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Hepatic phosphorylation status of serine/threonine kinase 1, mammalian target of rapamycin signaling proteins, and growth rate in Holstein heifer calves in response to maternal supply of methionine

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ABSTRACT

The study investigated whether methionine supply during late pregnancy is associated with liver mammalian target of rapamycin (MTOR) pathway phosphorylation, plasma biomarkers, and growth in heifer calves born to cows fed a control diet (CON) or the control diet plus ethylcellulose rumen-protected methionine (MET; 0.09% of dry matter intake) for the last 28 d prepartum. Calves were fed and managed similarly during the first 56 d of age. Plasma was harvested at birth and 2, 7, 21, 42, and 50 d of age and was used for biomarker profiling. Liver biopsies were harvested at 4, 14, 28, and 50 d of age and used for protein expression. Body weight, hip height, hip width, wither height, body length, rectal temperature, fecal score, and respiratory score were measured weekly. Starter intake was measured daily, and average daily gain was calculated during the first 8 wk of age. During the first 7 wk of age, compared with calves in the CON group, calves in the MET group had greater body weight, hip height, wither height, and average daily gain despite similar daily starter intake. Concentration of methionine in plasma was lower at birth but increased markedly at 2 and 7 d of age in MET calves. Plasma insulin, glucose, free fatty acids, and hydroxybutyrate did not differ. A greater ratio of phosphorylated α-serine/threonine kinase (AKT):total AKT protein expression was detected in MET calves, namely due to differences at 4 d of age. The phosphorylated MTOR:total MTOR ratio also was greater in MET calves due to differences at 28 and 50 d (8 d postweaning). The decrease in phosphorylated MTOR:total MTOR between 14 and 28 d in CON calves agreed with the increase in phosphorylated eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1):total EIF4EBP1 ratio during the same time frame. The overall expression of phosphorylated ribosomal protein S6 kinase B1 (RPS6KB1):total RPS6KB1 and phosphorylated eukaryotic translation elongation factor 2 (EEF2):total EEF2 was lower in MET calves. Regardless of methionine supply prepartum, there was an 11-fold temporal decrease from 4 to 50 d in phosphorylated AKT:total AKT. Similarly, regardless of methionine supply, there were overall decreases in phosphorylation ratios of AKT, MTOR, RPS6KB1, and eukaryotic translation initiation factor 2A (EIF2A) over time. Data provide evidence of a positive effect of methionine supply during the last month of pregnancy on rates of growth during the first 7 wk of age. Phosphorylation status of some components of the MTOR pathway in neonatal calf liver also was associated with greater maternal supply of methionine. Thus, the data suggest that molecular mechanisms in the liver might be programmed by supply of methionine during late pregnancy. The exact mechanisms coordinating the observed responses remain to be determined.
Key words: ruminant, amino acid, gluconeogenesis, insulin

INTRODUCTION

A key adaptation that neonatal animals undergo is a shift in the reliance for energy on glucose, lactate, AA, and fatty acids in utero to relying on digestion of lactose to glucose, protein to AA, and fat to fatty acids via intake of colostrum and milk (Girard et al., 1992; Hammon et al., 2012). Unlike monogastrics, ruminants absorb little glucose from the gut, and both the pregnant mother and the fetus rely to various degrees on propionate and AA as gluconeogenic substrates (Stevenson et al., 1976; Prior and Christenson, 1977; Prior and Scott, 1977). In fact, a unique feature of the bovine fetal liver is the marked reliance on AA (e.g., alanine) and lactate (utilization of propionate is very low)

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for gluconeogenesis as early as 80 d of gestation and through term (Prior and Scott, 1977). Because voluntary feed intake of pregnant dairy cows could decrease markedly during the last 3 wk before parturition (Loor et al., 2013), the potential effect of prepartal nutrition of the cow on the rapidly growing fetus (Ferrell, 1991) cannot be underestimated. Therefore, the beneficial effect on calf birth weight reported often when the supply of dietary protein and energy prepartum is increased is not surprising (Funston et al., 2010).

Recent data have provided some evidence that enhancing micronutrient supply (methionine, Zn, Cu, Co, Mn) in pregnant dairy cows during the last month of gestation can induce changes in the transcriptome of the neonatal liver (Jacometo et al., 2016, 2017) and circulating leukocytes (Jacometo et al., 2015) without markedly altering growth rate during the preweaning period. Whether the prenatal supply of micronutrients alters protein expression of nutrient-responsive signaling pathways in organs of the bovine neonate is unknown. There is evidence that methyl donor supply during pregnancy in pigs alters the epigenetic status of hepatic gluconeogenic (Cai et al., 2014b) and lipid metabolism–related genes (Cai et al., 2014a, 2016) in 1-d-old piglets. At least in ruminants, it is unknown whether such effects persist during postnatal life and whether they are related to physiologic measures of rumen development and growth.

Mammalian target of rapamycin (**MTOR**) is a key protein kinase that connects nutritional and hormonal signals to downstream proteins involved in various processes, including protein synthesis. Underfeeding of beef cows during early gestation reduced the concentrations of phosphorylated MTOR in both maternal and fetal skeletal muscle (Du et al., 2005). Although no published studies demonstrate a response to individual AA on MTOR signaling in neonatal bovine liver, exogenous arginine in neonatal nursing pigs increased muscle and liver protein synthesis rate, but only muscle had greater phosphorylation of MTOR (Yao et al., 2008). An in vitro study with hepatocytes also demonstrated that, as long as insulin was present, methionine and leucine both increased phosphorylation of α-serine/threonine kinase (**AKT**) coupled with a decrease in mRNA expression of gluconeogenic genes (Lansard et al., 2011).

Newborn ruminants lack a functional rumen and in current production systems rely on high-fat and high-protein milk replacers for nourishment for the first weeks of life. They are gradually introduced to solid feed that is high in fiber while milk replacer is removed from the diet and are fully weaned at approximately 42 d of age (Hammon et al., 2012). Thus, not only does the profile of nutrients reaching tissues change early in life, but so do the endocrine environment (e.g., insulin, glucagon) and activity of various key hepatic enzymes (Hammon et al., 2012). In light of the unique physiologic adaptations that neonatal ruminants undergo, the present study aimed to evaluate whether maternal methionine supply was associated with hepatic metabolic gene transcription, MTOR signaling, and overall growth patterns during early life. To address our objective, we used the offspring from dairy cows that were supplemented or not with rumen-protected methionine during the last month of pregnancy (Batistel et al., 2017a).

MATERIALS AND METHODS

Animals and Experimental Design

All procedures for the current study were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the University of Illinois (protocol no. 14270). Details of the maternal treatments were described previously (Batistel et al., 2017b). Briefly, 60 multiparous Holstein cows were used in a complete block design with 30 cows per treatment. Cows were fed with a basal control (**CON**; 1.47 Mcal/kg of DM, 15.6% CP) diet with no addition of methionine or the basal diet plus ethylcellulose rumen-protected methionine (**MET**; Mepron, Evonik Nutrition & Care GmbH, Hanau Wolfgang, Germany) during the last 28 ± 2 d of pregnancy. The supplemental methionine was top-dressed on the total mixed diet at a rate of 0.09% of DMI. The methionine was supplied to achieve a lysine:methionine ratio in the MP reaching the small intestine close to 2.8:1. This rate has been demonstrated to elicit beneficial effects in terms of production performance and health (Osorio et al., 2013; Zhou et al., 2016b).

Because of their economic importance in terms of milk production, only a subset of heifer calves born to cows in the CON (n = 8) or MET (n = 8) groups was used (Alharthi et al., 2017). Body weight, hip and wither height, hip width, and body length were measured at birth. The navel was disinfected with a 7% tincture of iodine solution (First Priority Inc., Elgin, IL), and calves were vaccinated with TSV II (Pfizer Inc., New York, NY) via nostril application. All calves were housed and managed in the same fashion during the first 7 wk of life. Per standard operating procedures, calves received 3.8 L of first-milking colostrum within 6 h after birth. Calves were housed in individual outdoor hutches bedded with straw and fed twice daily (0700 and 1800 h) with a milk replacer (Advance Excelerate, Milk Specialties, Carpentersville, IL; 28.5% CP, 15% fat) until 35 d of age. They were then switched to once-daily feeding at 0700 h until weaning (42 d of