercise before and during pregnancy plays a key role in reducing the risk of

offspring's obesity and the occurrence of diabetes [8]. Recent studies in animal models demonstrated that maternal exercise improved glucose and lipid metabolism in the offspring [9-11] and changed the amount of offspring's activity during adulthood [12]. Similar results were confirmed by human studies on maternal exercise during pregnancy [1, 13, 14]. According to a recent study, exercise experience of maternal mice presented good metabolic adaptation. More specifically, the use of voluntary exercise improved glucose homeostasis and body composition in pregnant female mice [10]. Exercise completely altered the metabolic effects of maternal obesity in chow fed offspring [15], avoiding maternal obesity caused by hyperleptinemia and hyperlipidemia.

Regular exercise can improve organismal energy metabolism. Recently, several studies have confirmed the positive effect of maternal exercise on offspring as well as maternal parent. Yet, whether exercise before and during pregnancy can alter energy metabolism and related mechanisms are unclear. This is the first study to investigate the relationship between energy metabolism and exercise during pregnancy. Therefore, exercise before and during pregnancy was tested to determine whether it could affect energy metabolism during pregnancy.

Material and methods

Animals and diet

For these experiments 3-week-old virgin female C57BL/6 mice and same aged male mice were obtained from the Shanghai Experimental Animal Center (SLAC). Animals were housed under SPF conditions in animal husbandry. The laboratory humidity and temperature were constant and were maintained in a 12 hours light/night cycle. After one week acclimation, the female mice were divided into four groups based on the dietary conditions: standard chow (SC) diet (12% kcal consisting of fat; Xietong, china), exercise-SC (SC-Ex), high-fat diet (HFD, 45% kcal consisting of fat, D12451, Research Diets, USA), and exercise-HFD (HFD-Ex), with no restrictions to food and water. Food and water intake were weighed every 24 hours to calculate the actual consumption of each mouse. During the period, male mice were fed with SC. All experiments were conducted in accordance with the requirements and obtained approval from the animal ethics committee of Shanghai University of sports (2017040).

Swimming exercise

The exercise intervention was done by swimming. The water temperature was controlled at 30 ± 1°C. In brief, the average active water area of each mouse was 0.04 m^2. Swimming was performed for 5 days in a week, and 2 days over the weekend. The adaptation period comprised of 7 days, with the initial swimming sessions of 10 minutes per day during the first week. The time was gradually increased (increased by 10 minutes each week) until the mice were able to swim without loading for 60 minutes during week 6. In week 7, the mice were loaded with 3% body weight for swimming [16]. The initial swimming time was maintained for 40 minutes, with an addition of 5 minutes per week until the mice were able to load swimming for 60 minutes in week 12. During the mating period and throughout pregnancy, the mice swam for 30 minutes without any load. The two sedentary control groups were placed in 3 cm deep water to soak for the same time in order to mimic the temperature, touch and other stresses of water on mice (**Figure 1**).

Mating and pregnancy calculation

Mating was performed after 13 weeks of intervention. Male to female ratio was 1:2, single cage rearing and mating was continued for 5 days, and the females were checked daily in the morning for a vaginal plug. During this period, if the plug was observed, the female was removed from the cage, and was considered as its first day of pregnancy (F1). Daily weight monitoring during pregnancy was performed, and if no significant increase in body weight after 14 days of pregnancy was observed, then the sample was removed.

Glycemic analysis

Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed on day 14 of gestation. In order to reduce excessive stress during pregnancy, half of the mice in each group underwent GTTs and the others ITTs. For GTTs, the dams were fasted for 12 hours, the blood was drawn from the tail vein, and the mice were intraperitoneally injected with 1 g glucose (Sinopharm, China) per kg body weight.

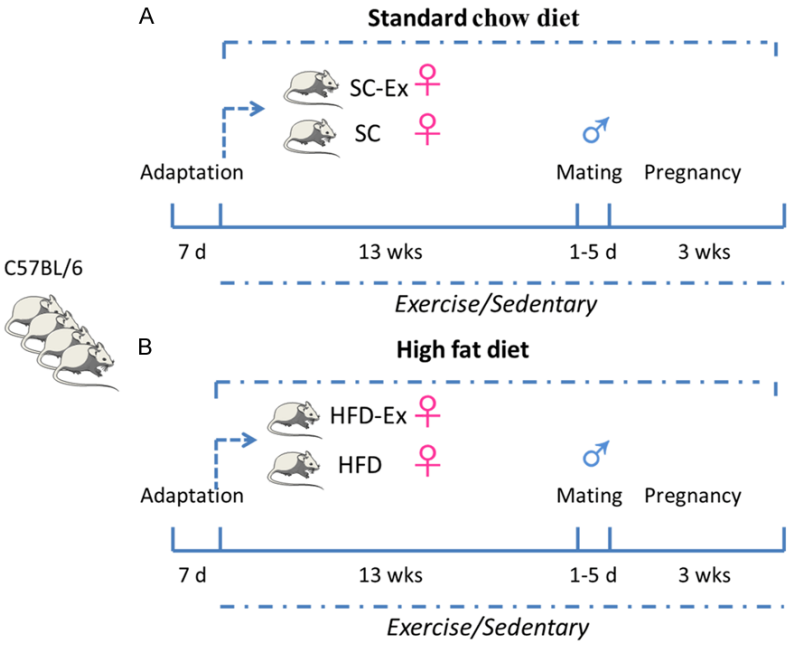

Figure 1. Experimental design. (A) Standard chow diet and (B) High-fat diet groups. Each group was divided into two subgroups, exercise and sedentary group. The mice were divided into four groups above after a week of adaptation. Swimming in mice begin when they are 4-weeks-old, and the duration is gradually increased, until it can swim for 1 hour against resistance. Exercise frequency was 5 times per week during pre-pregnancy and pregnancy. Then mating was performed for 1-5 days. During the mating period and throughout pregnancy, the mice swam without any load for 30 minutes. The pregnancy was judged by vaginal plug. The maternal metabolism and phenotypic indicators were observed during the third week of pregnancy. On day 19 of pregnancy, each group was randomly euthanized 8-10, and the remaining dams gave birth naturally.

For ITTs, the dams were fasted for 4 hours, and the mice were injected with 1 IU of human recombinant insulin (Novo Nordisk, Denmark) per kg body weight. GTTs were determined at 0, 15, 30, 60, and 120 minutes after glucose injection. ITTs were determined at 3, 15, and 30 minutes after insulin injection.

Indirect calorimetry and body composition

Energy metabolism was analyzed using the TSE Labmaster platform (TSE system) [17]. On day 17 of gestation, the mice were individually placed in a metabolic cabin for testing for the next 48 hours. Instrument debugging settings before the test were as follows: the temperature was controlled at 22 ± 1°C, contained 20.47% of O_2 and 0.52% of CO_2. The single cage inflow was set at 0.40 ml/min and the Samp was 0.25 ml/min. Sample interval was set to 3 minutes per box. During the test, the 12-hour light and 12-hour darkness were alternated, and no exercise intervention was performed. During the test, the activity data such as speed, DistD, and DistK, Calo data such as O_2, CO_2, VO_2, VCO_2, RER, and food intake data were recorded.

Body composition of the pregnant mice on gestation day of ~ 19 was analyzed live by using IRIS-CT (Inviscan SAS, France) [18]. The adipose tissue volume, lean body mass, etc. were detected in dams.

Tissue extraction and immunoblotting

Western blotting was used to analyze protein expression levels in the liver of pregnant mice on gestation day ~ 19. Dams were euthanized and tissues were collected. The blood was allowed to stand for 30 minutes, and then serum was obtained after centrifugation at 4000 rpm for 10 minutes at 4°C and frozen at -80°C. Liver protein was lysed with RIPA Lysis Buffer and the supernatant was extracted (P0013B, Beyotime biotechnology, china) [19]. The total protein concentration in the supernatant was determined with Bicinchoninic Acid assay (P0010S, Beyotime biotechnology, China) [20]. LGR4, Rspo1, PGC1α, β-Actin and GAPDH primary antibodies were purchased from Abcam (Cambridge, MA, USA) (ab137480, ab106556, ab54481, ab8227 and ab9485, respectively). PDK4 primary antibodies were purchased from Abways Technology (Shanghai, China) (AY 3551). PPARγ and UCP2 were obtained from Cell Signaling technology (San Antonio, TX, USA) (#2443 and #89326, respectively). Protein samples were separated by SDS-PAGE (8% or 10% polyacrylamide gels) and then transferred onto PVDF membranes. We blocked the membranes with 5% nonfat milk for 90 minutes at room temperature and then applied primary antibody overnight at 4°C. After several rinses, the membranes were incubated

with horseradish peroxidase-conjugated anti-rabbit secondary antibody for 1 hour and then rinsed again. Bands were imaged using the Fluorescence Chemiluminescence Imaging System (Tanon 5200 Multi, Shanghai, China).

Metabolic measurement

Glucose, free fatty acid concentrations (FFA), total cholesterol (TC), and triglycerides (TG) were measured in the clinical biochemistry department using a standard automatic biochemistry analyzer (Olympus AU2700, Hamburg, Germany). Homeostasis model assessment was calculated from HOMA-IR = FPG (mmol.L^{-1}) × FINS (mIU.L^{-1})/22.5 [11].

Luminex assay

Bio-Plex Pro™ mouse panels were used to analyze the levels of cytokines, such as IL-6, insulin, leptin (Bio-Rad, USA) etc. [21]. Frozen serum samples were thawed, and then the experimental steps were conducted in accordance with the manufacturer's instructions. Finally, the Bio-Plex 200 system (Bio-Rad, USA) was used to read the plate and presented as concentrations (pg/ml).

Histological

Liver was fixed in 10% formaldehyde for 48 hours. Paraffin was embedded after the tissues were dehydrated and transparent. The thickness of the film was 0.5 μm, followed by spreading in water at 37°C, and then baking for 1 hour after slicing at 60°C. After dewaxing in a solution of xylene for 30 minutes, the gradient alcohol was used to remove xylene and then rinsed with water. The film was then stained with PAS. In brief, 0.5% periodate was added dropwise, and the dark box was protected from light for 10 minutes. After rinsing with water, Schiff solution was added dropwise. The light should be protected in the box for 15 minutes, and then rinsed with water. The nuclei were stained using hematoxylin for 30 seconds. Gradient alcohol dehydration and xylene were allowed to transparence for 30 seconds before neutral gel encapsulation.

Statistical analyses

All data are presented as means ± SEM. Analysis of the effects of maternal diet and exercise intervention was evaluated by two-way analysis of variance. $P < 0.05$ was considered to be statistically significant. Area under the curve (AUC) for blood glucose levels were calculated using "Area Under the Curves" function in Sigma Plot 12.0.

Results

Food intake and weight during pregnancy

At week 4, the SC and SC-Ex groups showed significant differences (SC = 2.80 ± 0.1 g, SC-Ex = 3.22 ± 0.1 g, $P < 0.05$) in food intake, but demonstrated no significant differences between SC and SCEx group at weeks 8, 12 and 16. At week 16 (gestation), the food intake was similar in HFD and SC groups or HFD-Ex and SC-Ex groups (SC = 3.85 ± 0.08 g vs HFD = 3.84 ± 0.40 g, SC-Ex = 4.49 ± 0.50 g vs HFD-Ex = 4.64 ± 0.21 g). However, in the two HFD groups, a significant increase in food intake was observed in the HFD-Ex group (HFD-Ex = 4.64 ± 0.21 g vs HFD = 3.84 ± 0.40 g, $P < 0.05$), (**Figure 2A**). Gestation water intake showed no differences in SC and SC-Ex groups (**Figure 2B**). During pregnancy, the maternal body weight showed an increasing trend. Just pregnant (i.e., on day 1) HFD and HFD-Ex mice were 32.4 and 22% heavier than their respective SC group mice and at second week of pregnancy (i.e., on day 14) HFD and HFD-Ex mice were 31.5 and 29.3% heavier than mice in respective SC groups. However, this difference did not continue with the development of pregnancy (**Figure 2C**).

Pregnant female glucose tolerance and body composition

During the mid-gestation period, GTT and ITT were performed in pregnant dams (**Figure 3A**, **3B**). The GTT results showed a significant difference between the HFD and HFD-Ex groups than their respective SC groups at 15, 30, 60, and 120 minutes (**Figure 3A-C**), ($P < 0.05$). Similar results were observed for ITT at 3, 15 and 30 minutes (**Figure 3B**). Body composition was measured late in pregnancy, showing no significant differences in the total fat between SC and SC-Ex groups (SC = 4.01 ± 0.6 g, SC-Ex = 3.60 ± 0.64 g). Exercise protected HFD fed dams against the increase in fat (HFD = 7.56 ± 1.59 g, HFD-Ex = 6.10 ± 1.41 g, n = 8-10, $P < 0.05$ vs HFD group) (**Figure 3D**).

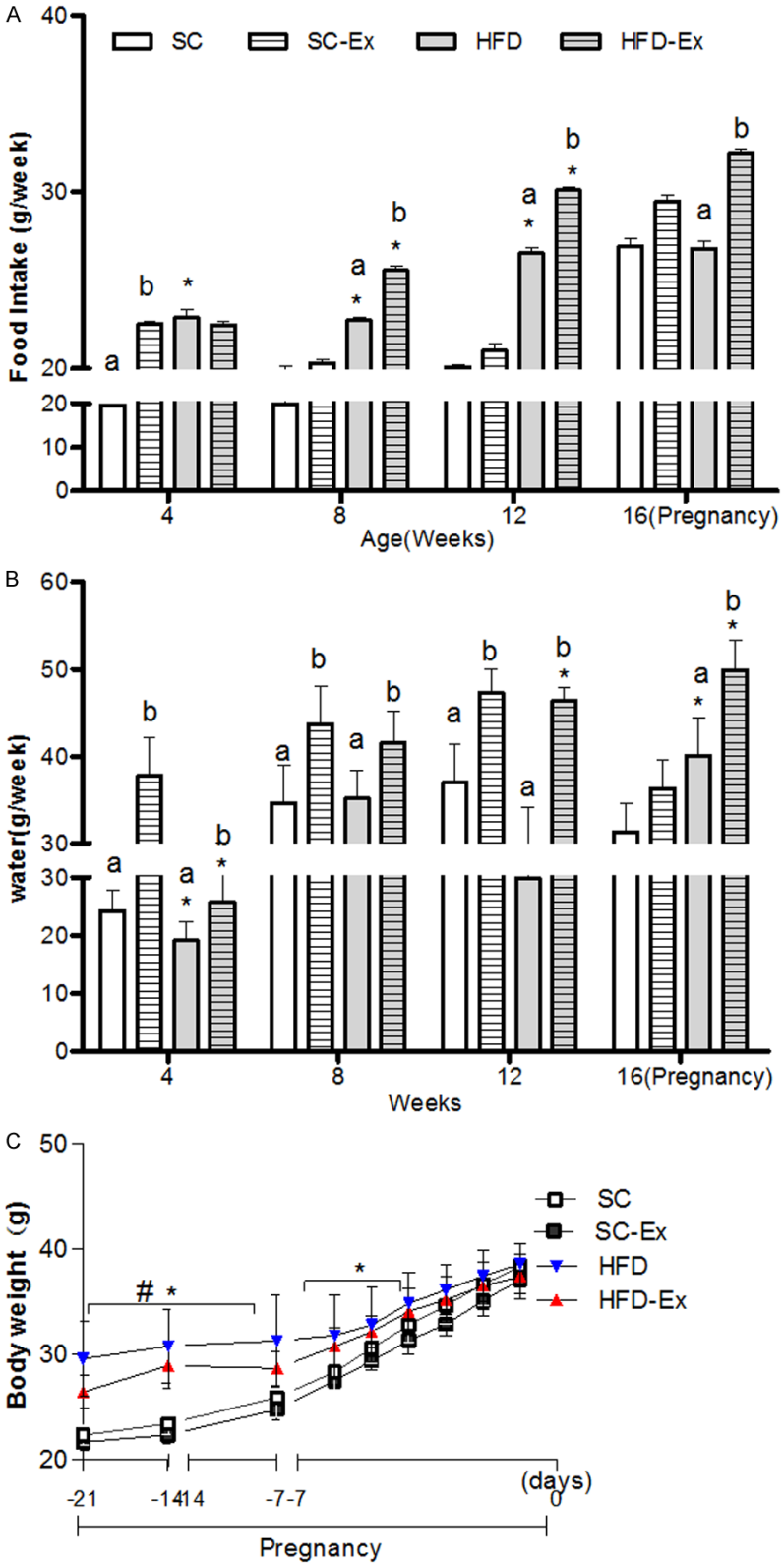

Figure 2. Maternal food intake and weight change during pregnancy. (A) Food intake (B) Water intake and (C) Maternal body weight during pregnancy. Data are presented as mean ± S.E.M, n = 8-10/group; *$P < 0.05$ vs effect of diet between SC and HFD or SC-Ex and HFD-Ex; $P < 0.05$ for different letters between SC and SC-Ex or HFD and HFD-Ex; #HFD vs HFD-Ex, SC and SC-Ex.

Pregnancy outcome

The remaining dams gave birth naturally and observed fertility rate and body height and weight as indicators after birth (**Figure 3E**, **3F**). The fertility rate of HFD was only 44%, and the exercise reversed this situation to a certain extent (60% in the HFD-Ex group), but was still lower than the SC group (76%). No significant intergroup differences were observed between the 4 groups in terms of birth height and body weight of the offspring.

Maternal metabolism at the end of pregnancy

At the end of pregnancy, the maternal cholesterol, FFA, triglycerides, IL-6, insulin, leptin, and HOMA-IR were elevated in HFD group (**Figure 4A-F**). Exercise did not affect these variables in SC diet groups. However, it could effectively improve these indicators in the HFD groups ($P < 0.05$). There was no significant difference between the two exercise groups in IL-6 and HOMA-IR levels (**Figure 4D**, **4G**).

Exercise increased energy consumption during pregnancy

To determine whether exercise and diet affected energy homeostasis, respiratory exchanges were determined by indirect calorimetry coupled with activity and energy consumption determination. The locomotor activity, RQ (VCO_2/VO_2), and energy expenditure were significantly lower in HFD th-

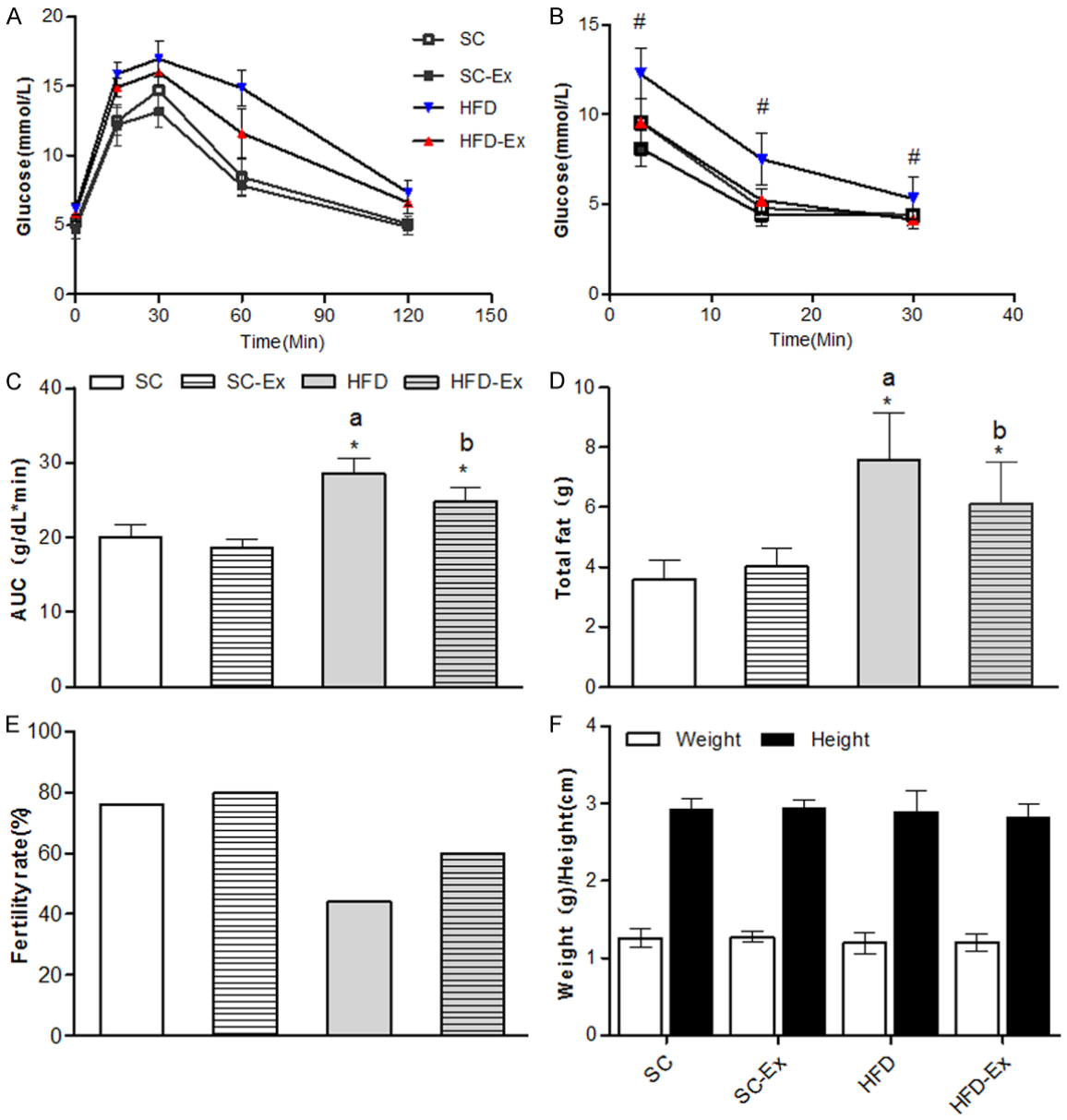

Figure 3. Exercise improves glucose tolerance and fertility in mice of HFD group. (A) pregnancy glucose tolerance test (B) insulin tolerance test (C) AUC (D) Total fat (E) Fertility rate (%) (F) Weight (g)/Height (cm). Data are presented as mean ± S.E.M, n = 8-10; *$P < 0.05$ vs effect of diet between SC and HFD or SC-Ex and HFD-Ex; $P < 0.05$ for different letters between SC and SC-Ex or HFD and HFD-Ex; #HFD vs HFD-Ex, SC and SC-Ex.

an HFD-Ex, while there was no significant difference between SC and SC-Ex groups (**Figure 5A-C**). Compared with SC, HFD was significantly lower than the former in either light or dark ($P < 0.05$).

Metabolic-related protein activity and liver histology

To evaluate the effects of long-term exercise and HFD on metabolism during pregnancy, the liver tissues from the late-pregnancy mice were isolated and analyzed LGR4 and its endogenous ligand Rspondin-1, PDK4, PGC1α, UCP2, and PPARγ protein expression. In addition, liver glycogen depletion pathology was observed. Compared with HFD, the expression of LGR4, PDK4, and UCP2 was significantly reduced in HFD-Ex group, and the expression of PGC1α was increased ($P < 0.05$), (**Figure 6A**). UCP2 protein was different between SC-Ex and SC ($P < 0.05$), but there was no significant difference

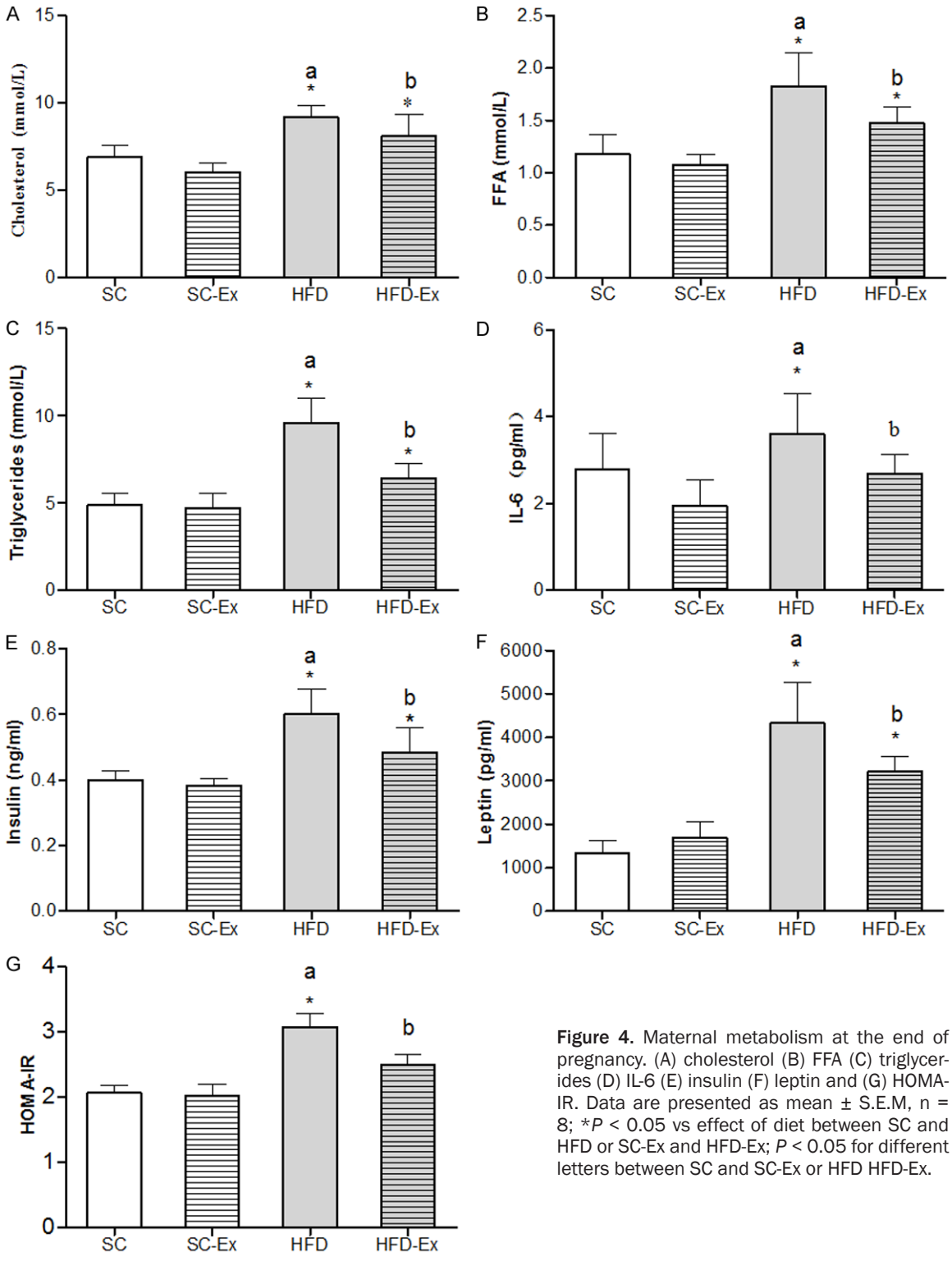

Figure 4. Maternal metabolism at the end of pregnancy. (A) cholesterol (B) FFA (C) triglycerides (D) IL-6 (E) insulin (F) leptin and (G) HOMA-IR. Data are presented as mean ± S.E.M, n = 8; *$P < 0.05$ vs effect of diet between SC and HFD or SC-Ex and HFD-Ex; $P < 0.05$ for different letters between SC and SC-Ex or HFD HFD-Ex.

between others. Liver periodic acid Schiff staining revealed that hepatic glycogen was depleted in HFD pregnant mice, and the HFD-Ex group showed normal glycogen expression except hepatic steatosis (**Figure 6B**).

Discussion

A series of physiological and psychological changes occur during pregnancy, leading to low levels of PA or increased sedentary behavior.

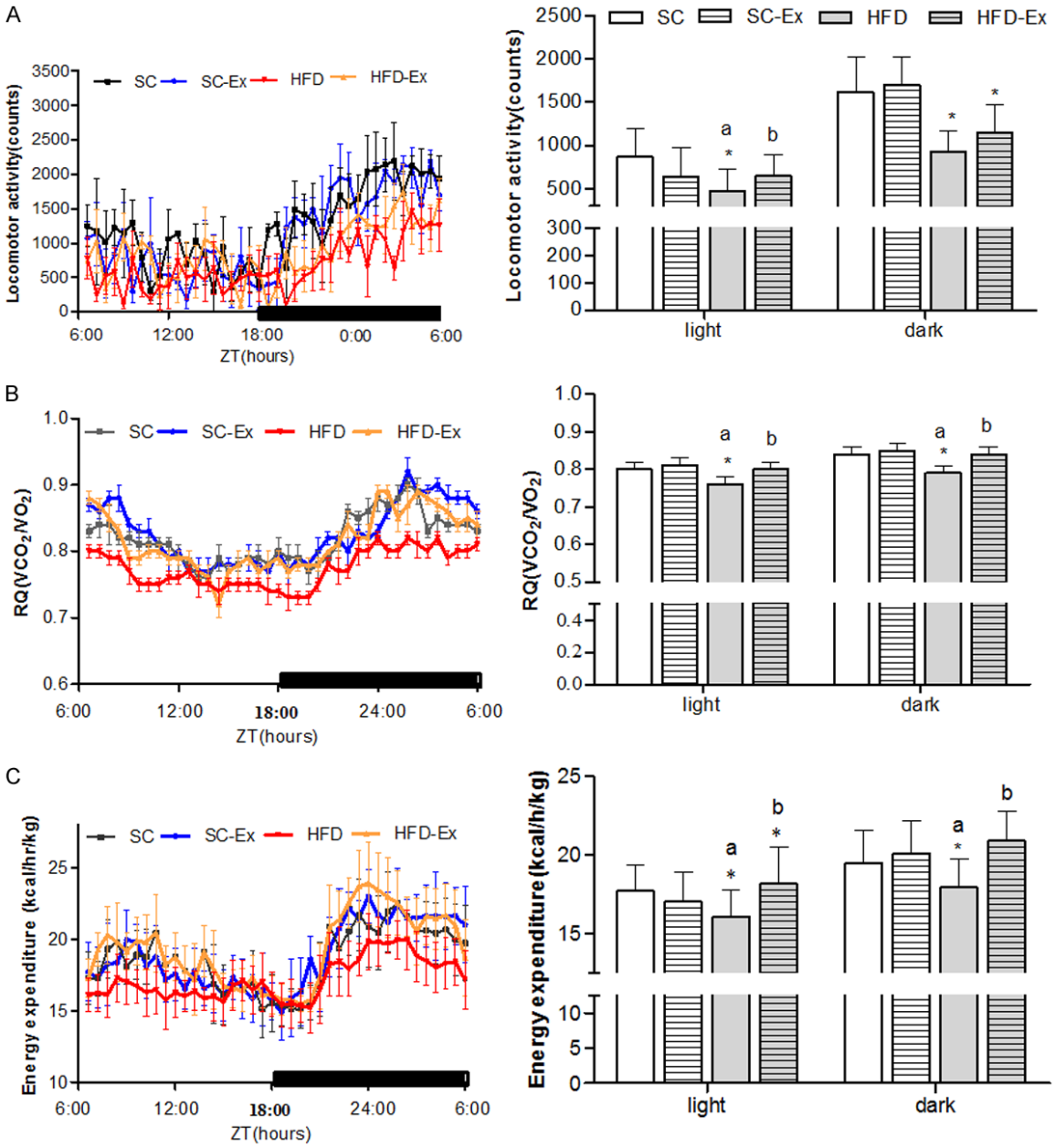

Figure 5. Exercise increases energy consumption during the end of pregnancy. (A) Locomotor activity (B) RQ (VCO_2/VO_2) and (C) Energy expenditure. Data are presented as mean ± S.E.M, n = 6; *P < 0.05 vs effect of diet between SC and HFD or SC-Ex and HFD-Ex; P < 0.05 for different letters between SC and SC-Ex or HFD HFD-Ex.

These in turn causes maternal obesity, gestational diabetes, pregnancy hypertension and other diseases. According to the latest study, the intrauterine fetal exposure to maternal obesity increases the risk of abnormal glucose tolerance, overweight, or obesity in the offspring [8, 15]. It is well known that obesity and diseases caused by imbalances in energy metabolism are worldwide scientific problems and research hotspots. Exercise consumes a lot of energy, and requires contraction of skeletal muscles. Scientifically, regular exercise plays an important role in promoting and improving the body's energy metabolism [22]. The health promotion effects of exercise on pregnancy have also been reported in several literatures [1, 23]. A recent randomized controlled trial concluded that moderate-intensity exercise during pregnancy improved fitness, but did not affect the birth weight or other clinical out-

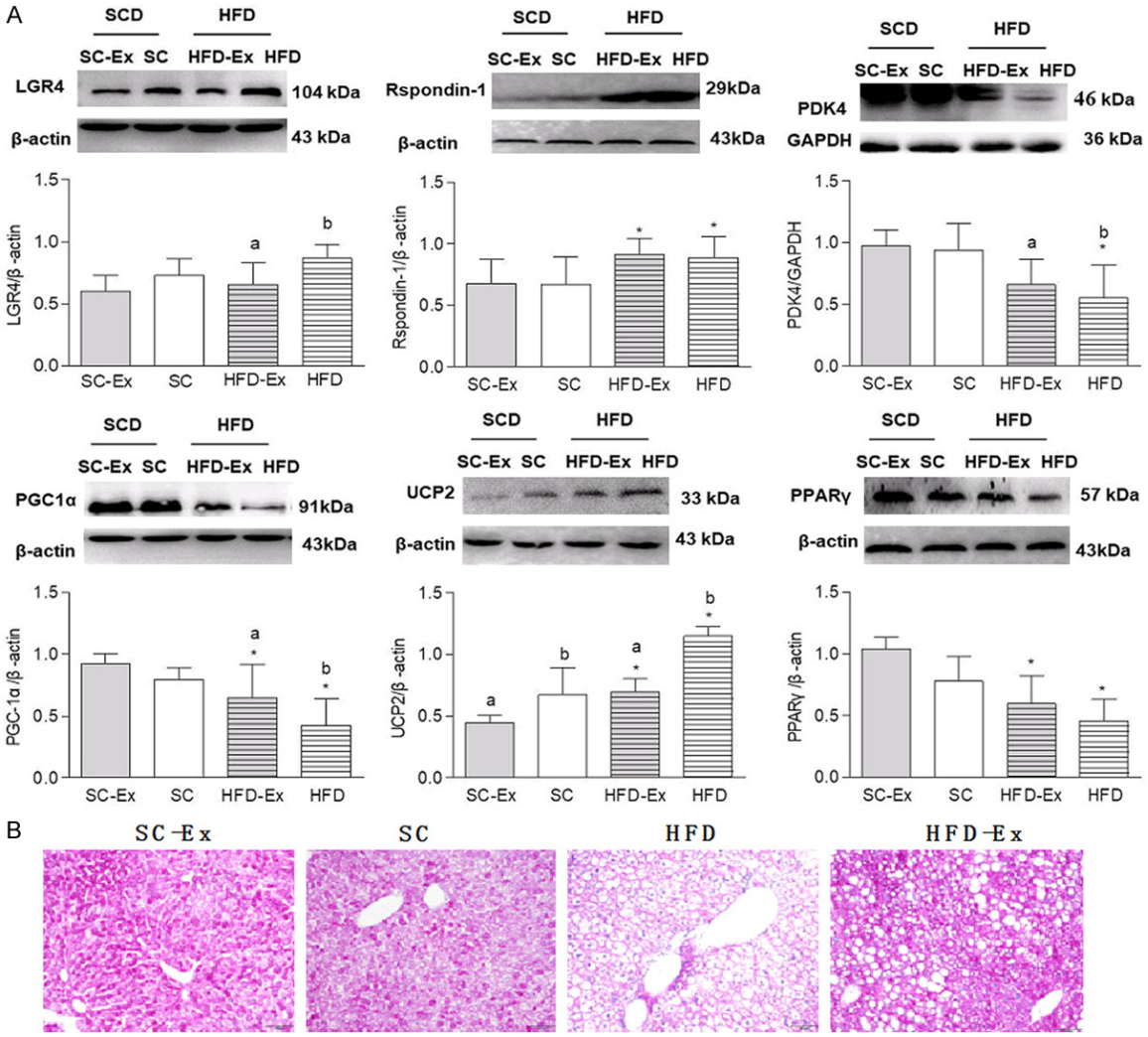

Figure 6. Late in pregnancy, livers were collected for *in vitro* analyses. A. Western blot analysis of relevant active proteins during glycolipid metabolism (n = 6). B. Periodic acid Schiff staining of liver in pregnant mice. *$P < 0.05$ vs effect of diet between SC and HFD or SC-Ex and HFD-Ex; $P < 0.05$ for different letters between SC and SC-Ex or HFD and HFD-Ex.

comes [24]. Exercise during pregnancy is a non-invasive treatment that can effectively manage maternal body weight and improve pregnancy outcomes of pregnant women with gestational diabetes [13]. Another study indicated that exercise in obese female rats showed beneficial effects on male and female offspring metabolism [11]. These studies showed that reasonable exercising before and during pregnancy can effectively improve maternal and offspring health, but the effects of long-term exercise on maternal metabolism during pregnancy has not been confirmed by these studies. Therefore, further studies are needed to determine the interactive effects of long-term exercise, dietary intervention, and energy expenditure during pregnancy.

Maternal diet, body composition and glucose homeostasis

Although there was a significant difference in the weight between the groups before gestation at day ~ 19, the HFD group still showed the highest. But in the last week of pregnancy, there was no significant difference between the 4 groups. Interestingly, this was consistent with Vega's findings [11]. The two groups of HFD showed a steady increase in body weight throughout pregnancy, whereas the two groups of SC did not have a large increase in the magnitude before gestation day ~ 14, but was increased rapidly in the last week. This study showed that pre-pregnancy and post-pregnancy swimming significantly improved glucose

homeostasis and body composition of high-fat fed pregnant mice. It is well known that obesity can lead to insulin resistance. In **Figure 6B**, hepatic glycogen deposition was less than in the HFD group, and serum leptin levels and HOMA-IR were significantly increased. This revealed the presence of insulin resistance in the HFD group. With the increase of fat mass, macrophages were accumulated in the adipose tissue, leading to pro-inflammatory environment [25, 26]. Adipose tissue releases many pro-inflammatory hormones and cells/adipokines, which further reduces the systemic insulin sensitivity by inactivating key proteins [25]. A certain intensity and duration of physical activity plays a positive role in improving the insulin sensitivity of obese pregnant women induced by long-term diet. This has also been confirmed in other animal experiments. The researchers found that voluntary exercise during pregnancy can significantly improve glucose tolerance and insulin sensitivity in pregnant female mice [10]. Long-term contraction of muscles consumes energy, further activating AMP-activated protein kinase (AMPK) during exercise. Exercise regulates glucose uptake through an AMPK-mediated insulin-independent mechanism. Exercise regulates GLUT4 transport to the plasma membrane and lateral tubules, increasing glucosamine uptake in the skeletal muscle [7]. Two major intracellular mechanisms have been proposed to explain the contraction-dependent glucose transport. The first hypothesis is that glucose transportation can be related to the metabolic strain imposed on skeletal muscles during exercise [27]. The second explanation is that glucose uptake increases due to depolarization of sarcolemma and T-tubule membrane via calcium-mediated secondary messengers [28].

Maternal metabolism and energy consumption

In the experiments reported here, indirect calorimetry was used to assess energy metabolism during pregnancy. Considering that the energy consumption was calculated based on oxygen uptake, no significant difference was observed between SC-Ex and SC, but the energy consumption of HFD-Ex was significantly higher than that of HFD. Similar phenomenon existed under light and dark conditions. In addition, the energy consumption of the SC group mice was also higher than that of the HFD group mice. Interestingly, we also found a similar phenomenon in our research. The mice in the HFD group demonstrated less locomotor activity than the SC mice, and the effect of exercise was only observed during the day. In both the HFD groups, there was a change in the RQ (VCO_2/VO_2) in the exercise group. Adipokines including leptin, resistin, and adiponectin played a role in energy metabolism. Leptin is an important energy sensor in the body [29]. Obese mice showed a significant reduction in energy expenditure due to lack of leptin on energy metabolism, which was related to an increase in body weight and fat mass [30]. Leptin can reflect the entire body's energy storage situation to the central nervous system. As shown in **Figure 4F**, exercise suppression expressed leptin. This is because the central nervous system is the most important direct target organ of leptin, causing increased sympathetic activity and a series of metabolic changes, such as increased body temperature, increased oxygen consumption, etc. The specific mechanisms may be achieved through molecular regulatory function of the central leptin receptor signal transduction pathway. Resting energy metabolism is the key to energy consumption in the body, and has a relatively wide range of positive health effects [31]. Whether exercise can improve and regulate the levels of resting energy metabolism, which in turn improves the health problems caused by lack of PA is still unclear. This study did not evaluate and measure the resting metabolic rate (RMR) in pregnant mice, and this can be investigated in future studies.

In our study, although exercise improved cholesterol, FFA, triglycerides, IL-6, insulin, leptin, and HOMA-IR in HFD pregnant mice, the signs of dysfunction of carbohydrate metabolism still persisted. This suggested that long-term exercise does not completely counteract the metabolic hazards associated with long-term HFD.

Metabolism related protein expression

Reasonable PA has a positive effect on maternal health during the perinatal period, especially in terms of glucose and lipid metabolism [1]. However, most of the molecular mechanisms involved have focused on exercise to improve insulin sensitivity [10]. LGR4, which is also known as G protein-coupled receptor 48 (GPR48), is a newly discovered member of the G protein

family in the recent years, and has been shown to play an important role in many tissues [32, 33]. LGR4 has a wide range of metabolic functions, including food intake, lipid metabolism, energy expenditure, and beta cell proliferation, and insulin secretion [32].

Respondin is identified as an endogenous ligand of LGR4 and is found to regulate energy metabolism and cell proliferation [34, 35]. Whether exercise regulates respondin1-LGR4, especially, in other metabolic organs other than adipose tissue is still a question. With this research idea, we conducted verification and found that exercise can reduce liver LGR4 expression in HFD group, while ligand respondin-1 differs only in different diet groups and exercise has no effect on it.

PGC1α is closely related to energy metabolism. Overexpression of PGC-1α in murine animal model enhanced the oxidative capacity of fatty acids and the process of mitochondrial biosynthesis and angiogenesis [36]. PGC1α co-activates PPAR, enhancing lipolysis and heat production [37]. It also promotes PDK4 expression and inhibits pyruvate from entering into the tricarboxylic acid cycle [38]. PDK4 is a key enzyme in the process of glucose oxidation. PDK4 can phosphorylate and inactivate PDH. It also inhibits oxidative decarboxylation of pyruvate in the mitochondria, thereby inhibiting glucose oxidation. At the same time, reduced acetyl-CoA also inhibited the production of malonyl-CoA, where the latter inhibited lipid oxidation and promoted lipid metabolism. Increased expression of PDK4 plays a crucial role in the energy balance of the body [38]. In this study, protein expression of PDK4, PGC1, and PPARγ in the liver of pregnant mice was up-regulated after exercise. This may be due to the down-regulation of LGR4 by exercise, activation of PGC1α and PPARγ, and increased fat decomposition and heat production. During this process, LGR4 acts as an intermediary to participate in the body's metabolism of glucose and lipids.

Mitochondrial UCP2 is a type of carrier protein that is present on the mitochondrial inner membrane. It plays an important role during the energy metabolism of the liver. Expression of mitochondrial UCP2 in non-alcoholic fatty liver was increased [39]. Fatty acids can activate proton leak activity of UCP2 via peroxide. UCP2 expression increases and voltage-dependent calcium channel closure reduces insulin secretion [40]. The mechanism of down-regulation after exercise is likely to be mediated by this proton leakage activity, but its specific mechanism needs further investigation.

Conclusion

Overall, these findings suggest that maternal swimming has a positive effect on energy homeostasis during pregnancy, as it can partially reduce adverse metabolic effects of HFD. On the other hand, there were no significant metabolic differences between SC and SC-Ex groups, suggesting that exercise intensity and load during pregnancy should be re-considered. Different interventions (eg, type, intensity, duration of exercise, etc.) may experience inconsistent results for different models of metabolic diseases during pregnancy. These are still questions that require exploration and should be studied in the areas of future sports and pregnancy health promotion.

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Disclosure of conflict of interest

None.

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