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EFFECTS OF MATERNAL FOLIC ACID SUPPLEMENTATION AND INTRAUTERINE GROWTH RETARDATION ON EPIGENETIC MODIFICATION OF HEPATIC GENE EXPRESSION AND LIPID METABOLISM IN PIGLETS

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ABSTRACT

The present study was conducted to investigate the effect of maternal folic acid supplementation and intrauterine growth retardation (IUGR) on epigenetic modification of hepatic gene expression and lipid metabolism in weanling piglets. Thirty-two primiparous sows were randomly divided into two dietary groups: control group (C, 1.3 mg/kg folic acid) and folic acid supplemented group (FS, 30 mg/kg folic acid) during gestation. Blood and liver samples of piglets were collected at d 28 postnatal. Intrauterine growth retardation pigs had lower plasma triglyceride, liver triglyceride and total fat than that of normal birth weight (NBW) pigs. Although plasma nonesterified fatty acid (NEFA) was higher for NBW piglets, the birth weight effect was negated in IUGR piglets born to sows fed a FS diet. Hepatic fatty acid synthase (FAS) and Acyl-CoA oxidase (AOX) activities were lower and higher in IUGR piglets, respectively. Methylation status of peroxisomal proliferator-activated receptor-α (PPARα) promoter was lower in IUGR pigs from sows fed a control diet than other groups. Folic acid supplementation reduced the promoter methylation status of glucocorticoid receptor (GR). The mRNA expressions of PPARα, AOX, FAS, phosphoenolpyruvate carboxykinase (PEPCK), and leptin were differentially affected by birth weight. However, mRNA expression of PPARα and FAS in IUGR pigs from sows fed a FS diet was not different from that of NBW pigs. Expression of GR was higher in pigs from mothers fed a FS diet than their counterpart. The present study demonstrated that IUGR changed lipid metabolism and mRNA expression of genes related to this process, and maternal folic acid supplementation was an effective way to prevent the changes by regulating DNA methylation of promoter.

Key words*:* DNA methylation; folic acid; intrauterine growth retardation; lipid metabolism; maternal nutrition

INTRODUCTION

Intrauterine growth retardation (IUGR) is defined as impaired growth and development of the mammalian embryo/fetus or its organs during gestation (Wu *et al.,* 2004; Wang *et al.,* 2008). Pigs, as multifetal domestic animals, exhibit the most severe, naturally occurring IUGR due to the uteroplacental insufficiency (Wu *et al.,* 2004). A series of epidemiological and animal studies have demonstrated that IUGR is closely related to metabolic-related diseases such as diabetes, hypertension, insulin resistance, dyslipdemia and obesity (Barker *et al.,* 1993). Previous studies showed that lipid metabolic process was altered in IUGR offspring, which is caused by uteroplacental insufficiency or maternal undernutrition (Detmer *et al.,* 1992; Lane *et al.,* 2001; Qasem *et al.,* 2010). Interestingly, although the initial insult of the IUGR offspring occurs during pregnancy period, these IUGR animals are characterized by postnatal alterations in metabolic process (Gluckman *et al.,* 2008; Joss-Moore *et al.,* 2010; Neitzke *et al.,* 2011; Nissen and Oksbjerg, 2011). The persistent change in lipid metabolism implies that IUGR induces a relative stable alteration in gene mRNA transcriptional process by regulating DNA methylation (Davis and Uthus, 2004). In the rat, previous studies showed that IUGR leads to abnormal hepatic one-carbon metabolism, DNA methylation, and subsequent gene mRNA expression (MacLennan *et al.,* 2004; Lillycrop *et al.,* 2007).

Folic acid serves as coenzymes in a number of essential one-carbon transfer reactions, which is a key factor to regulate DNA methylation (Bailey and Gregory, 1999; Waterland and Michels, 2007). Furthermore, results from animal models showed that dietary folic acid supplementation during critical periods in early development prevents negative effects of IUGR on metabolic phenotype by regulating promoters DNA methylation status of some specific genes and subsequent mRNA expression abundance (Lillycrop *et al.,* 2005; Burdge *et al.,* 2008, 2009). However, it is noteworthy that there are some variances in gene expression regulation patterns in IUGR offspring from different experimental models, such as uteroplacental insufficiency by ligating uterine arteries or maternal undernutrition (Nüsken *et al.,* 2011). Thus, the results from rat models, especially maternal undernutrition induced IUGR, could not be applied to naturally occurring IUGR piglets.

Our group has demonstrated that maternal folic

acid supplementation was an effective way to improve hepatic one-carbon metabolism in newborn IUGR piglets (Liu *et al.,* 2011a). However, whether the changes in DNA methylation of promoters, mRNA expression abundance of genes, and subsequent lipid metabolic process were ameliorated by this nutritional intervention still warrants further investigations. The present study was to investigate the effects of maternal folic acid supplementation on lipid metabolism-related enzyme activities, hormone and metabolite concentrations, hepatic DNA methylation status and mRNA expression levels of target genes in IUGR piglets vs. NBW piglets.

MATERIALS AND METHODS

Animals and experimental design: Thirty-two Yorkshire primiparous sows at an average body weight of 120 kg were used in this study. All sows had similar body condition and were housed in individual feed stalls in a breeding facility. Oestrus detection was conducted in the morning and afternoon by backpressure testing with fence-line contact of a mature boar until the expected date of the third oestrus. The sows were artificially inseminated twice with fresh semen by the same well-trained person at 12 and 24 h after the observation of standing heat. All the used semen was from the same boar. All serviced sows were randomly divided into two groups, control (C, folic acid 1.3 mg/kg) and folic acid supplementation (FS, folic acid 30 mg/kg) group, 16 sows each. The sows from C and FS groups were fed a basal diet or a folic acid supplemented diet during gestation, respectively. During lactation, all the dams were fed a common diet *ad libitum*. The diet (Table 1) was formulated to meet or exceed the nutrient requirements of pregnant and lactation gilts (NRC, 1998). Pregnant sows were fed twice daily (0800 and 1400 h) at an allowance of 2.5 kg/d during gestation and had free access to water. After farrowing, the litter size was adjusted to 10 to 11 piglets per litter within 24 h and birth weight for each newborn piglet was recorded. IUGR and NBW piglets could be defined followed the criteria that as we described previously (Liu *et al.,* 2011b). Finally, sixteen male IUGR piglets and sixteen male NBW piglets from each dietary group were used. This produced 4 experimental groups (birth weight/maternal diet): NBW/C: normal birth weight pigs from sows fed a control diet, NBW/FS: normal birth weight pigs from sows fed a folic acid-supplemented diet, IUGR/C: intrauterine growth retarded pigs from sows fed a control diet, and IUGR/FS: intrauterine growth retarded pigs from sows fed a folic acid-supplemented diet. Routine farm feeding and management procedures were followed. Creep feed was not provided for the piglets.

Slaughtering and sample collection: Piglets were weaned and weighed at d 28 postnatal. Blood samples were taken from the jugular vein into tubes containing lithium heparin at the end of the experiment and all the thirty-two piglets were humanely sacrificed at the same age. Plasma was separated from cells by centrifugation and stored at -20°C for further analysis. The liver samples were taken within 5 min after sacrifice and rapidly frozen in liquid nitrogen before being stored at -80°C for further analysis.

Biochemical analysis: Plasma triglyceride, nonesterified fatty acid (NEFA), cholesterol, and glucose concentrations were measured using a Konelab 20 autoanalyser (Labmedics Limited, Salford Quays, Manchester, UK) as described previously (Burdge *et al*., 2006). Liver lipids were extracted using the chloroform/methanol (2:1) method (Folch *et al.,* 1957), and the extracts were taken to dryness under vacuum by a rotary evaporator. Triglyceride and cholesterol were measured by an enzymatic method using a flow analysis device (Technicon DAX-24; Bayer Diagnostic, Pittsburgh, PA) (Hercberg *et al.,* 2004). Leptin concentrations in plasma were measured with commercial RIA kits (Beijing North Institute of Biotechnology, Beijing, China). The detection limits was 0.45 ng/mL. The intra- and inter-assay coefficients of variations were 5% and 10%, respectively.

The activities of Acyl-CoA oxidase (AOX), fatty acid synthase (FAS), and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) in liver were determined in the present study. Weighed amounts of samples (approximately 2 g) were homogenized in ice-cold 0.25 M sucrose buffer containing 1 mM dithiothreitol, 1 mM EDTA and protease inhibitors. After centrifuged at 30,000 × *g* at 4°C for 60 min, the supernatant was obtained for enzyme activities assay. NADPH was used as oxidative substrate to assess the activity of FAS at 37°C by absorbance at 340 nm (Bazin and Ferré, 2001). The AOX assay was based on the determination of H_2O_2-dependent oxidation of leuko-dichlorofluorescein by absorption at 502 nm (Reubsaet *et al.,* 1988). The HMG-CoA reductase activities were measured as described previously with some modifications (Shapiro *et al.,* 1974). The incubation mixtures containing microsome and NADPH were incubated at 37°C for 15 min after [^{14}C]-HMG-CoA was added, and 6 mol/L of HCl was used to terminate the reaction. The ^{14}C radioactivity was measured by a liquid scintillation counter (Packard Tricarb 1600TR; Packard Instrument, Meriden, CT, USA).

DNA isolation and DNA methylation assay: To evaluate the promoter methylation status of PPARα, PPARγ, and GR, genomic DNA was extracted from liver tissue samples using a commercial Kit (TaKaRa, Dalian, China) according to the manufacturer's protocol. One microgram of purified DNA was used for bisulfate treatment using a Kit (QIAGEN, USA) according to the manufacturer's

protocol. The bisulfate-treated genomic DNA was then used as a template for the amplification of promoters of GR, PPARα, and PPARγ. Gene promoters were amplified by two nested primers, as shown in Table 2. The PCR products were purified, cloned into pMD 19-T Vector (TaKaRa, Dalian, China), and sequenced. Ten clones were sequenced for each sample. Levels of DNA methylation were determined by comparison with the sequence after bisulfited DNA sequencing PCR, and data of each piglet were normalized by the average value of that in NBW piglets from sows fed a control diet, therefore, the DNA methylation status was a relative value against that in NBW piglets from sows fed a control diet.

RNA extraction, reverse transcription and Real-Time PCR: Total RNA in the liver was extracted using Trizol Reagent (TaKaRa, Dalian, China). cDNA synthesis was performed using PrimeScript^{TM} reagent kit (TaKaRa, Dalian, China) according to manufacturer's instructions. Real-time quantitative PCR was performed in a CFX-96 Real-Time PCR detection System (Bio-Rad, USA).

The sequence of primers used is shown in Table 3. The RT-PCR protocol used in this study was described elsewhere (Liu *et al.,* 2013). Relative gene mRNA expression levels to the reference gene *β*-actin were performed in order to correct for the variance in amounts of RNA input in the reactions. The efficiency of PCR amplification of target genes and reference gene ranged from 95% to 105% in the present study. Therefore, the $2^{-\triangle\triangle Ct}$ method was used to analyze the results from real-time PCR as follows (Livak and Schmittgen, 2001).

$$\text{Ratio} = 2^{(Ct,\ \text{target} - Ct,\ \beta\text{-actin})\ \text{NBW/C} - (Ct,\ \text{target} - Ct,\ \beta\text{-actin})\ \text{sample}}$$

Statistical analyses: Data were analyzed by the Two-way ANOVA procedure of SAS (SAS Institute, Carry, NC, USA). Maternal diet, birth weight and their interactions were tested. Differences between treatment groups were compared using least significant difference. Results were presented as means with pooled SEM. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance, plasma and hepatic metabolites and enzyme activity: The birth weight and weanling weight of the IUGR piglets were significantly lower than that of NBW piglets ($P < 0.05$). Concentrations of plasma triglyceride and NEFA ($P < 0.05$) were significantly lower in IUGR piglets, but folic acid supplementation during pregnancy eliminated the difference in plasma NEFA between NBW and IUGR piglets. Total fat and triglycerides in the liver were significantly lower in IUGR piglets compared with NBW offspring ($P < 0.05$). The concentrations of plasma glucose, cholesterol, leptin, and hepatic cholesterol were not affected by birth weight or maternal diet ($P > 0.05$). The activities of hepatic FAS and AOX were significantly lower and higher in IUGR piglets, respectively. There is no effect of birth weight and folic acid supplementation on the activity of hepatic HMG-CoA reductase ($P > 0.05$, Table 4).

Gene promoter DNA methylation status: The methylation status of the PPARα promoter was lower in IUGR piglets from sows fed a control diet compared with NBW piglets ($P < 0.05$), but maternal folic acid supplementation eliminated the difference between birth weight groups ($P < 0.05$). PPARγ promoter methylation status did not differ across treatment groups ($P > 0.05$). In contrast, the methylation status of GR promoter was not influenced by birth weight and significantly reduced by folic acid supplementation during gestation period ($P < 0.05$, Table 5).

mRNA expression of target genes: Greater PPARα and PEPCK mRNA expressions were observed in the liver of IUGR piglets from sows fed a control diet during gestation compared with other groups ($P < 0.05$). Maternal folic acid supplementation increased the expression levels of GR ($P < 0.05$). In contrast, FAS expression was decreased in IUGR piglets compared with NBW piglets, whereas maternal folic acid addition retards this effect to some extent ($P < 0.05$). The mRNA expression abundance of AOX and leptin were significantly higher and lower in the liver of IUGR offspring, respectively ($P < 0.05$). There is no difference in hepatic PPARγ and HSL expression levels across treatment groups ($P > 0.05$, Table 5).

In the current study, results are similar with previous studies (Burdge *et al.*, 2008; Liang *et al.*, 2011). Although increasing dietary folic acid level offered during pregnancy prevented the negative effects of IUGR on gene promoter methylation and subsequent mRNA expression levels, hepatic levels of lipid metabolite and enzymes activities were not affected by our nutritional strategy. The variance between the present investigation and previous studies from maternal protein restriction rat model suggests that maternal folic acid supplementation modify the lipid metabolic process of IUGR offspring depends on the cause of IUGR (Burdge *et al.*, 2008, 2009).

The growth performance of NBW and IUGR piglets during lactation was not influenced by maternal folic acid supplementation, which is different with the results from rat's model (Burdge *et al.*, 2008). Previous study showed that growth performance of IUGR offspring from protein restricted dams was depressed by maternal folic acid supplementation during pregnancy (Burdge *et al.*, 2008). Although the addition of folic acid to maternal diet normalized the concentration of plasma NEFA in the IUGR piglets, the concentrations of glucose, triglyceride, and cholesterol in the blood and liver of the offspring were not affected by maternal folic acid

supplementation in this study. Our results differ from a previous study, which reported that folic acid supplementation during pregnancy altered plasma and hepatic concentrations of lipid metabolite and glucose in the progeny of protein restricted dams (Burdge *et al.*, 2008). The difference in results may be caused by the methods used to induce IUGR. Leptin is an important factor in the regulation of lipid metabolism (Ramsay 2003, 2005). Consistent with previous research in pigs (Poore and Fowden, 2004), serum leptin concentration was not affected by birth weight or folic acid supplementation during pregnancy in the present experiment.

In agreement with the results from maternal protein restriction rat models (Burdge *et al.*, 2009), IUGR reduced the concentrations of triglyceride in plasma and liver of the weanling piglet suggesting that the lipid metabolism process was affected by birth weight. Fatty acid synthase is a key lipogenic enzyme that catalyzes the conversion of acetyl-CoA and malonyl-CoA to palmitic acid. AOX regulates the utilization of acetyl-CoA for energy expenditure. The lower activity of FAS and greater activity of AOX were observed in the liver of IUGR piglets, which suggest the reduced capacity for deposition of fatty acid in liver. Furthermore, previous study also found that the mRNA expression levels of FAS were downregulated in the IUGR weanling piglets, induced by maternal protein restriction (Liang *et al.*, 2011). It is noteworthy that changed activities of hepatic FAS and AOX could partially explain the decreased total fat and triglyceride content in the liver of IUGR offspring. The association between lipid metabolism-related enzyme activities and concentrations of metabolite may provide a possible mechanism responsible for the effect of IUGR on energy metabolic status of the offspring.

One potential mechanism causing long-term changes in gene expression is abnormal DNA methylation modification, and DNA methylation patterns are largely established in utero (Davis and Uthus, 2004). GR and PPAR play a central role in maintaining lipid homeostasis (Desvergne and Wahli, 1999; Yang and Zhang, 2004). The observation that maternal folic acid supplementation prevented the changes in the methylation status of PPARα promoter in the liver of IUGR offspring is consistent with a previous report (Lillycrop *et al.*, 2005). Although the methylation status of PPARγ promoter was not affected by birth weight and folic acid supplementation, lower methylation status of hepatic GR promoter in the offspring from mothers fed a folic acid supplemented diet were observed in the present study.

Table 1. Composition of experimental diets (as-fed basis)

Item	Pregnancy period		Lactation period
	Control diet	Folic acid supplemented diet	
Ingredient, %			
Corn	76.3.	76.3	66.3
Soybean meal	17.0	17.0	23.0
Soybean oil	1.0	1.0	3.0
Fish meal	2.0	2.0	4.0
Sodium chloride	0.4	0.4	0.4
Calcium carbonate	0.9	0.9	0.9
Dicalcium phosphate	1.7	1.7	1.7
Choline chloride	0.1	0.1	0.1
Vitamin and mineral premix [a]	0.5	0.5	0.5
Folic acid premix	0.1 [b]	0.1 [c]	0.1 [b]
Total	100.0	100.0	100.0
Calculated nutrient content			
Digestible energy (MJ/kg)	14.49	14.49	14.97
Crude protein (%)	15.73	15.73	18.94
Lysine (%)	0.81	0.81	1.05
Ca (%)	0.95	0.95	1.08
P (%)	0.71	0.71	0.79

[a] Provided the following amount per kilogram of diet：Cu: 30 mg; Fe: 160 mg; Mn: 30 mg; Zn: 160 mg; I: 0.6 mg; Se: 0.5 mg; vitamin A: 12800 IU; vitamin D_3: 2600 IU; vitamin E: 44 IU; menadione: 4 mg; thiamin: 2.4 mg; riboflavin: 8.8 mg; pyridoxine: 3.2 mg; vitamin B_{12}: 0.028 mg; niacin: 32 mg; pantothenic acid: 4 mg; biotin: 0.5 mg

[b] Provided the following amount per kilogram of diet： folic acid: 1.3 mg

[c] Provided the following amount per kilogram of diet： folic acid: 30 mg

Table 2. Primers sequence used for gene promoter amplification

Gene	Primer (5'-3')		Product size (bp)	Reannealing temperature, ^{o}C
GR	GR-BF(571-593)	TTGGGATAGGGTTATTTGTAATG	328	53.8
	GR-BR1(954-976)	TATAAACAACCTCAACAACCAAA		
	GR-BR2(878-898)	AATCCCATCCAAAAAAAAAAA		
PPARα	PPARα-BF1(238-261)	GGATTGGTGTTAGGTTAAGGTTAT	210	55.1
	PPARα-BR1(759-780)	CCAAAAACAAAAACAAAAAACA		
	PPARα-BF2(352-371)	TAGGGTTGTTTTGATAGGGG		57
	PPARα-BR2(541-561)	AACACATCAAAAAAAACACCA		
PPARγ	PPARγ-BF1(74-96)	GAAGATTAGAGGTTTTTTGGTTG	439	55.1
	PPARγ-BF2(83-104)	AGGTTTTTTGGTTGGGTATTTA		
	PPARγ-BR(499-521)	AATTTTTTTTTTCCCAAACTCTT		57

GR - glucocorticoid receptor; PPAR - peroxisomal proliferator-activated receptor

Table 3. Primers sequence used for target genes amplification

Gene	Primer (5'-3')	Gene Bank Accession no.	Product size (bp)
PPARα	F: GACACATTGAGAGAATGCAGGAG	NM001044526	99
	R: GCAGGAGTTTGGGGAAGAGA		
PPARγ	F: AGGACTACCAAAGTGCCATCAAA	AF059245	142
	R: GAGGCTTTATCCCCACAGACAC		
GR	F: ACGATAACTTGACTTCCTTGG	AF141371	250
	R: AGATAATTGTGCTGTCCTTCC		
AOX	F: AGGCAGCGTGGAACCTAACTT	DQ842227	156
	R: ACAGAGCATAGAGGAGACACAGG		
HSL	R: ACCCTCGGCTGTCAACTTCTT	AY686758	84
	R: TCCTCCTTGGTGCTAATCTCGT		
FAS	F: GTCCT GCTGA AGCCT AACTC	EF589048	206
	R: TCCTTGGAACCGTCTGTG		
PEPCK	F: AGCTCTTCCAGGTCTCCAAGG	NM_001123158	138
	R: TCTGGCTGATTCTCTGCTTCAG		
Leptin	F: CCCTCATCAAGACGATTGTCA	AF102856	213
	R: GGTTCTCCAGGTCATTCGATA		
β-actin	F: TCTGGCACCACACCTTCT	DQ178122	114
	R: TGATCTGGGTCATCTTCTCAC		

GR - glucocorticoid receptor; PPAR - peroxisomal proliferator-activated receptor; FAS - fatty acid synthase; PEPCK - phosphoenolpyruvate carboxykinase; AOX - Acyl-CoA oxidase; HSL - hormone-sensitive lipase; F - forward primer; R - reverse primer

Hepatic lipid metabolic pathways are regulated by expression of several genes. The mRNA expression of PPARα and GR was changed correspondingly according to their promoter methylation. This is consistent with previous studies on PPARα and GR expression in rat model (Bertram *et al.,* 2001; Lillycrop *et al.,* 2005). Increased PPARα activity induces enhanced AOX expression (Tugwood *et al.,* 1992). However, the expression patterns of AOX and PPARα were not changed consistently as previously described (Lillycrop *et al.,* 2005). One possible interpretation is that the increase in the level of AOX mRNA is not only controlled by PPARα, but also by other transcriptional factors. It has been reported that leptin mRNA was downregulated in the offspring from maternal malnutrition in different IUGR models (Xu *et al.,* 2010; Liang *et al.,* 2011). The decreased expression levels of leptin mRNA in the liver of IUGR offspring is consistent with previous studies, which suggested that the nutrient intake of the fetal during gestation has pronounced effects on the expression abundance of leptin. In agreement with the results of a previous study (Morise *et al.,* 2009), mRNA expression abundance of FAS was reduced in the IUGR offspring. However, maternal folic acid addition improved the expression levels of this gene in IUGR pigs in the present study. The lower mRNA expression of FAS concurred with decreased activity of this enzyme in IUGR piglets means that IUGR modulates lipid metabolism by transcriptional regulation. Consistent with a previous report (Burdge *et al.,* 2009), dietary folic acid

supplementation reversed the mRNA expression of PEPCK in IUGR offspring, which might be caused by modulating DNA methylation status of PEPCK promoter. Although maternal folic acid supplementation regulated promoter methylation status of PPARα and GR, and mRNA expression abundance of genes involved in lipids metabolism, few effects of this nutritional intervention on enzyme activities and metabolite concentrations were observed in the current study. One possible explanation is that the enzyme activities and metabolite content related to lipid metabolism were affected by multiple molecular pathways. Maternal folic acid supplementation prevents the detrimental effects of IUGR on mRNA expression of some genes involved in this process by regulating gene promoter methylation. However, IUGR could also influence the enzyme activities and metabolite concentrations through other molecular pathways, thus possibly counteracting the effects of folic acid supplementation.

Table 4. Effects of folic acid supplementation during pregnancy and birth weight on piglet growth performance, plasma and hepatic metabolites and enzyme activity in piglets at 28 days of age

	NBW		IUGR			*P*		
Item	**C**	**FS**	**C**	**FS**	**SEM**	**Birth weight**	**Diet**	**Interaction**
Growth performance								
Birth weight (kg)	1.53^{a}	1.52^{a}	0.94^{b}	0.95^{b}	0.04	<0.01	1.000	0.796
Weaning weight (kg)	8.47^{a}	8.34^{a}	6.93^{b}	6.55^{b}	0.23	<0.01	0.315	0.620
Metabolite								
Plasma glucose (mmol/L)	6.12	5.96	5.78	6.29	0.25	0.984	0.488	0.189
Plasma triglyceride (mmol/L)	0.40^{a}	0.41^{a}	0.31^{b}	0.30^{b}	0.01	<0.01	1.000	0.417
Plasma cholesterol (mmol/L)	2.14	2.43	2.20	2.39	0.13	0.940	0.081	0.709
Plasma NEFA (μmol/L)	274.43^{a}	283.14^{a}	202.18^{b}	241.48^{ab}	14.67	<0.01	0.112	0.307
Liver total fat (%)	7.90^{a}	8.11^{a}	6.37^{b}	6.42^{b}	0.32	<0.01	0.836	0.755
Liver triglyceride (mmol/g)	0.31^{a}	0.32^{a}	0.23^{b}	0.22^{b}	0.01	<0.01	0.983	0.562
Liver cholesterol (μmol/g)	3.25	2.83	3.17	3.05	0.26	0.364	0.472	0.551
Metabolic hormones								
Leptin (ng/mL)	0.14	0.16	0.14	0.15	0.01	0.385	<0.05	0.385
Liver enzymes activities (U/mg protein)								
Fatty acid synthase	30.67^{a}	28.76^{a}	23.17^{b}	24.33^{b}	0.73	<0.01	0.596	<0.05
Acyl-CoA oxidase	4.59^{a}	4.34^{a}	5.80^{b}	5.73^{b}	0.21	<0.01	0.453	0.671
HMG-CoA reductase	5.12	4.89	5.53	5.28	0.28	0.157	0.390	0.971

Means within the same row with different letters differ significantly ($P < 0.05$); IUGR - intrauterine growth retardation; NBW - normal birth weight; C – control diet; FS – folic acid supplemented diet; NEFA - nonesterified fatty acid; HMG-CoA reductase – 3-hydroxy-3-methyl-glutaryl-CoA reductase

Table 5. Effects of maternal folic acid supplementation and birth weight on hepatic DNA methylation status of gene promoter and mRNA expression of genes related to lipids metabolism

	NBW		IUGR			*P*		
Item	**C**	**FS**	**C**	**FS**	**SEM**	**Birth weight**	**Diet**	**Interaction**
DNA methylation								
GR	1.00^{a}	0.52^{b}	0.93^{a}	0.46^{b}	0.08	0.437	<0.01	0.952
PPARα	1.00^{a}	1.13^{a}	0.62^{b}	1.06^{a}	0.10	<0.05	<0.01	0.142
PPARγ	1.00	0.95	1.14	1.03	0.07	0.272	0.422	0.762
mRNA expression								
GR	1.00^{b}	1.63^{a}	0.95^{b}	1.57^{a}	0.11	0.608	<0.01	0.962
PPARα	1.00^{b}	1.09^{b}	2.14^{a}	0.94^{b}	0.14	<0.01	<0.01	<0.01
PPARγ	1.00	0.94	1.08	1.03	0.10	0.424	0.601	0.953
AOX	1.00^{a}	1.12^{a}	3.16^{b}	2.92^{b}	0.23	<0.01	0.795	0.439
HSL	1.00	0.94	1.13	1.10	0.08	0.477	0.879	0.681
FAS	1.00^{a}	0.97^{a}	0.27^{c}	0.53^{b}	0.06	<0.01	0.080	<0.05
PEPCK	1.00^{a}	1.09^{a}	2.34^{c}	1.67^{b}	0.11	<0.01	<0.05	<0.01
Leptin	1.00^{a}	1.14^{a}	0.42^{b}	0.53^{b}	0.05	<0.01	<0.05	0.782

Different small letters denote statistical differences between groups ($P < 0.05$); IUGR - intrauterine growth retardation; NBW - normal birth weight; C – control diet; FS – folic acid supplemented diet; GR - glucocorticoid receptor; PPAR - peroxisomal proliferator-activated receptor; FAS - fatty acid synthase; PEPCK - phosphoenolpyruvate carboxykinase; AOX - Acyl-CoA oxidase; HSL - hormone-sensitive lipase

Conclusions: Overall, the present findings showed that IUGR altered most aspects of lipid metabolism and associated gene mRNA expression levels. However, the mRNA expression levels of some lipid metabolic-related genes, such as PPARα, FAS and PEPCK, in IUGR pigs were normalized to that of NBW offspring through regulating gene promoter methylation by folic acid supplementation during pregnancy. The observations are different with a previous study in the rats to some extent (Burdge *et al.*, 2008), which supported that fetal programming of gene expression in IUGR offspring depends on the cause of low birth weight (Nüsken *et al.*, 2011). Our findings suggest this hypothesis could be extended to include lipid metabolic changes in the offspring induced by maternal folic acid supplementation.

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