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Annu Rev Anim Biosci. Author manuscript; available in PMC 2020 February 20.

Published in final edited form as:

Annu Rev Anim Biosci. 2019 February 15; 7: 309–335. doi:10.1146/annurev-animal-020518-115130.

Regulation of Muscle Growth in Early Postnatal Life in a Swine Model

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Abstract

Skeletal muscle growth during the early postnatal period is rapid in the pig and dependent on the capacity of muscle to respond to anabolic and catabolic stimuli. Muscle mass is driven by the balance between protein synthesis and degradation. Among these processes, muscle protein synthesis in the piglet is exceptionally sensitive to the feeding-induced postprandial changes in insulin and amino acids, whereas muscle protein degradation is affected only during specific catabolic states. The developmental decline in the response of muscle to feeding is associated with changes in the signaling pathways located upstream and downstream of the mechanistic target of rapamycin protein complex. Additionally, muscle growth is supported by an accretion of nuclei derived from satellite cells. Activated satellite cells undergo proliferation, differentiation, and fusion with adjacent growing muscle fibers. Enhancing early muscle growth through modifying protein synthesis, degradation, and satellite cell activity is key to maximizing performance, productivity, and lifelong pig health.

Keywords

amino acids; insulin; mechanistic target of rapamycin; neonatal pig; satellite cell; skeletal muscle protein synthesis

INTRODUCTION

Growth rate during the neonatal period is greater than at any other stage of postnatal life. Although all body tissues contribute to growth during this period, the gain in protein mass in skeletal muscle exceeds most, if not all, other tissues. Carcass protein, composed primarily of muscle protein, increases eightfold from birth to weaning in the newborn pig, whereas visceral protein increases fivefold (1). Similarly, the contribution of muscle protein to whole-body protein in the rat increases from 30% at birth to 45% at weaning (2). Although whole-body growth across the life span of a pig is largely regulated by hormone status and nutrient supply, skeletal muscle is particularly sensitive to these factors during the neonatal period.

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Moreover, the rapid postnatal increase in muscle protein mass implies that dietary energy and protein are used efficiently for growth in the piglet.

The neonatal pig is also a suitable model organism for the study of pediatric nutrition and metabolism (3). Advances in our understanding of piglet feeding have dual benefits for agriculture and biomedical research. First, we can improve the efficiency of pig production in the pre- and immediate post-weaning periods. Second, we can improve the nutritional support of human infants, especially of low–birth weight and preterm infants, whose growth has been compromised.

This review reports recent findings on the regulation of skeletal muscle growth in the neonatal pig. The key mechanisms that regulate muscle protein synthesis and degradation are discussed, and the role of prenatal myogenesis and contribution of muscle satellite cells (SCs) to postnatal muscle growth are outlined.

SKELETAL MUSCLE PROTEIN ACCRETION IN THE NEONATAL PIG

Feeding Increases Protein Synthesis Through Elevated Postprandial Levels of Insulin and Amino Acids

Protein deposition occurs when the rate of protein synthesis exceeds the rate of degradation. In the newborn pig, feeding increases whole-body protein synthesis and decreases whole-body protein degradation (4). However, in skeletal muscle, feeding rapidly stimulates protein synthesis without affecting protein degradation (5, 6). The greater responsiveness of protein synthesis in skeletal muscle relative to other tissues suggests that unique mechanisms regulate the response of the immature muscle to feeding.

Circulating levels of insulin and amino acids (AAs) increase after a meal. These postprandial changes in both insulin and AAs are correlated with the increase in muscle protein synthesis rates in neonatal pigs (7). Moreover, it appears that a cyclical pattern in insulin and AA levels, produced when feeding complete meals several times daily, is necessary to optimize muscle protein synthesis and lean growth (8, 9). Studies using the pancreatic hormone-substrate clamp technique to elicit fasting, intermediate, and fed plasma levels of insulin and AAs have shown that insulin and AAs independently stimulate muscle protein synthesis in the neonatal pig (7, 10). Conversely, AAs but not insulin stimulate protein synthesis in most, but not all, visceral organs (10). The ability of insulin to increase protein synthesis in skeletal muscle appears to be unique to the immature muscle. Studies in adult humans have shown that insulin does not stimulate muscle protein synthesis, even when basal levels of AAs are maintained (11). Instead, insulin reduces muscle protein degradation (12). The ability of skeletal muscle in the neonate to respond to stimulation by both insulin and AAs contributes to the rapid gain in muscle mass during the neonatal period.

The mTORC1 Signaling Pathway Regulates Translation Initiation

The distinct signals from insulin and AAs to stimulate protein synthesis in skeletal muscle are integrated through the mechanistic target of rapamycin (mTOR), a serine/threonine protein kinase that forms the catalytic subunit of mTOR complex 1 (mTORC1; see Reference 13 for review) (Figure 1*a*). This complex is composed of several subunits that are

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required for mTORC1 translocation to the surface of the lysosome and its subsequent activation. Raptor is a critical component of mTORC1 that facilitates the recruitment of two key substrates, eukaryotic initiation factor (eIF)4E binding protein 1 (4E-BP1) and p70 ribosomal protein S6 kinase 1 (S6K1), to mTOR (14). The activation of mTORC1 by insulin and AAs promotes translation initiation, the rate-limiting step in protein synthesis, largely through the phosphorylation of 4E-BP1 and S6K1 (Figure 1*b*). The mTORC1 substrate 4E-BP1 binds to and sequesters eIF4E, preventing the formation of the active eIF4E · eIF4G complex required for recruiting messenger RNA (mRNA) to the 43S preinitiation complex (15). The inactivation of 4E-BP1 by mTORC1 releases eIF4E and increases the abundance of the active eIF4E · eIF4G complex. S6K1 regulates several additional targets involved in translation initiation and elongation (15). The phosphorylation of rpS6 (a component of the small ribosomal subunit), eIF4B (which enhances the RNA helicase activity of eIF4A), and eukaryotic elongation factor (eEF)2 kinase (which prevents the inactivation of eEF2) is dependent on S6K1 activation. Lastly, the recruitment of methionyl–transfer RNA (tRNA) to the ribosome requires GTP-bound eIF2 (15). The GTP loading state of eIF2 is mediated by eIF2B, whose guanine nucleotide exchange factor (GEF) activity is enhanced by growth factors and diminished by AA deprivation or cellular stress through its inactivation by phosphorylation of the α subunit of eIF2. In neonatal pigs, the phosphorylation of mTORC1 and its downstream targets S6K1 and 4E-BP1, and the abundance of the active eIF4E · eIF4G complex, is increased with feeding or when insulin and AAs are increased from fasting to fed levels (5, 16). Conversely, eEF2 and eIF2α phosphorylation are not affected by feeding, insulin, or AAs.

Growth Factors Regulate mTORC1 Signaling

Growth factors regulate mTORC1 signaling through inhibition of the tuberous sclerosis complex (TSC), a key negative regulator of mTORC1 signaling. TSC is a heterotrimeric complex composed of TSC1, TSC2, and TBC1D7 and functions as a GTPase activating protein (GAP) toward Rheb, a small GTPase and potent activator of mTOR (13, 17). Insulin and insulin-like growth factor (IGF)-I inhibit TSC through Akt-dependent phosphorylation of TSC2 on Thr1462 (18). Other growth factors, such as Wnt, can stimulate mTORC1 signaling through TSC independently of Akt by inhibiting glycogen synthase kinase-3β (GSK3β) (19), but the relative contribution of the Akt- and GSK3β-dependent pathways in muscle to enhance protein synthesis in the neonatal pig has not been determined. Negative control of mTORC1 activity is also exerted through TSC. For example, cellular energy stress is conveyed to mTORC1 through AMP-activated protein kinase (AMPK)-mediated phosphorylation of TSC2 on Thr1227 and Ser1345 (20).

Amino Acids Regulate mTORC1 Signaling

AAs facilitate mTORC1 recruitment to the lysosome, where it is activated by Rheb. Among all AAs, leucine (Leu) stimulates protein synthesis to the greatest extent. Cellular Leu concentration is dependent on its appearance from endogenous protein degradation, the plasma concentration, and its ability to enter cells through specific transporters. Leu transport into cells, therefore, is an essential step, upstream of mTORC1, in Leu-induced mTORC1 activation. The large neutral AA transporter 1 (LAT1) system, which imports Leu, and the alanine, serine, cysteine–preferring transporter 2 (ASCT2), which exports glutamine

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(Gln), is implicated in mediating mTORC1 activation (21). In some circumstances, the inhibition of the sodium-coupled neutral AA transporter 2 (SNAT2) depletes intracellular Gln levels and may prevent mTORC1 signaling, because Leu transport through LAT1 is dependent on Gln (21). In newborn pigs continuously fed a milk replacement formula with pulsatile Leu administration to simulate the rise in Leu after a meal, the abundance of LAT1 and SNAT2 in skeletal muscle was increased (22).

An important breakthrough in our understanding of mTORC1 regulation by AAs came with the discovery of Rag proteins. The Rag proteins RagA/B and RagC/D are heterodimeric proteins with GTPase activity (23). The mTORC1 component Raptor links Rag proteins to mTOR, and the binding of Rag proteins to Raptor is strongly dependent on GTP-bound RagA/B and GDP-bound RagC/D. Rag proteins are tethered to the lysosome through their association with the Ragulator complex, which functions as a GEF to facilitate the exchange of GDP with GTP upon AA stimulation (24).

The GTPase activity of Rag proteins is regulated by the GATOR complex. This complex is composed of two distinct proteins: GATOR1, a GAP toward RagA/B, and GATOR2, a negative regulator of GATOR1 (25). The protein complex KICSTOR is required for the association between GATOR1 and GATOR2 and between GATOR1 and the Rag proteins (26). An additional component of the Rag-Ragulator complex is vacuolar-type H^+-ATPase (v-ATPase), a lysosomal transmembrane protein (27). Upon AA stimulation, v-ATPase generates a proton gradient across the lysosomal membrane. The subsequent H^+-dependent efflux of AAs from the lysosomal lumen to the cytosol enhances the GEF activity of Ragulator toward Rag proteins. Recently, Gln has been reported to also stimulate mTORC1 signaling through a Rag-independent and v-ATPase-dependent mechanism (28).

Our understanding of AA sensing by mTORC1 was further advanced with the identification of Sestrin2 as a key sensor for intracellular Leu. Leu binds Sestrin2 and disrupts the Sestrin2–GATOR2 interaction, which leads to the inhibition of GATOR1 by GATOR2 and the subsequent activation of mTORC1 (29). In newborn pigs, the diminished association of GATOR2 with Sestrin2 parallels increased association of Rag proteins with mTOR upon AA stimulation (30). Leucyl-tRNA synthetase (LRS) has been reported as a second Leu sensor acting in parallel to Sestrin2 that potentiates the GTPase activity of RagD (31). It is unlikely that LRS plays a major role in mTORC1 activation in the neonatal pig because physiological conditions preclude the level of uncharged leucyl-tRNA that suppresses mTORC1 signaling (30).

Arginine (Arg) has been demonstrated to activate mTORC1 through the GATOR1/2-Rag pathway (32). Analogous to Sestrin2, CASTOR1 inhibits GATOR2, and this interaction is perturbed by Arg. Arg-mediated transport of Leu derived from lysosomal proteolysis through SLC38A9 located on the lysosomal membrane may facilitate mTORC1 activation (33). In the neonatal pig, it is more likely that mTORC1 activation by Arg is through CASTOR1 rather than SLC38A9, because the relative contribution of extracellular AAs to the muscle-free AA pool, particularly after feeding, is likely far higher than the lysosomal degradation of endogenous proteins.

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Although many of the components involved in AA signaling to mTORC1 have been identified in neonatal pig skeletal muscle (30), it is unclear if they retain the same functions in vivo as they have in vitro. However, in newborn pigs, feeding and AA stimulation alone elicit responses in these components consistent with their defined function in vitro (30). This is a compelling sign that their putative roles in the AA signaling pathway are intact.

Leucine, Its Metabolites α-KIC and HMB, and Arginine Mediate mTORC1 Signaling in the Neonatal Pig

The significance of Leu-mediated signaling through mTORC1 to promote translation initiation and protein synthesis in a live animal model was first established in adult rats (34). Subsequently, we showed that the physiological rise in Leu that occurs after a meal activates translation initiation and skeletal muscle protein synthesis in the neonatal pig (35, 36). It is almost certain that the Leu-induced activation of mTORC1 in the newborn pig occurs through the GATOR1/2-Rag pathway. Feeding increases the association of RagA and RagC with mTOR and lowers the association of Sestrin2 with GATOR2 (37), and the provision of AAs or Leu alone is able to mirror this response (22, 30).

The ability of Leu to sustain the feeding-induced stimulation of muscle protein synthesis is dependent on the availability of other AA substrates for protein synthesis (38, 39). Otherwise, protein synthesis declines as the free AA pool becomes depleted despite sustained anabolic signaling through mTORC1. Thus, when newborn pigs are fed a protein-restricted diet, Leu supplementation alone has a limited capacity to restore lean growth (40). Moreover, when newborn pigs are fed an energy- and protein-restricted diet, Leu supplementation does not improve body weight gain although mTORC1 activation is sustained, suggesting that low energy intake supersedes an anabolic effect of Leu (41, 42).

The Leu metabolites α-ketoisocaproic acid (α-KIC) and β-hydroxy-β-methylbutyrate (HMB) also have anabolic properties. The initial step in Leu catabolism is the reversible transamination of Leu to α-KIC by mitochondrial branched-chain AA transferase (BCATm), which is primarily expressed in skeletal muscle. Skeletal muscle has high BCATm activity but low activity of other enzymes required for subsequent α-KIC oxidation. As a result, α-KIC is released from muscle to be further metabolized in other tissues (43). The vast majority of α-KIC is catabolized to isovaleryl-CoA by mitochondrial branched-chain α-ketoacid dehydrogenase in the liver, whereas α-KIC catabolism to HMB by cytosolic KIC dioxygenase (4-hydroxyphenylpyruvate dioxygenase) accounts for only 5–10% of total Leu intake (44). In neonatal pigs, α-KIC increases anabolic signaling and muscle protein synthesis to a similar extent as Leu (45). This response is likely due to reamination of α-KIC to Leu, as both plasma and muscle intracellular Leu concentrations are elevated following α-KIC administration. Similarly, parenteral or enteral administration of HMB at a dose similar to that used in human studies (46) augments muscle protein synthesis in neonatal pigs (47, 48). These changes in protein synthesis occur without affecting Leu or α-KIC levels but are still paralleled by changes in the phosphorylation of S6K1 and 4E-BP1 and the abundance of the eIF4E · eIF4G complex.

Despite clear increases in the activation of the mTORC1 signaling pathway in pigs, it is unknown how HMB signals to mTORC1. The phosphorylation of Akt and AMPK, and the

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association of RagA with mTOR, are not affected by HMB in neonatal pigs (A. Suryawan & T.A. Davis, unpublished data). There is some evidence to suggest that HMB reduces the phosphorylation of eIF2α and eEF2 in cachectic mice, thus promoting translation initiation and elongation (49), but this mechanism does not appear to be present in healthy newborn pigs (48).

Arg signals to mTORC1 in a GATOR1/2-Rag-dependent manner (32), but this AA may have other benefits in addition to enhancing mTORC1 activation in the newborn pig. Although neonatal piglets can synthesize Arg from citrulline and proline, there is an upper limit to its synthesis (50). Supplementing Arg to piglets improves growth, increases plasma insulin, and reduces plasma urea and ammonia levels (51). Arg and N-carbamylglutamate, an analog of endogenous N-acetylglutamate and activator of Arg synthesis, enhance mTORC1 activation and protein synthesis in skeletal muscle of neonatal pigs, leading to higher body weight gain (52, 53). Similarly, supplementing Arg to lactating sows increases AA content in milk and improves piglet growth, mainly during the first week of postnatal life when piglet sensitivity to AAs is highest (54). These findings suggest that Arg is a limiting AA in sow milk, and Arg supplementation to piglets increases its supply as a substrate and signal for protein synthesis.

Direct and Indirect Effects of Colostrum and Milk Intake on Skeletal Muscle Growth

Colostrum and milk intake in neonatal pigs is essential for optimal whole-body and skeletal muscle growth. Mammary secretions are a complex fluid containing carbohydrates, fats, proteins, vitamins, minerals, and various biologically active factors (55). Colostrum in particular contains several components that benefit the newborn pig. These include peptide growth factors that stimulate tissue growth, especially that of gastrointestinal and liver tissues, and immunoglobulins that confer passive immunity. The early growth and maturation of the gut have direct implications for skeletal muscle growth because the efficient digestion and absorption of milk nutrients by the gut enables a robust postprandial insulin response and facilitates nutrient use for protein synthesis. Among these growth factors, insulin and IGF-I are more concentrated in colostrum than mature milk but are not present in formula. Both insulin and IGF-I have been reported to be absorbed from the gut intact, but the contribution of colostrum-derived insulin to stimulating muscle protein synthesis is minimal compared with the feeding-induced increase in insulin (56). Greater plasma IGF-I following colostrum intake, however, may sustain muscle protein synthesis for a longer period compared with an equivalent intake of formula (56). The increase in muscle protein synthesis following colostrum intake is almost entirely restricted to the synthesis of myofibrillar proteins (57).

Skeletal Muscle Protein Degradation

Because skeletal muscle protein deposition can occur only when protein synthesis exceeds protein degradation, it is necessary to delineate the contribution of protein degradation to muscle growth in the newborn pig. The three major protein degradation pathways in skeletal muscle are the calpain, autophagy-lysosome, and ubiquitin (Ub)-proteasome pathways. The autophagy-lysosome system is responsible for the degradation of cytosolic and membrane proteins and organelles, whereas the calpain and Ub-proteasome systems mediate the

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degradation of the majority of muscle (i.e., sarcomeric) proteins (58). In the case of sarcomeric proteins, the rate-limiting step in their degradation is their calpain-mediated dissociation from the intact myofibril, which must occur before they are conjugated with Ub and degraded by the proteasome (59). The key enzymes in the conjugation process are the E3 Ub protein ligases. The main muscle-specific Ub protein ligases atrogin-1 and muscle ring finger protein 1 (MuRF1) were first identified in rats (60) and were later recognized as key determinants of muscle atrophy in numerous catabolic states, including muscle unloading, nutrient deprivation, glucocorticoid administration, and sepsis (61). Both the autophagy-lysosome and Ub-proteasome systems are regulated in part by the insulin/PI3K/Akt signaling pathway through the forkhead box protein O (FoxO) transcription factor family. Insulin signaling promotes Akt activation and subsequent phosphorylation of FoxO proteins (62). This prevents FoxO protein translocation to the nucleus, where they function to increase the expression of atrogin-1 and MuRF1 as well as several autophagy-related genes.

The relative contributions of the autophagy-lysosome and Ub-proteasome systems to total skeletal muscle protein degradation in the neonatal pig are unclear. Despite no change in protein degradation across the hind limb of piglets with feeding (measured as in vivo net AA release), the muscle microtubule-associated protein light chain 3 (LC3)-2 to total LC3 ratio, a putative index of autophagic activity, is diminished (6). The LC3–2 to total LC3 ratio is in fact consistently reduced after feeding in pigs, mirroring the increase in mTORC1 activity (40, 63). The phosphorylation of ULK1 by mTORC1 is required for autophagosome biogenesis and is enhanced during postprandial insulin and AA levels (64). However, the abundance of atrogin-1 and MuRF1 does not appear to be affected by feeding (6, 40) or by insulin and AAs (64) in neonatal pigs. This is consistent with the established role of these proteins as agonists of proteolysis during specific atrophic conditions. Discrepancies between markers of protein degradation and actual rates of protein degradation underline the importance of measuring, and developing new and straightforward methods to measure, absolute rates of protein degradation in skeletal muscle, particularly in catabolic states when proteolysis is known to increase (65–67). It is important that changes in protein degradation signaling and absolute whole-body or muscle protein degradation rates are reconciled. However, few studies in any species have directly compared measurements of protein degradation rates to protein degradation signaling.

Negative Regulators of Muscle Protein Mass

Skeletal muscle protein synthesis in the newborn pig is remarkably sensitive to positive regulation by insulin and AAs through the mTORC1 signaling pathway. Not surprisingly, this signaling pathway is also subject to negative regulation. A key negative regulator of muscle protein mass is myostatin, a myokine belonging to the transforming growth factor (TGF)-β family. Mutations in the *MSTN* gene locus or therapeutic inhibition of myostatin protein produces a distinct double-muscled phenotype in multiple species. The Belgian Blue and Piedmontese cattle breeds are classical examples of naturally occurring double-muscled cattle (68). Similarly, mice lacking a functional copy of the *Mstn* gene have substantial increases in body weight and muscle mass (69). Myostatin plays a key role in the regulation of postnatal muscle fiber hypertrophy through suppressing Akt activation, and thus

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mTORC1 signaling, in a Smad2/3-dependent manner (70). However, myostatin does not affect the Ub-proteasome system (70). Transgenic Meishan pigs carrying a loss-of-function mutation in the *MSTN* gene have increased skeletal muscle mass at 4 and 16 months of age, enhanced activation of the insulin signaling pathway, and greater whole-body insulin sensitivity (71). In mice, *Mstn* gene deletion increases total muscle abundance of Akt and rpS6 proteins (72), which may contribute to greater muscle hypertrophy. However, the potential ramifications on insulin-mediated regulation of protein synthesis and degradation have not been determined in the neonatal period.

Skeletal muscle protein metabolism is also regulated by inflammation, proinflammatory cytokines, and glucocorticoids. This is of particular interest for any livestock species as disease disrupts normal whole-body protein and AA metabolism to preferentially partition AAs from growth toward the immune response. Skeletal muscle represents a reservoir of relatively labile AAs for gluconeogenesis and acute-phase protein synthesis and is sensitive to a range of inflammatory insults. Sepsis and bacterial lipopolysaccharide (LPS) models of inflammation lead to decreases in muscle AA uptake and protein synthesis (65, 66, 73, 74) and a specific reduction in the contribution of muscle to whole-body protein synthesis (75). In neonatal pigs, LPS blunts muscle protein synthesis (76, 77). Experimental colitis, which better represents common gastrointestinal pathologies during suckling and weaning in pigs, also attenuates muscle protein synthesis (78). At the same time, sepsis and LPS increase skeletal muscle protein degradation (65, 66).

The distinct reduction in skeletal muscle protein synthesis by sepsis and LPS can be reproduced with proinflammatory cytokines or glucocorticoids alone and is associated with suppressed mTORC1 signaling. This is in part due to a decline in voluntary feed intake and subsequent changes in local and circulating growth factor levels. However, there are also direct effects of cytokines and glucocorticoids on mTORC1 activation (79). Proinflammatory cytokines increase the expression of myostatin and suppressor of cytokine signaling proteins, which blunts insulin/IGF signaling (80). Moreover, glucocorticoid-mediated transcription of REDD1 inhibits mTORC1 signaling through promoting the release of TSC2 from inhibitory 14–3–3 proteins (81). Myostatin also appears to be required for glucocorticoid-induced muscle proteolysis and atrogin-1 and MuRF1 expression (82).

Acute resistance to the Leu-induced increase in skeletal muscle protein synthesis during inflammation is a phenomenon first reported in adult rats (73, 74). In this context, the ability of Leu to increase muscle S6K1 and 4E-BP1 phosphorylation and protein synthesis is impaired, whereas mTORC1 activation and protein synthesis can still be stimulated by insulin or IGF-I. The LPS-mediated reduction in mTORC1 signaling and muscle protein synthesis in newborn pigs is rescued by insulin and AAs, as well as Leu, indicating that short-term resistance to Leu is not present in the highly anabolic immature muscle of the neonatal pig (83, 84). The Leu-induced increase in skeletal muscle protein synthesis during endotoxemia is linked with enhanced association of Raptor with eIF3B, a subunit of eIF3 that facilitates substrate recruitment to activated mTORC1 (77, 85).

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Skeletal Muscle Protein Accretion Is Developmentally Regulated in the Neonatal Pig

In the newborn pig, muscle protein synthesis rates are high and exceed protein degradation rates, thus driving rapid rates of protein deposition. Although these opposed processes are coordinately regulated in the older pig, such that when muscle protein synthesis is increased, degradation is suppressed, and vice versa, the neonatal pig may not respond in the same manner (6). Nonetheless, there is a rapid postnatal decline in maximal rates of muscle protein synthesis and a slower decline in degradation from birth to weaning (57). The rapid postnatal decline in protein synthesis also appears to be unique to skeletal muscle, as the protein synthesis rate of most visceral organs remains unchanged from birth to weaning (86).

A key determinant of protein deposition in skeletal muscle is the maximal rate at which it can synthesize protein. The upper limit to protein synthesis in muscle is dependent on the abundance of ribosomes and the efficiency with which ribosomes translate mRNA into protein. Ribosome abundance in muscle declines from birth to weaning, and the extent to which feeding can enhance the translational efficiency of ribosomes is greater in younger than in older piglets (57, 87). The abundance and activity of positive and negative regulators of mTORC1 signaling are also affected by age (16, 30, 64, 88–90) (Table 1; see sidebar titled Prematurity Blunts Muscle Protein Synthesis). For example, the abundance of Raptor, and its association with mTOR in muscle, declines with age. Conversely, the abundance of Deptor, a negative regulator of mTOR, increases with age. This ultimately corresponds to lower phosphorylation of S6K1 and 4E-BP1 in older pigs despite similar total abundance of these proteins in muscle. The TGF-β family of ligands is also developmentally regulated. In neonatal rats, the abundance of myostatin and bone morphogenetic protein (BMP)-2 and BMP-7 proteins, negative regulators of muscle growth, increases with age (91).

Likewise, protein degradation is subject to postnatal regulation in neonatal pigs (64). Both the abundance and phosphorylation of ULK1 are lower in older than in younger pigs. The extent to which insulin and AAs decrease the LC3–2 to total LC3 ratio is greater in younger pigs than in older pigs, which is consistent with the heightened sensitivity of immature muscle to these stimuli. In the context of the Ub-proteasome system, FoxO1 phosphorylation is lower, and atrogin-1, but not MuRF1, abundance is higher in older pigs than in younger pigs (64, 67).

SKELETAL MUSCLE MYONUCLEAR ACCRETION IN THE NEONATAL PIG

Satellite Cells: A Population of Resident Muscle Stem Cells

The muscle fiber is a single cell whose cytoplasmic volume is maintained by many nuclei distributed along its length. Muscle growth in the neonate is dependent on rapid increases in myofiber size and muscle nuclear content, whereas the addition of new fibers contributes minimally (92). The nuclei within the myofibers are postmitotic and unable to divide. The addition of nuclei to support the increasing metabolic activity and volume of the growing myofiber is accomplished by SCs, skeletal muscle stem cells that reside in a niche between the sarcolemma and the basal lamina. Myonuclear accretion occurs when SCs proliferate and give rise to myogenic progenitor cells, which differentiate and fuse with the adjacent myofiber. In newborn pigs, SCs account for 30–35% of total muscle nuclei (93, 94).

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However, this proportion progressively decreases to approximately 5% in older pigs, reflecting both the increase in differentiated myonuclei and the diminishing rate of SC replication.

Characterizing Satellite Cells in the Myogenic Lineage

SCs are characterized by the expression of the paired box transcription factor Pax7 or Pax3, depending on the embryonic origin of the muscle (95, 96). While in the activated state, they also express one or more of the myogenic determination/differentiation factors Myf5, MyoD, myogenin, and/or Mrf4 (Figure 2). A majority of SCs (approximately 80% in the rat; 97) continue to express the *Myf5* transcript and are believed to represent a population primed for rapid activation. A second population of SCs that do not express the *Myf5* transcript are thought to represent the true quiescent pool necessary for long-term muscle growth and repair. Upon activation, Pax7+/Myf5− SCs divide asymmetrically to generate Pax7+/Myf5+ and Pax7+/Myf5− cells. The basal daughter cells are Pax7+/Myf5− and maintain the reservoir population, whereas apical daughter cells are Pax7+/Myf5+ and commit to the myogenic lineage. Activated SCs continue to express MyoD and proliferate further to expand the muscle progenitor cell pool. Some of these SCs lose expression of MyoD and/or Myf5 and withdraw from the cell cycle, replenishing the reservoir SC pool, whereas others go on to express myogenin and Mrf4, undergoing terminal differentiation and fusion (98).

SCs isolated from neonatal pigs also display a distinct heterogeneity (99). Similar to rats, one subset highly expresses Myf5, proliferates rapidly, and is thought to be the main source of myonuclei for growth of existing muscle fibers. A second subset exhibits slower proliferation and expresses markers for terminal differentiation. Miersch et al. (99) propose that this population contributes to tertiary fiber formation during the first week of postnatal life. The modest decline in fusion potential of SCs isolated from low–birth weight pigs, despite no change in myogenin expression, does not fully account for impaired skeletal muscle growth (100). This implies that muscle growth in these pigs is blunted from fewer total muscle fibers or a diminished response to anabolic stimuli.

Contribution of Satellite Cells to Postnatal Muscle Growth

SCs are essential for normal postnatal muscle growth. When the Pax7+ SC pool is depleted, neonatal mice have stunted growth and die before weaning, despite appearing normal at birth (95). Conversely, Pax7 is not required for SC-dependent muscle regeneration in adult mice but instead directs SCs toward quiescence (101). SCs are also required for the hypertrophic response to muscle overload in young rodents (102). Some evidence suggests that other cell types, such as pericytes, contribute to skeletal muscle regeneration and have an active role in modulating muscle growth (103). These putative progenitor cells alone, however, are unable to compensate for a depleted Pax7+ SC pool following muscle stretch or injury and are thus insufficient for postnatal muscle growth (95, 101, 104).

Postnatal muscle hypertrophy in the short term may occur without SCs, but the extent to which this occurs is controversial. McCarthy et al. (105) demonstrated that in adult mice with an inducible ablation of Pax7+ SCs, mechanical overload of the plantaris muscle

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caused an increase in muscle mass without a concurrent increase in myonuclear number. However, when Egner et al. (106) repeated these experiments, they failed to demonstrate the same hypertrophic response. Mice with inducible muscle expression of constitutively active Akt exhibit rapid muscle hypertrophy after three weeks in the absence of myonuclear accretion (107).

The Satellite Cell Niche

The opposing processes of SC quiescence and activation are tightly regulated and highly dependent on their location in the SC niche, where they are influenced by the structural and biochemical cues derived from this environment. Interactions with host muscle fibers, the extracellular matrix (ECM), adjacent vasculature, and various interstitial cells all play a role in niche-dependent SC regulation. These interactions largely govern whether the quiescent state is maintained or the activated state is induced (108).

Direct contact with the basal lamina is required for many stem cell types to remain dormant. Cultured SCs are invariably activated, which strongly suggests that SC interaction with their immediate surroundings provides signals needed to induce and maintain quiescence. In the postnatal period, rapid muscle hypertrophy is paired with rapid ECM deposition (109). Various ECM constituents are able to sequester growth factors to effectively lower their availability to activate receptors on SCs (110). As a result, the relatively labile ECM during the postnatal period may contribute to the sustained activation of SCs in the neonate. When muscles are approaching their adult size, the ECM is likely more stable and provides the signals needed to support SC quiescence while sequestering the signals needed for SC activation. In fact, it appears that SCs have an active role in ECM turnover through downregulating collagen synthesis in fibrogenic cells in an exosome-dependent manner (109). Moreover, the enzymatic activity of matrix metalloproteinase (MMP)-2 is increased in SCs activated by muscle stretch, promoting ECM turnover (111).

Extrinsic Factors Regulate Satellite Cell Activation

SC activity is regulated by numerous factors, including chemical and mechanical interactions with the host muscle fiber. The close proximity of SCs to muscle capillaries suggests these cells are sensitive to growth factors derived locally and systemically (112). Many different signaling pathways have been implicated in SC activation, proliferation, differentiation, and fusion. The immature muscle itself is a major source of these growth factors, and the developmental decline in their expression likely contributes to diminished SC activation and onset of quiescence.

It is important to note, however, that most animal models that investigate SC activation employ muscle stretch, overload, or injury to induce muscle regeneration during adulthood or senescence. This is not directly comparable to postnatal muscle growth in young animals. Stretch and overload without muscle damage are likely the most pertinent to muscle growth. Because muscle injury leads to the infiltration of immune cells and subsequent production of pro- and anti-inflammatory cytokines, this model is not pertinent for the immature healthy muscle.

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Hepatocyte growth factor.—Hepatocyte growth factor (HGF) is a potent activator of SCs, and its expression during the early postnatal period is high (113). HGF is produced and secreted by both muscle and nonmuscle cells, including SCs themselves, and is sequestered within the ECM (110). Tatsumi (114) proposed a cascade of events in the SC following muscle stretch or overload. Briefly, an influx of calcium ions in the SC promotes calcium-calmodulin formation, NOS activation, and NO radical production. NO diffuses from the SC and activates MMPs within the basal lamina (111). The MMPs degrade the proteoglycans that harbor inactive HGF, freeing HGF to be cleaved and activated. HGF subsequently binds to c-Met receptors on SCs to initiate a cascade of events promoting the expression of cell cycle–related genes. HGF also inhibits myoblast differentiation by upregulating Twist protein, an inhibitor of MyoD (115).

Fibroblast growth factors.—SC activation is mediated by fibroblast growth factors (FGFs), which are potent regulators of cell survival, proliferation, and differentiation. Similar to HGF, FGFs are sequestered in the basal lamina surrounding muscle fibers (116). FGF signaling to quiescent SCs, which express FGF receptor (FGFR)1 and −4 (117), may thus follow a similar order of events as HGF. Increasing the availability of FGFR1 potentiates SC proliferation and delays differentiation, whereas ablating FGFR1 signaling promotes SC differentiation, suggesting a key role of FGF signaling during early-phase myogenesis (118). Among the FGFs present in skeletal muscle, FGF-2 and −4 are expressed primarily in rapidly proliferating SCs, whereas FGF-6 is produced within the myofiber, in response to stretch-induced hypertrophy (117). No individual FGF is strictly required for muscle growth owing to the functional redundancy of other FGF proteins.

Insulin-like growth factors.—Local production of IGFs by the immature muscle is considered to be a predominant driver of muscle growth (119). In pigs, muscle IGF-I expression peaks at birth and subsequently declines, whereas IGF-II expression is maximal during fetal development (120). Both IGF-I and IGF-II signal to SCs through the IGF-I receptor, and IGF binding proteins can potentiate or inhibit IGF activity. The IGFs are unique among growth factors through biphasic effects on SC proliferation and differentiation (121). The IGF-mediated early inhibition and later stimulation of myogenin expression occur through a time-dependent switch from Erk1/2 to Akt signaling (122). The late inhibition of Erk1/2 signaling is thought to be dependent on upregulation of phosphatase activity specific to Erk1/2.

The *Igf1* gene can yield different splice variants. In rodents, the *Igf1a* transcript is the predominant form expressed by the liver and muscle, whereas *Igf1b* expression is transiently elevated in muscle following stretch or injury (123). The *Igf1b* transcript is initially translated and subsequently processed to form two peptides: mature IGF-I and mechano-growth factor (MGF) peptide. MGF stimulates SC proliferation but inhibits differentiation, whereas mature IGF-I promotes both proliferation and differentiation (121, 124). In porcine SCs, MGF does not affect long-term MyoD expression but suppresses myogenin expression, reinforcing its putative role in delaying SC differentiation (125). The MGF peptide has also been reported to have IGF-I-independent effects on muscle growth and repair (124), but the physiological role for MGF has been questioned recently (126).

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Notch and Wnt.—In skeletal muscle, Notch signaling pathways are involved in SC activation and proliferation and are implicated in SC self-renewal (127, 128). Notch signaling is activated after Notch ligands (Delta-like 1 and 4 and Jagged 1 and 2) bind to one of three Notch receptors expressed on SCs (127). Among Notch receptors, only Notch-1 has been reported to mediate isolated porcine SC activity, leading to upregulation of cell cycle–related genes and downregulation of MyoD and myogenin (129). FoxO3, a target of Akt phosphorylation, has been shown to promote SC quiescence by activating Notch signaling, suggesting cross talk between the Notch and insulin/IGF signaling pathways (130).

The Wnt signaling pathway also has a critical role in regulating stem cell function, including that of SCs. Wnt proteins are expressed by skeletal muscle and signal through the Frizzled receptor family (131). Brack et al. (132) explained that a switch from Notch to Wnt signaling is necessary for the transition of SCs from proliferation to differentiation. This transition is mediated through GSK-3β, which is maintained in the active state by Notch signaling and in the inactive state by Wnt signaling (133). Conversely, noncanonical Wnt signaling is independent of GSK-3β and is required for the symmetric expansion of the Pax7+/Myf5− SC pool (134). Bentzinger et al. (135) reported a role for the ECM protein fibronectin to potentiate SC proliferation through noncanonical Wnt signaling, reinforcing the key role of the ECM on SC fate.

Interleukin-4 and interleukin-6.—Local muscle production of interleukin (IL)-4 and IL-6 is essential for SC-mediated muscle hypertrophy. IL-6 regulates SC proliferation, and IL-4 promotes the fusion of terminally differentiated SCs with myofibers (136, 137). This cytokine-dependent SC proliferation and fusion are independent of the insulin/IGF signaling pathway and are mediated by the transcription factor Srf (138). Overexpression of IL-6 and IL-4 restores SC proliferation and fusion, respectively, in adult mice lacking muscle Srf during overload-induced hypertrophy.

Transforming growth factor-β family.—Despite the absolute requirement of SCs for long-term postnatal muscle growth, SCs do not appear to be needed during myostatin inhibition–induced muscle hypertrophy (139, 140). The lack of SC requirement described in these studies is likely specific for periods of acute muscle fiber hypertrophy. However, there appears to be a role for myostatin, and potential cross talk between the myostatin and insulin/IGF signaling pathways, in activated SCs (141). Myostatin suppresses Akt activation in a Smad2/3-dependent manner, leading to reduced MyoD expression and SC differentiation. Conversely, the insulin/IGF signaling pathway is dominant over the myostatin pathway and promotes SC differentiation by enhancing the sequestration of unphosphorylated Smad3 in an Akt kinase-independent manner, which prevents the formation of the active Smad2/3 complex (141).

Additional members of the TGF-β superfamily have been reported to mediate SC activity. Follistatin antagonizes myostatin activity, and there appears to be a role for follistatin, specific to SCs and independent of myostatin, in driving muscle growth (142). Follistatin expression in SCs has recently been shown to be myogenin dependent and induced by canonical Wnt signaling, enhancing SC differentiation and fusion (143). Impaired BMP

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signaling blunts SC proliferation, myonuclear accretion, and muscle growth in juvenile mice (144).

Glucocorticoids.—The elevation in glucocorticoids during various pathologic conditions is associated with reduced muscle growth, but the mechanisms through which they mediate SC activity are not fully understood. Exposure of fetal rats to glucocorticoids diminishes the reservoir pool of SCs and total myonuclear number at birth (145). Dexamethasone administration decreases SC proliferation and differentiation by suppressing MyoD and myogenin expression in a myostatin-dependent manner in adult mice (146).

Nutritional Regulation of Myonuclear Accretion

Much attention has been directed to the response of the translational machinery in muscle to nutrient intake, whereas the nutritional regulation of myonuclear accretion has largely been ignored. This is especially concerning because the relationship between muscle protein accretion and number of myonuclei is tightly correlated during rapid postnatal muscle growth (92). In rats, nutrient restriction during lactation reduces muscle protein content and, to a lesser extent, DNA content compared with well-nourished controls (147). Similarly, protein restriction in neonatal pigs results in a proportional decrease in myonuclei number and myofiber hypertrophy compared with pigs fed a diet adequate in protein (148).

Specific AAs and their metabolites have roles in regulating myonuclear accretion. Leu has been reported to enhance isolated porcine SC proliferation and differentiation in an mTOR-dependent manner (149). However, Leu supplementation is unable to restore myonuclear accretion in neonatal pigs fed a protein-restricted diet despite enhancing skeletal muscle protein synthesis (148). HMB increases myonuclear accretion in parallel to muscle protein synthesis in neonatal pigs (48). In contrast to the HMB-mediated increase in protein synthesis, which occurs without Akt activation in piglets, HMB appears to enhance SC proliferation and differentiation through both the MAPK/ERK and insulin/IGF signaling pathways (150).

Lastly, there is a potential relationship between Arg status and HGF/NO-mediated SC activation in piglets during postnatal growth. Consumption of sow milk, which is known to be limiting in Arg content, may compromise lean growth in piglets through depressing NO-dependent myonuclear accretion in addition to muscle protein synthesis. Although nutritional deficiencies have been shown to reduce SC activation and subsequent muscle growth, a clear link between nutrient and SC proliferation and differentiation in growing animals, and in particular whether specific nutrients signal directly or indirectly to SCs, should be established.

PRENATAL DETERMINANTS OF POSTNATAL MUSCLE GROWTH IN THE NEONATAL PIG

Litter size is an important economic trait in any multiparous livestock animal species, including the pig. The trend over the last several decades has been to select for increasing litter size, as this trait is responsive to genetic selection. The substantial increases in litter

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size, however, have not been without consequence. Higher ovulation rate and embryonic survival have largely outpaced uterine capacity in reproductive sows, leading to greater incidence of low–birth weight piglets (151). Low–birth weight piglets have higher preweaning morbidity and mortality, lower growth performance (i.e., body weight gain, feed conversion), and altered body composition (152). These poor performance attributes can be ascribed to in utero nutrient restriction resulting from limited sow feed intake during gestation, intrauterine crowding, and placental insufficiency and have substantial implications for prenatal myogenesis.

Total muscle fiber number is directly correlated with muscle mass and postnatal muscle growth in pigs (153). In general, small piglets have fewer secondary muscle fibers compared with their normal-sized littermates (154). Primary muscle fiber formation in pigs occurs early in gestation, from days 35 to 55, through fusion of myogenic precursor cells into myotubes (151). This is followed by a longer period of secondary muscle fiber formation, between days 55 and 90, which employs the primary myofiber as a scaffold. This stage involves rapid proliferation and differentiation of myoblasts to form multinucleated myotubes and secondary muscle fibers, which account for the majority of skeletal muscle fibers (155). During gestation, primary muscle fiber formation is generally thought to be resistant to hormonal and nutritional influences, whereas secondary muscle fiber formation is susceptible to extrinsic regulation (154, 156). A third generation of muscle fiber formation has been described in pigs (157). These tertiary fibers are smaller in diameter than either primary or secondary fibers and form in the early postnatal period. Following the formation of this final generation of myofibers, total fiber number remains largely unchanged, and environmental influences after this time may permanently impact the development of these muscle fibers and postnatal pig growth potential.

Restricted feed allowance for pregnant sows is routinely practiced to avoid excessive maternal weight gain and subsequent farrowing and lactation difficulties. In pigs, underfeeding pregnant sows leads to piglets with fewer secondary fibers but no change in primary fiber number (158). When pregnant ewes are underfed during the period of rapid fetal myoblast proliferation, the consequent reduction in fetal myofiber number persists well after birth (159). Increasing sow feed intake prior to the onset of secondary fiber formation increases total muscle fiber number and postnatal piglet growth and feed efficiency (158), but no effect of sow nutrition on myofiber formation has also been reported (160). Conversely, feed restriction during late gestation, after myofibers are largely permanent, leads to offspring with smaller rather than fewer myofibers (159). Protein restriction alone also affects fetal weight and fetal myogenesis in pigs (161). Similar reductions in fetal weight occur when protein is restricted during early or late, or throughout, gestation, suggesting that protein intake may initially limit myogenesis and later limit myofiber hypertrophy. Competition for energy and protein substrates among littermates in utero, particularly when nutrient supply is limited, likewise contributes to deficits in myogenesis, because fetal and birth weights are inversely correlated with litter size (152). Despite the direct link between maternal nutrient status and fetal growth, the extent to which mobilization of maternal fat and protein stores contributes to fetal growth during periods of insufficient maternal nutrient intake warrants further investigation.

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The placenta has a key role in mediating the impact of maternal nutrient status on fetal growth. The placenta is a core component of the maternal-fetal interface, and its function and size are critical determinants of fetal growth (162). Placental insufficiency, however, is a major cause of intrauterine growth restriction in pigs by lowering fetal nutrient and oxygen supply throughout gestation. Although embryos are initially distributed uniformly throughout each uterine horn in the sow, embryonic losses can exceed 30% such that the final intrauterine allocation of the remaining live fetuses can vary considerably (151). This variation in fetal distribution, or high fetal number, can further limit blood supply to individual fetuses and impair secondary myogenesis (163). Restricted maternal nutrient intake can also impede placental function. Although the underlying mechanisms are not well understood, it appears that downregulation of placental AA transporters through diminished insulin/IGF and mTOR signaling is involved (164). Placental insufficiency–induced intrauterine growth restriction also limits myoblast proliferation independently of maternal nutrient intake (165). This appears to be due not to intrinsic deficiencies in the myoblast per se but instead to changes in fetal factors that regulate myoblast proliferation (166).

Arg promotes placental growth by activating the mTORC1 signaling pathway and as a precursor for NO and polyamine synthesis, which regulate vascular function and angiogenesis (167). Arg, therefore, may increase myofiber hyperplasia by enhancing placental growth and improving fetal nutrient supply. Increased Arg intake in sows throughout gestation increases the total number of piglets born alive and litter weight (168). Madsen et al. (169) recently reported that Arg provision to sows during early gestation increases total myofiber number and myofiber cross-sectional area in the semitendinosus muscle in piglets exposed to intrauterine crowding. The provision of citrulline, an Arg precursor, restores fetal weight and fetal muscle protein synthesis and enhances placental efficiency in pregnant rats with intrauterine growth restriction induced by protein deprivation (170, 171).

CONCLUSION

Rapid skeletal muscle growth in the neonatal pig is driven by enhanced sensitivity of muscle to feeding and the accretion of nuclei derived from SCs in muscle fibers. Feeding induces a postprandial rise in circulating insulin and AAs, which stimulates the insulin and AA signaling pathways, activates mTORC1, and increases translation initiation and protein synthesis. Developmental changes in several key components of these pathways are responsible for the postnatal decline in muscle protein synthesis rates. Although the contribution of SCs is fundamental to postnatal muscle growth, the regulation of SC proliferation and differentiation and the establishment of the adult pool in growing animals are less understood.

In the short term, quantification of the recently identified KICSTOR and CASTOR1 proteins involved in AA signaling toward mTORC1, and Rag-independent activation of mTORC1 by Gln, should be carried out in neonatal pig muscle. Additionally, the regulation of postnatal muscle growth by microRNAs (miRNAs) is warranted. These small, noncoding RNAs have important roles in regulating SC proliferation and differentiation (172, 173) and adult muscle hypertrophy (174). However, less is known about miRNA-mediated regulation of

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muscle growth during the postnatal period, whether miRNAs contribute to the rapid developmental decline in mTORC1 signaling and muscle protein synthesis, and if their expression is influenced by nutrients and growth factors.

In the long term, transgenic pig models will provide the opportunity to advance our understanding of postnatal muscle growth beyond what can be achieved separately by nontransgenic pigs and transgenic mice. Recent advances in genome editing technologies have made complex genetic editing possible in pigs where it was previously unfeasible (175). Through manipulating the expression and activity of specific factors that regulate muscle protein synthesis and degradation, or SC proliferation and differentiation, muscle growth in pigs can potentially be optimized. This is relevant for both the agricultural and biomedical fields. For agriculture, the sustainability of livestock production is always in the public eye. Farmers routinely seek to improve the efficiency of pig production, especially now as growth-promoting antibiotics are phased out and unconventional feed ingredients are increasingly used. For medicine, improving lean growth of infants is associated with lower lifetime risk of developing diabetes, obesity, and cardiovascular disease, which otherwise impose a substantial economic burden on society.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) AR44474, NIH HD072891, US Department of Agriculture (USDA) NIFA 2013-67015-20438, and USDA/ARS 6250-51000-055. The authors wish to acknowledge the contribution of Dr. Agus Suryawan in the preparation of the table and figures in this manuscript.

Glossary

SC	satellite cell
AA	amino acid
mTORC1	mechanistic target of rapamycin complex 1
eIF	eukaryotic initiation factor
4E-BP1	eukaryotic initiation factor 4E binding protein 1
S6K1	p70 ribosomal protein S6 kinase 1
eEF	eukaryotic elongation factor
IGF	insulin-like growth factor
GSK3β	glycogen synthase kinase-3β
Leu	leucine
Arg	arginine
HMB	β-hydroxy-β-methylbutyrate
HGF	hepatocyte growth factor

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PREMATURITY BLUNTS MUSCLE PROTEIN SYNTHESIS

The developmental regulation of skeletal muscle protein accretion occurs not only postnatally but also prenatally. Piglets born prematurely have a blunted response to feeding, with lower rates of protein synthesis and lower activation of key signaling proteins in skeletal muscle, compared to older piglets (37). This response is fundamentally different from that exhibited by runt piglets that have impaired myogenesis and fewer muscle fibers. Lower protein synthesis may be mediated through diminished insulin signaling and/or amino acid signaling toward mechanistic target of rapamycin 1. Identification of the major factors that contribute to this phenomenon could provide further insight into postnatal control of muscle protein synthesis.

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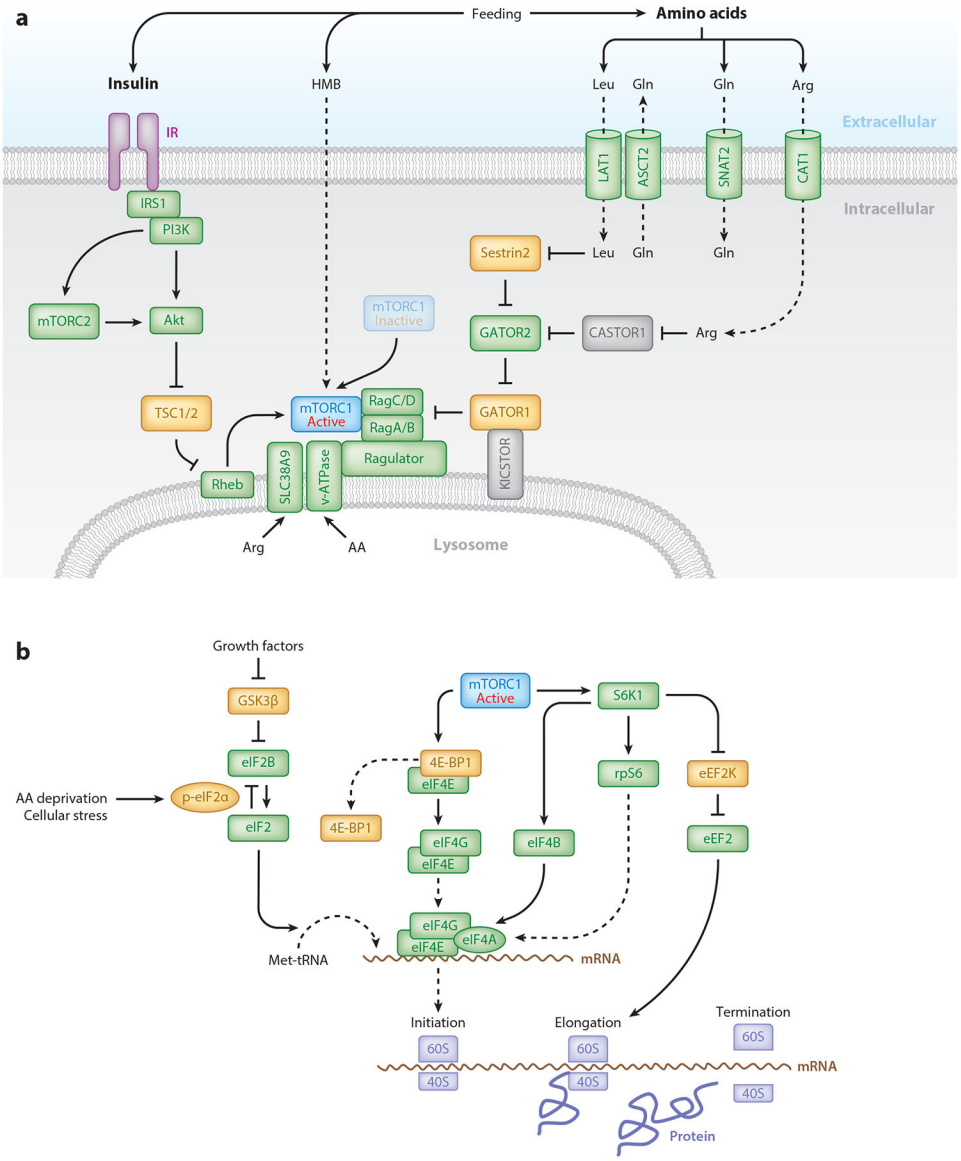

Figure 1.

Insulin and amino acid (AA) signaling pathways upstream of mechanistic target of rapamycin complex 1 (mTORC1) (*a*) and regulation of translation and protein synthesis downstream of mTORC1 (*b*) in neonatal pig skeletal muscle. Positive regulators are shown in green; negative regulators are shown in yellow; components of these pathways not currently quantified in neonatal pigs are gray. The dashed arrows indicate movement/transport of the amino acids from outside to inside the cell, or vice versa, and within the cell. Abbreviations: 4E-BP1, eukaryotic initiation factor 4E binding protein 1; Arg, arginine; ASCT2, alanine, serine, cysteine–preferring transporter 2; CAT1, cationic amino acid transporter 1; eEF2, eukaryotic elongation factor 2; eEF2K, eukaryotic elongation factor 2 kinase; eIF, eukaryotic initiation factor; Gln, glutamine; GSK3β, glycogen synthase kinase-3β; HMB, β-hydroxy-β-methylbutyrate; IR, insulin receptor; IRS1, insulin receptor substrate 1; LAT1, large neutral amino acid transporter 1; Leu, leucine; mRNA, messenger

RNA; PI3K, phosphatidylinositol-3-kinase; rpS6, ribosomal protein s6; S6K1, p70 ribosomal protein S6 kinase 1; SNAT2, sodium-coupled neutral amino acid transporter 2; tRNA, transfer RNA; TSC1/2, tuberous sclerosis complex 1/2; v-ATPase, vacuolar-type H^+ ATPase.

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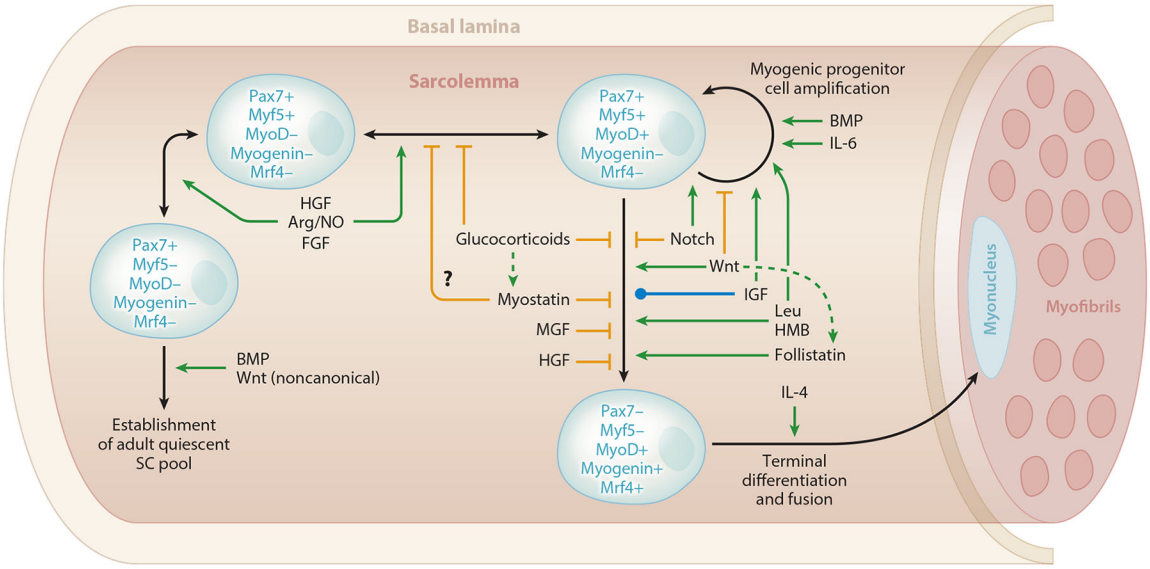

Figure 2.

Progression and regulation of skeletal muscle satellite cells (SCs) through the myogenic lineage during postnatal growth in neonatal pigs. SC proliferation and differentiation events are subject to regulation by factors derived from the host muscle fiber, extracellular matrix, nearby interstitial cells and vasculature, and systemic circulation. Positive regulators are shown in green, negative regulators are shown in yellow, time-dependent switch from negative to positive regulation is shown in blue, and indirect regulation is shown as a dashed line. Abbreviations: Arg, arginine; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HMB, β-hydroxy-β-methylbutyrate; IGF, insulin-like growth factor; IL, interleukin; Leu, leucine; MGF, mechano-growth factor; NO, nitric oxide.

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Table 1

Developmental changes in components upstream and downstream of mTORC1 in neonatal pig skeletal muscle

Item	Effect on protein synthesis	Effect of development	Reference
Insulin signaling			
IR	↑	Declines with age	89
p-IR (Tyr1185)	↑	Declines with age	89
IR substrate 1 (IRS-1)	↑	None	30
p-IRS1 (multiple Tyr residues)	↑	Declines with age	30
Total Akt	↑	Declines with age	16
p-Akt (Ser473)	↑	Declines with age	16
Total TSC2	↓	Increases with age	16
P-TSC2 (Thr1462)	↑	Declines with age	16
Total Rheb	↑	None	16
Rheb · mTOR	↑	Declines with age	30
Amino acid signaling			
GATOR1	↓	None	30
GATOR2	↑	None	30
Sestrin2	↓	Declines with age	30
Sestrin2 · GATOR2	↓	Increases with age	30
RagA · mTOR	↑	Declines with age	30
RagC · mTOR	↑	Declines with age	30
v-ATPase	↑	Declines with age	30
SLC38A9	↑	None	30
Amino acid transporters			
LAT1	↑	Declines with age	91
LAT2	↑	Declines with age	91
ASCT2	↑	Declines with age	91
SNAT2	↑	Declines with age	91
mTOR and associated proteins			
Total mTOR	↑	Declines with age	16
p-mTOR (Ser2448)	↑	Declines with age	16
Deptor	↓	Increases with age	30
Raptor	↑	Declines with age	90
Raptor-mTOR	↑	Declines with age	16
Ragulator (LAMTOR1/2)	↑	Declines with age	30
Downstream of mTORCl			
Total S6K1	↑	None	16
P-S6K1 (Thr389)	↑	Declines with age	16
Total rpS6	↑	None	64
p-rpS6 (Ser235/236 and Ser240/244)	↑	Declines with age	64

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Item	Effect on protein synthesis	Effect of development	Reference
Total 4E-BP1	↓	None	16
P-4E-BP1 (Thr70)	↑	Declines with age	16
Inactive eIF4E · 4E-BP1	↓	Increases with age	16
Total eIF4E	↑	Declines with age	64
Active eIF4E · eIF4G	↑	Declines with age	16
Total eEF2	↑	None	16
p-eEF2 (Thr56)	↑	None	16

Abbreviations: eEF, eukaryotic elongation factor; eIF, eukaryotic initiation factor; IR, insulin receptor; mTOR, mechanistic target of rapamycin; TSC, tuberous sclerosis complex; 4E-BP1, eIF4E binding protein 1.