J. Dairy Sci. 97:383–391
http://dx.doi.org/10.3168/jds.2013-7042

Effects of level of nutrient intake and age on mammalian target of rapamycin, insulin, and insulin-like growth factor-1 gene network expression in skeletal muscle of young Holstein calves

P. Wang,*† J. K. Drackley,† J. A. Stamey-Lanier,†[1] D. Keisler,‡ and J. J. Loor†[2]
*College of Animal Science and Technology, Northwest A&F University, Yangling, 712100, China
†Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801
‡Department of Animal Sciences, University of Missouri, Columbia 65211

ABSTRACT

The molecular mechanisms by which level of nutrient intake enhances skeletal muscle growth in young ruminants are not fully understood. We examined mammalian target of rapamycin (mTOR), insulin, and insulin-like growth factor-1 (*IGF-1*) gene network expression in semitendinosus muscle tissue of young male Holstein calves fed a conventional milk replacer plus conventional starter (CON) or an enhanced milk replacer plus high-protein starter (ENH) for 5 wk followed by a conventional starter or a high-protein starter until 10 wk of age. Feeding ENH led to greater concentration of plasma IGF-1 and leptin and greater carcass protein and fat mass throughout the study. Despite the greater plasma IGF-1 and protein mass at wk 5, calves fed ENH had lower expression of *IGF1R*, *INSR*, and *RPS6KB1* but greater expression of *IRS1* and *PDPK1* in muscle tissue. Except for *IGF1R* expression, which did not differ at wk 10, these differences persisted at wk 10, suggesting a long-term effect of greater nutrient intake on physiological and molecular mechanisms. Components of mTOR complex (mTORC)1 and mTORC2 (*RICTOR* and *RPTOR*) and *FOXO1* expression decreased by wk 10 regardless of diet. Overall, the present data revealed that greater nutrient intake throughout the milk-fed and early postweaning phase alters body mass composition partly by altering hormonal and molecular profiles of genes associated with glucose and amino acid signaling. Those networks may play a crucial role in coordinating neonatal muscle growth and metabolism in response to level of nutrient intake.

Key words: growth, metabolism, dairy calf, protein synthesis

INTRODUCTION

Physiological and molecular studies primarily in nonruminants have provided evidence of higher growth rates in neonates attributed to the feeding-induced stimulation of protein synthesis in muscle via insulin- and AA-mediated signaling pathways (Davis and Fiorotto, 2009). The mammalian target of rapamycin (**mTOR**) signaling pathway, which is downstream of insulin and AA signaling, plays a central role in enhancing protein synthesis and cell growth (Wullschleger et al., 2006). Signaling through IGF-1 and the IGF-1 receptor also appears to play a role in skeletal muscle cell differentiation during the neonatal period (Dickson et al., 1991). Furthermore, hypertrophy of skeletal muscle myotubes is enhanced through the activation of the IGF-1–mTOR signaling pathways (Rommel et al., 2001).

Dietary protein ingestion affects the expression of growth hormone receptor (*GHR*) or *IGF1* in muscle (Brameld et al., 1996), and the concentration of both hormones in blood (Daniels et al., 2009). Therefore, the greater growth rate of dairy calves (Blome et al., 2003; Bartlett et al., 2006) fed higher-protein diets could involve not only hormonal changes but also alterations in mRNA expression of genes associated with mTOR, insulin, and IGF-1 signaling pathways. From a nutritional standpoint, however, alterations in expression of the negative feedback loop encompassing tuberous sclerosis 2 (**TSC2**)–mTOR–ribosome protein S6 kinase (**RPS6KB1**)–insulin receptor substrate 1 (**IRS1**) (Harrington et al., 2004; Um et al., 2006; Tremblay et al., 2007) are important. In humans, nutrient overload or obesity leads to altered insulin sensitivity (Um et al., 2006), with potential involvement of this feedback loop in coordinating such a response. Relatively little is known in livestock species about the expression pattern of mTOR, insulin, and IGF-1 signaling pathways and the feedback loop as it relates to level of nutrient intake and physiological state. Therefore, it is important to understand the molecular events whereby diet coordinates growth and metabolism.

Received May 17, 2013.
Accepted September 15, 2013.
[1]Current address: Prince Agri Products Inc., 229 Radio Road, Quincy, IL 62306.
[2]Corresponding author: jloor@illinois.edu

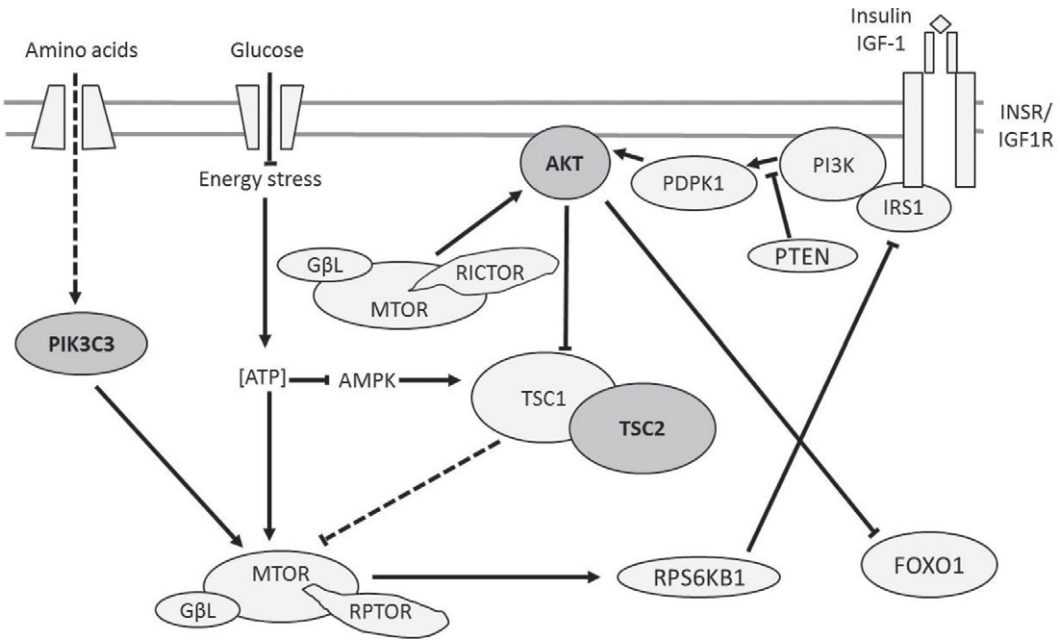

Figure 1. Model depicting the mammalian target of rapamycin (mTOR) signaling pathway in response to nutrients and growth factors. The mTOR complex (mTORC)1, including mTOR, regulatory-associated proteins of mTOR (RPTOR), and G protein β-subunit-like protein (GβL), plays central roles in modulating cell growth and metabolism in response to extracellular growth stimuli, including nutritional signals (amino acids and glucose) and growth factors. On one hand, the mTOR pathway responds to growth factors via the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway. Binding of insulin or IGF to their receptor (i.e., INSR or IGF1R) leads to recruitment and phosphorylation of insulin receptor substrate 1 (IRS-1) and subsequent recruitment of PI3K, and PI3K bound to IRS coverts phosphatidylinositol-4,5-phosphate (PIP2) in the cell membrane to phosphatidylinositol-3,4,5-phosphate (PIP3). Accumulation of PIP3 is antagonized by the lipid phosphatase phosphatase and tensin homolog (PTEN). PIP3 co-recruits PDK1 and Akt to the membrane, resulting in the phosphorylation and activation of Akt by PDK1. mTOR is wired to the PI3K pathway through tuberous sclerosis complex (TSC)1/TSC2 (Wullschleger et al., 2006). The TSC1/TSC2 complex acts as a negative modulator of mTORC1 relaying growth factors signals in addition to energy status. Energy stress conditions leads to activation of AMP-activated protein kinase (AMPK), which phosphorylates TSC2 (Inoki et al., 2003). The TSC1/TSC2 complex is also required for maintaining insulin signaling to PI3K by repressing a negative feedback loop from mTOR/S6K (also known as ribosomal protein S6 kinase beta-1, RPS6KB1) to the adaptor molecule IRS-1 (Harrington et al., 2004). On the other hand, AA seem to be the most crucial signals for mTORC1 activation (see review by Jewell and Guan, 2013). Nutrients such as AA and glucose increases hVps 34 (also known as phosphatidylinositol 3-kinase, catalytic subunit type 3, PIK3C3) activity, stimulating the production of PI3P; PI3P, in turn, recruits proteins containing FYVE or PX domains to endosomal membranes (see review by Um et al., 2006). Additionally, Akt seems to play a pivotal role in the regulation of skeletal muscle hypertrophy and atrophy in rodents and cells through mTOR and FOXO1 (Léger et al., 2006). Moreover, Akt is suggested to be a downstream of mTORC2, which contains mTOR, rapamycin-insensitive companion of mTOR (RICTOR), and GβL (Sarbassov et al., 2005).

Components of cell growth and metabolism related to the mTOR, insulin, and IGF-1 signaling pathways that were measured in this study are shown in Figure 1. The figure was developed primarily based on the review of Wullschleger et al. (2006). The main objective of this study was to examine the effect of level of nutrient intake (Naeem et al., 2012; Stamey et al., 2012) on expression of genes with relevance to the mTOR, insulin, and IGF-1 signaling pathways in skeletal muscle. Our general hypothesis was that both plane of nutrition and stage of growth would alter mRNA expression of genes associated with these pathways. Quantitative PCR (**qPCR**) was used as a tool for in-depth study of the relationships among the 15 genes chosen and hormones in response to different plane of nutrition from birth through the first week after weaning. The present data provide evidence of coordination between growth and metabolism when different levels of nutrition are provided to young calves.

MATERIALS AND METHODS

Experimental Animals and Sampling

Details of diets and general management have been published (Stamey et al., 2012). Briefly, male calves were fed 1 of 2 different diets: (1) control (**CON**), conventional milk replacer (**MR**; 20% CP, 20% fat, 4.95 Mcal/kg of DM) plus conventional starter (19.6% CP, 3.16 Mcal/kg of DM); and (2) enhanced (**ENH**) MR (28.5% CP, 15% fat, 4.72 Mcal/kg of DM) plus high-CP starter (25.5% CP, 3.21 Mcal/kg of DM). Conventional MR was reconstituted to 12.5% solids and fed at 1.25% of birth BW daily in 2 feedings from wk 1 to 5 and at