Livestock Science 136 (2011) 157–163

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of maternal protein restriction on lipid metabolism in Meishan piglets at weaning

Jing Liang [a], Xiaowei Zhang [a], Ruqian Zhao [a], Steffen Maak [b], Xiaojing Yang [a,*]

[a] Key Laboratory of Animal Physiology and Biochemistry, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Leibniz-Institute for Farm Animal Biology, D-18196 Dummerstorf, Germany

ARTICLE INFO

Article history:
Received 23 April 2010
Received in revised form 22 August 2010
Accepted 23 August 2010

Keywords:
Pigs
Maternal protein restriction
Offspring
Lipid metabolism
Perilipin

ABSTRACT

The present study aimed to determine the effects of maternal protein restriction on lipid metabolism of piglets at weaning and the associated underlying mechanism employing Meishan pigs as model. Sixteen Meishan sows were assigned to the control group and the maternal protein restriction group (MPR). The treated group was fed a low-protein diet containing 6% protein during pregnancy followed by 7% protein during lactation, whereas the control group received 12% and 14% protein during pregnancy and lactation, respectively. Blood and subcutaneous fat of piglets were sampled at weaning. The results showed that in MPR piglets' body weight and backfat thickness were significantly decreased compared to control piglets ($P<0.05$). Leptin mRNA expression was significantly down-regulated and the leptin content showed a decreased tendency ($P=0.10$) in subcutaneous fat of MPR group though the serum leptin concentration was unchanged. The lipolytic lipase activity (including Hormone sensitive lipase, HSL and adipose tissue triglyceride lipase, ATGL) in subcutaneous fat was significantly increased in MPR group ($P<0.05$). The fatty acid synthase (FAS) mRNA expression decreased significantly ($P<0.05$) although CCTTA enhancer-binding protein (C/EBP-β) and peroxisome proliferators-activated receptor γ (PPAR-γ) mRNA expression showed no obviously changes in MPR group compared with the control. Furthermore, though no alteration was detected for total perilipin protein level, the mRNA expression of perilipin was significantly decreased and the phosphorylation level of perilipin protein demonstrated an increased tendency ($P=0.09$) in the MPR group. The present study indicates that the lower lipid deposition in piglets of maternal protein restriction group at weaning may be mediated by the increased lipolysis in the subcutaneous fat demonstrated by increased lipolytic lipase activity and higher perilipin phosphorylation level. These alterations may influence the lipid metabolism of later life.

1. Introduction

Extensive epidemiological and experimental evidence demonstrate that nutritional factors exerting their influence during embryonic development or infancy may have the potential to induce a variety of chronic diseases (such as diabetes and obesity) later in life. This phenomenon is called "metabolic programming" (Ravelli et al., 1999; Petry and Hales, 2000; Zambrano et al., 2006; Metges, 2009). Though a few studies showed that malnutrition during pregnancy may program adult obesity of offspring (Valdez et al., 1994; Ravelli et al., 1999; Langley-Evans, 2006), the influence of maternal protein restriction on offspring lipid metabolism is still on debate (Erhuma et al., 2007; Cheim et al., 2009). Furthermore, the majority of such investigations on offspring's lipid metabolism are restricted to the adulthood period (Erhuma et al., 2007; Cheim et al., 2009; Zhang et al., 2007; Bol et al., 2008; Fagundes et al., 2009) whereas there are relatively few studies focusing on the changes at early period which is just a

* Corresponding author. Tel.: +86 2584395047; fax: +86 2584398669.
E-mail address: xiaojingyang2000@yahoo.com.cn (X. Yang).

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doi:10.1016/j.livsci.2010.08.013

crucial time for determining long-term weight gain and obesity (McMillen et al., 2005; Holzhauer et al., 2009).

Most of triacylglycerols (TAG) are deposited in lipid droplets of adipocytes. Investigations over the past decade revealed lipid droplets as regulated organelles with surprising complexity. They are coated by specific proteins, belonging to the so-called PAT family named after the three initially identified members perilipin (PLIN1), adipocyte differentiation-related protein (ADRP), and tail-interacting protein of 47 kDa (TIP47) (Tansey et al., 2003; Wolins et al., 2005; Bickel et al., 2009). The best characterized family member is perilipin which has been shown to play a crucial role both in lipid storage and in lipolysis (Moore et al., 2005; Miyoshi et al., 2008). At basal state, perilipin surrounds the lipid droplet to block the access of intracellular lipases to the lipids. However, once phosphorylated, it triggers a massive remodeling of the lipid droplets by increasing the surface area available to lipases and it assists the hormone sensitive lipase (HSL) in gaining access to lipid substrates (Greenberg et al., 1991; Egan et al., 1992), and promotes the activity of the adipose tissue triglyceride lipase (ATGL). Although perilipin is thoroughly investigated in rats, there are only few reports in pigs (Li et al., 2008; Tao et al., 2008).

Leptin, a circulating hormone secreted mainly by adipose tissue, is involved in the control of body fat stores through coordinated regulation of feeding behavior, metabolism, and body energy balance (Barb et al., 1998; Harris, 2000). It has been documented that programming of leptin concentrations by early diet may be one mechanism that links early nutrition with later obesity (Vickers et al., 2005; Singhal et al., 2002). A postnatal surge in plasma leptin occurs in rodents during the suckling period and might serve as a key developmental signal to the hypothalamus to exert influence over subsequent food intake and body weight throughout life (Ahima et al., 1998; Delahaye et al., 2008). However, these studies are focused on the rats, yet the leptin content in the piglets treated by maternal protein restriction and its role in the lipid metabolism regulation are still largely unknown.

The Meishan pig is a Chinese indigenous pig breed known for its high reproductive capacity and good meat quality (Bidane et al., 1993). However, purebred Meishan pigs are not widespread in the market as a result of their low growth rate and abundance of fat. In the present study we employed the Meishan pig as a model to investigate the effect of maternal protein restriction on the lipid metabolism in the piglets at weaning and the associated underlying mechanism by analyzing leptin content, lipase activities, and the perilipin protein level, as well as the gene expression of lipid regulating factors in subcutaneous fat. The aim of present study was to clarify the lipid metabolism changes of offspring piglets immediately after the maternal protein restriction for elucidating possible programming pathways on the lipid metabolism in the offspring.

2. Materials and methods

2.1. Animals and sample collection

Sixteen purebred, first parity Meishan sows were randomly assigned to two groups (7 to the control and 9 to the treatment group). Pregnant dams were fed a low-protein (6% protein) or an isocaloric control (12% protein) diet. During lactation, a low-protein (7% protein) or an isocaloric control (14% protein) diet was provided. The diet composition is shown in Table 1. Sows were housed in individual pens, fed feed twice daily (0800 and 1400 h) with the rations of 1.8 kg/day and 2.6 kg/day during gestation and lactation respectively, and had free access to water. Litter size was adjusted to 7 to 8 pigs per litter at 24 h post farrowing. Sows and piglets were maintained under identical feeding conditions. Routine farm management procedures were followed. For consistency, only male offspring were used for the study. The piglets were weaned and weighed at day 35. Then the male piglet which was closest to the average weight in the litter was selected and immediately sacrificed for tissue sampling just at day 35. The serum was collected and the backfat depth over the first rib, last rib, and last lumbar vertebrae were measured before sampling. The subcutaneous fat was taken within 5 to 10 min after death and rapidly frozen in dry ice, then stored at −80 °C until use.

The experiment was carried out following the guidelines of the regional Animal Ethics Committee.

2.2. Radioimmunoassay for leptin content

Leptin concentrations in serum and subcutaneous fat were measured with commercial multispecies RIA kits purchased from 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.

2.3. Lipase (including HSL and ATGL) activity assay

A modification of the procedure of Zhang et al. (2008) was used to extract and assay the two enzymes. Briefly,

Table 1
Composition of the diets and nutrient content.

	Pregnancy period		Lactation period	
	Control	Maternal protein restriction	Control	Maternal protein restriction
Corn	58	52.8	61	55.8
Soybean meal	12	0	17	0
Bran	15	11	12	15
Bone meal	1	0.5	1	0.5
Corn sugar	10	27	5	22
$CaHPO_4$	0	0.7	0	0.7
Fibre [1]	0	1	0	1
Attapulgite	0	3	0	1
Premix [2]	4	4	4	4
Calculated composition				
Digestible energy (MJ/kg)	13.1	13.1	13.1	13.0
Crude protein (%)	12.1	6.1	14.0	6.9
Crude fiber (%)	2.7	2.3	2.8	2.6
Lysine (%)	0.22	0.60	0.26	0.71
Methionine and cystine (%)	0.25	0.45	0.28	0.51
Ca (%)	1.2	1.2	1.2	1.2
P (%)	0.4	0.4	0.4	0.4

[1] The fiber concentrate ARBOCEL® was purchased from JRS (Germany).
[2] The premix contains (per kg of diet): vitamin A: 240,000 IU; vitamin D3: 6000 IU; vitamin E: 720 IU; vitamin K3: 30 mg; vitamin B1: 30 mg; vitamin B2: 120 mg; vitamin B6: 60 mg; vitamin B12: 360 mg; niacin: 66 mg; pantothenic acid: 600 mg; folic acid: 6 mg; manganese sulphate: 1.0 g; zinc oxide: 2.5 g; iron sulphate: 4 g; copper sulphate: 4 mg; selenuim: 6 mg; calcium: 150 g; phosphorus: 15 g; sodium chloride: 40 g; lysine: 30 g.