Published in final edited form as:

Nutr Today. 2008 ; 43(4): 143–149. doi:10.1097/01.NT.0000303337.37556.17.

Insulin and Amino Acids Are Critical Regulators of Neonatal Muscle Growth

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Abstract

Newborn humans and animals grow at very rapid rates because they use the protein that they eat very efficiency to increase body protein mass. This high efficiency of protein deposition in neonates is largely due to their ability to markedly increase the amount of protein synthesized in their muscles when they eat. This enhanced stimulation of muscle protein synthesis after eating is mediated by the rise in the hormone, insulin, and the rise in amino acids, which are the building blocks of protein. Intracellular signaling components that respond to insulin and amino acids have been identified and these have been shown to be involved in the feeding-induced stimulation of protein synthesis in skeletal muscle of the neonate. The enhanced activation of these intracellular signaling components in neonatal muscle contributes to the high rate of muscle protein synthesis and rapid gain in skeletal muscle mass in newborns.

Growth rate is higher during the neonatal period than at any other stage of postnatal life.^{1,2} The rate at which protein is deposited in the body of healthy newborns is very rapid. The gain in protein mass is more rapid in skeletal muscle than in other tissues in the body of the neonate. The high rate of protein deposition is due to their ability to use the protein that they eat very efficiently to synthesize body protein, with little of the dietary protein lost through catabolic processes.³ [Insert Text Box 1]

Growth of Low Birth Weight Infants

Many infants, however, are born at a low birth weight, i.e., 2500 g or less.⁴ In fact, as many as 8% of all births in the United States are of low birth weight infants. Improvements in drug treatment and neonatal technology have increased the survival of these infants, but morbidity still remains high. The nutritional management of low birth weight infants, aimed at achieving the intrauterine rate of growth and protein accretion, has improved. However, it still remains a challenge. Most low birth weight infants are discharged from the hospital weighing less than the tenth percentile of intrauterine growth standards.⁵ Many of these infants remain small even to adulthood and some exhibit adverse, long-term developmental outcomes. These include learning impairment and a reduced capacity to perform work.^{6,7} Therefore, new strategies need to be developed to optimize the nutritional management of low birth weight infants and to improve their outcome.

Text Box 1: Skeletal muscle is the most rapidly growing tissue in neonates.

Feeding Stimulates Muscle Protein Synthesis

The neonatal pig has become a common research model for the human infant due to their similarities in physical size, physiology, and metabolism.^{8,9} Using the neonatal pig as a model of the human infant, we found that dietary amino acids, which are the building blocks of protein, are used very efficiently for growth during the neonatal period. Furthermore, this high efficiency is due to a marked increase in the synthesis of body protein after eating. These results are consistent with limited studies performed in the human infant in which infants were shown to have higher rates of protein synthesis in the whole body after eating.¹⁰ Because ethical considerations preclude obtaining tissue samples from the human neonate, more detailed work was performed in the piglet model. These studies showed that feeding stimulates protein synthesis in all tissues of the neonate, however, the magnitude of the increase is greater in skeletal muscle than in other tissues of the body.^{11,12} In addition, the response of protein synthesis to feeding decreases with age and this age-related decline is more pronounced in skeletal muscle than in other tissues. The enhanced ability of protein synthesis in skeletal muscle of neonates to respond to the provision of nutrients should not be surprising, because the rate of protein deposition during the postprandial period must be higher than the rate of protein loss during the postabsorptive period to permit growth of skeletal muscle during early postnatal life. [Insert Text Box 2]

Effects of Insulin on Protein Synthesis

Insulin increases rapidly in response to feeding, and this hormone is widely known to promote the uptake of glucose from the blood into different tissues of the body, including skeletal muscle. Studies performed in incubated muscles obtained from rodents have also demonstrated that insulin stimulates the synthesis of protein.¹³ Because of these findings, studies to identify the mechanism responsible for the increase in protein synthesis in skeletal muscle after eating have focused on insulin. The infusion of insulin into overnight fasted, weaned rats stimulates protein synthesis in skeletal muscle and this response to feeding can be blocked by anti-insulin serum.^{14,15} Insulin has also been shown to stimulate the synthesis of body protein in fetal sheep and the synthesis of protein in the hindlimbs of young lambs.^{16,17} In marked contrast to studies conducted in growing animals, most studies performed in adult animals and humans show little, if any, response of muscle protein synthesis to insulin.^{18,19} This suggests that the ability of the protein synthetic process in skeletal muscle to respond to insulin decreases with age.

Insulin Is a Critical Regulator of Neonatal Muscle Protein Synthesis

Experimentally-induced hyperinsulinemia reduces circulating amino acid levels. A decrease in amino acids, which are the substrates for protein synthesis, during the infusion of insulin, could limit the ability of insulin to stimulate protein synthesis. To enable examination of the role of insulin in the regulation of protein synthesis, independent of changes in circulating amino acids as well as glucose, we developed a novel technique, called the hyperinsulinemic-euglycemic-euaminoacidemic clamp.²⁰ This technique allows the maintenance of amino acids and glucose at fasting (or other desired levels) during the infusion of insulin. Using this hyperinsulinemic-euglycemic-euaminoacidemic clamp technique in overnight-fasted 7- and 26-day-old pigs, we showed that when amino acids and glucose are maintained at fasting levels, the infusion of insulin increases the uptake and utilization of amino acids by the body. Furthermore, less insulin is required to stimulate amino acid utilization in the younger compared to the older pigs. The maximum increase in amino acid utilization in response to high levels of insulin also is greater in the younger pigs. This suggests that the age-related

Text Box 2: Feeding increases the rate of synthesis of protein in skeletal muscle of neonates.

decline in the sensitivity of whole-body amino acid utilization to insulin may underlie the age-related decline in the efficiency with which dietary protein is utilized for protein deposition.

Our studies further showed that raising circulating insulin concentrations in the neonatal pig from fasting to fed levels using the hyperinsulinemic-euglycemic-euaminoacidemic clamp, can increase the rate of protein synthesis in skeletal muscle, even when amino acids and glucose are maintained at fasting levels. The rate of muscle protein synthesis achieved is similar to that normally present in the fed state.²¹ This response to insulin, like the response to feeding, is greater the younger the animal.²² Furthermore, insulin stimulates protein synthesis in muscle in a dose-dependent manner within the physiological range. Maximal rates of protein synthesis are achieved at the level of insulin normally present in the fully-fed state.²³ Insulin stimulates protein synthesis even when amino acids are allowed to fall to half of the level normally present in the fasting state. This suggests that insulin mediates the stimulation of protein synthesis by feeding in skeletal muscle of the neonate and this effect of insulin is independent of amino acids. [Insert Text Box 3] By contrast, protein synthesis in the visceral tissues, like the liver, intestine, pancreas, and kidney, does not respond to insulin infusion, even when amino acids and glucose are maintained at fasting levels.²² Feeding stimulates protein synthesis in visceral tissues as well as in peripheral tissues, like skeletal muscle. Therefore, our studies suggest that the feeding-induced stimulation of protein synthesis in visceral tissues is not mediated by insulin and that other anabolic factors must be involved.

Effects of Amino Acids on Protein Synthesis

Circulating amino acids increase after a meal and are utilized, in part, for the synthesis of body proteins. Amino acids appear to have the ability to stimulate protein synthesis in skeletal muscle throughout the life cycle, in contrast to the age-related decline and loss of the ability of insulin to stimulate muscle protein synthesis. In growing rats, adult humans, and elderly people, amino acid infusion stimulates protein synthesis in skeletal muscle.^{15,24,25} Recent studies suggest, however, that the magnitude of the stimulation of muscle protein synthesis by amino acids may decrease with age.²⁶

Amino Acids Are Critical Regulators of Neonatal Muscle Protein Synthesis

Our studies in neonatal pigs have shown that infusion of an amino acid mixture that is similar in composition to the amino acids present in body protein, increases protein synthesis in skeletal muscle. The response to the infusion of this balanced amino acid mixture is greater the younger the animal. The age-related decrease in the response of muscle protein synthesis to amino acids parallels the age-related decline in the response of muscle protein synthesis to eating.²⁶ The stimulation of muscle protein synthesis by amino acids occurs in the presence of fasting levels of insulin and at levels of insulin that are intermediate between the fasting and the fully-fed levels.²⁷ Amino acids also stimulate muscle protein synthesis even when circulating insulin levels are reduced to undetectable levels using the hormone, somatostatin, which blocks insulin secretion. The magnitude of the increase in muscle protein synthesis in response to fed levels of amino acids is similar to the magnitude of the increase in protein synthesis in response to fed levels of insulin. Infusion of fed levels of amino acids also increases muscle protein synthesis to rates that are similar to that which occurs after a meal.

Together, the results suggest that insulin and amino acids act independently to stimulate protein synthesis in skeletal muscle of the neonate after a meal. The ability of skeletal muscle to respond to both insulin and amino acids likely contributes to the more rapid gain in protein mass in skeletal muscle, compared to other tissues of the body, in the neonate. By contrast, amino acids,

Text Box 3: The increase in protein synthesis in muscle when neonates eat is mediated by the rise in the hormone, insulin.

but not insulin, stimulate protein synthesis in most visceral tissues of the neonate. Thus, the rise in amino acids after a meal appears to largely mediate the post-prandial increase in protein synthesis in visceral tissues of the neonate. [Insert Text Box 4]

Insulin Signaling Pathway Leading to Protein Synthesis

To stimulate protein synthesis, insulin initiates its signal by binding to its receptor. This activates the insulin receptor (InR) and insulin receptor substrate-1 (IRS-1), followed by the activation of phosphoinositide 3-kinase (PI 3-kinase)²⁸ (Figure 1). Activated PI 3-kinase then stimulates the activation of phosphoinositide-dependent kinase 1 (PDK-1) and protein kinase B (PKB). PKB phosphorylates and inactivates an inhibitor of cell growth, called tuberous sclerosis complex 1 and 2 (TSC2), thereby inactivating the function of the TSC1/2 complex, and inducing the activation of the master protein kinase, mammalian target of rapamycin (mTOR).²⁹ mTOR regulates the translation of mRNA by phosphorylating ribosomal protein S6 kinase 1 (S6K1) and eIF4E binding protein-1 (4EBP1).³⁰ Phosphorylated 4EBP1 releases eIF4E from an inactive eIF4E•4EBP1 complex, allowing the formation of the active eIF4E•eIF4G complex which mediates the binding of mRNA to the 43S ribosomal complex. Insulin signaling can be attenuated by the action of a number of phosphatases including protein tyrosine phosphatase-1B (PTP-1B) which dephosphorylates the InR and IRS-1, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) which inactivates PI 3-kinase, and PP2A which attenuates the action of PKB and S6K1.³¹

Amino Acid Signaling Pathway Leading to Protein Synthesis

The signaling pathway by which amino acids stimulate protein synthesis is less well understood. A number of studies suggest that mTOR integrates amino acid and insulin signals via multiple mechanisms including phosphorylation of S6K1 and 4EBP1, resulting in the modulation of protein synthesis and cell growth (Figure 1).³² However, little is known as to the mechanism by which amino acids modulate the activation of signaling components upstream of mTOR. Under energy starvation conditions, AMP-activated protein kinase (AMPK) phosphorylates TSC2 on specific sites that enhance its activity. This results in an inhibition of mTOR activation and a reduction in protein synthesis.³³ However, it is not clear as to whether amino acids modulate mTOR through TSC2. Recent findings suggest that amino acids regulate the binding of raptor to mTOR, resulting in the activation of S6K1 and 4EBP1.³⁴

Regulation of the Insulin Signaling Pathway Leading to Protein Synthesis in Neonatal Muscle

Most of the studies that have examined the intracellular mechanisms by which insulin and amino acids stimulate protein synthesis have been conducted in cell culture systems. Little is known about how the insulin and amino acid signaling components respond to physiological changes in insulin and amino acids in the intact animal. Using the neonatal pig as a model of the human neonate, we showed that the activation of the insulin receptor, IRS-1, PI 3-kinase, PDK-1, and PKB in skeletal muscle increases after eating.^{35,36} The activation of these early steps in the insulin signaling pathway in response to eating decreases with age. The decrease in this activation parallels the age-related decline in the ability of feeding to stimulate muscle protein synthesis. Furthermore, we showed that the infusion of physiological levels of insulin increases the activation of these insulin signaling components in a dose-dependent manner.³⁷ However, physiological changes in the levels of amino acids have no effect on the activation

Text Box 4: The rise in amino acids after eating stimulates protein synthesis in most tissues, including skeletal muscle.

of these early steps in the insulin signaling pathway. Furthermore, amino acids do not alter the response of these insulin signaling components to insulin. [Insert Text Box 5]

Regulation of Translation Initiation by Insulin and Amino Acids in Neonatal Muscle

Our studies have shown that the activation of translation initiation factors in skeletal muscle of the neonatal pig increases after a meal. After eating, the phosphorylation and activation, of mTOR increases. However, the association of mTOR with raptor is not altered.^{38,39,40} Feeding also increases the phosphorylation of the downstream effectors of mTOR, i.e., S6K1 and 4EBP1, resulting in a decrease in the association of eIF4E with 4EBP1 in an inactive complex. This increases the abundance of the active eIF4E•eIF4G complex. The active complex regulates the binding of mRNA to the ribosome, thus, resulting in an increase in translation initiation. All of these responses to eating decline with age in skeletal muscle. The infusion of either insulin or amino acids, within the physiological range, increases the phosphorylation of mTOR, S6K1 and 4EBP1, decreases the binding of 4E-BP1 to eIF4E, and increase eIF4E binding to eIF4G.⁴¹ [Insert Text Box 6] This suggests that the rise in both insulin and amino acids after a meal activates signaling components that are downstream of PKB, including mTOR, S6K1, 4EBP1, and eIF4E-eIF4G, and, thus, that both insulin and amino acids utilize a common signaling pathway downstream of PKB. However, only the rise in insulin after a meal, but not amino acids, activates PKB and the signaling components that are upstream of PKB, i.e., the insulin receptor, IRS-1, and PI 3-kinase.

Negative Regulators of Insulin and Amino Acid Signaling in Neonatal Muscle

Negative regulators of the insulin and amino acid signaling pathways that lead to translation initiation also play a role in the age-dependent regulation of protein synthesis. The activation of PTP1B, PTEN, PP2A, and TSC2, all negative regulators of insulin signaling, are low in muscle of neonatal animals and increase with age. These changes are consistent with the age-related decrease in muscle protein synthesis.^{40,42} The activation of TSC2 decreases after eating. However, feeding has no effect on PTP1B, PTEN, and PP2A activation. Interestingly, the activation of AMPK, a negative regulator of the mTOR pathway and a sensor of cellular energy, does not change with feeding and is not affected by age. Together, the results suggest that the age-related changes in the activation of both positive and negative regulators of the insulin and amino acid signaling pathways contribute to the high rate of protein synthesis in neonates.

Summary

Neonates deposit protein at a very high rate and efficiently utilize dietary amino acids for protein deposition. The high efficiency is due to an elevated stimulation of protein synthesis in skeletal muscle after eating. This enhanced stimulation of muscle protein synthesis after feeding is mediated by the rise in both insulin and amino acids. Insulin and amino acid signaling components have been identified that are involved in the feeding-induced stimulation of protein synthesis in skeletal muscle. The enhanced activation of these signaling components in neonatal muscle contributes to the high rate of protein synthesis and more rapid gain in skeletal muscle mass in neonates. The results highlight the multiple factors that affect protein synthesis, and thus, growth in newborns. These studies provide fundamental new knowledge about the

Text Box 5: The rise in insulin after eating activates the intracellular insulin signaling pathway that leads to the translation of mRNA in neonatal muscle.

Text Box 6: The rise in amino acids after eating activates the intracellular protein kinase called mammalian target of rapamycin (mTOR).

mechanisms by which nutrients and hormones regulate muscle protein synthesis, and thus, growth, in neonates.

Acknowledgements

This work is a publication of the United States Department of Agriculture/Agriculture Research Service (USDA/ARS) Children's Nutrition Research Center, Department of Pediatric, Baylor College of Medicine. This project was supported in part by National Institute of Health (NIH) grants AR-44474 and by the USDA/ARS under Cooperative Agreement no. 6250510000-33. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

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Figure 1.

Insulin and amino acid signaling pathways leading to the stimulation of protein synthesis. Insulin binds to its receptor and activates the insulin receptor (InR) and insulin receptor substrate-1 (IRS-1), followed by the activation of phosphoinositide-3 kinase (PI 3-kinase). Activated PI3 kinase stimulates the activation of phosphoinositide-dependent kinase 1 (PDK-1) and protein kinase B (PKB). Phosphorylation of PKB inactivates tuberous sclerosis complex 1 and 2 (TSC1/2), inducing the activation of mammalian target of rapamycin (mTOR). Both amino acids and insulin activate mTOR, which exists in a complex with raptor. Activated mTOR phosphorylates ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor (eIF) 4E binding protein-1 (4EBP1). Phosphorylation of S6K1 enhances the activation of ribosomal subunit S6 (rpS6), which increases the translation of specific mRNA. Phosphorylated 4EBP1 releases eIF4E from an inactive complex with 4EBP1, enabling the formation of the active eIF4E•eIF4G complex that mediates mRNA binding to the ribosome. Insulin signaling can be attenuated by protein tyrosine phosphatase-1B (PTP-1B) which dephosphorylates the insulin receptor and IRS-1, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) which inactivates PI 3-kinase, and protein phosphatase 2A (PP2A) which decreases activation of PKB and S6K1. An increase in adenosine monophosphate (AMP) levels enhances AMP kinase activation, increasing TSC1/2 complex activation, and decreasing mTOR activation.