Eur J Nutr (2012) 51:19–27
DOI 10.1007/s00394-011-0185-4

ORIGINAL CONTRIBUTION

The beneficial effect of fiber supplementation in high- or low-fat diets on fetal development and antioxidant defense capacity in the rat

Yan Lin · Xing-fa Han · Zheng-feng Fang · Lian-qiang Che · De Wu · Xiu-qun Wu · Cai-mei Wu

Received: 24 July 2010 / Accepted: 8 March 2011 / Published online: 22 March 2011

Abstract
Background There is mounting evidence that an imbalance in oxidant/antioxidant activities plays a pivotal role in fetal development.
Aim of the study To determine the effects of maternal intake of fat and fiber on fetal intrauterine development and antioxidant defense systems of rats.
Methods Virgin female Sprague–Dawley rats were randomly assigned to 4 groups according to diet: the low-fat, low-fiber group (LL); the low-fat, high-fiber group (LH); the high-fat, low-fiber group (HL); and the high-fat, high-fiber group (HH). The diets were fed 4 weeks prior to breeding through day 17.5 of pregnancy. Dietary intakes of fiber (wheat bran and oat) and fat were quantitatively varied, while intakes of energy and essential nutrients were kept constant among the diets.
Results Rats fed a fiber-rich diet had significantly improved fetal numbers, as well as enhanced activity of superoxide dismutase (SOD) and capacity of scavenging free radicals ($p < 0.05$). Meanwhile, the placental malondialdehyde and protein carbonyl levels were affected by the diet fat and fiber levels ($p < 0.05$). Compared with the LL group, the mRNA abundance of hypoxia-inducible factor 1α (HIF-1α) and thioredoxin-2 (Trx2) in the maternal liver and glutathione peroxidase 1 (GPx1) in the placenta and fetus were significantly downregulated in the HL group ($p < 0.05$). Furthermore, rats fed a fiber-rich diet had significantly upregulated mRNA expressions of Cu,Zn-SOD, Mn-SOD, and HIF-1α in the maternal liver ($p < 0.05$); Cu,Zn-SOD and Mn-SOD in the placenta ($p < 0.05$); and Cu,Zn-SOD in the fetus ($p < 0.05$).
Conclusion When energy intakes are equivalent, consumption of fiber in high- or low-fat diets benefits fetal development and growth, through improvements in maternal, placental, and fetal antioxidant defense capacities.

Keywords Dietary fiber · Redox state · Antioxidant defense

Y. Lin · X. Han · Z. Fang · L. Che · D. Wu (✉) · X. Wu · C. Wu
Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education, and Animal Nutrition Institute of Sichuan Agricultural University, Ya'an 625014, China
e-mail: pig2pig@sina.com

Introduction

The oxidative stress programming hypothesis was proposed by Luo et al. [1]. This hypothesis suggests that oxidative stress could be a key to fetus or development programming of disease. The cell redox status varies based on embryonic development processes, and there are critical set points for the redox status at critical periods of development. An excessively oxidative status could be detrimental to embryonic development [2]. The cell redox status acts as a primary or secondary messenger of embryonic development through regulation of key transcription factors that influence cell signaling pathways, affecting cell proliferation and differentiation [3, 4].

Human diets high in fat are characteristic of many Western nations. When these diets predominate during pregnancy and lactation, they have long-term physiological effects on adult offspring [5]. Diets high in fat induce oxidative stress in mice [6]; furthermore, they disturb the balance of the oxidative and antioxidant defense systems in the mother, placenta, and embryo, which can retard embryonic progression, cause embryonic toxicity and miscarriages [4, 7], and increase oxidative stress in the

offspring brain [8]. In addition, abnormal intrauterine development of an embryo caused by oxidative damage may program the risk of the offspring to develop metabolic diseases later in life [5, 9]. Oxidative damage to the trophoblast and placental tissues induced by the disorganized and early onset of maternal blood to the placenta is reportedly a key factor in early fetal loss [7, 10]. This indicates that maternal oxidative stress can transfer to the fetus through the placenta, and fetal growth retardation and metabolic syndromes later in life can be associated with placental dysfunction during pregnancy [9, 11].

It is generally accepted that fiber may interfere with fat digestion and absorption and facilitate the excretion of dietary fat into the feces [12, 13]. The products of short-chain fatty acids during fermentation of dietary fiber in the gut could provide energy to colonocytes and other tissues [14]. Epidemiologic studies indicated that increased consumption of dietary fiber was inversely associated with the risk of metabolic syndromes later in life [15], suggesting that diets supplemented with fiber may reduce the oxidative stress of the organism [16]. It is therefore plausible to hypothesize that dietary fiber added to a high- or low-fat diet has beneficial effects on maternal as well as fetal health. However, few studies have elucidated whether maternal fiber intake in combination with a high- or low-fat diet affects fetal development and the redox state. The aim of the present study was to determine whether fiber supplementation in a high- or low-fat diet effectively ameliorates maternal and placenta oxidative stress, maintaining an appropriate fetal redox environment and improving fetal development.

Methods

Animal protocol

All protocols were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Eighty 90-day virgin female Sprague–Dawley rats weighing 260 ± 10 g were purchased from Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital Experimental Animal Research Institute. The rats were housed individually in galvanized steel cages and maintained at a controlled temperature (22 °C) with a 12-h light/dark cycle. One week later, each rat was randomly assigned to 1 of 4 groups: the low-fat, low-fiber group (LL); low-fat, high-fiber group (LH); high-fat, low-fiber group (HL); or high-fat, high-fiber group (HH). The diets were fed 4 weeks prior to breeding through day 17.5 of pregnancy according to a 2 × 2 factorial experimental design. Pregnancy was confirmed by the observation of vaginal plugs. Experimental diets were formulated as described by Malville-Shipan & Fleming [17]. The composition of the experimental diets is presented in Table 1. Insoluble fiber concentrations were determined with neutral and acid detergent methods after treatment with amylase [18]. Total soluble fiber was determined gravimetrically after base treatment [19], α-amylase digestion of starch, and dialysis [20]. Dietary intakes of fiber and fat by rats were quantitatively varied, while intakes of energy and essential nutrients were kept constant by controlling the feed intake. The rats were killed on day 17.5 of pregnancy. Maternal blood was collected and centrifuged at 4,000*g* for 10 min at 4 °C for serum collection. The fetus and placenta were rapidly removed, weighed, frozen in liquid nitrogen, and stored at −70 °C until analysis.

Placenta histology

Small 0.5 × 0.5 cm segments were cut and rinsed in ice-cold PBS. Placental tissues were fixed in 10% neutral buffered formalin, processed routinely for histological examination, cut to 5- to 6-μm-thick sections, and stained with hematoxylin and eosin.

Blood sample biochemical assay

Glucose, triglyceride, and total cholesterol serum concentrations were determined using a BMD/Hitachi 705 auto-analyzer (Japan). The kit was provided by Leadmanbio (Beijing, China). Concentrations of free fatty acids were determined based on ELISA analysis using a kit provided by RD (USA).

Total superoxide dismutase, copper-zinc superoxide dismutase, and manganese superoxide dismutase activity assay

Methods for the measurements of the total superoxide dismutase (T-SOD), copper-zinc superoxide dismutase (Cu,Zn-SOD), and manganese superoxide dismutase (Mn-SOD) activities in the serum and placenta were based on the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by the xanthine/xanthine oxidase system, according to the procedures described by Sun et al. [21] and Spitz and Oberley [22]. Activity units were determined by defining 1 unit of SOD activity as that amount of sample protein capable of inhibiting the reduction of NBT by 50% of maximum inhibition. The data were normalized by basing them on protein content as measured by the Bradford method.

Hydroxyl radical and superoxide anion assay

The ability of the serum and placenta to scavenge hydroxyl radicals was determined as described by Halliwell [23]

Table 1 Composition of experimental diets

Ingredient (g/kg)	Low fat		High fat	
	Low fiber (LL)	High fiber (LH)	Low fiber (HL)	High fiber (HH)
Corn starch	669.691	280.492	414.417	34.947
Wheat bran		250		250
Oat		250		250
Casein	227.5	121.5	267	158.5
Soybean oil	50	50	50	50
Lard			100	100
Rapeseed oil			100	100
Vitamin mixture[a]	11	10	14.286	11.78
Mineral mixture[b]	38.5	35	50	41.23
Choline bitartrate	1.1	1	1.429	1.178
L-Cysteine	2.2	2	2.857	2.356
t-BHQ[2]	0.009	0.008	0.011	0.009
Fat (%)	5	5	25	25
Soluble fiber (%)	0.17	2.46	0.17	2.46
Insoluble fiber (%)	2.29	10.94	2.29	10.94
Food intake level (%)	90	100	70	85

[a] Provided per kg of diet: Calcium 5,000 mg; Phosphorus 1,561 mg; Kalium 3,600 mg; Natrium 1,019 mg; Chlorine 1,517 mg; Magnesium 510 mg; Ferrum 35 mg; Zinc 30 mg; Manganese 10 mg; Copper 6 mg; I 0.2 mg; Selenium 0.15 mg

[b] Provided per kg of diet: VD_3 1,000 IU; VK_3 0.75 mg; VB_1 6.0 mg; VB_2 7.0 mg; VB_6 6.0 mg; VB_{12} 0.02 mg; nicotinic acid 30.0 mg; D-calcium pantothenate 15.3 mg; folic acid 2.0 mg; biotin 0.2 mg

with a slight modification. The ability of serum and placenta to scavenge superoxide anion was measured by the reduction of NBT according to a previously reported method [24]. Placentas (0.5 g) were homogenized in ice-cold 0.25 M Trizma base buffer with a pH of 7.4 (containing 0.2 M sucrose and 5 mM dithiothreitol) using a Teflon glass homogenizer. After centrifugation (12,000*g*, 10 min, 4 °C), supernatants were collected for analysis. All tests were performed 5 times, and the percentage of relative inhibition was evaluated by comparing the 4 groups.

Assay of malondialdehyde and protein carbonyl concentrations

The malondialdehyde (MDA) concentrations in the serum and placenta were determined by the TBA method using a commercial kit (LPO Assay Kit, Colorimetric) [25], which is based on the reaction of MDA with thiobarbituric acid to form a pink chromogen. Results were expressed as nmol/mL. The level of protein carbonyl formation in the placenta was determined using an Oxiselect™ protein carbonyl ELISA kit purchased from Cell Biolabs (San Diego, CA) according to the manufacturer's instructions. The carbonyl content (nmol/mg protein) was calculated using a molar extinction coefficient of 22 000 M/cm at 370 nm after subtraction of the blank absorbance.

RNA reparation and determination of mRNA level of antioxidant-related genes

The maternal liver, placental, and whole fetus samples in each litter ($n = 6$ per treatment group) were used for RNA extraction in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Determination of the ratio of the absorbance at 260 and 280 nm (A260/280) and agarose gel electrophoresis were performed to facilitate quantitative and qualitative analyses of the isolated RNA. Total RNA (5 μg) was used as a template to synthesize the cDNA. Aliquots of the cDNA were used as a template for the subsequent quantitative real-time PCR according to manufacturer's instructions. Real-time PCR was performed to analyze the expression of the genes listed in Table 2 using SYBR Green PCR Mix (TaKaRa, Japan) and the BioRad Research DNA Engine Opticon System as follows: 95 °C for 10 s, 40 cycles of 95 °C for 5 s, and 60 °C for 25 s. The relative levels of gene expression were normalized to the amount of the eukaryotic housekeeping gene β-actin mRNA.

Statistical analysis

Data were presented as means and their standard deviations. Only 52 pregnant rats were included in the analysis;

Table 2 Sequences of primers for PCR amplifications

Gene	Primers	Length	Gene Bank
GPx1	F: 5′ -GGCTCCCTGCGGGGCAAGGT -3′	112	NM_030826.3
	R: 5′-TGTACTTGGGGTCGGTCATG-3′		
SOD1	F: 5′-GAACCAGTTGTGGTGTCAGGA-3′	169	NM_017050
	R: 5′-CTCCAACATGCCTCTCTTCATC-3′		
SOD2	F: 5′-AGAGTTGCTGGAGGCTATCAAG-3′	166	NM_017051
	R: 5′-CAGTGGGTCCTGATTAGAGCA-3′		
Trx1	F: 5′-CCCTTCTTTCATTCCCTCTGTG-3′	150	NM_053800
	R: 5′-GAACTCCCCAACCTTTTGACC-3′		
Trx2	F: 5′-CGTACAATGCTGGTGGTCTAAC-3′	110	NM_053331.2
	R: 5′-GTCTTGAAAGTCAGGTCCATCC-3′		
HIF-1α	F: 5′-CATCTCCACCTTCTACCCAAGT-3′	110	AF057308
	R: 5′-GACTCTCTTTCCTGCTCTGTCTG-3′		
β-actin	F: 5′-CAC AGC TGA GAG GGA AAT-3′	155	NM_031144
	R: 5′-TCA GCA ATG CCT GGG TAC-3′		

GPx1 glutathione peroxidase 1, *Cu,Zn-SOD* Cu,Zn superoxide dismutase, *Mn-SOD* Mn superoxide dismutase, *Trx1* thioredoxin-1, *Trx2* thioredoxin-2, *HIF-1α* hypoxia-inducible factor 1 alpha, *β-actin* beta-actin

28 rats were found to be non-pregnant or had spontaneous abortions. The average fetal and placental weights within litters were used in the analysis.

Data were analyzed by LSD multiple comparison for the 2 × 2 factorial experimental design using the General Linear Model (GLM) procedure of the SAS software package (1990) based on the following model: $yijk = \mu + ai + bj + (ab)ij + eijk$ ($i = 1, 2, 3$, $j = 1, 2$, $k = 1, 2,\ldots, nij$), where *yijk* is the dependent variable; μ is the overall mean; *ai* is the fat level; *bj* is the fiber level; (*ab*)*ij* is the interaction between the fat and fiber levels; and *eijk* is the error term.

Results

Effect of the different diets on reproduction performance and metabolism

There were 4, 4, 5, and 3 rats in the LL, LH, HL, and HH groups, respectively, that were not pregnant in the detection progress (8 days) because of different diets or other uncertain reasons. At the end of the experiment, 4, 4, 3, and 1 pregnant rats in the LL, LH, HL, and HH groups, respectively, had abortions; these results suggest that the HH group had a lower abortion rate than that of the other groups.

As presented in Table 3, fetal weight was affected by fat level × fiber level interaction, and was significantly lower in the LL group than in the HH group ($p < 0.05$). However, placental weight was not affected ($p > 0.05$) by fat level, fiber level, or fat level × fiber level interaction. The presence of fiber in LH or HH diets significantly increased the fetal number ($p < 0.05$). At the end of the experiment, the triglyceride and total cholesterol contents were not affected by maternal diet intake ($p > 0.05$), while the serum glucose level was significantly affected by fat level ($p < 0.05$) and tended ($p = 0.08$) to be affected by fat level × fiber level interaction.

Effect of the different diets on placental histology

Consumption of the HH diet had a deleterious effect on placental tissue integrity (Fig. 1c). The placental trophoblasts exhibited an evident malformation, and were even partly necrotic in the HL group. However, dietary fiber supplementation alleviated the phenomenon (Fig. 1d). There was no obvious change in the LL or LH groups (Fig. 1a, b).

Effect of the different diets on antioxidant-related parameters

In the present study, activities of T-SOD, Cu,Zn-SOD, and Mn-SOD in maternal serum were significantly affected by dietary fiber ($p < 0.05$; Table 4). Compared with the LL group, activities of T-SOD in maternal serum and Cu,Zn-SOD in placenta were increased significantly in the LH group ($p < 0.05$). Similarly, the activities of T-SOD and Mn-SOD in maternal serum, as well as T-SOD in placenta, were significantly elevated in the HH group compared with those in the HL group ($p < 0.05$). However, T-SOD, Cu,Zn-SOD, and Mn-SOD in maternal serum and placenta, were not affected ($p > 0.05$) by fat level × fiber level interaction with the exception of the Mn-SOD activity in placenta ($p < 0.05$). Furthermore, the levels of MDA in maternal serum were significantly affected by dietary fiber ($p < 0.05$) and fat level × fiber level interaction

Table 3 Effects of experimental diets on reproductive performance and metabolism

	Treatment				*p* value		
	LL	LH	HL	HH	Fat	Fiber	Fat × fiber
No. of rats	12	12	12	16			
Fetal weight (g)	0.714 ± 0.043^{a}	0.864 ± 0.052^{b}	0.913 ± 0.210^{b}	0.815 ± 0.080^{ab}	0.103	0.562	0.010
Placental weight (g)	0.340 ± 0.069	0.366 ± 0.026	0.347 ± 0.046	0.351 ± 0.032	0.802	0.404	0.526
Fetal number	9.83 ± 1.83^{a}	11.82 ± 1.40^{bc}	10.14 ± 1.46^{ab}	12.00 ± 1.58^{c}	0.684	0.004	0.916
Glucose[1]	4.40 ± 0.91^{b}	5.50 ± 0.52^{b}	7.50 ± 0.97^{a}	4.75 ± 0.99^{b}	0.020	0.108	0.078
Triglyceride[1]	1.88 ± 0.42^{a}	1.93 ± 0.40^{a}	2.54 ± 0.77^{b}	2.39 ± 0.46^{a}	0.042	0.864	0.701
Total cholesterol[1]	1.79 ± 0.38	1.59 ± 0.32	2.13 ± 0.59	1.66 ± 0.17	0.312	0.089	0.502

LL low-fat and low-fiber diet, *LH* low-fat and high-fiber diet, *HL* high-fat and low-fiber diet, *HH* high-fat and high-fiber diet

Data are presented as means ± standard deviation, and means in the same row with different letters are different at ($p < 0.05$)

[1] mmol/L

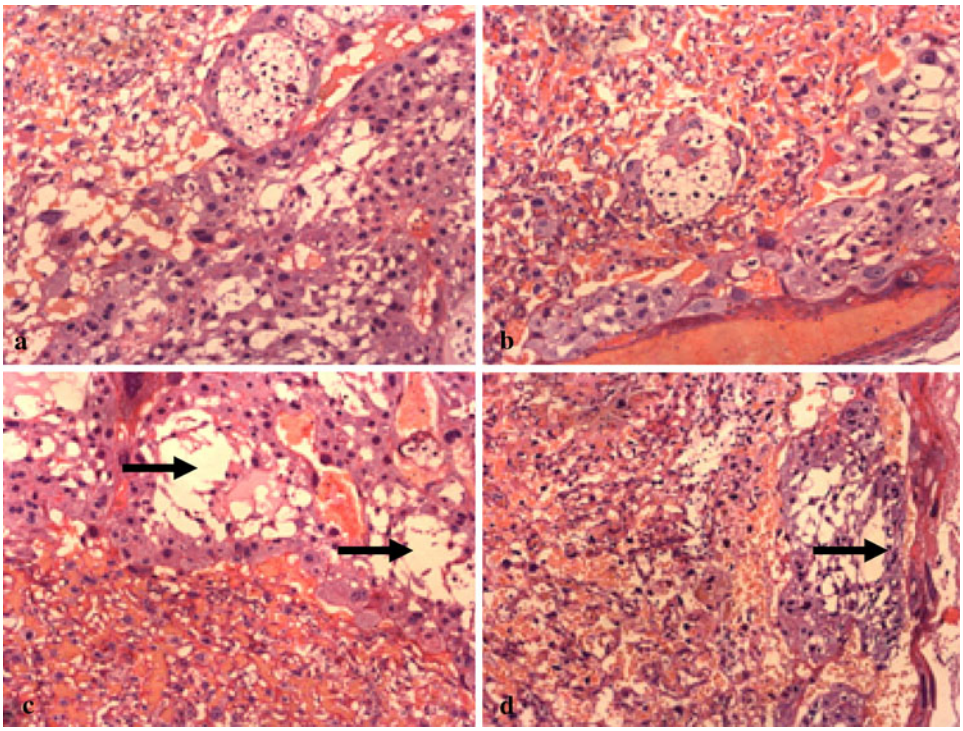

Fig. 1 Effects of experimental diets on placental structure (magnification × 200). **a** Low-fat, low-fiber diet; **b** low-fat, high-fiber diet; **c** high-fat, low-fiber diet; **d** high-fat, high-fiber diet. The histological findings in the LL group were typical of normal placenta; pronounced placental pathology could be seen in the HL group and aided restoration after fiber supplement. Placental morphological changes are identified by *black arrows*

($p < 0.05$), and the levels of MDA and protein carbonyl in placenta were affected by the dietary fat and fiber levels ($p < 0.05$). As shown in Fig. 2a and b, the superoxide anion and hydroxyl radical scavenging capacities of the maternal serum in the LH and HH groups were significantly higher than those in the LL and HL groups ($p < 0.05$). The regulations for the placenta were very similar to those found in the maternal serum, in that the superoxide anion and hydroxyl radical scavenging capacities were improved in the HH group compared with those in the HL group ($p < 0.05$).

Effect of the different diets on critical gene expressions related to redox regulation

To further confirm the action of the treatment diets on the antioxidant defense capacity, we performed quantitative PCR analysis on key gene expressions in redox regulation. The mRNA expressions of Cu,Zn-SOD, Mn-SOD, and HIF-1α in maternal liver were affected by dietary fat and fiber levels ($p < 0.05$; Fig. 3a), while glutathione peroxidase 1 (GPx1) expression was affected only by dietary fiber level ($p < 0.05$). Furthermore, mRNA expressions of

Table 4 Effects of experimental diets on antioxidant-related parameters in maternal serum and placenta

Parameters	Treatment				*p* value		
	LL	LH	HL	HH	Fat	Fiber	Fat × fiber
Serum							
T-SOD[1]	25.00 ± 1.26[a]	30.13 ± 1.04[b]	26.48 ± 3.34[a]	30.39 ± 0.74[b]	0.387	0.001	0.542
Cu,Zn-SOD[1]	16.29 ± 0.57[a]	18.94 ± 0.50b[c]	17.40 ± 1.30[ab]	18.18 ± 0.68[b]	0.703	0.004	0.065
Mn-SOD[1]	8.72 ± 0.71[a]	11.10 ± 1.45[ab]	9.09 ± 2.24[a]	12.21 ± 1.12[b]	0.366	0.006	0.645
MDA[2]	58.01 ± 2.39[b]	27.06 ± 3.02[a]	62.93 ± 3.87[c]	23.27 ± 0.73[a]	0.723	0.000	0.018
Placenta							
T-SOD[1]	34.26 ± 4.36[ab]	37.28 ± 1.16[b]	31.35 ± 2.13[a]	37.16 ± 1.34[b]	0.337	0.018	0.377
Cu,Zn-SOD[1]	23.62 ± 3.27[a]	30.57 ± 0.63[b]	22.57 ± 1.90[a]	26.64 ± 1.76[ab]	0.075	0.002	0.272
Mn-SOD[1]	10.64 ± 2.27	6.71 ± 0.64	8.78 ± 2.65	10.51 ± 2.27	0.449	0.393	0.048
MDA[2]	3.58 ± 0.57[b]	2.35 ± 0.26[a]	4.84 ± 0.36[c]	2.97 ± 0.58[ab]	0.008	0.000	0.268
Protein carbonyl[2]	34.91 ± 1.39[b]	26.58 ± 3.13[a]	60.29 ± 1.46[d]	42.02 ± 1.54[c]	0.000	0.000	0.003

Data are presented as means ± standard deviation, and means in the same row with different letters are different at ($p < 0.05$)

LL low-fat and low-fiber diet, *LH* low-fat and high-fiber diet, *HL* high-fat and low-fiber diet, *HH* high-fat and high-fiber diet

[1] U/mg protein

[2] nmol/mg protein

Cu,Zn-SOD, thioredoxin-1 (Trx1), and HIF-1α were affected by fat level × fiber level interaction ($p < 0.05$). Conversely, when energy intakes were equivalent, there was no significant effect on mRNA expression of Mn-SOD, Trx1, Trx2, or GPx1 in maternal liver. Meanwhile, the HH group showed significant upregulations in the mRNA levels of Cu,Zn-SOD, Trx1, and GPx1 compared with the HL group ($p < 0.05$).

As shown in Fig. 3b, the mRNA expressions of GPx1 and HIF-1α in placenta were affected by dietary fat and fiber levels ($p < 0.05$). Rats fed a fiber-rich diet significantly upregulated the mRNA expressions of Cu,Zn-SOD and Mn-SOD ($p < 0.05$) and tended to upregulate the mRNA expressions of Trx1 and Trx2 ($p > 0.05$). Compared with the LL group, mRNA expressions of GPx1 and Cu,Zn-SOD were significantly upregulated in the LH group ($p < 0.05$). Compared with the HL group, the HH group showed significant upregulations in the mRNA levels of HIF-1α, Cu,Zn-SOD, and Mn-SOD ($p < 0.05$).

The mRNA expression of GPx1 in the fetus was significantly affected by dietary fat level ($p < 0.05$; Fig. 3c), whereas the dietary fiber level significantly upregulated the mRNA expression of Cu,Zn-SOD ($p < 0.05$) and tended to increase the mRNA expression of HIF-1α ($p = 0.08$). However, the mRNA expressions of detected genes were not affected by fat level × fiber level interaction ($p > 0.05$). Compared with the LL group, the LH group showed significant upregulations in the mRNA levels of HIF-1α, GPx1, and Cu,Zn-SOD ($p < 0.05$). When compared with the HL group, the HH group also exhibited significant upregulations in the mRNA levels of HIF-1α, Mn-SOD, and Trx2 in the fetus.

Discussion

Prenatal nutritional programming of postnatal physiological functions has been experimentally demonstrated in a wide variety of laboratory and farm animals. Previous reports have shown the consequences for the progeny of feeding high-fat diets during gestation only or during both gestation and lactation [26, 27]; thus, our study focused on the effect of fiber supplementation in high- or low-fat diets on fetal development and the antioxidant defense capacity of the rat. Our results showed that maternal intake of a fat-rich diet increased fetal weight, while fiber supplementation in both low- and high-fat diets enhanced litter size when energy intake was equivalent. Other studies have indicated that high-fat diets increase the concentrations of circulating glucose, by elevating the tolerance of peripheral tissues to circulating insulin and preventing entrance of the circulating glucose to peripheral tissue cells [28, 29, 29], which may lead to fetal overgrowth.

It is well known that reactive oxygen species (ROS), such as the superoxide radical and hydroxyl radical, are inevitable byproducts of embryonic development and metabolism; they have been implicated in impaired embryonic development in vitro by inducing lipid and protein peroxidation [30]. The present study showed that maternal and placental radical scavenging capacities decreased and the end products of lipid and protein peroxidation increased in the HL group, which was in accordance with the results of Milagro et al. [31]; thus, structural membrane damage occurred, as evidenced by the placental tissue slice. Fetal numbers and SOD activity were markedly increased by inclusion of fiber in the diet, as

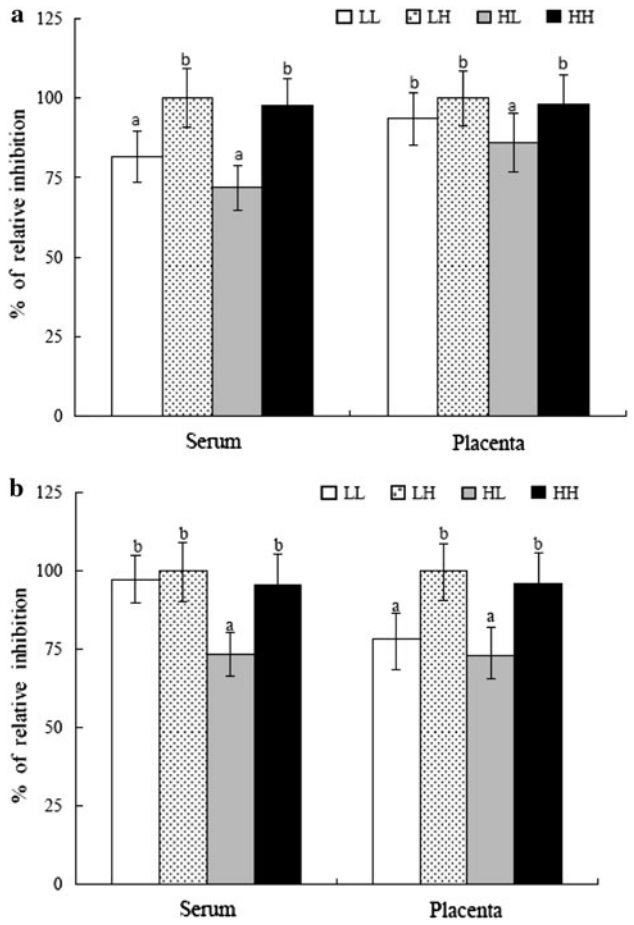

Fig. 2 Effects of experimental diets on superoxide anion (**a**) and hydroxyl radical (**b**) scavenging capacities in maternal serum and placenta. *LL* low-fat, low-fiber diet; *LH* low-fat, high-fiber diet; *HL* high-fat, low-fiber diet; *HH* high-fat, high-fiber diet. The data represent the percent of relative inhibition compared with the LH group. Means (6 rats per group) with standard deviations are depicted by *vertical bars*. Means with different letters are significantly different ($p < 0.05$)

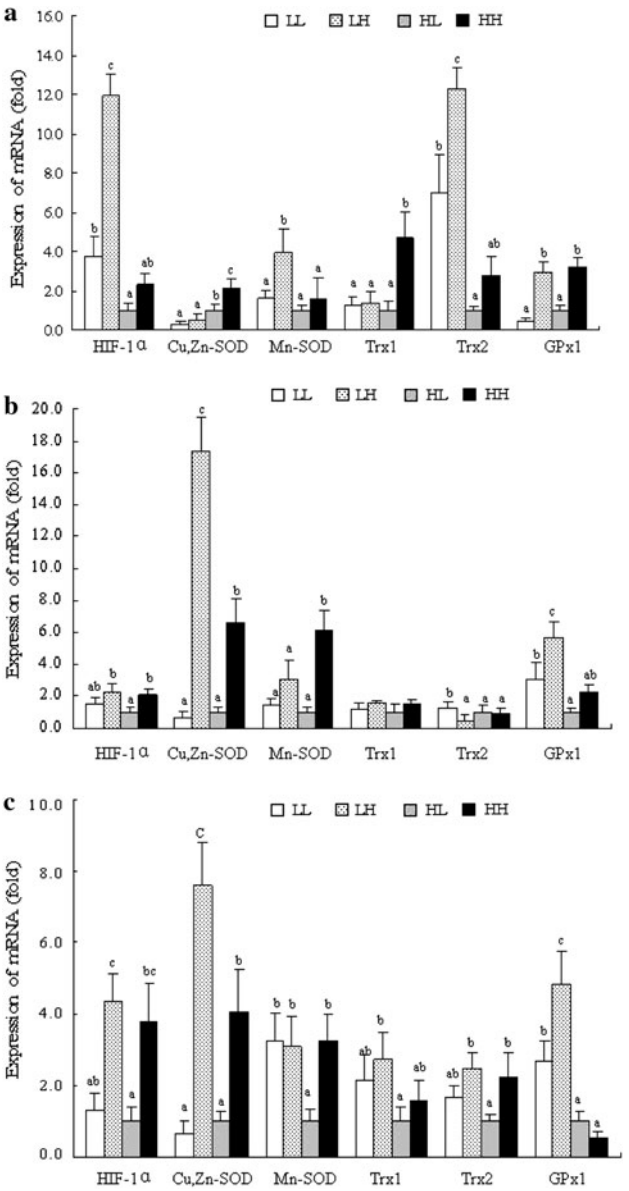

Fig. 3 Effects of experimental diets on the mRNA levels of antioxidant-related genes in the maternal liver. **a** Maternal liver; **b** placenta; **c** fetus. *LL* low-fat, low-fiber diet; *LH* low-fat, high-fiber diet; *HL* high-fat, low-fiber diet; *HH* high-fat, high-fiber diet. *HIF-1a* hypoxia-inducible factor 1 alpha; *Cu,Zn-SOD* Cu,Zn superoxide dismutase; *Mn-SOD* Mn superoxide dismutase; *Trx1* thioredoxin-1; *Trx2* thioredoxin-2; *GPx1* glutathione peroxidase 1. Means (6 rats per group) with standard deviations are depicted by *vertical bars*. Means with different letters are significantly different ($p < 0.05$)

evidenced by the lower levels of lipid and protein peroxidation products in maternal serum and placenta. Other studies demonstrated that diets supplemented with cocoa or soluble cocoa fiber products had significantly decreased serum MDA levels [18, 32] and diminished DEN-induced hepatic protein carbonyl contents [33], revealing the protective role of fiber against oxidative stress.

Extensive human epidemiological findings have provided support for the hypothesis that oxidative stress may be a common link underlying the superficial programming associations between fetal growth and elevated risks of certain adult metabolic diseases [1]. Maternal/embryonic redox-homeostasis is of major importance; dysregulation in the equilibrium of pro- and antioxidative processes retards embryo development, leading to organ malformation, and embryo lethality [34]. In the present study, the similar changes in antioxidant-related gene expressions in the maternal liver and the fetus suggest that maternal nutrition may affect oxidative stress of the pregnant female, and subsequently change the redox status of fetus. It was recently reported that maternal high-fat, protein- and calorie-restricted diets induced oxidative stress in the offspring [34, 35]. Dietary fiber decreased oxidative stress [18], and whole bran could effectively reduce the damage derived from H_2O_2 to human lymphocyte DNA molecules

[36]; this suggests that dietary fiber protects DNA molecules against H_2O_2-induced oxidative damage. Meanwhile, it was reported that the products of short-chain fatty acids during fermentation of dietary fiber in the gut, such as butyrate and acetate, could prevent DNA and cell damage induced by H_2O_2 [37], and that butyrate could induce the activity of glutathione S-transferase [38], resulting in an enhanced antioxidant capacity of the organism. In addition, antioxidant supplements such as vitamin C and E reduced oxidative stress-induced mouse embryo toxicity and improved the blastocyst in vitro culture [39]. More importantly, the maternal oxidative status is closely related to the fetal redox status [40]. Taken together, diets supplemented with fiber appear to alleviate high-fat-diet-induced oxidative stress by regulating the mRNA expressions of antioxidant-related genes in the maternal liver and fetus, maintaining an anti-prooxidant balance.

The placenta is both the target of maternal oxidative stress and a modulator of fetal oxidative stress [41]. There is increasing experimental evidence to indicate that the oxidative stress in placental tissue is involved in fetal development and miscarriage [7, 10]. Furthermore, placental and/or systemic antioxidant systems may regulate placental development and function with consequential effects on fetal programming [42]. Our results were the first to demonstrate that maternal exposure to a high-fat diet downregulated SOD activities and antioxidant-related gene expressions of the placenta, while fiber supplementation upregulated antioxidant-related gene expressions; this suggests that maternal nutrition may affect oxidative stress of the placenta, and thus affect the redox status of the fetus. Placental cells are reportedly sensitive to oxidative stress because of their extensive cell divisions and the concomitant exposure of their DNA [43], but the concentrations of the principal antioxidant enzymes in the placenta are very low [44]. Indeed, placental oxidative damage has a close relationship with fetal growth retardation and metabolic syndromes of later life [9, 45]. Therefore, the placental redox state plays an important role in embryonic development. Although additional studies are needed, we suggest that there might be close relationships among redox states for maternal, placental, and fetal tissues, and a high degree of correlation between dietary fiber and oxidative stress.

HIF-1α plays important roles in redox regulation [46], which is essential for normal embryonic development [47]. Compared with the LL group, the HL diet tended to downregulate the mRNA level of placental HIF-1α, whereas fiber supplementation elevated the mRNA expressions of HIF-1α in maternal liver, placental, and fetal tissues. ROS enhanced HIF-1 protein and mRNA levels [48], while treatment with the antioxidant GSH ethyl ester and N-acetylcysteine inhibited HIF-1α mRNA and protein expressions [49]. In addition, it is known that HIF-1α is involved in glucose transport [50] and placental development and function [51], which may affect fetal development. However, if and how dietary fiber affects placental development and function needs to be researched.

In conclusion, maternal intake of a fat-rich diet increased the oxidative stress states of maternal, placental, and fetal tissues, while dietary fiber appeared to enhance the antioxidant defense capacity of maternal, placental, and fetal tissues, alleviating oxidative stress derived from a high-fat diet, therefore improving fetal development when energy intakes were equivalent.

Acknowledgments The present research was supported by the Educational Commission of Sichuan Province of China (09ZB056) and the National Natural Science Foundation of China (31072044). All authors read and approved the final manuscript.

References

1. Luo ZC, Fraser WD, Julien P, Deal CL, Audibert F, Smith GN, Xiong X, Walker M (2006) Tracing the origins of "fetal origins" of adult diseases: programming by oxidative stress? Med Hypotheses 66:38–44
2. Castagné V, Lefèvre K, Natero R, Clarke PG, Bedker DA (1999) An optimal redox status for the survival of axotomized ganglion cells in the developing retina. Neuroscience 93:313–320
3. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30:1191–1212
4. Salas-Vidal E, Lomelí H, Castro-Obregón S, Cuervo R, Escalante-Alcalde D, Covarrubias L (1998) Reactive oxygen species participate in the control of mouse embryonic cell death. Exp Cell Res 238:136–147
5. Taylor PD, Poston L (2007) Developmental programming of obesity in mammals. Exp Physiol 92:287–298
6. Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T, Ando H, Yokoyama M, Honda M, Zen Y, Nakanuma Y, Miyamoto K, Kaneko S (2007) Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. Hepatology 46:1392–1403
7. Hempstock J, Jauniaux E, Greenwold N, Burton GJ (2003) The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol 34:1265–1275
8. White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim SO, Hise TL, Keller JN, Ingram DK, Morrison CD, Bruce-Keller AJ (2009) Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. Neurobiol Dis 35:3–13
9. Barker DJ (1995) Fetal origins of coronary heart disease. Br Med J 311:171–174
10. Jauniaux E, Greenwold N, Hempstock J, Burton GJ (2003) Comparison of ultrasonographic and Doppler mapping of the intervillous circulation in normal and abnormal early pregnancies. Fertil Steril 79:100–106
11. Baker DJP, Gluckman PD, Godfrey KM, Harding J, Owens JA, Robinson JS (1993) Fetal nutrition and adult disease. Lancet 341:938–941
12. Dégen L, Halas V, Tossenberger J, Szab CS, Babinszky L (2009) The impact of dietary fiber and fat levels on total tract digestibility of energy and nutrients in growing pigs and its

consequence for diet formulation. Acta Agriculturae Scandinavica 59:150–160
13. Deuchi K, Kanauchi O, Imasato Y, Kobayashi E (1994) Decreasing effect of chitosan on the apparent fat digestibility by rats fed on a high-fat diet. Biosci Biotech Biochem 58:1613–1616
14. Ichikawa H, Shineha R, Satomi S, Sakata T (2002) Gastric or rectal instillation of short-chain fatty acids stimulates epithelial cell proliferation of small and large intestine in rats. Dig Dis Sci 47:1141–1146
15. Galisteo M, Duarte J, Zarzuelo A (2008) Effects of dietary fibers on disturbances clustered in the metabolic syndrome. J Nutr Biochem 19:71–84
16. Lecumberri E, Goya L, Mateos R, Alía M, Ramos S, Izquierdo-Pulido M, Bravo L (2007) A diet rich in dietary fiber from cocoa improves lipid profile and reduces malondialdehyde in hypercholesterolemic rats. Nutrition 3:332–341
17. Malville-Shipan K, Fleming SE (1992) Wheat bran and corn oil do not influence proliferation in the colon of healthy rats when energy intakes are equivalent. J Nutr 122:37–45
18. Mertens DR (2002) Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J AOAC Int 85:1217–1240
19. Bertuzzi MA, Armada M, Gottifredi JC (2007) Physiochemical characterization of starch based films. J Food Eng 82:17–25
20. Hatfield RD (1991) Alfalfa-stempectins: enzymic degradation and structural characterization of a buffer-soluble fraction. Carbohydr Res 212:177–186
21. Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34:497–500
22. Spitz DR, Oberley LW (1989) An assay for superoxide dismutase activity in mammalian tissue homogenates. Anal Biochem 179:8–18
23. Elizabeth K, Rao MNA (1990) Oxygen radical scavenging activity of curcumin. Int J Pharmaceut 58:237–240
24. Fontana M, Mosca L, Rosei MA (2001) Interaction of enkephalines with oxyradicals. Biochem Pharmacol 61:1253–1257
25. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310
26. Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T (2009) High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. FASEB J 23:271–278
27. Khan IY, Dekou V, Douglas G, Jensen R, Hanson MA, Poston L, Taylor PD (2005) A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. Am J Physiol Regul Integr Comp Physiol 288:127–133
28. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW (1997) Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 46:1768–1774
29. Han DH, Hansen PA, Host HH, Holloszy JO (1997) Insulin resistance of muscle glucose transport in rats fed a high-fat diet: a reevaluation. Diabetes 46:1761–1767
30. Nasr-Esfahani MH, Aitken JR, Johnson MH (1990) Hydrogen peroxide levels in mouse oocytes and early cleavage stage embryos developed invitro or invivo. Development 109:501–507
31. Milagro FI, Campión J, Martínez JA (2006) Weight gain induced by high-fat feeding involves increased liver oxidative stress. Obesity 14:1118–1123
32. Ramos S, Moulay L, Granado-Serrano AB, Vilanova O, Muguerza B, Goya L, Bravo L (2008) Hypolipidemic effect in cholesterol-fed rats of a soluble fiber-rich product obtained from cocoa husks. J Agric Food Chem 56:6985–6993
33. Granado-Serrano AB, Martín MA, Bravo L, Goya L, Ramos S (2009) A diet rich in cocoa attenuates N-nitrosodiethylamine-induced liver injury in rats. Food Chem Toxicol 47:2499–2506
34. Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC, Bateman AC, Clough GF, Poston L, Hanson MA, McConnell JM, Byrne CD (2009) Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology 50:1796–1808
35. Theys N, Bouckenooghe T, Ahn MT, Remacle C, Reusens B (2009) Maternal low-protein diet alters pancreatic islet mitochondrial function in a sex-specific manner in the adult rat. Am J Physiol Regul Integr Comp Physiol 297:1516–1525
36. Wang J, Sun BG, Cao YP, Song HL, Tian Y (2008) Inhibitory effect of wheat bran feruloyl oligosaccharides on oxidative DNA damage in human lymphocytes. Food Chem 109:129–136
37. Abrahamse SL, Pool-Zobel BL, Rechkemmer G (1999) Potential of short chain fatty acids to modulate the induction of DNA damage and changes in the intracellular calcium concentration by oxidative stress in isolated rat distal colon cells. Carcinogenesis 20:629–634
38. Ufer C, Wang CC, Borchert A, Heydeck D, Kuhn H (2010) Redox control in mammalian embryo development. Antioxid Redox Signal. [Epub ahead of print]
39. Wang X, Falcone T, Attaran M, Goldberg JM, Agarwal A, Sharma RK (2002) Vitamin C and vitamin E supplementation reduce oxidative stress–induced embryo toxicity and improve the blastocyst development rate. Fertil Steril 78:1272–1277
40. Arikan S, Konukoglu D, Arikan C, Akcay T, Davas I (2001) Lipid peroxidation and antioxidant status in maternal and cord blood. Gynecol Obstet Invest 51:145–149
41. Liguori A, D'Armiento FP, Palagiano A, Balestrieri ML, Williams-Ignarro S, de Nigris F, Lerman LO, D'Amora M, Rienzo M, Fiorito C, Ignarro LJ, Palinski W, Napoli C (2007) Effect of gestational hypercholesterolaemia on omental vasoreactivity, placental enzyme activity and transplacental passage of normal and oxidised fatty acids. BJOG 114:1547–1556
42. Myatt L (2006) Placental adaptive responses and fetal programming. J Physiol 572:25–30
43. Burton GJ, Hempstock J, Jauniaux E (2003) Oxygen, early embryonic metabolism and free radical-mediated embryopathies. Reprod Biomed Online 6:84–96
44. Watson AL, Skepper JN, Jauniaux E, Burton GJ (1998) Susceptibility of human placental syncytiotrophoblast mitochondria to oxygen-mediated damage in relation to gestational age. J Clin Endocrinol Metab 83:1697–1705
45. Martyn CN, Barker DJP, Osmond C (1996) Mothers pelvic size, fetal growth and death from stroke in men. Lancet 348:1264–1268
46. Huang LE, Arany Z, Livingston DM, Bunn HF (1996) Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 271:32253–32259
47. Kozak KR, Abbott B, Hankinson O (1997) ARNT-deficient mice and placental differentiation. Dev Biol 191:297–305
48. Bonello S, Zähringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, Kietzmann T, Görlach A (2007) Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. Arterioscler Thromb Vasc Biol 27:755–761
49. Tajima M, Kurashima Y, Sugiyama K, Ogura T, Sakagami H (2009) The redox state of glutathione regulates the hypoxic induction of HIF-1. Eur J Pharmacol 606:45–49
50. Isoe T, Makino Y, Mizumoto K, Sakagami H, Fujita Y, Honjo J, Takiyama Y, Itoh H, Haneda M (2010) High glucose activates HIF-1-mediated signal transduction in glomerular mesangial cells through a carbohydrate response element binding protein. Kidney Int 78:48–59
51. Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Wallace JM, Caton JS, Redmer DA (2005) Animal models of placental angiogenesis. Placenta 26:689–708