Domestic Animal Endocrinology 58 (2017) 63–75

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Moderate high or low maternal protein diets change gene expression but not the phenotype of skeletal muscle from porcine fetuses

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 3 August 2016
Accepted 5 August 2016

Keywords:
Fetus
Insulin-like growth factor
Maternal protein intake
Myogenic regulatory factors
Skeletal muscle

ABSTRACT

The aim of our study was to characterize the immediate phenotypic and adaptive regulatory responses of fetuses to different in utero conditions reflecting inadequate maternal protein supply during gestation. The gilts fed high- (250% above control) or low- (50% under control) protein diets isoenergetically adjusted at the expense of carbohydrates from the day of insemination until the fetuses were collected at day 64 or 94 of gestation. We analyzed body composition, histomorphology, biochemistry, and messenger RNA (mRNA) expression of fetal skeletal muscle. Both diets had only marginal effects on body composition and muscular cellularity of fetuses including an unchanged total number of myofibers. However, mRNA expression of myogenic regulatory factors (*MYOG*, *MRF4*, $P \leq 0.1$), IGF system (*IGF1*, *IGF1R*, $P \leq 0.05$) and myostatin antagonist *FST* ($P = 0.6$, in males only) was reduced in the fetal muscle exposed to a maternal low-protein diet. As a result of excess protein, *MYOD*, *MYOG*, *IGF1R*, and *IGFBP5* mRNA expression ($P \leq 0.05$) was upregulated in fetal muscle. Differences in muscular mRNA expression indicate in utero regulatory adaptive responses to maternal diet. Modulation of gene expression immediately contributes to the maintenance of an appropriate fetal phenotype that would be similar to that observed in the control fetuses. Moreover, we suggest that the modified gene expression in fetal skeletal muscle can be viewed as the origin of developmental muscular plasticity involved in the concept of fetal programming.

1. Introduction

During pregnancy, the developing fetus is completely dependent on the maternal organism for nutrient supply and removal of metabolic by-products. Thus, long-term inadequacy of the maternal nutrient supply may have serious consequences for fetal development and is a major factor contributing to intrauterine growth retardation (IUGR; reviewed in [1]). Moreover, inadequate maternal nutrition may impair postnatal animal physiology and health in a process known as fetal programming [2,3]. In farm animals, changes in animal performance are of particular interest [4]; fetal programming may explain postnatal growth retardation and has associated

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http://dx.doi.org/10.1016/j.domaniend.2016.08.003

far-reaching implications for animal sciences. Furthermore, the ability of a given genotype to exhibit alternative structures, physiological states, or behaviors in response to environmental conditions is defined as developmental plasticity [5]. Programming or plasticity is associated with epigenetic alterations such as DNA methylation, histone modification, chromatin remodeling, and action of non-coding RNA (reviewed in [6]), all of which act to modulate gene expression.

Skeletal muscle tissue comprises approximately 50% of body mass in pigs and forms the basis of meat. It has been shown that fetal myogenesis is an important process for postnatal skeletal muscle growth and development in farm animals, and by extension, for the meat quality of pigs at market weight [7–9].

Previous studies of pigs exposed to a severe (0%–0.5%) or a moderate (8.5%) protein deficit either during selected periods or throughout gestation found decreased fetal growth in the offspring [10–12]. Furthermore, protein-free maternal diets resulted in changes in the body composition of the offspring [13–16]. However, detailed investigations of the influence of maternal protein supply on skeletal muscle development are scarce. In addition, studies in pigs exposed to excessive protein intake throughout gestation or at defined periods of gestation are lacking. We previously reported that both moderate low (6.5%) and high (30%) maternal protein diets led to lowered birth weights due to IUGR [17], but only the offspring exposed to a maternal low-protein diet exhibited less myofibers [18]. The relationship between prenatal development in utero and postnatal phenotypic appearance of skeletal muscle has been insufficiently investigated. The aim of the present study was to determine the immediate effects of a moderate low-protein (6.5% crude protein or 50% of requirement) or a high-protein (30% crude protein or 250% of requirement) diet fed to gilts during gestation on BW and composition as well as cellular properties of skeletal muscle in fetuses at day 64 and 94 of gestation. Both fetal ages analyzed in our study are of special interest for porcine skeletal muscle development and reflect the formation of primary myofibers (until day 60 of gestation) and of secondary fibers (until day 90 of gestation), as described by Wigmore and Stickland [19].

We tested 2 hypotheses: (1) that limited or excess maternal protein nutrition impairs fetal growth and skeletal muscle development, as assessed by IUGR, and (2) that programming or plasticity resulting from maternal nutrition leads to the modified expression of genes associated with myogenesis and skeletal muscle metabolism.

2. Materials and methods

All procedures involving animal handling and treatment were in accordance with the German animal protection law and were approved by the Committee on Animal Care and Use of the Agricultural Department of Mecklenburg-Vorpommern, Germany; LVL M-V/TSD/7221.3-1.1-006/04; LALLF M-V/TSD/7221.3-1.2-05/06; LALLF M-V/TSD/7221.3-1.2-013/06.

2.1. Animals and experimental procedures

A total of 56 German Landrace gilts, bred and raised in the institute's pig breeding facility, were used. The experiment included 5 successive replicates of 1 to 3 gilts per diet. Housing and breeding management have been described in detail by Rehfeldt et al [20]. Briefly, 1 day before the first insemination, gilts were randomly allocated into dietary groups. Beginning on the day of the first insemination, gilts were fed isoenergetic corn barley and soybean meal diets (∼13.7 MJ ME/kg) containing an adequate (AP, 12.1%; n = 18), high (HP, 30%; n = 19) or low (LP, 6.5%; n = 19) crude protein level throughout gestation. The adequate protein level corresponded to the recommendations for gilts, and the AP diet was used as a control in our experiment. In the AP, HP, and LP diets, the protein to carbohydrate ratio was 1:5, 1:1.3, and 1:10.4, respectively. Diets were fed between 2.3 and 2.9 kg/d to achieve an average target energy intake of ∼34 MJ ME/d during gestation, following the recommendations for primiparous sows [21]. The gilts were fed twice daily, and water was provided ad libitum.

2.2. Fetus and tissue collection

On days postconception (dpc) 64 and 94, fetuses from 28 (AP = 9; HP = 9; and LP = 10) and 28 (AP = 9; HP = 10; and LP = 9) gilts, respectively, were collected by Caesarean section lege artis under general anesthesia by the intravenous application of azaperone (0.05-mL/kg BW, Stresnil, Janssen-Cilag GmbH, Neuss, Germany) and ketamine (0.15-mL/kg BW, Ursotamin, Serumwerk Bernburg AG, Bernburg, Germany). Fetuses were removed and immediately euthanized by an intracardial injection of T61 (200-mg embutramide, 50-mg mebezonium iodide, and 5-mg tetracaine hydrochloride per 10-kg BW, Intervet, Unterschleissheim, Germany). The BW and crown-rump length of all fetuses were recorded (number of fetuses at dpc 64: AP = 115, HP = 112, and LP = 124; dpc 94: AP = 114, HP = 117, and LP = 108). From a subset of fetuses (dpc 64: AP = 54, HP = 54, and LP = 59; dpc 94: AP = 54, HP = 60, and LP = 54), skeletal muscles (*M. longissimus*–LM, *M. semitendinosus*–ST, and *M. psoas major*–PM) were quickly dissected, immediately snap-frozen in liquid nitrogen, and stored at −80°C until biochemical and gene expression analyses. The weight, length, and circumference of the ST as standard muscle for the determination of total fiber number (TFN) in pigs [7,22] were recorded. A slice of the muscle midbelly was mounted on a cork chuck and frozen in isopentane cooled with liquid nitrogen. Not all analyses could be done in ST muscle due to its limited size in fetuses (histomorphological analyses–ST, gene expression–LM, biochemical analyses–ST and LM [and in parts in PM]). Both, LM and ST are large, mixed muscles with similar metabolic and contractile properties and form economically important meat cuts. From the same fetuses, selected organs (brain, heart, kidneys, spleen, lungs, and liver) were weighed. The remaining fetuses were used to analyze body composition (dpc 64: AP = 61, HP = 58, and LP = 65; dpc 94: AP = 30, HP = 27, and LP = 27).