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Effect of folic acid supplementation on hepatic antioxidant function and mitochondrial-related gene expression in weanling intrauterine growth retarded piglets

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ABSTRACT

The aim of the present study was to investigate the effect of dietary folic acid supplementation on antioxidant function and mRNA expression levels of genes involved in mitochondrial biogenesis and function in the liver of piglets affected by intrauterine growth retardation (IUGR). Sixteen piglets at normal birth weight and 16 IUGR piglets were fed either a control diet (C) or a folic acid-supplemented diet (FS, C+5 mg/kg folic acid) from 14 d of age to 35 d of age postnatal. Blood and liver samples were collected at the end of the study. The results showed that body weight was lower for IUGR piglets than that of NBW piglets at 14 d of age and 35 d of age postnatal ($P<0.05$). Dietary folic acid supplementation increased plasma folic acid concentrations and hepatic GPx activity, but decreased plasma homocysteine levels ($P<0.05$). Reduced activities of Mn-SOD and T-AOC, mitochondrial DNA (mtDNA) content, and ATP concentration ($P<0.05$) were observed in the liver of IUGR pigs fed a control diet. However, the changes in mtDNA content and activities of Mn-SOD and T-AOC were negated in IUGR pigs fed a FS diet ($P<0.05$). Also, dietary folic acid supplementation decreased protein carbonyls and MDA concentration in the liver of IUGR piglets ($P<0.05$). Expression levels of genes encoding for PPARγ coactivator-1α (PGC-1α), mammalian silencing information regulator-2α (SIRT-1), nuclear respiratory factor-1 (NRF-1), mt transcription factor A (TFAM), mt single-strand DNA-binding protein (mt SSB), mt polymerase r (mt polr), glucokinase, citrate synthase (CS), ATP synthase (ATPS), and cytochrome c oxidase (CcOX) subunit I and V were decreased in IUGR piglets compared with NBW piglets. However, mRNA levels of PGC-1α, SIRT-1, NRF-1, TFAM, and mt polr in the liver of IUGR pigs fed FS diet were not different from that of NBW pigs. Gene mRNA expression abundance of CcOX IV was enhanced by folic acid supplementation ($P<0.05$) regardless of birth weight. The present study indicates that IUGR impaired hepatic antioxidant function and mRNA expression levels of genes are involved in mitochondrial biogenesis and function. Dietary folic acid supplementation prevented the harmful effect of IUGR on hepatic antioxidant function and mtDNA biogenesis, but had no effect on mRNA expression levels of genes involved in mitochondrial function.

1. Introduction

The concept of "fetal programming" suggested that the inadequate uptake of nutrients during intrauterine development leads to abnormal growth of the fetus and causes

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long-term influence on the offspring (Barker, 2000; Gluckman et al., 2008). Intrauterine growth retardation (IUGR), characterized by impaired development of the fetus during pregnancy, is a major threat in animal production because of the harmful effect on postnatal growth performance and health status, which could be due to the disturbed growth of the fetus (Wu et al., 2006). There are abundant evidences that IUGR confers permanent stunting effect on postnatal growth, morbid physiological functions, and impaired energy homeostasis (He et al., 2011; Wu et al., 2004). Mitochondria are central to regulating energy metabolism and modulating reactive oxygen species (ROS) production. Mitochondrial dysfunction was observed in IUGR offspring evidenced by decreased ATP synthesis, reduced activity of antioxidant enzyme, increased production of ROS, and impaired expression levels of mitochondrial DNA-encoded genes responsible for oxidative phosphorylation and electron transport chain process (Lee and Wei, 2005; Ogata et al., 1990; Park et al., 2003; Simmons et al., 2005). Previous studies demonstrated that IUGR led to mitochondrial oxidative phosphorylation dysfunction in liver and skeletal muscle of rat offspring (Peterside et al., 2003; Selak et al., 2003). Proteome analysis also revealed that IUGR altered expression levels of proteins involved in mitochondrial energy metabolism (Sparre et al., 2003; Wang et al., 2008). Furthermore, IUGR changed gene expression patterns in fetal tissue and 10% of these altered genes were involved in mitochondrial protein synthesis (Reusens et al., 2008).

It is well-recognized that dietary folic acid administration plays a central role in regulating mitochondrial biogenesis and function (Chang et al., 2007; Chou et al., 2007). Folic acid was involved in one-carbon metabolism and DNA replication and repair (Anguera et al., 2006; Bailey and Gregory, 1999; Shane, 2001). Folic acid exhibited antioxidant function through decreased pro-oxidant homocysteine level (Huang et al., 2001). In addition, folic acid supplementation could ameliorate some of the harmful effects that cancer chemotherapy confers upon the mtDNA deletions and mtDNA content in the liver of rat (Branda et al., 2002). Furthermore, dietary folic acid deficiency altered mtDNA content, deletions and biogenesis in different tissues of young rats and changed mRNA levels of genes related to mitochondrial functions (Chou et al., 2007).

Therefore, we hypothesized that mitochondrial dysfunction may provide a possible link between IUGR and impaired antioxidant function or abnormal energy metabolism. The aim of the present study was to test whether dietary folic acid supplementation during early life could alleviate the negative effects that IUGR confers upon hepatic mitochondrial functions, mtDNA biogenesis, and subsequent antioxidant function in the pig offspring.

2. Materials and methods

The experimental protocols used were approved by the Animal Care Advisory Committee of the Sichuan Agricultural University.

2.1. Animals, experimental design and sample collection

Landrace × Yorkshire sows used in the present study were of the same parity. The sows were fed the same diet during gestation period as suggested by National Research Council (1998). All sows were artificially inseminated with the semen pooled from three Duroc boars. After natural farrowing, birth weight of each newborn piglet was carefully recorded. The definition of IUGR followed the criteria that birth weight of IUGR pig was 2SD below the average birth weight of all piglets in the same litter. When one IUGR piglet was selected from a litter, the other NBW piglet (weight >1.4 kg) was also chosen from the same litter. One sow provided one IUGR piglet and one NBW piglet. The lactating sows were fed the same diet (National Research Council, 1998) throughout the lactation period. Sixteen IUGR (average birth weight was 0.98 kg) and 16 NBW (average birth weight was 1.44 kg) male DLY piglets on 14 d of age were selected. Both IUGR and NBW piglets were fed a control (C, no folic acid supplementation) diet or folic acid supplemented diets (FS, 5 mg/kg folic acid). Thus, a 2×2 factorial experimental design was used and each group (NBW/C, NBW/FS, IUGR/C, IUGR/FS) had 8 piglets. The piglets were individual-housed and pens were assigned randomly. The animals were given free access to both water and feed. The time of the experiment was from 14–35 d postnatal. All the pigs were fasted 8 h before the collection of blood samples and killed by electrical stunning and exsanguinations at the end of the experiment. Blood samples were collected by venipuncture and EDTA-plasma samples were stored at -20 °C for further analyses. The liver samples were taken within 5 min after sacrifice and snap-frozen in liquid nitrogen, then stored at -80 °C until further analysis.

2.2. Folic acid and homocysteine assay

Folic acid concentrations in plasma were measured with commercial SimulTRAC-SNB Radioassay Kit [^{57}Co] Vitamin B_{12}/[^{125}I] Folate (MP Biomedicals, Diagnostics Division, USA). Plasma homocysteine concentration was determined by HPLC using the kit supplied by Alpco Diagnostics (USA).

2.3. Analysis of mtDNA content

The relative mtDNA content was measured by coamplifying the mt D-loop and the nuclear-encoded β-actin gene using real-time PCR assay. Total DNA was extracted from the liver of each piglet using a DNAiso Reagent (TaKaRa, China). The amount of mt D-loop and β-actin gene was quantified by fluorescent probes. The mt D-loop (Accession number: AF276923) was amplified using a set of primers: forward 5′-GATCGTACATAGCACATATCATGTC-3′, reverse 5′-GGTCCTGAAGTAAGAACCAGATG-3′, yielding a 198-bp product. The β-actin (Accession number: DQ452569) was amplified using a set of primers: forward 5′-CCCCTCCTCTCTTGCCTCTC-3′, reverse 5′-AAAAGTCCTAGGAAAATGGCAGAAG-3′, yielding a 74-bp product. The fluorescent probes of mt D-loop and β-actin gene were 5′-(FAM)

CCAGTCAACATGCGTATCACCACCA (Eclipse)-3′ and 5′-(FAM) TGCCACGCCCTTTCTCACTTGTTCT (Eclipse)-3′, respectively. PCR amplification was carried out in a 20-μl reaction volume consisting of 8 μl TaqMan Universal Master mix, 1 μl enhance solution, 1 μl each of forward and reverse primers, 1 μl probe, 7 μl ddH_2O and 1 μl DNA. The cycling conditions were as follows, 95 °C for 10 s, 50 cycles involving a combination of 95 °C for 5 s and 60 °C for 25 s, and 95 °C for 10 s. Each sample was amplified in triplicate. The fluorescence spectra were monitored by CFX-96 Real-Time PCR detection System (Bio-Rad, USA). The ratio of mtDNA to genomic DNA content was calculated with ΔCt (mt $\Delta Ct_{D\text{-}loop}$–nuclear $Ct_{\beta\text{-}actin}$). The relative expression (RE) indicates the factorial difference in mtDNA content between each group. RE was calculated as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = \Delta Ct_{\text{mtDNA content in other group}} - \Delta Ct_{\text{mtDNA content in the control}}$.

Table 1
Composition of the diets and nutrient content.

Ingredient composition (%)		Nutrition composition	
Corn	39.5	DE/(MJ/kg)	14.9
Puffing soybean	9.0	Crude protein (%)	21.9
Decupled soybean meal	2.0	Total lys (%)	1.7
Soybean protein concentrate	6.5	Total methionine (%)	0.6
Animal fat	1.0	Methionine+cysline (%)	1.0
Sucrose	2.0	Ca (%)	0.9
Glucose	3.0	P (%)	0.7
Lactose	3.0	AP (%)	0.6
Whole milk powder	4.5	Crude fiber (%)	2.2
Whey	16.0		
Fish meal	4.0		
Plasma protein	6.5		
L-Lys	0.3		
DL-Met (98%)	0.14		
L-Try	0.01		
L-Thr (98.5%)	0.08		
Sodium chloride	0.1		
Calcium carbonate	0.85		
Dicalcium phosphate	0.9		
Choline chloride	0.1		
Vitamin and mineral premix[a]	0.52		

[a] Provided per kilogram of diet: 20.0 mg of Cu as $CuSO_4 \cdot 5H_2O$, 115 mg of Fe as $FeSO_4 \cdot 7H_2O$, 55 mg Mn of as $MnSO_4 \cdot H_2O$, 115 mg of Zn as$ZnSO_4 \cdot H_2O$, 0.3 mg of Se as Na_2SeO_3, 0.3 mg of I as KI; vitamin A, 8.000IU; vitamin D_3, 2000IU; vitamin E, 12IU; vitamin K_3, 1.2 mg; vitamin B_1, 1.5 mg; vitamin B_2, 4 mg; vitamin B_6, 2 mg; vitamin B_{12}, 0.02 mg; Biotin 0.08 mg; Pantothenic acid 12 mg; Niacin 20 mg. No folic acid was added in vitamin premix.

2.4. Total RNA extraction and mRNA quantification

Total RNA was extracted using Trizol Reagent (TaKaRa, Dalian, China). mRNA was reverse-transcribed using PrimeScript™ reagent kit (TaKaRa, Dalian, China) according to manufacturer's instructions. The concentration of RNA in the final preparation was calculated from the value of OD260 and dilution with Rnase Free dH_2O (TaKaRa, Dalian, China) for RT-PCR. The integrity of RNA was verified by denaturing agarose gel electrophoresis. The cDNA used for real-time PCR was dissolved by easy dilution H_2O (TaKaRa, Dalian, China) (Table 1).

cDNA transcription from mRNA was used for real-time PCR of mammalian silencing information regulator-2α (SIRT-1), peroxisomal proliferator-activated receptor-γ coactivator-1α (PGC-1α), nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (TFAM), mitochondrial single-strand DNA-binding protein (mt SSB), mitochondrial polymerase r (mt polr), glucokinase, citrate synthase (CS), adenosine triphosphate synthase (ATPS), cytochrome c oxidase I (CcOX I), cytochrome c oxidase IV (CcOX IV), cytochrome c oxidase V (CcOX V), Cytochrome c (Cyt c), and NADH dehydrogenase subunit 4 (ND4). Sequence of primers used for the RT-PCR was shown in Table 2. β-actin was used as internal standard. All primers were synthesized commercially by TaKaRa Biotechnology (Dalian, China). Quantitative Real-time PCR used was 96-well CFX-96 Real-Time PCR detection System (Bio-Rad, USA). The SYBR Green PCR reaction system

Table 2
Primer sequences of the target genes.

Gene	5′-Primer (F)	3′-Primer (R)	Accession number	Length
PGC-1α	CCCGAAACAGTAGCAGAGACAAG	CTGGGGTCAGAGGAAGAGATAAAG	NM_213963	111
TFAM	GGTCCATCACAGGTAAAGCTGAA	ATAAGATCGTTTCGCCCAACTTC	AY923074.1	167
NRF-1	GCCAGTGAGATGAAGAGAAACG	CTACAGCAGGGACCAAAGTTCAC	AK237171.1	166
mt SSB	CTTTGAGGTAGTGCTGTGTCG	CTCACCCCTGACGATGAAGAC	AK352341.1	143
mt polr	CTTTGAGGTTTTCCAGCAGCAG	GCTCCCAGTTTTGGTTGACAG	XM_001927064.1	119
ND4	TTATTGGTGCCGGAGGTACTG	CCCAGTTTATTCCAGGGTTCTG	NM_001097468	112
CcOX I	ATTATCCTGACGCATACACAGCA	GCAGATACTTCTCGTTTTGATGC	AJ950517.1	127
CcOX IV	CCAAGTGGGACTACGACAAGAAC	CCTGCTCGTTTATTAGCACTGG	AK233334.1	131
CcOX V	ATCTGGAGGTGGTGTTCCTACTG	GTTGGTGATGGAGGGGACTAAA	AY786556.1	160
Cyt c	TAGAAAAGGGAGGCAAACACAAG	GGATTCTCCAGGTACTCCATCAG	NM_001129970.1	154
ATPS	TGTCCTCCTCCCTATCACACATT	TAGTGGTTATGACGTTGGCTTGA	AK230503	116
SIRT-1	TGACTGTGAAGCTGTACGAGGAG	TGGCTCTATGAAACTGCTCTGG	EU030283.2	143
Glucokinase	CTTTTCCCTCCCACACTGCTAT	GACTCCTCTTCCTGAGACCCTCT	AK233298.1	119
CS	CCTTTCAGACCCCTACTTGTCCT	CACATCTTTGCCGACTTCCTTC	M21197.1	127
β-actin	TCTGGCACCACACCTTCT	TGATCTGGGTCATCTTCTCAC	DQ178122	114

PGC-1α: peroxisomal proliferator-activated receptor-γ coactivator-1α; TFAM: mitochondrial transcription factor A; NRF-1: nuclear respiratory factor-1; mt SSB: mitochondrial single-strand DNA-binding protein; mt polr: mitochondrial polymerase r; ND4: NADH dehydrogenase subunit 4; CcOX: cytochrome c oxidase; Cyt c: Cytochrome c; ATPS: adenosine triphosphate synthase; SIRT-1: mammalian silencing information regulator-2α; CS: citrate synthase.

was 10 μl in total, it was composed of 5 μl SYBR Premix Ex Taq™ (2 ×), 1 μl each of forward and reverse primers, 2 μl ddH_2O and 1 μl cDNA. Each sample was amplified in triplicate. The parameters of real-time PCR were as follows: a pre-run at 95 °C for 10 s, 40 cycles of denaturation step at 95 °C for 5 s, followed by a 60 °C annealing step for 25 s and a 72 °C extension step for 15 s. Melting curve conditions were: 1 cycle of denaturation at 95 °C for 10 s, then 65 °C change to 95 °C with temperature change velocity at 0.5 °C/s. Gene expression data from replicate samples were averaged and analyzed using the Pfaffl method (Pfaffl, 2001) to between the cassava and maize cycle threshold values.

2.5. Biochemical assay

Fifty milligram of frozen liver in 5 ml of homogenization buffer (0.05 M Tris-HCl, pH 7.4 1 mM EDTA, and 0.25 M sucrose) was homogenized on ice with an Ultra-Turrax homogenizer for 5 s at 13,500 rpm. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C and the supernatant was stored at −80 °C until further analysis.

The activities of Mn-superoxide dismutase (Mn-SOD), total antioxidative capability (T-AOC), glutathione peroxidase (GPx) and the contents of glutathione (GSH), adenosine triphosphate (ATP), malondialdehyde (MDA), and protein carbonyls in homogenization were determined using colorimetric methods with spectrophotometer. All the assay kit used in the assay was purchased from the Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China) and the assay was conducted following the instructions of the kits. All samples were measured in triplicate to determine the activities of the enzymes. Bradford method was used to measure total protein content in the homogenate. Hepatic enzymes activities were expressed as unites per milligram (U/mg) of protein.

2.6. Statistical analysis

Statistical analyses were carried out using SAS software package (1990). Difference between groups was determined by ANOVA with birth weight and diet as factors. An alpha level of 0.05 was considered as significant. All data are presented as group means ± SEM.

3. Results

3.1. Growth performance of weanling piglets

The growth performance of piglets was shown in Fig. 1. Body weight on 14 d and 35 d of age was significantly lower for IUGR piglets compared with NBW piglets ($P < 0.05$). Folic acid supplementation did not improve the growth performance.

3.2. Plasma folic acid and homocysteine concentration

Plasma folic acid concentration was increased significantly in pigs fed a FS diet compared with that fed a control diet regardless of birth weight (Fig. 2A). On the contrary, homocysteine levels were decreased significantly by dietary folic acid supplementation (Fig. 2B).

3.3. Activities of Mn-SOD, T-AOC, and GPx in liver

Without folic acid supplementation, hepatic Mn-SOD (Fig. 3A) and T-AOC (Fig. 3B) activities were lower in IUGR piglets compared with NBW piglets ($P < 0.05$). With folic acid supplementation, IUGR pigs exhibited no difference with NBW pigs in hepatic Mn-SOD and T-AOC activities. Hepatic GPx activity (Fig. 3C) was enhanced by dietary folic acid addition ($P < 0.05$).

3.4. Content of ATP, GSH, MDA and protein carbonyls in liver

Hepatic GSH content (Fig. 4A) was not affected by birth weight and dietary folic acid supplementation ($P > 0.05$). Protein carbonyls concentration (Fig. 4B) and MDA content (Fig. 4C) in the liver was higher in IUGR pigs fed a control diet compared with NBW pigs fed a FS diet ($P < 0.05$). However, there was no difference in hepatic protein carbonyls concentration and MDA content between IUGR pigs fed a FS diet and NBW pigs. Hepatic ATP content (Fig. 4D) was decreased in IUGR piglets compared with NBW piglets ($P < 0.05$), and was not affected by folic acid supplementation ($P > 0.05$).

3.5. mtDNA content in liver

Hepatic mtDNA content was presented in Fig. 5, the mtDNA content in the liver was decreased in IUGR piglets fed a control diet compared with NBW pigs ($P < 0.05$). However, the mtDNA content in the liver of IUGR pigs fed a FS diet was not different with that in NBW pigs.

3.6. mRNA levels of mitochondrial biogenesis and function-related genes in liver

Relative mRNA expression of genes related to mitochondrial biogenesis and functions in liver of piglets were presented in Fig. 6. Expression levels of genes encoding for PGC-1α ($P < 0.05$), SIRT-1 ($P < 0.05$), NRF-1 ($P < 0.05$), TFAM ($P < 0.05$), and mt polr ($P < 0.05$) were decreased in IUGR piglets fed a control diet compared with NBW piglets. However, there was no difference in hepatic mRNA abundance of PGC-1α, SIRT-1, NRF-1, TFAM, and mt polr between IUGR pigs fed a FS diet and NBW piglets. mRNA expression levels of mt SSB, glucokinase, CS, ATPS, CcOX I, and CcOX V were reduced in IUGR offspring compared with NBW pigs ($P < 0.05$), and were not affected by dietary folic acid supplementation ($P > 0.05$). Increased folic acid content in diet significantly enhanced mRNA abundance of CcOX IV regardless of birth weight ($P < 0.05$). Cyt c and ND4 mRNA abundance were not affected by birth weight or diet.

4. Discussion

It has been demonstrated that the metabolic status of IUGR offspring was altered, and abnormal mitochondrial

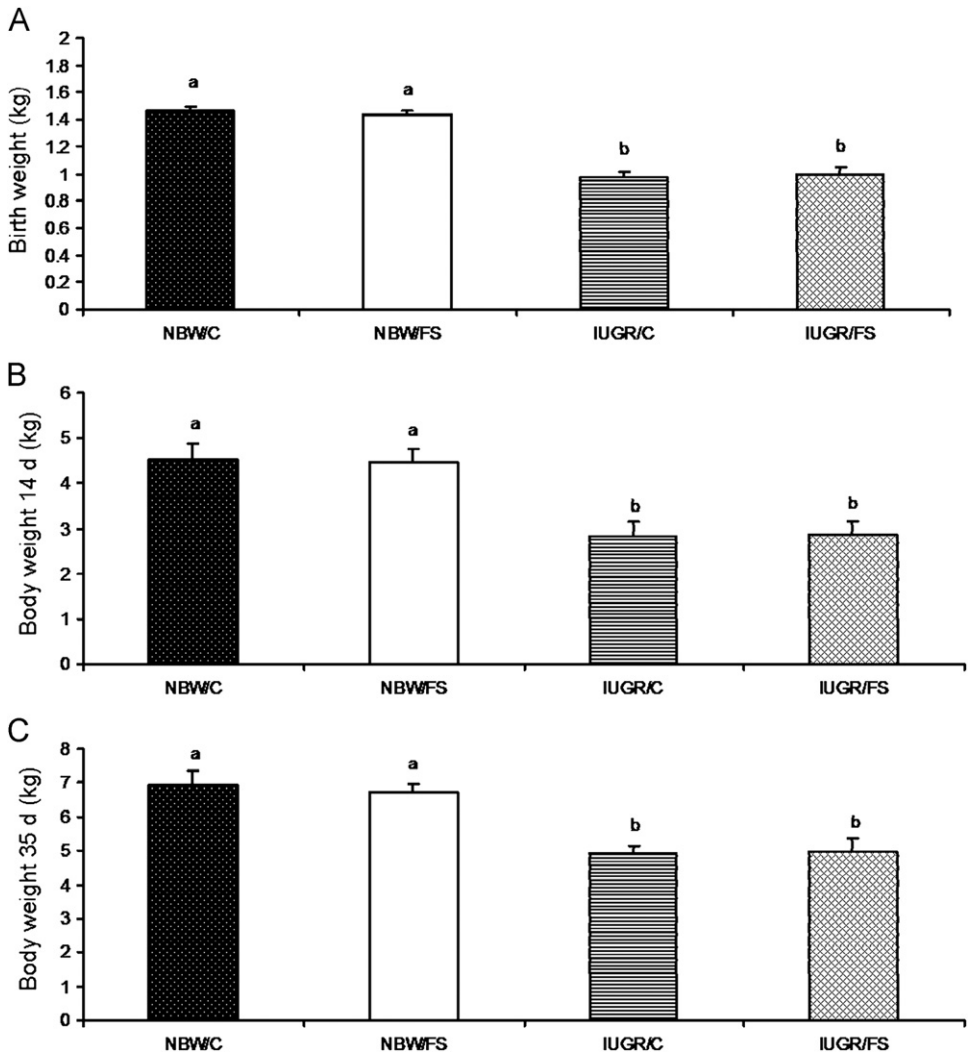

Fig. 1. Effect of folic acid supplementation on growth performance. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

biogenesis and function was a widely accepted mechanism for that phenomenon (Park et al., 2003; Reusens et al., 2008; Sparre et al., 2003; Taylor et al., 2005). Increased folic acid intake could ameliorate the extent of mitochondrial damage after cancer chemotherapy, indicating that folic acid may protect mitochondria from other insults (Branda et al., 2002). In this study, we investigated whether folic acid supplementation after weanling could prevent some of the negative effects that IUGR confers upon hepatic mitochondrial functions, mtDNA biogenesis and subsequent antioxidant function in the offspring.

Previous study showed that IUGR induced by maternal protein malnutrition was associated with an impaired antioxidant defense system and increased oxidative stress through mitochondrial pathway (Park et al., 2003). In the present study, we found that hepatic Mn-SOD and T-AOC activities were decreased in IUGR piglet. However, increased dietary folic acid content could enhance hepatic GPx, Mn-SOD and T-AOC activities. The concentrations of MDA and protein carbonyls in liver were inversely correlated with hepatic Mn-SOD and T-AOC activities in our investigation, which is consistent with previous study where maternal protein restriction-induced IUGR reduced hepatic Mn-SOD activity and increased MDA concentration (Park et al., 2003). Indeed, rats that fed a folic acid-deficient diet showed decreased activities of Mn-SOD and GPx in the liver, which was observed in a previous study (Chang et al., 2007). The antioxidant function of folic acid might be caused by decreased pro-oxidant homocysteine levels and enhanced antioxidant enzymatic activity pathway instead of mitochondrial respiratory functions (Huang et al., 2004; Handy et al., 2005). In the current study, plasma homocysteine levels were decreased by dietary folic acid supplementation, which is in agreement with previous study (Huang et al., 2001). Furthermore, folic acid supplementation increased hepatic activities of antioxidant enzymes and decreased concentration of peroxidation products in IUGR piglets, but had no effect

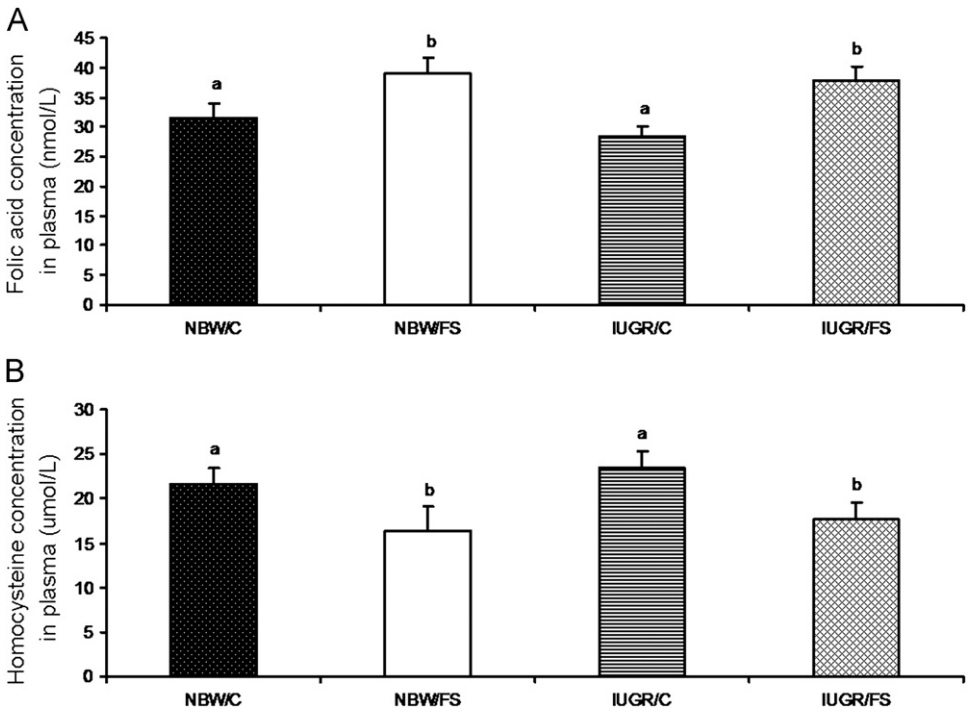

Fig. 2. Effect of folic acid supplementation on plasma folic acid and homocysteine concentrations. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

on expression levels of genes involved in mitochondrial function. Our results, supported by previous studies, suggested that the antioxidant function of folic acid mainly depends upon homocysteine pathway instead of modulating mitochondrial function.

In the present study, hepatic mtDNA content in IUGR individual was significantly reduced compared with NBW offspring. But folic acid supplementation could prevent this alteration. Reduced mtDNA content in IUGR piglets in our study is in agreement with previous researches (Park et al., 2003, 2004; Theys et al., 2009). In these studies, IUGR was induced by bilateral uterine artery ligation or maternal protein restriction during gestation, where the mtDNA content in the IUGR individual was significantly decreased (Park et al., 2003, 2004; Theys et al., 2009). It has been shown that folic acid had a potential role on antioxidant action and was responsible for mitochondrial DNA repair and replication (Huang et al., 2004; Crott et al., 2005). It has been reported that dietary folic acid deficiency led to reduction in mitochondrial DNA content and mtDNA biogenesis, deletion of mitochondrial DNA, and destruction of mitochondrial electron transport chain (Chang et al., 2007; Chou et al., 2007). Folic acid can protect against the mtDNA deletion occurred in the liver of chemotherapeutic drug-treated rats and aging rats, demonstrating that folic acid played a beneficial role in protecting mitochondrial biogenesis compromise (Branda et al., 2002). In the present study, folic acid supplementation after weanling prevented mtDNA content reduction in IUGR piglets, which might be explained by the above mechanism.

In this trial, alteration in hepatic mtDNA content may be caused by abnormal mitochondrial biogenesis process, which was regulated by multiple transcriptional factors (Puigserver and Spiegelman, 2003). PPARγ coactivator-1α (PGC-1α) and nuclear respiratory factor-1 (NRF-1), transcriptional coactivators of nuclear receptors to modulate mitochondrial biogenesis, were found to be down-regulated in IUGR piglets in the present study. Expression of mitochondrial transcriptional factor A (TFAM), a nuclear-encoded regulator of mtDNA replication and transcription that could be regulated by PGC-1a and NRF-1 to initiate mitochondrial biogenesis (Ekstrand et al., 2004; Maniura-Weber et al., 2004), were decreased in IUGR piglets compared with NBW piglets. Mitochondrial DNA replication and repair were also affected by mt single-strand DNA-binding protein (mt SSB) and mt polymerase r (mt polr, Scarpulla, 2006). Expression levels of mt SSB and mt polr were reduced in the liver of IUGR offspring; whereas hepatic expressions of NRF-1, TFAM and poly r were reversed in the IUGR offspring after folic acid supplementation. In liver, increased expression of PGC-1α in the IUGR offspring was observed, which is consistent with previous study using rats as the IUGR model induced by uterine artery ligation (Lane et al., 2002; Selak et al., 2003). SIRT-1 could enhance the role of PGC-1α in the mitochondrial biogenesis (Gerhart-Hines et al., 2007; Hock and Kralli, 2009) and they were similarly expressed in the liver of the piglets from each group in the current study. The changes in expression levels of genes involved in mitochondrial replication and repair may account for, at least in part, the regulation of mitochondrial DNA

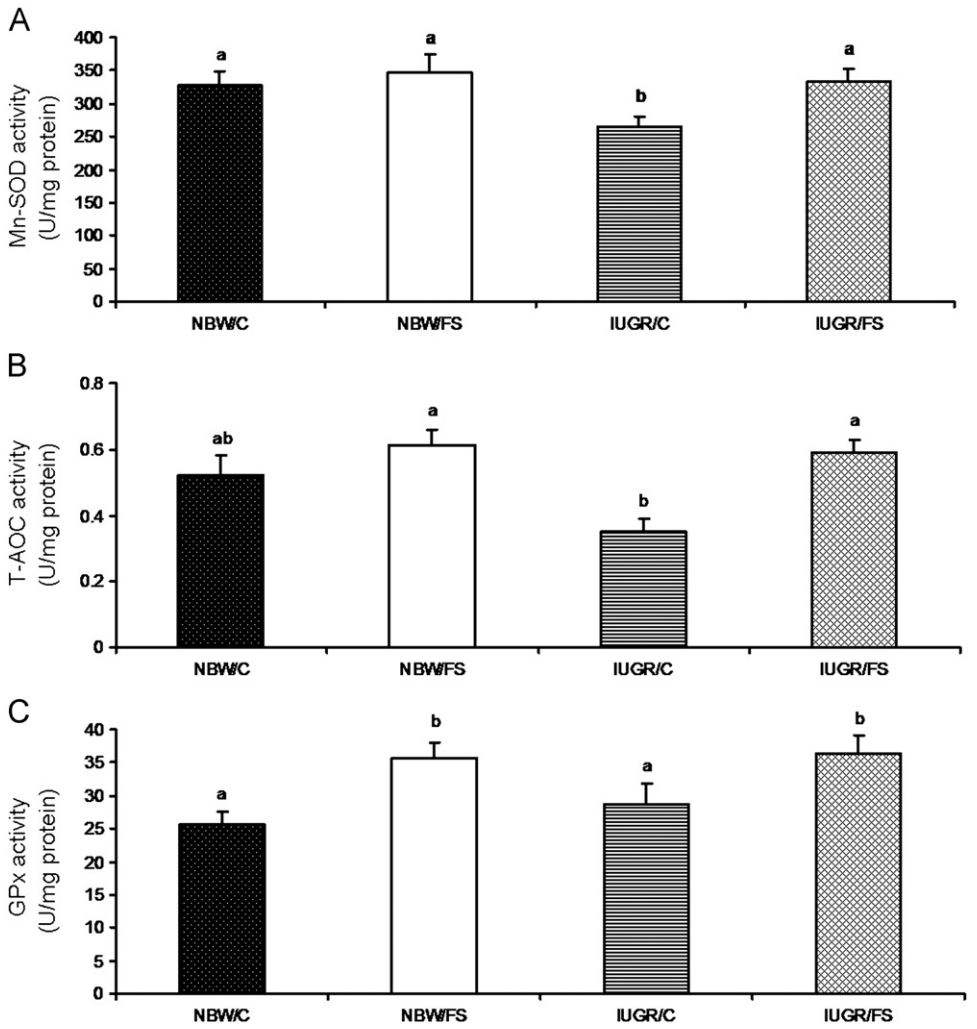

Fig. 3. Effect of folic acid supplementation on hepatic enzymes activity. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

biogenesis. Moreover, the differences in mtDNA content among groups were consistent with the expression pattern of genes involved in mtDNA biogenesis in our experiment. Mitochondrial dysfunction could lead to changes in glucose utilization, oxidative phosphorylation, TCA cycle, and ATP production (Lim et al., 2010; Petersen et al., 2003). The reduction of ATP concentration in the liver of IUGR offspring reflected that the process of glycolysis, TCA cycle, electron transport, and ATP formation might be impaired. Folic acid addition did not reverse the harmful effects of IUGR on ATP formation in this study. Glucokinase and CS determine the rate of glycolysis and TCA cycle and subsequent ATP formation (Matschinsky, 1996; Wallace, 2005). Intrauterine growth retardation decreased the expression abundance of glucokinase and CS in liver. Moreover, gene coding for enzyme involved in ATP formation, ATPS, encoded by mtDNA, was down-regulated in IUGR offspring. Nuclear-encoded and mt-encoded respiratory subunits (CcOX I, IV, V, Cyt c) were responsible for the function of electron transport and reactive oxygen species (ROS) scavenge (Hoffbuhr et al., 2000; Sue et al., 2000). Intrauterine growth retardation decreased the expression levels of CcOX I and V, but had no effect on Cyt c and ND4. The present study found that the influence of folic acid supplementation on mRNA level of CcOX IV was independent of birth weight. Evidence from rats also indicated that dietary folic acid deficiency led to specific reduction of CcOX IV activity, whereas other respiratory chain activities were not altered (Chang et al., 2007). In vitro study confirmed the finding that CcOX IV is the site of electron donation of 10-formyl-tetrahydrofolate in mitochondria of isolated rat liver (Brookes and Baggott, 2002). These data indicated that folic acid supplementation might have specific beneficial role on different CcOX subunit.

In conclusion, the present study demonstrated that hepatic antioxidant function, mtDNA biogenesis and mitochondrial function were impaired in IUGR piglets. This was

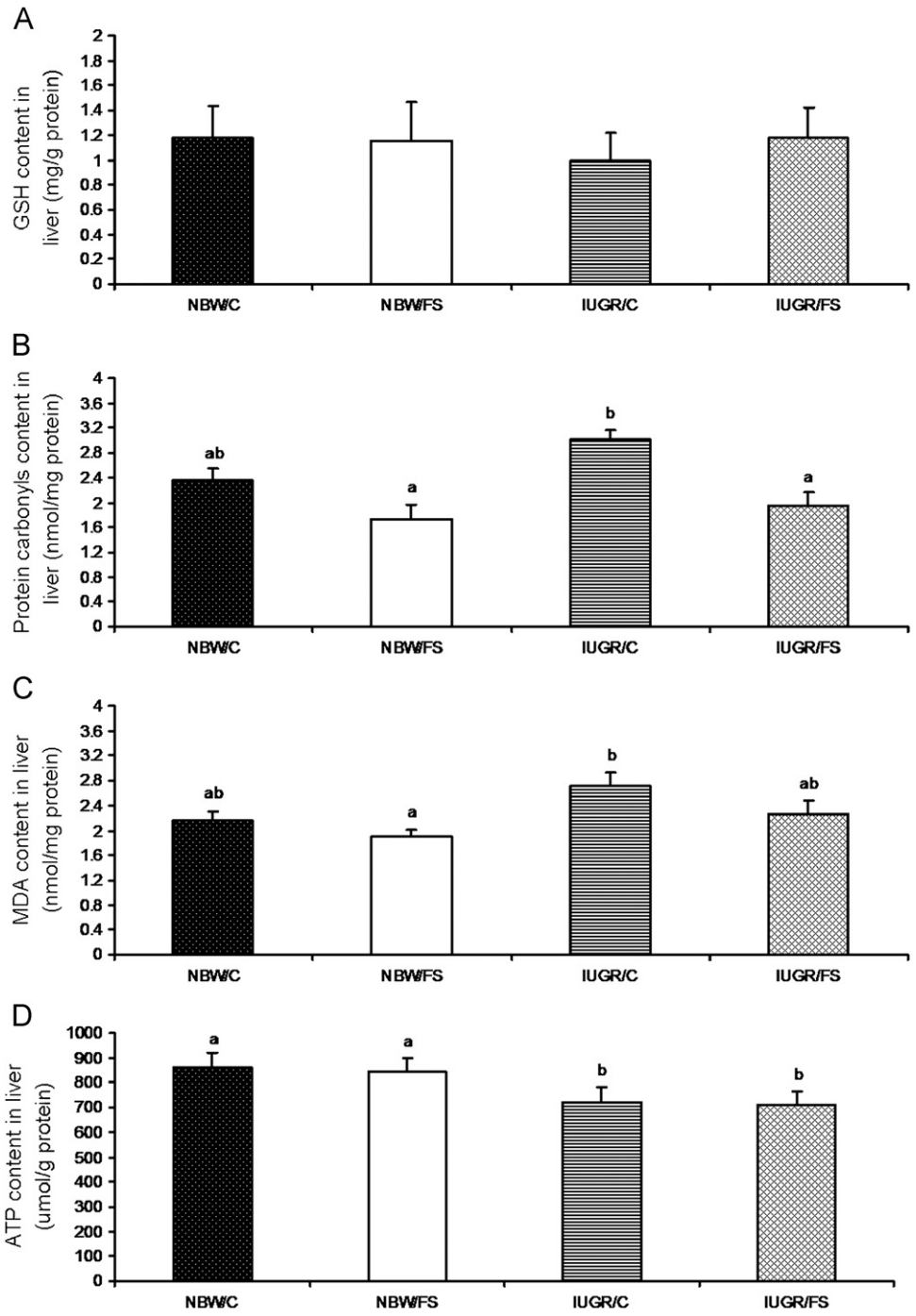

Fig. 4. Effect of folic acid supplementation on hepatic metabolite concentrations. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

accompanied by increased oxidative stress in liver. However, dietary folic acid supplementation after weanling prevented the harmful effect of IUGR on antioxidant function and mtDNA biogenesis. Based on these findings, our results suggested that dietary folic acid fortification during early period of life ameliorate the susceptibility of IUGR offspring to oxidative stress through homocysteine pathway instead of regulating mitochondrial function. This might provide some explanation for the improved growth performance of piglets resulted from the maternal folic acid supplementation during lactation (Wang et al., 2011).

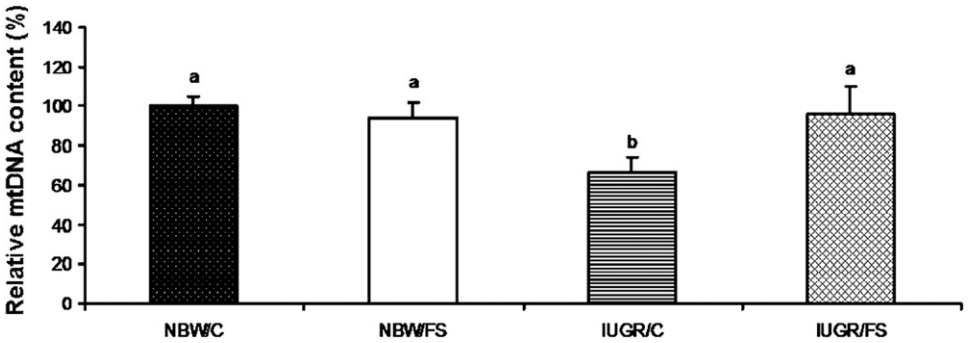

Fig. 5. Effect of folic acid supplementation on hepatic mtDNA content. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

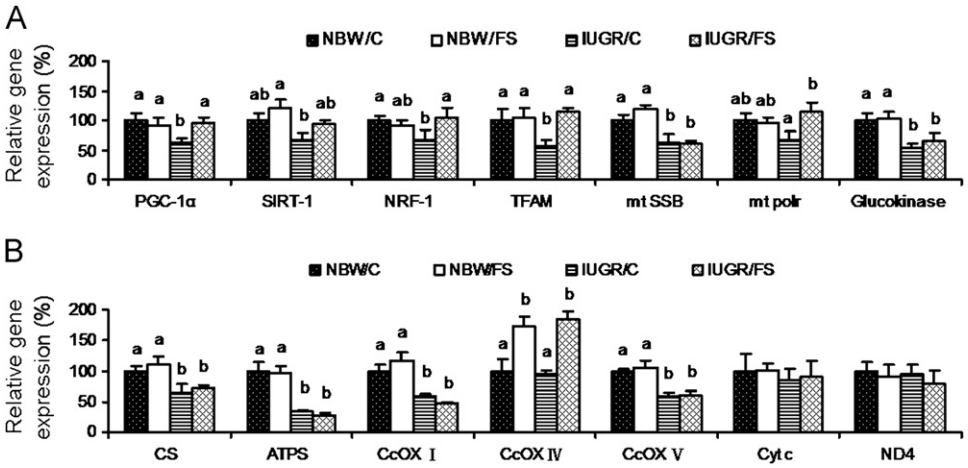

Fig. 6. Effect of folic acid supplementation on mRNA expression levels of mitochondrial-related genes. NBW: normal birth weight; IUGR: intrauterine growth retardation; C: control group; FS: folic acid supplemented group. Values are means ± SEM, $n=8$/group. Different small letters denote statistical differences of $P<0.05$ between groups.

Conflict of interest statement

No conflict of interest exists as regarding for this paper.

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