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Impact of diet deprivation and subsequent over-allowance of gestating sows on mammary gland and skeletal muscle development of their offspring at puberty

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ABSTRACT

The impacts of diet deprivation and subsequent over-allowance during gestation on mammary development, skeletal muscle histochemistry and gene expression in mammary and muscle tissue of offspring at puberty were determined. Twenty three pubertal gilts (11 control, CTL, and 12 treated, TRT) were used. These gilts were born from sows that were reared under a conventional or an experimental dietary regimen during gestation. The experimental regimen provided 70% (restriction diet, RES) and 115% (over-allowance diet, OVER) of the protein and digestible energy contents provided by the conventional diet. The RES diet was given during the first 10 weeks of gestation followed by the OVER diet until farrowing. Female offspring from these dams were grown until puberty using standard commercial practices and were slaughtered at 212 ± 4 days of age. Mammary tissue and the semitendinosus (ST) muscle were collected. Weights of TRT gilts was less than those from CTL gilts at birth ($P < 0.05$) but were similar thereafter (until puberty, $P > 0.1$). Mammary composition and mammary expression for the genes *IGF1*, *IGF2*, *ODC1*, *PRLR-LF*, *STAT5A*, and *STAT5B* were not affected by treatment ($P > 0.1$). Maternal dietary regime during gestation did not influence skeletal muscle microstructure or relative mRNA abundance for *IGF1* and *IGF2* ($P > 0.1$). In conclusion, restricted feeding followed by overfeeding of gestating sows has no harmful effects on the development of skeletal muscle and mammary tissue of their female offspring up to puberty.

1. Introduction

Mammary development is a key factor determining potential sow milk yield and it is of interest to develop management and feeding strategies that will optimize mammary development in swine. One avenue which has received attention is the feeding of gestating sows. It was shown that a too high energy intake in late gestation has negative effects on mammary development (Head and Williams, 1991; Weldon et al., 1991), whereas protein intake has no effect (Kusina et al., 1999; Weldon et al., 1991). Restricted feeding followed by overfeeding of gestating sows in order to induce compensatory growth was also studied as a tool to improve mammary development and subsequent milk yield (Crenshaw et al., 1989; Farmer et al., 2014). Yet, the potential impact of such a treatment on the mammary gland of the offspring postnatally is not known. This is of importance since it was demonstrated that feeding of gestating rats (Hilakivi-Clarke et al., 1997), ewes (Van der Linden et al., 2009; Blair et al., 2010), and gilts (Farmer and Palin, 2008) can alter the development of mammary tissue from their

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offspring. Several studies in rats also showed that postnatal mammary expression of specific genes or proteins in the offspring can be affected by gestational feeding (Fernandez-Twinn et al., 2007; Kovacheva et al., 2009; Polanco et al., 2011; Zheng and Pan, 2011). Nutritional intervention during gestation can also alter development and/or gene expression patterns in skeletal muscle of pig offspring (McNamara et al., 2011; Rehfeldt et al., 2012a). The present study was therefore conducted as a corollary to a previous trial where gestating sows were fed a dietary regimen consisting of diet deprivation and subsequent over-allowance in order to potentially stimulate their mammary development (Farmer et al., 2014). In that earlier study, however, the dietary regimen had unfavorable effects on sow BW, backfat, mammary development and mammary gene expression at the end of gestation. The goals of the current project were to determine if such a feeding regimen in gestation has a long-lasting impact on development of skeletal muscle and mammary tissue of their female offspring at puberty.

2. Materials and methods

2.1. Animals and treatments

Animals were raised according to a recommended code of practice (Agriculture and Agri-Food Canada, 1993) and to the guidelines of the Canadian Council on Animal Care (1993). Procedures were approved by the institutional animal care committee. Twenty three pubertal gilts (11 control, CTL, and 12 treated, TRT) were used in the study. They were born from Yorkshire x Landrace sows that were fed either a conventional or an experimental dietary regimen during gestation. The experimental regimen was designed to restrict growth and then induce compensatory growth by providing 70% (restriction diet, RES) and 115% (over-allowance diet, OVER), respectively, of the crude protein (CP) and digestible energy (DE) contents provided by the conventional diet. The RES diet was given for 10 weeks starting at mating, followed by the OVER diet until farrowing, and they were fed at a rate of 2.27 kg/day until farrowing. These diets and their compositional analyses were previously described (Farmer et al., 2014). Sows in the Farmer et al. (2014) study were housed in individual gestation pens and were moved to farrowing crates on day 110 of gestation. They were fed a commercial lactation diet (3170 kcal/kg DE, 13.0% CP, 0.58% total lysine) in 2 equal meals at a rate of 1.81 kg/day on the day of farrowing, 2.72 kg/day on day 1, 4.08 kg/day on day 2, 5.90 kg/day on day 3, 7.71 kg/day on day 4, and then ad libitum for the remainder of lactation. Litter size was standardized to 11 ± 1 piglets (within treatment group) within 24 h of birth and piglets were weighed at birth and on days 7, 14 and 18.

After weaning (19 days), female pigs from each treatment (13 CTL and 13 TRT) were housed in separate 2.96×4.57 m pens with a 1.22 m four-hole feeder and free access to water. They were fed, consecutively, seven standard commercial diets for growing pigs (starting at 22.05% CP, 3653 kcal/kg DE and ending at 16.32% CP, 3530 kcal/kg DE) ad libitum (average amount fed per pig for each diet consecutively: 0.8, 18, 12, 50, 23, 57 and 136 kg) until 100 kg BW (approximately 147 days of age) and were weighed at 27, 67, 102 and 147 days of age. These gilt offsprings were then transferred to a gestation room and were housed 5 per pen of 3.20×2.44 m, including four individual feeding stalls. A boar was present at all times, and heat was checked twice daily. Age at first estrus was noted. Upon arrival in the gestation room, gilts were fed a 13% CP gestation diet ad libitum until they were slaughtered at 151.6 and 150.0 kg (SEM = 7.1) for CTL and TRT gilts, respectively (212 ± 4 days of age). Three gilts had not cycled by that time and were not slaughtered so that 11 CTL and 12 TRT gilts remained and were included in the present study. Gilts were weighed at 176 days of age and at slaughter. Gilts were never slaughtered on the first two days of standing heat.

At slaughter, mammary glands were excised from the abdominal wall and parenchymal tissue was obtained from the fourth anterior pair of teats for molecular biology measures. These parenchymal samples were immediately frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until measurements of the relative mRNA abundance for insulin-like growth factor 1 (*IGF1*), *IGF2*, the long form of the prolactin receptor (*PRLR-LF*), signal transducers and activators of transcription 5A (*STAT5A*), *STAT5B*, and ornithine decarboxylase (*ODC1*) were performed. The rest of the glands were stored at $-20\,^{\circ}C$ until dissection and analyses for tissue composition. The right ST muscle was removed as a whole, weighed, and the circumference at the muscle mid-belly was recorded. Samples from the central portion of the ST were taken for histological and gene expression analyses of *IGF1* and *IGF2* within 10 min postmortem, snap-frozen in liquid nitrogen and stored at $-80\,^{\circ}C$.

2.2. Total RNA extraction and cDNA synthesis

For the parenchymal tissue, total RNA was extracted and reverse-transcribed as previously described (Labrecque et al., 2009). Integrity and purity of extracted RNA was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). For the ST muscle tissue, total RNA was isolated using the RNeasy fibrous Mini Kit (Qiagen, Hilden, Germany), as recommended by the supplier. This procedure includes the removal of genomic DNA with RNase-free DNase. Extracted RNA was quantified with a NanoDrop instrument (Peqlab, Erlangen, Germany). Quality of all RNA samples was assessed by the A_{260}/A_{280} ratio and by denaturing agarose (1%) gel electrophoresis. Reverse transcription was carried out as previously reported (Kalbe et al., 2013) using a mixture (2:1) of random primer p $(dN)_6$ and anchored-oligo $(dT)_{18}$ primer (Roche, Mannheim, Germany).

2.3. qPCR analyses of studied genes

For the parenchymal tissue, the relative mRNA abundance of studied genes (*IGF1, IGF2, ODC1, PRLR-LF, STAT5A* and *STAT5B*) was determined using real-time qPCR amplifications. Primers were designed using the Primer Express software 3.0 (PE Applied BioSystems, Foster City, CA, USA). The description