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Sexually dimorphic effects of maternal dietary protein restriction on fetal growth and placental expression of 11β-HSD2 in the pig

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ABSTRACT

Placental 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) inactivates glucocorticoids (GCs) to protect fetuses from over-exposure to maternal GCs, yet how maternal malnutrition affects placental 11β-HSD2 expression is unknown. In this study, Meishan sows were fed standard-protein (SP) or low-protein (LP, 50% of SP) diets and fetuses/newborn piglets were weighed and the corresponding placenta and umbilical cord blood were collected on gestational day 70 and the day of parturition. Significant growth retardation was observed in female, but not male, fetuses ($P<0.05$) and the newborns ($P<0.01$) of the LP group, which was accompanied by sexually dimorphic expression of 11β-HSD2 in placentas. Female fetuses in LP group showed significant decrease in placental 11β-HSD2 protein content ($P<0.05$) and enzyme activity ($P<0.05$), whereas male fetuses demonstrated significantly enhanced placental 11β-HSD2 activity ($P<0.05$). Serum cortisol levels were significantly higher ($P<0.05$) in male piglets compared to females, and the effects of maternal protein restriction on thyroid hormones (T3 and T4) in the umbilical cord blood were also sex dimorphic. Male piglets in LP group had significantly higher T3 ($P<0.01$) and lower T4 ($P<0.01$), whereas female piglets showed significantly lower T4 ($P<0.01$) with no change in T3. As a result, male piglets in LP group exhibited significantly higher T3/T4 ratio compared to female counterparts. These results indicate that the effects of maternal protein restriction on placental 11β-HSD2 expression are gender-dependent in the pig, and thyroid hormones may be involved in such effects.

1. Introduction

The interface between the mother and fetus, the placenta, is pivotal for the optimal fetal growth and development of livestock. Maternal undernutrition or malnutrition may disrupt placental function (Bloomfield et al., 2013) and thereby increase the risk for intrauterine growth retardation (IUGR) (Fowden et al., 2008; Wu et al., 2004). Placental 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol to inactive cortisone to protect the fetus from over-exposure to maternal glucocorticoids (GCs) (Seckl and Holmes, 2007). Maternal low-protein diet attenuates the expression and/or the activity of placental 11β-HSD2 in rats (Bertram et al., 2001; Langley-Evans et al., 1996; Stocker et al., 2004), and low placental 11β-HSD2 activity in rats and humans

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is associated with smaller fetuses (Seckl and Meaney, 2006). Maternal undernutrition in rats increases the maternal and/or fetal corticosterone concentration (Blondeau et al., 2001; Lesage et al., 2001), which is associated with decreased placental 11β-HSD2 mRNA expression (Lesage et al., 2001). Therefore, it is presumed that elevated maternal or fetal cortisol (or corticosterone in rodents) levels mediate the down-regulation of placental 11β-HSD2 caused by maternal nutritional deficiency (Cottrell and Seckl, 2009; Reynolds, 2010). However, not all the reports support this. Maternal undernutrition during gestation decreases expression and/or activity of placental 11β-HSD2 in sheep (Gnanalingham et al., 2007; McMullen et al., 2004; Whorwood et al., 2001) and in this study was accompanied by significantly lower cortisol:cortisone ratio in the fetal plasma (McMullen et al., 2004). In human and baboon, GCs were reported to increase, rather than to decrease, the 11β-HSD2 expression and/or activity *in vivo* (Clifton et al., 2006; Ma et al., 2003) and *in vitro* (van Beek et al., 2004). These inconsistent findings may be attributed, to some extent, to the striking differences among species in placenta structure. Porcine placenta is classified as epitheliochorial, while human and rodent placentas are haemochorial (Yang, 1997). Up to date, studies concerning the effects of maternal nutrition on placental 11β-HSD2 expression in pigs are scarce. The only report so far is that maternal protein restriction-induced IUGR was not associated with alterations in placental 11β-HSD2 expression and activity in 94-day-old German Landrace pig fetuses (Kanitz et al., 2012).

Several lines of evidence suggest that maternal low-protein diet during pregnancy leads to sexually dimorphic IUGR and developmental programming of adult diseases, such as hypertension, type 2 diabetes and obesity (Goyal and Longo, 2013; Moritz et al., 2010). In rats a maternal low-protein diet during pregnancy also reported to markedly reduce the birth weight of females but not males (Zambrano et al., 2006). It is presumed that placentas of male and female fetuses might differentially respond to the maternal low-protein diet (Rosenfeld, 2012). However, direct experimental evidence is lacking as to whether maternal dietary protein restriction affects placental gene expression and function in a sexually dimorphic manner.

Thyroid hormones play important roles in the regulation of fetal growth (Shields et al., 2011). Sows subjected to protein deprivation during pregnancy produced offspring of low birth weight with consistently lower thyroid hormone levels (Atinmo et al., 1978). Tri-iodothyronine (T3) is reported to markedly inhibit 11β-HSD2 expression and activity in the liver and pituitary, but not kidney and distal colon, of rats in vivo, suggesting that the action is tissue-specific (Whorwood et al., 1993). So far, it remains unclear whether thyroid hormones are involved in the effects of maternal dietary protein restriction on placental 11β-HSD2 activity in pigs.

In this study, we used primiparous purebred Meishan sows to investigate the effects of maternal protein restriction on fetal growth and placental 11β-HSD2 expression and activity. Hormones, including cortisol, leptin and thyroid hormones, as well as their receptors, were also determined to investigate the possible involvement of these hormones.

Table 1
Composition and nutrient content of experimental diets.

	SP	LP
Ingredient (%)		
Corn	58.0	52.8
Soybean meal	12.0	0.0
Bran	15.0	11.0
Bone meal	1.0	0.5
Corn sugar	10.0	27.0
$CaSPO_4$	0.0	0.7
Fiber[a]	1.0	1.0
Attapulgite	0.0	3.0
Premix[b]	4.0	4.0
Nutritional composition		
Digestible energy (MJ/kg)	13.1	13.1
Crude protein (%)	12.1	6.1
Crude fiber (%)	2.7	2.3
Calcium (%)	1.2	1.2
Phosphorous (%)	0.4	0.4

[a] The fiber concentrate ARBOCEL® was purchased from JRS (Germany).

[b] The premix contains (per kilogram): vitamin A: 240,000 IU; vitamin D3: 60,000 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: 600 mg; pantothenic acid: 300 mg; folic acid: 6 mg; manganese sulfate: 1.0 g; zinc oxide: 2.5 g; iron sulfate: 4 g; copper sulfate: 4 g; sodium selenite: 6 mg; calcium: 150 g; phosphorus: 15 g; sodium chloride: 40 g.

2. Materials and methods

2.1. Animals and placenta sampling

Animal experiments were carried out in the National Meishan Pig Preservation and Breeding Farm at Jiangsu Polytechnic College of Agriculture and Forestry, Jurong, Jiangsu Province, China. Twenty-three primiparous purebred Meishan gilts (body weight: 36.1 ± 1.8 kg) were assigned randomly into standard protein (SP, $n = 11$) and low protein (LP, $n = 12$) diet groups. The composition of the diets is shown in Table 1. One month prior to artificial insemination, sows of SP group were fed a diet containing 12.1% crude protein, while LP sows were fed a diet containing 6.1% crude protein, twice daily (0800 and 1400 h) with the ration of 1.8 kg/day throughout gestation. Seven (3 from the SP, 4 from the LP) sows were sacrificed on 70 days of gestation; the uteri were removed immediately for further processing (Vonnahme and Ford, 2004). The uterine horn was opened over each conceptus along the antimesometrial side. Fetuses were removed from the uterine horn and weighed. Each fetus and the corresponding placenta were carefully marked. The fetuses of both genders close to the average fetal weight of the litter were considered to be representative and their corresponding placentas were selected for further analysis. A small portion of placenta surrounding the umbilical cord was collected from each conceptus and snap-frozen in liquid nitrogen, then stored at −80 °C until further analysis. The ends of each placenta were fixed with dissection pins placed in the uterine wall, and each placenta was peeled from the endometrium and weighed. To obtain a measure of placental efficiency, the fetus weight was divided by the weight of its placenta. The remaining sows (8 for each group) were used for blood