

Dietary Energy Levels from Middle to Late Gestation of Guangdong Small-Ear Spotted Pig: Effects on Body Weight, Muscle Development, and Glucose Metabolism of its Newborn Piglets

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Research

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Abstract

Background: Guangdong Small-ear Spotted pigs are Chinese indigenous pig that have the characteristic of desired meat quality and resistance to coarse feeding. However, no study has elucidated the effects of dietary energy levels on body weight, muscle development and glucose metabolism of its newborn piglets. Therefore, a total of 66 pregnant gilts with an average body weight of 80.6 ± 6.6 kg at day 60 of gestation were randomly divided into two groups: control group (CON group; 11.50 MJ/kg digestible energy), and high-energy diet group (HE group; 13.42 MJ/kg digestible energy).

Results: The results showed that the maternal HE diet was shown to decrease the birth weight of piglets that from the gilts with total or alive litter size of 12 to 13. Additionally, the HE diet group were shown to impair the glucose tolerance of newborn piglets, as evidenced by the glucose tolerance test and the inhibition of insulin signaling pathway in liver and soleus muscle. Despite no significant change in the muscle weight in the two groups, the maternal HE diet was shown to downregulate the protein level of slow-twitch fiber myosin heavy chain I (MyHC I), and upregulate the protein levels of fast-twitch fiber myosin heavy chain IIb (MyHC IIb) and IIx (MyHC IIx) in soleus muscle of their progeny. Furthermore, the newborn piglets in HE group were showed a decrease in mitochondrial biogenesis in liver and soleus muscle when compared to that in CON group.

Conclusions: Maternal high-energy diet from middle to late gestation decreases the birth weight of piglets that from the gilts with total or alive litter size of 12 to 13, induces the formation of glycolytic muscle fibers, and impairs glucose tolerance of their newborn piglets.

Introduction

In the last decade, the selection for high prolificacy in modern sow herds has led to a marked increase in litter sizes. One consistent outcome of this strategy was the increased number of low birth weight piglets with less vitality and mature. Maternal nutrition plays a vital role in this performance. For instance, increased maternal feed intake from 1.8 kg/d to 3.5 kg/d during days 90 to 110 of gestation was reported to reduce the occurrence of lightweight piglets [1]. These results suggest that an optimal nutrition level of sows during gestation is important to the improvement of birth weight and its heterogeneity of piglets.

Adequate energy intake during the gestation can improve reproductive performance, litter performance, and colostrum quality [2]. However, excessive energy intake during gestation can reduce feed intake during lactation and pig weight [2, 3], increase body weight and backfat thickness loss [4]. In addition, maternal energy intake is closely associated with fetus skeletal muscle development. Study showed that the skeletal muscle differentiation and maturity of fetus were delayed under maternal high energy intake during the gestation [5]. Therefore, the energy intake of gestating sows needs to satisfy both of fetal growth and body condition maintenance.

Guangdong small-ear spotted pigs are Chinese indigenous pig breed that have many unique characteristics, such as desired meat quality and adaptability to crude fiber. However, this breed also

possesses negative economic features including slow growth rate, low lean-meat percentage, and low feed utilization rate. To our knowledge, no study has elucidated the energy requirement of this breed during pregnancy. Therefore, the aim of this study was to investigate the effects of maternal energy diet feeding from middle to late gestation of Guangdong small-ear spotted pigs on birth weight, muscle development and glucose metabolism of its newborn piglets.

Methods

Experimental design and diets

A total of 66 pregnant gilts (Guangdong Small-ear Spotted pig) were assigned to either a control group (CON group, 11.50 MJ/kg digestible energy) or a high-energy diet group (HE group; 13.42 MJ/kg digestible energy) during gestation, with 11 replicate pens per treatment and 3 gilts per pen, respectively. The nutrient level for the control diet (Table 1) met the requirement of the Chinese National Feeding Standard (2004) for gestating gilts. No difference was observed in the maternal body weight (80.8 ± 1.1 kg VS 80.0 ± 1.8 kg) and backfat thickness (28.4 ± 0.6 mm VS 28.2 ± 0.4 mm) in the two groups at the start of the study. Each pen (3 gilts) was fed 3.6, 4.5, and 7.5 kg of diet per day at day 60–85, 86–90, and 91–110 of gestation, respectively, with free access to drinking water. On day 110 of gestation, gilts were cleaned with warm water and moved to individual farrowing crates (2.2m × 1.5 m). The experimental design and procedure presented in this study are reviewed and approved by the Animal Care and Use Committee of the South China Agricultural University.

Table 1
Composition and nutrient levels of the experimental diets (as-fed basis) ¹

Items	Gestation diet		Lactation diet
	Con	HE	
Ingredient, %			
Corn	32.1	59.4	48.7
Soybean meal	10.0	14.2	19
Barley	13.0	-	-
Rice bran			10
Wheat brain	24.0	16.0	17
Soybean hull	16.3	2.8	-
Soybean oil	-	3.0	-
Calcium carbonate	0.5	0.5	-
Limestone			0.4
Dicalcium phosphate			0.8
Premix ¹	4.1	4.1	4.1
Total	100.00	100.00	100.00
Calculated composition ²			
DE, MJ/kg	11.5	13.4	12.7
CP, %	13.5	13.4	16.2
EE, %	2.9	5.9	4.1
CF, %	9.4	4.1	4.1
Ca, %	1.1	1.0	1.2
P, %	0.6	0.6	0.7
Lys, %	0.6	0.6	0.8

¹Premix provided for 1 kg of complete diet: Cu,75 mg; Fe,750 mg; Se, 0.20 mg; Zn, 750 mg; Mn, 187 mg; vitamin A, 150,000 IU; vitamin B₂, 140 mg; vitamin D₃, 25,000 IU; vitamin E, 72 IU; vitamin K₃, 35 mg; vitamin B₂, 140 mg; vitamin B₆, 70 mg; calcium pantothenate, 350 mg; niacin, 500 mg; and vitamin B₁₂, 0.4 mg.

² Calculated value using values for feed ingredients from the Nutrient requirements of swine (2012).

Items	Gestation diet		Lactation diet
	Con	HE	
Met + Cys, %	0.6	0.5	0.6
Trp, %	0.2	0.2	0.2
Thr, %	0.5	0.5	0.6
¹ Premix provided for 1 kg of complete diet: Cu,75 mg; Fe,750 mg; Se, 0.20 mg; Zn, 750 mg; Mn, 187 mg; vitamin A, 150,000 IU; vitamin B ₂ , 140 mg; vitamin D ₃ , 25,000 IU; vitamin E, 72 IU; vitamin K ₃ , 35 mg; vitamin B ₂ , 140 mg; vitamin B ₆ , 70 mg; calcium pantothenate, 350 mg; niacin, 500 mg; and vitamin B ₁₂ , 0.4 mg.			
² Calculated value using values for feed ingredients from the Nutrient requirements of swine (2012).			

Performance measurement of gilts and piglets

After parturition, the birth weight of piglets was measured within 6 h after birth. No difference was observed in the birth weight of the piglets (645 ± 23 g VS 604 ± 8 g) between the two groups. Backfat thickness of gilts was measured at parturition. Backfat thickness was measured at the P2 position using a mode ultrasound scanner (Renco Lean-Meater®, Minneapolis, MN, USA). The numbers of total born, born alive, and stillborn piglets were recorded at farrowing.

Glucose tolerance test (GTT)

After an overnight fast, an intraperitoneal glucose tolerance test (GTT) was performed. Briefly, neonatal piglets with a similar average birth weight ($n = 8$) were given an intraperitoneal injection of glucose (1 g/kg body weight) over a period of 120 min. Blood glucose levels in tail vein were measured at 10, 20, 30, 45, 60, 90, and 120 min after the initial glucose injection using a glucometer (Sannuo, Changcha, China).

Sample collection

After parturition, piglets ($n = 8$) with a similar average birth weight were selected for sample collection. Blood samples were collected into 10-mL centrifuge tubes from the jugular vein, followed by centrifugation at $3,000 \times g$ and 4 °C for 15 min to recover the serum. Meanwhile, liver and soleus muscle (about 2 g each) were collected from the carcass and weighed, followed by snap-freezing in liquid nitrogen and storage at -80 °C for further analysis.

Biochemical parameter

The levels of serum insulin (CSB-E06829p, Cusabio Biotech, Wuhan, China) and non-esterified fatty acid (NEFA) (A042-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were determined using commercial kits according to the manufacturer's instructions. Homeostasis model assessment-insulin resistance (HOMA-IR) = [(fasting insulin, mIU/L)] × (fasting glucose, mmol/L)]/22.5.

Triglyceride (A110-1-1, Jiancheng Bioengineering Institute, Nanjing, China), Glycogen (BC0345, Solarbio, China), and glycogen synthase (H388, Jiancheng Bioengineering Institute, Nanjing, China) were determined by using commercially available kits according to the manufacturer's instructions.

ATP levels

ATP levels in muscle and liver were determined using the commercial kits (Beyotime, Beijing, China) according to the manufacturer's instructions.

Malic dehydrogenase (MDH) and succinate dehydrogenase (SDH) enzyme activity assay

The MDH and SDH activities in muscle and liver were measured using the commercial assay kits (Solarbio, China) according to the manufacturer's instructions.

Mitochondrial DNA (mtDNA) copy number

Total genomic DNA was isolated from muscle and liver using the QIAamp DNA Mini Kit (Qiagen, USA) according to the manufacturer's instructions. Mitochondrial DNA copy number was determined as previously described [6]. The primers were presented in supplemental Table 1.

Real-time quantitative RT-PCR.

Total RNA was isolated from the muscle and liver tissues using the TRIzol reagent (Invitrogen, Carlsbad, USA). cDNA synthesis was performed using 1000 ng RNA according to the manufacturer's instructions of the PrimeScript RT reagent kit (Takara, Dalian, China). Primers used are listed in supplemental Table 1. Real-time quantitative PCR was performed in the total reaction volume of 15 μ L containing 2 μ L of cDNA template solution, 7.5 μ L of RealStar Green Fast Mixture (GenStar, Beijing, China), 4.7 μ L of water, and 0.8 μ L of each primer using ABI QuantStudio™ 6 Flex system (Applied Biosystems, Carlsbad, CA). The thermal cycling consisted of a 10-min incubation at 95 °C, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/extension for 30 s at 60 °C. Relative gene expression was expressed as a ratio of the target gene to the control gene and calculated using the formula $2^{-\Delta\Delta CT}$ values ($\Delta Ct = Ct^{gene} - Ct^{control}$).

Western blotting

Total protein was extracted from muscle and liver tissues using the protein extraction kit (Beyotime Biotechnology, Jiangsu, China). The protein concentration of lysates was measured using BCA protein Assay kit (Beyotime Biotechnology, Jiangsu, China). Immunoblotting analysis was performed as previously described.[7] Blots were incubated overnight at 4°C with the following primary antibodies:

(IRS1) antibody (Proteintech, USA, 1:1000 dilution), phospho-IRS-1 (Ser636/639) antibody (CST, USA, 1:1000 dilution), Akt antibody (Proteintech, USA, 1:1000 dilution), phospho-Akt (Ser473) antibody (CST, USA, 1:1000 dilution), and β -Actin (CST, USA, 1:1000 dilution). The density of bands was quantified using Image J software and then normalized to β -Actin levels.

Statistical analysis

Data are presented as mean \pm SEM. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL) software. The differences between the two groups were analyzed by the Student's *t* test. $P < 0.05$ was considered as statistically significant.

Results

Performance of gilts and piglets

The litter weight, total piglets born, piglets born alive and stillbirth are not difference ($P > 0.05$) between the CON and HE groups. The HE group showed an increase trend ($P = 0.07$) in the birth weight variation when compared to the CON group (Table 2). Interestingly, the HE diet was shown to decrease ($P < 0.05$) the piglet birth weight of gilts with total or alive litter size of 12 to 13 (Fig. 1A, B). No difference ($P > 0.05$) was observed in the muscle weight of newborn piglets in the two groups (Fig. 2).

Table 2
Effects of maternal dietary energy levels during gestation on performance of gilts

Items	CON	HE	<i>P</i> value
Replicates ¹	11	11	
Litter weight (g)	7057.6 \pm 397.3	6872.1 \pm 253.0	0.69
No. of pigs per litter			
Total piglets born	11.2 \pm 0.6	11.7 \pm 0.5	0.50
Piglets born alive	10.6 \pm 0.6	10.4 \pm 0.4	0.58
Stillbirth	0.5 \pm 0.2	0.9 \pm 0.3	0.19
Birth weight variation	18.9 \pm 0.9	22.2 \pm 1.5	0.07
Backfat thickness at parturition day	31.1 \pm 0.8	33.8 \pm 0.6	0.01
Value are mean \pm SEM. The differences between the two groups were analyzed by the Student's <i>t</i> test.			
¹ There were 11 replicate pens (3 gilts /pens) per group.			

Gilts from the HE group showed a higher ($P < 0.05$) backfat thickness at parturition day (Table 2).

Maternal high-energy diet feeding impairs glucose tolerance in offspring

We performed a GTT (glucose tolerance test) in neonatal piglets and the results are shown in Fig. 3. The two groups showed no difference ($P > 0.05$) in the fasting glucose level at baseline (Fig. 3A). However, the HE piglets were higher ($P < 0.05$) than the CON piglets in the area under the curve (AUC) analysis of GTT (Fig. 3B). Additionally, the HE piglets were shown to be higher ($P < 0.05$) than the CON piglets in HOMA-IR value, fasting serum insulin level, and triglyceride concentration (Fig. 3C, D, F). However, no difference ($P > 0.05$) was observed in the serum NEFA concentration between the two groups (Fig. 3E).

Maternal high-energy diet feeding induces TG accumulation, but prevents glycogen deposition in the muscle and liver of offspring

Compared with the CON group, the HE piglets displayed a higher ($P < 0.05$) concentration of TG, while a lower ($P < 0.05$) content of glycogen and glycogen synthase in the muscle (Fig. 4A-D).

Maternal high-energy diet feeding inhibits the activation of insulin signaling pathway in the muscle and liver of offspring

In Fig. 5, when compared to the CON group, the HE piglets showed a reduced activation of insulin signaling pathway in the liver and muscle, as evidenced by the decreased protein levels of p-IRS-1 ($P < 0.05$) and p-AKT ($P < 0.05$).

Maternal high-energy diet feeding induces the transition of skeletal muscle fiber types in offspring

Here, we investigated the effects of maternal HE diet on the skeletal muscle fiber type in offspring. In soleus muscle, we found that maternal HE diet downregulated the slow-twitch fiber-associated genes of MyHC I ($P < 0.05$) and MyHC IIa ($P < 0.05$), with a trend ($P = 0.08$) to upregulate the fast-twitch fiber associated gene of MyHC IIb (Fig. 6A). Accordingly, the protein level of MyHC I ($P < 0.05$) was downregulated, in contrast to the upregulation in the protein levels of MyHC IIb ($P < 0.05$) and MyHC IIx ($P < 0.05$) in the soleus muscle of HE piglets versus the CON piglets (Fig. 6B).

Maternal high-energy diet feeding impairs mitochondrial biogenesis in the muscle and liver of offspring

In Fig. 7, maternal HE diet was shown to reduce ($P < 0.05$) the soleus muscle ATP levels (Fig. 7D), with a trend ($P = 0.08$) to decrease the mtDNA content (Fig. 7E). When compared with the CON group, the HE piglets showed a downregulation ($P < 0.05$) in the protein levels of peroxisome proliferator-activated receptor gamma coactivator (PGC-1 α) ($P < 0.05$) and nuclear respiratory factor-1 (NRF-1) ($P < 0.05$) in the muscle (Fig. 7A-C). Additionally, maternal HE diet decreased the SDH ($P < 0.05$) and MDH ($P < 0.05$) activities in the muscle of offspring (Fig. 7F).

In Fig. 8, maternal HE diet was seen to reduce ($P < 0.05$) the hepatic ATP levels (Fig. 8E). Additionally, the protein levels of PGC-1 α ($P < 0.05$) and NRF-1 ($P < 0.05$) were also downregulated in the liver of the HE piglets versus the CON piglets (Fig. 8A-C). Furthermore, the HE piglets also showed a decrease ($P < 0.05$) in the SDH activity in the liver due to maternal HE diet treatment (Fig. 8F).

Discussion

Maternal dietary energy level during pregnancy plays an important role in reproductive performance. Guangdong Small-ear Spotted pigs are Chinese indigenous pig breed that have many advantages, which are highly valuable to the local pig industry. However, few studies have focused on the energy requirement of this breed during pregnancy. Therefore, the present study was to investigate the effects of dietary energy levels from middle to late gestation on reproductive performance of Guangdong Small-ear Spotted pig. In the present study, Maternal HE diet had no effect on litter weight, total piglets born, piglets born alive and stillbirth, which was in line with previous study [8]. Interestingly, maternal HE diet was shown not effect on average birth weight of piglets, but was shown to increase birth weight variation and decrease the average birth weight of piglets that from gilts with total or alive litter size of 12 to 13. This is a novel finding which suggests that increased maternal dietary energy levels from 11.50 MJ/kg to 13.42 MJ/kg decreased the reproductive performance of gilts with total or alive litter size of 12 to 13. However, the muscle weight of neonatal piglets was not affected by dietary energy treatment. Similarly, the increased backfat thickness in late gestation was observed in gilts by increasing dietary energy levels [3]. In addition, the glucose tolerance in neonatal offspring was investigated, and maternal HE diet was shown to impair the glucose tolerance of neonatal offspring, as evidenced by GTT test, fasting serum insulin, and HOMA-IR index, which was in line with previous studies reporting that maternal high-fat diet impaired glucose metabolism in offspring [9].

Skeletal muscle consumes nearly 80% of glucose [10], and liver regulates glycogen storage, glucose uptake and output [11], indicating skeletal muscle and liver play an important role in glucose homeostasis. Glucose homeostasis is disrupted when the insulin signal pathway is suppressed in liver and muscle [12, 13]. In the present study, the insulin signaling pathway in the liver and muscle of the HE offspring was examined to explore the molecular mechanism underlying their impaired glucose tolerance. In their liver and muscle, the protein levels of p-IRS1 and p-AKT were seen to be decreased, implying the insulin signaling pathway was impaired. Lipotoxicity is characterized by excessive accumulation of lipid in non-adipose tissues, including liver and muscle, leading to cellular dysfunction. Excessive triglyceride accumulation in muscle is an important contributor to obesity and insulin resistance, and it is also known

as a marker of insulin resistance [14]. The increased triglyceride content in the liver and muscle of the HE offspring further confirmed that maternal low-energy diet is beneficial to the glucose homeostasis of the offspring. Additionally, we observed a decrease in the glycogen content in liver and muscle, as well as a decrease in the content of glycogen synthase in muscle. Glycogen synthesis is a major fate for glucose uptake in the body, and glycogen synthase is a key enzyme related to whole-body insulin sensitivity. Previous studies have shown impaired glucose tolerance and metabolism in liver or muscle glycogen synthase knock-out mice [15, 16]. Therefore, we speculate that suppressing the insulin signal pathway in liver and muscle may lead to a reduction in glucose utilization, thus impairing the glucose tolerance in the offspring under maternal HE diet treatment.

Skeletal muscle fiber types, including MyHC I, MyHC IIa, MyHC IIb, and MyHC IIx, play a role in glucose homeostasis. Slow-twitch muscle fibers are more sensitive than fast-twitch muscle fibers to insulin stimulation [17]. Additionally, type I and IIa fibers are more abundant than type IIb and IIx fibers in the protein levels of insulin receptor, glucose transport-4 (GLUT4), and glycogen synthase [18], and the former two fibers play a more important role in maintaining glucose homeostasis in response to insulin [19]. Furthermore, MyHC IIx fiber exerts a negative effect on glucose uptake [20]. To explore the mechanism underlying glucose homeostasis, we determined the protein levels of MyHC I, MyHC IIa, MyHC IIb, and MyHC IIx in soleus muscle. Under maternal HE diet treatment, the protein levels of type I fibers (MyHC I) and MyHC IIa were downregulated in the offspring, in contrast to up-regulation in the protein levels of type II fibers (MyHC IIb and MyHC IIx). Based on the results in previous and present studies, impairment of glucose tolerance in offspring under HE diet treatment may be attributed to the decreased protein levels of slow-twitch muscle fibers.

Mitochondrial function plays an important role in energy metabolism. Previous studies have shown that mitochondrial function and biogenesis are compromised in liver and muscle in type 2 diabetes, obesity, and insulin resistance [21–25]. PGC1 α is a transcription factor coactivator that improves mitochondrial biogenesis via activation of various transcription factors including NRF1 [26]. Type I muscle fibers have more mitochondria and higher oxidative metabolism than type II fibers [27]. Increased type I muscle fiber was observed in PGC-1 α overexpressing mice [28]. Consistent with the increase of type I muscle fiber, the protein levels of PGC-1 α and NRF1 were downregulated in the muscle and liver of the HE offspring. Furthermore, ATP content was also observed to decrease in the liver and muscle of the HE offspring. It has been reported that impaired mitochondrial biogenesis and function result in decreased ATP production [29]. The above findings indicate that maternal HE diet feeding impaired mitochondrial biogenesis in the liver and muscle of the offspring, which can be supported by previous studies reporting that maternal high-energy diet impaired mitochondrial biogenesis in the skeletal muscle of the offspring in pigs [30]. SDH and MDH are respiratory enzymes and exert an important role in mitochondrial function, and the decreased SDH and MDH activities in the HE group further support that maternal HE diet impairs mitochondrial biogenesis in the liver and muscle of the offspring.

In conclusion, maternal high-energy diet from middle to late pregnancy was shown to decrease the birth weight of piglet that from the gilts with total or alive litter size of 12 to 13. In addition, maternal high-

energy diet impairs the glucose tolerance in the neonatal offspring, with the mechanisms involved in the downregulation of slow-twitch muscle fibers in soleus muscle and the decrease in hepatic and muscular mitochondrial biogenesis. These results contribute to a better understanding of the role of maternal energy diet in the reproductive performance of Guangdong Small-ear Spotted pig, and glucose metabolism and muscle fiber transformation in offspring.

Abbreviations

ATP: adenosine triphosphate; AUC: area under the curve; GTT: glucose tolerance test; HOMA-IR: Homeostasis model assessment-insulin resistance; IRS1: insulin receptor substrate 1; MDH: malic dehydrogenase; mtDNA: mitochondrial DNA; NEFA: non-esterified fatty acid; NRF-1: nuclear respiratory factor-1; SDH: succinate dehydrogenase; PGC-1 α : peroxisome proliferator-activated receptor gamma coactivator.

Declarations

Authors' contributions

HCJ and TCQ designed the study; HCJ, HXY, WSQ and YLF acquired the data; HCJ, YYY, CMX, HXY, and WSQ carried out the experiments and data analysis; HCJ, TCQ, and ZSH drafted and revised the manuscript. All authors have read and approved the final version of the manuscript.

Availability of data and materials

The datasets are available from the corresponding author upon reasonable request.

Consent for publication

All authors approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval

The experimental design and procedure presented in this study are reviewed and approved by the Animal Care and Use Committee of the South China Agricultural University. All procedures performed in studies

involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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Figures

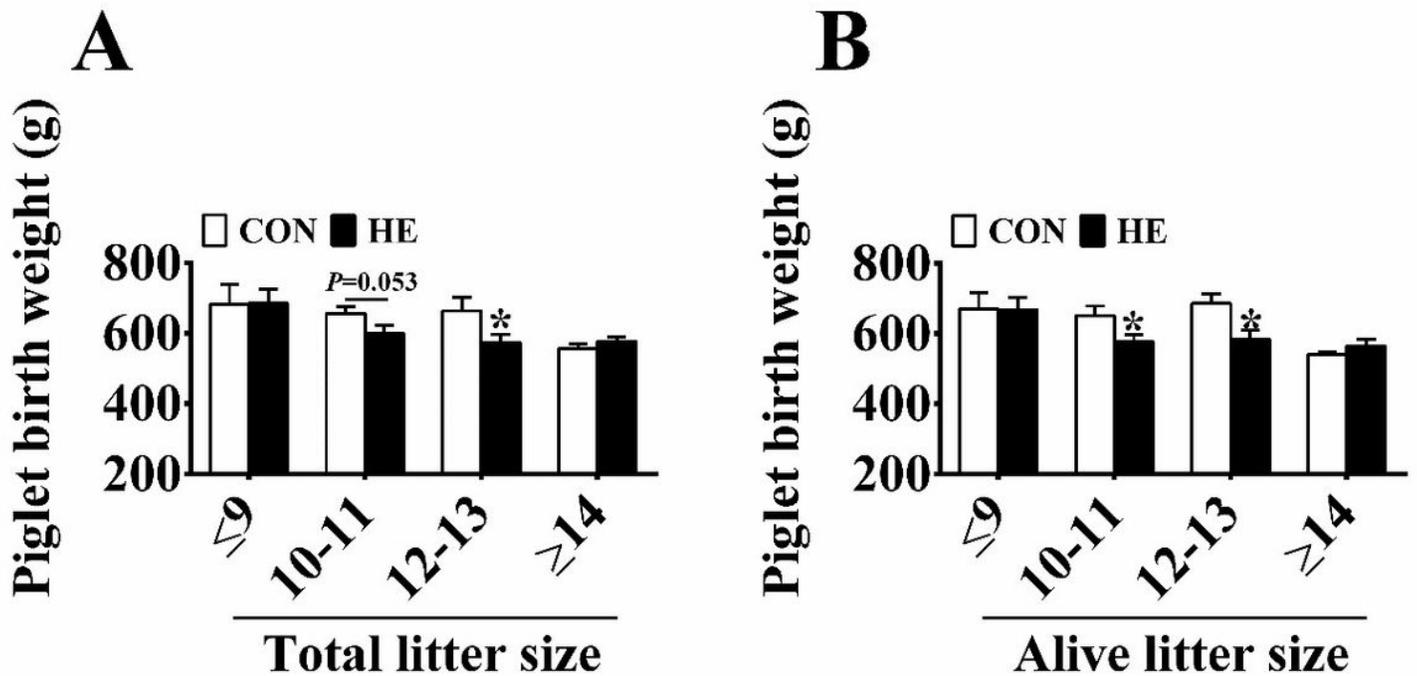


Figure 1

The birth weight of piglets of sows with different total (A) or alive (B) litter size. Values are mean \pm SEM. The difference between two means was analyzed using the Student's test. * indicates $P < 0.05$.

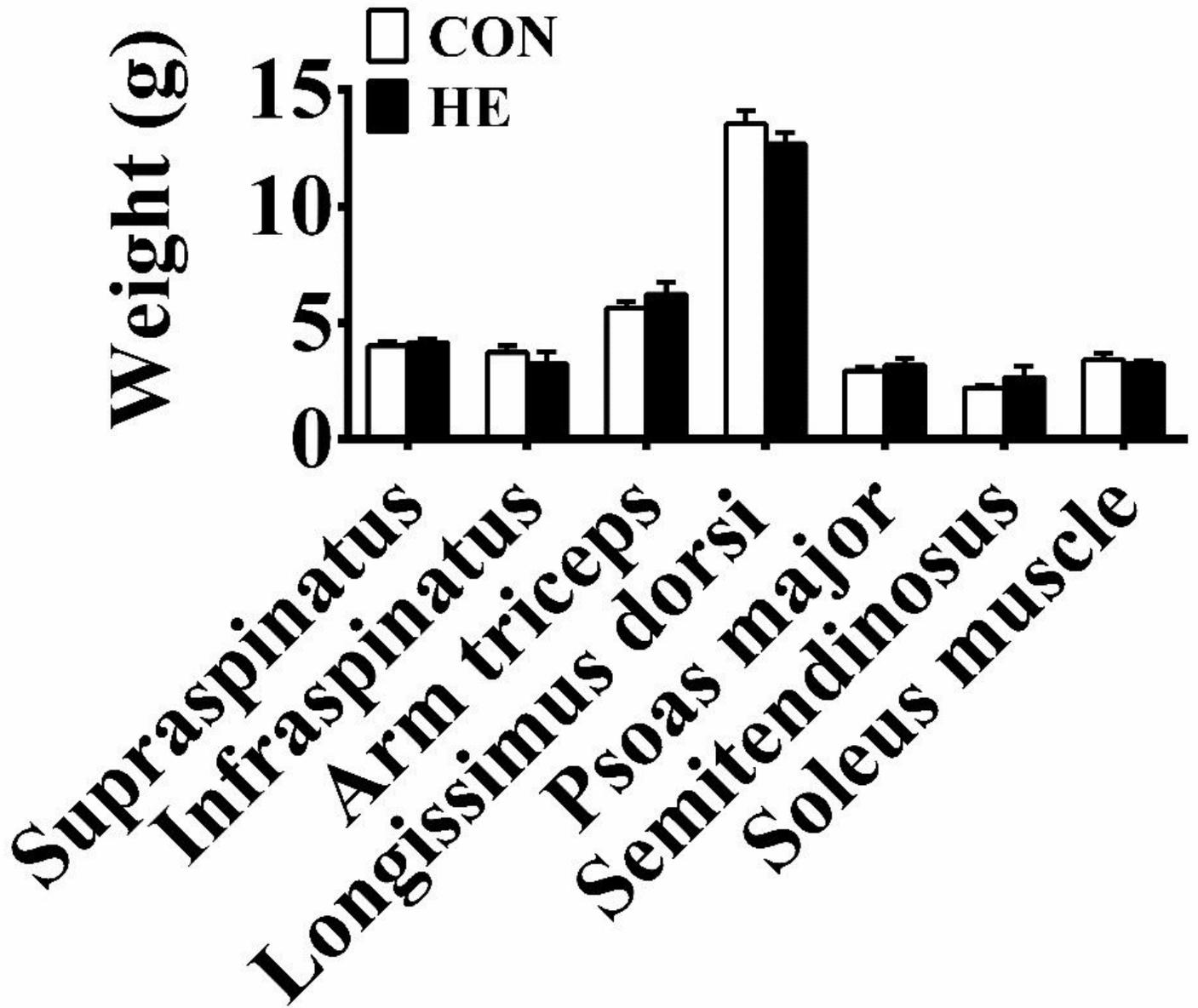


Figure 2

Effects of maternal dietary energy levels on muscle weight of newborn piglets. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test.

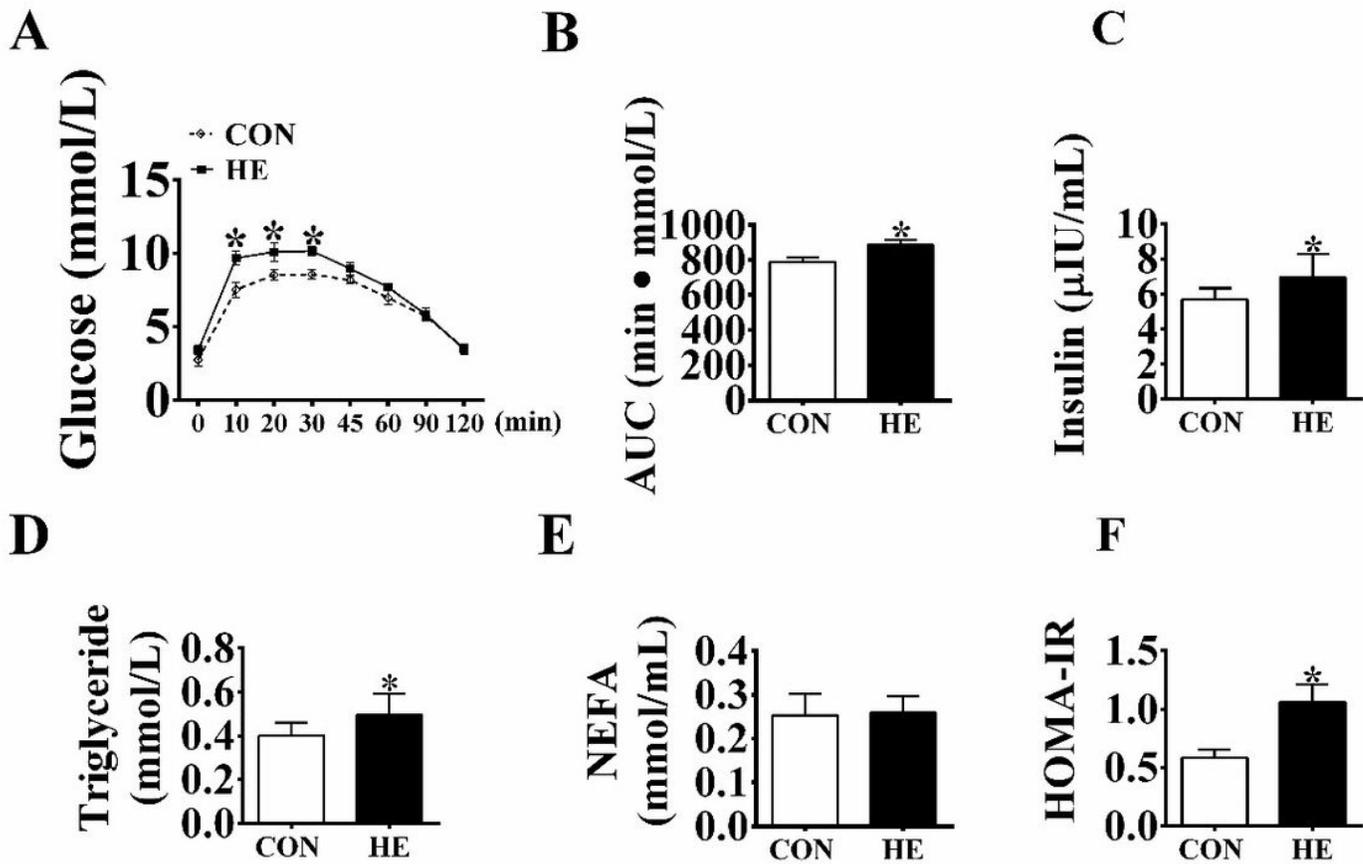
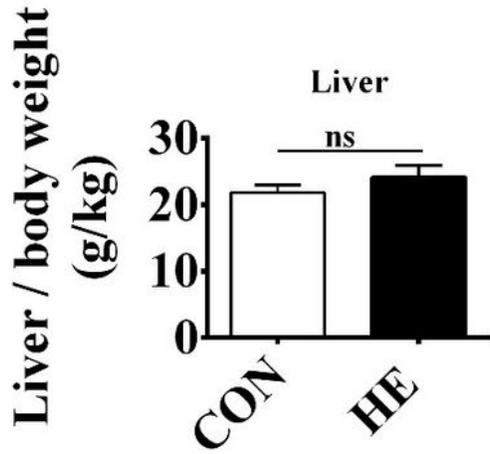
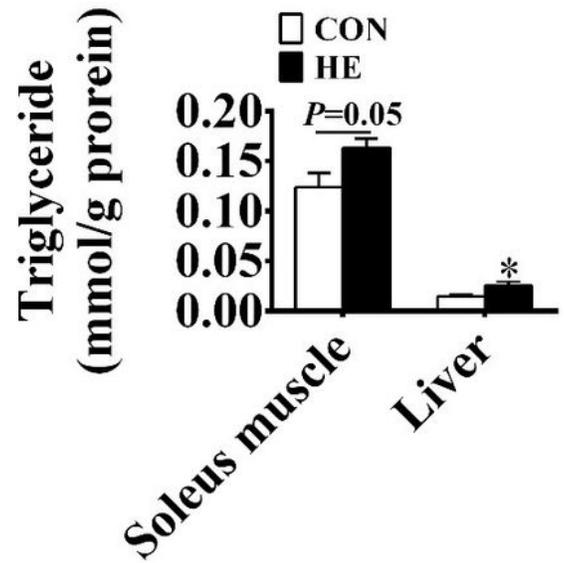
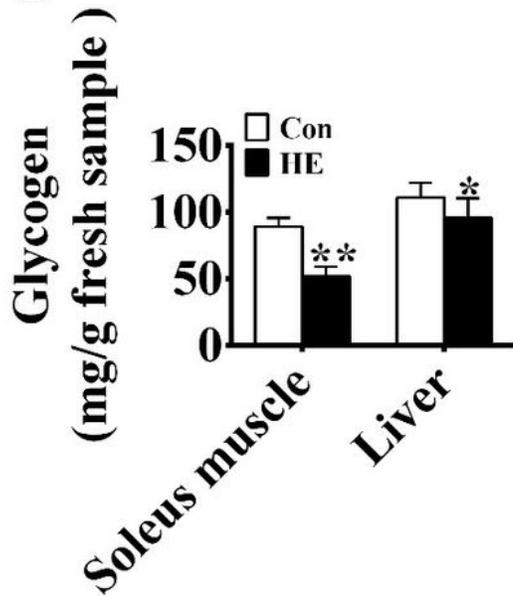
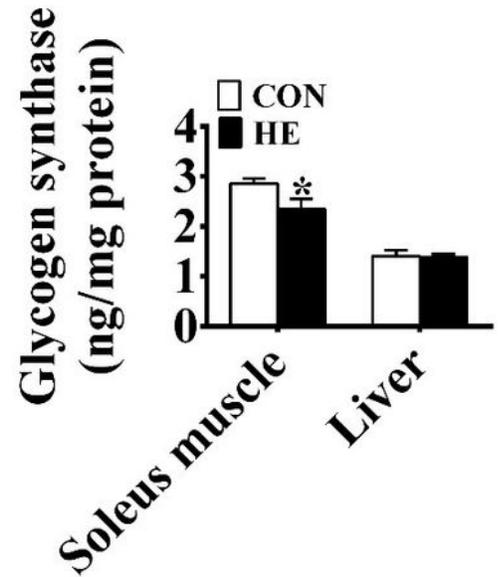


Figure 3

Effects of maternal dietary energy levels on glucose tolerance of newborn piglets. GTT curve (A) and AUC (B) of piglets after intraperitoneal injection with 1 mg/kg glucose. (C-E) Insulin, triglyceride, and NEFA levels in the serum of piglets. (F) Insulin resistance index (HOMA-IR), defined as fasting insulin ($\mu\text{IU/ml}$) \times fasting glucose (mmol/L)/22.5. Values are mean \pm SEM, $n=7-8/\text{group}$. The difference between two means was analyzed using the Student's test. * indicates $P < 0.05$.

A**B****C****D****Figure 4**

Effects of maternal dietary energy levels on TG accumulation and glycogen deposition in the muscle and liver of piglets. Liver index (A) of piglets. (B-D) The contents of triglyceride, glycogen, and glycogen synthase in the soleus muscle and liver of piglets. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test. * indicates $P < 0.05$; ** indicates $P < 0.01$; ns, no significant difference.

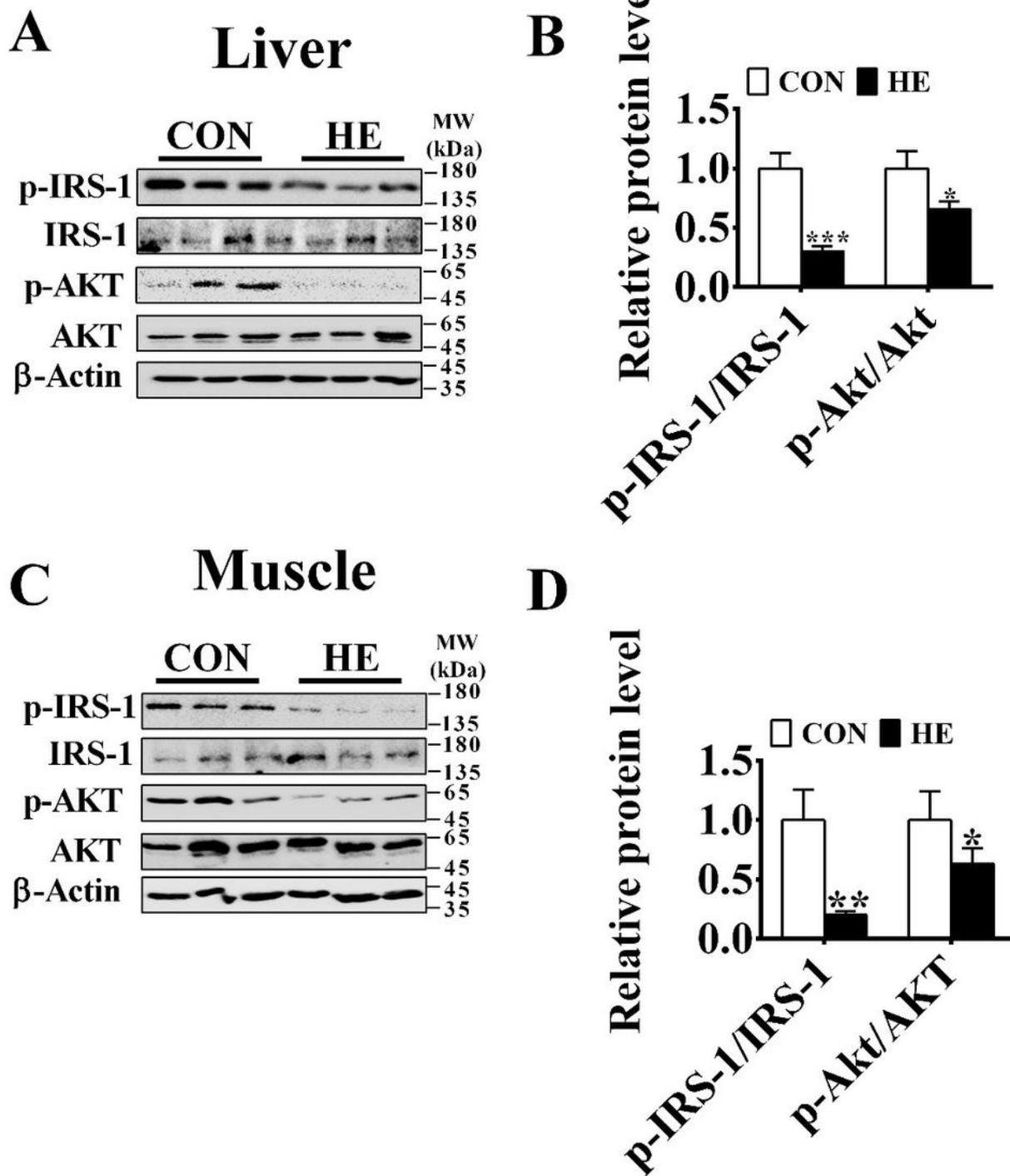


Figure 5

Effects of maternal dietary energy levels on the inulin signaling pathway activation in the muscle and liver of piglets. Western blotting analysis was used to determine the protein levels of p-IRS-1, IRS-1, p-AKT, and AKT in the liver (A) and soleus muscle (C) of piglets. (B, D) Summarized data. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test. * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.

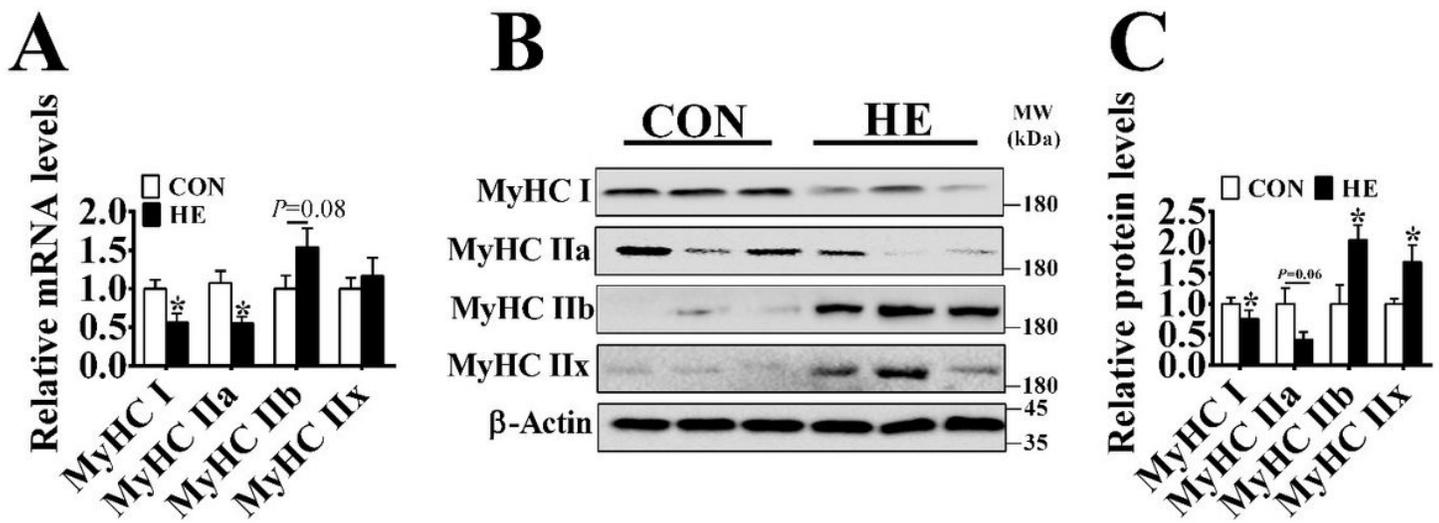


Figure 6

Effects of maternal dietary energy levels on the transition of skeletal muscle fiber types in piglets. qPCR and Western blotting analyses were employed to measure the mRNA (A) and protein (B, C) levels of MyHC I, IIa, IIb, and IIx in soleus muscle, respectively. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test. * indicates $P < 0.05$.

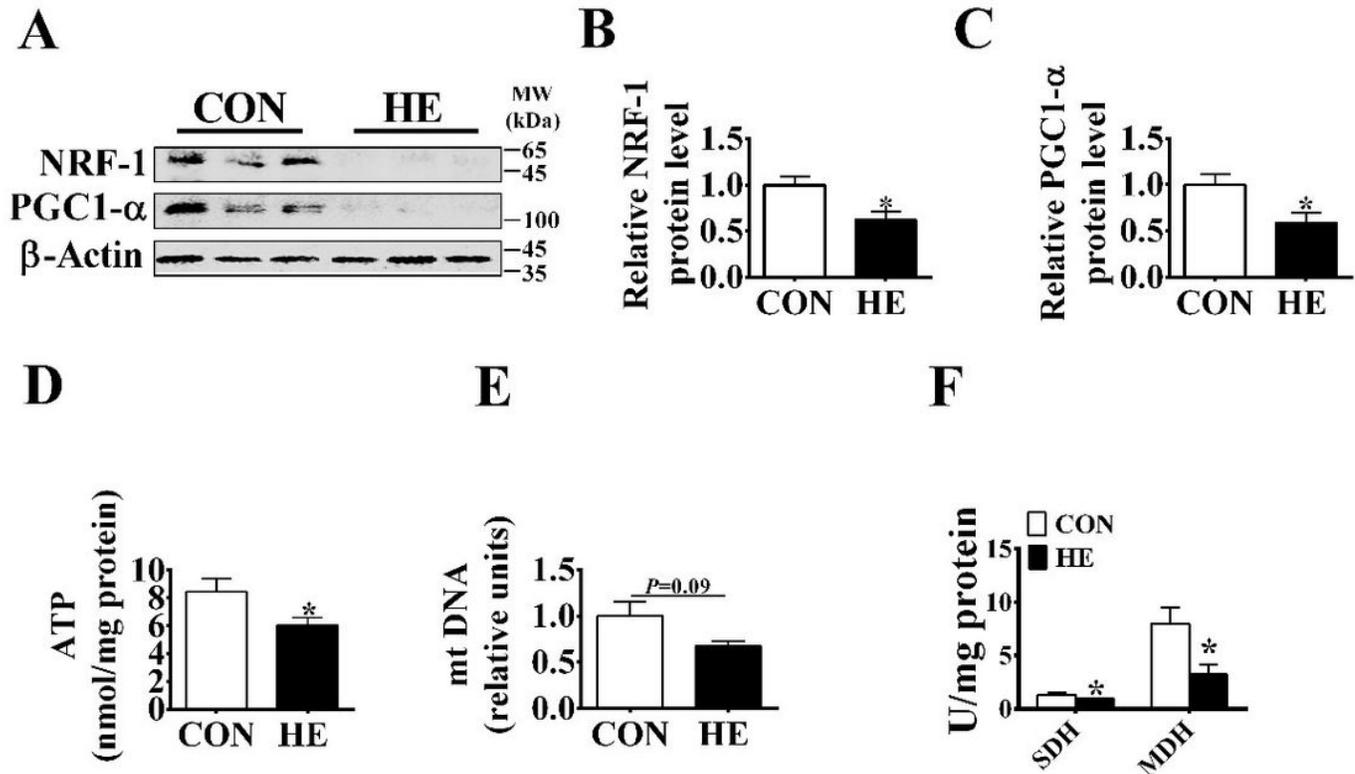


Figure 7

Effects of maternal dietary energy levels on the muscle mitochondrial biogenesis of piglets. Western blotting analysis was used to determine the protein levels of NRF-1 and PGC1- α in soleus muscle (A). The ATP level (B), mtDNA content (C), and activities of SDH and MDH (D) in soleus muscle. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test. * indicates P < 0.05.

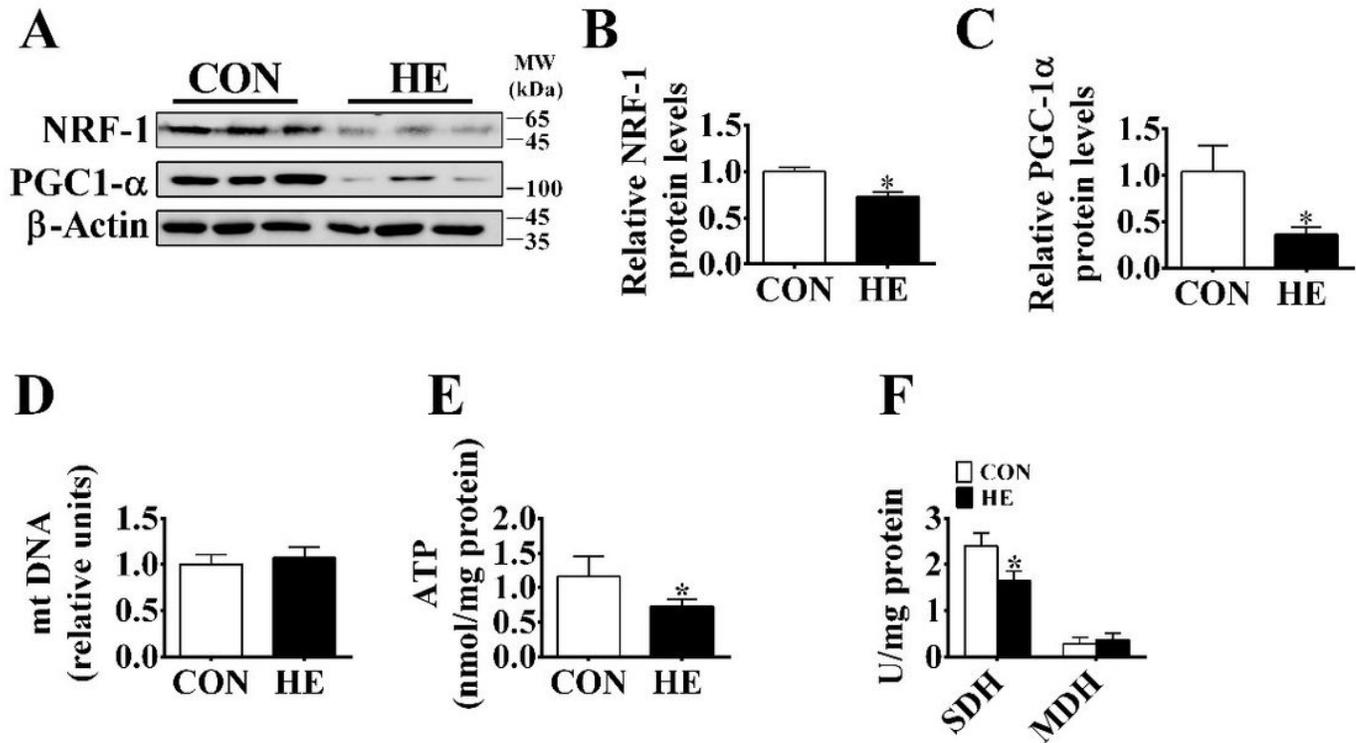


Figure 8

Effects of maternal dietary energy levels on the hepatic mitochondrial biogenesis of piglets. Western blotting analysis was used to determine the protein levels of NRF-1 and PGC1- α in liver (A). The ATP level (B), mtDNA content (C), and activities of SDH and MDH (D) in liver. Values are mean \pm SEM, n=7-8/group. The difference between two means was analyzed using the Student's test. * indicates P < 0.05.

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