Developmental regulation of protein metabolism

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Growth and development are characterized by high rates of protein turnover that support rapid rates of protein accretion. The relative rates of protein deposition vary among tissues, with the growth rate of the skeletal musculature being amongst the highest. The efficiency with which dietary amino acids are utilized for protein deposition is high in the neonatal animal and decreases as the animal matures. This high efficiency in the neonate is enabled by an enhanced capacity for feeding to stimulate protein synthesis. The rise in protein synthesis in response to feeding and its developmental decline are more pronounced in skeletal muscle than in other tissues. In the neonatal pig, the postprandial rises in insulin and amino acids independently stimulate protein synthesis in skeletal muscle, whereas amino acids are the principal anabolic stimulus in the liver. The developmental changes in muscle protein synthesis are regulated by alterations in the expression and activity of components of the intracellular signaling pathway that controls the initiation of translation.

Key Words: Muscle, Pigs, Neonate, Protein synthesis, Insulin, Amino acids
Introduction

The relative rates of growth and protein deposition are higher during the neonatal period than at any other stage of postnatal life (Denne and Kalhan, 1987; Goldspink and Kelly, 1984). The rapid rate of protein deposition is sustained by elevated fractional rates of protein synthesis. With the advancement of development, fractional rates of growth and protein synthesis decline. During the neonatal period, more rapid gains in protein mass occur in skeletal muscle than in the body as a whole (Young, 1970). Fractional rates of protein synthesis in skeletal muscle are high immediately after birth and decline more rapidly than in other tissues of the body during the first month of life (Davis et al., 1993, 1996). This developmental decline in skeletal muscle protein synthesis varies with fiber type (Davis et al., 1989). Marked changes in the composition of muscle also occur during the early postnatal period, including a specific accumulation of myofibrillar proteins (Fiorotto et al., 2000).

Role of Nutrient Intake in the Developmental Regulation of Protein Metabolism

The efficiency with which dietary amino acids are utilized for protein deposition is extremely high in the neonatal animal, and declines rapidly with development (Davis et al., 1993; Fiorotto et al., 1991; McCracken et al., 1980). Our work in rats and pigs suggests that neonatal animals utilize their dietary amino acids more efficiently for growth because they are capable of a greater increase in tissue protein synthesis in response to feeding than older animals, thereby reducing the loss of amino acids by catabolism (Davis et al., 1991, 1993, 1996, 1997). Although feeding increases protein synthesis in all tissues of the neonatal pig and rat, the postprandial rise in protein synthesis and the developmental decline in the response to feeding are most pronounced in skeletal muscle, particularly in those muscles that contain predominately fast-twitch muscle fibers (Burrin et al., 1991, 1992, 1995, 1997; Davis et al., 1993, 1996, 1997). About 50% of the decline in skeletal muscle protein synthesis from birth to adulthood occurs during the first month of life.
The postprandial stimulation of protein synthesis has been observed in the whole body of the newborn human and in skeletal muscle of the post-weaned growing rat and lamb (Denne et al., 1991; Garlick et al., 1983; Oddy et al., 1987; Wester et al., 2000). The response is present to a lesser extent, if at all, in adult mammals (Baille and Garlick, 1992; Melville et al., 1989; Teleni et al., 1986; Tessari et al., 1996). By contrast, liver protein synthesis rates can be modulated by changes in food intake in both growing and adult animals (Burrin et al., 1992, 1997; Mosoni et al., 1996; Preedy et al., 1988).

Thus, there appear to be fundamental differences between the mechanisms that regulate protein deposition in the adult and the neonate. In the adult, protein mass is gained and lost equally between the postprandial and postabsorptive periods and changes in protein synthesis with feeding and fasting are minimal. In the neonate, protein is deposited more rapidly in the fed state than the amount that is lost between meals, and, thus, high postprandial rates of protein synthesis are required. The enhanced stimulation of protein synthesis in skeletal muscle in response to eating serves as an efficient mechanism that channels dietary amino acids into muscle protein, thereby accelerating muscle protein accretion in the neonate.

Role of Insulin in the Developmental Regulation of Protein Metabolism

Insulin likely plays a key role in the regulation of protein deposition in the neonatal animal. Examination of the relationship between skeletal muscle protein synthesis and plasma insulin concentrations in fasted and fed suckling pigs shows a positive relationship between the postprandial changes in protein synthesis and changes in circulating insulin concentrations (Davis et al., 1997). However, the postprandial rise in amino acids and glucose potentially may influence the relationship between protein synthesis and insulin. Therefore, to determine the effects of insulin, independent of changes in circulating amino acids and glucose, we developed a novel amino acid clamp technique (Wray-Cahen et al., 1997) for use in conjunction with the glucose clamp technique (DeFronzo et al.,
1979), to maintain constant blood amino acids and glucose concentrations during the infusion of insulin. Use of the amino acid clamp during insulin infusion studies is particularly important in young animals, as experimentally induced systemic hyperinsulinemia, in the absence of amino acid administration, results in a fall in amino acid concentrations that is more profound and more rapid the younger the animal. A decrease in circulating amino acid levels during the infusion of insulin could limit the ability of insulin to stimulate protein synthesis.

Results of studies using the hyperinsulinemic-euglycemic-euaminoacidemic clamp technique showed that insulin stimulates the utilization of amino acids for protein deposition in the neonatal pig, and that both the sensitivity and responsiveness of amino acid disposal to insulin decline markedly with development (Wray-Cahen et al., 1997). This response suggests that the enhanced insulin sensitivity of whole body amino acid disposal may underlie the more efficient use of dietary amino acids for growth in the neonatal animal. Furthermore, raising insulin concentrations in neonatal pigs to reproduce the levels in the fed state, while amino acids and glucose are clamped near fasting levels, increases the rate of skeletal muscle protein synthesis to that present in the fed state (Wray-Cahen et al., 1998). This response to insulin is attenuated as the pig matures, in parallel with the developmental change in the stimulation of muscle protein synthesis by feeding. The stimulation of muscle protein synthesis by insulin, like that in response to feeding, is greater in fast-twitch muscle and is not specific to myofibrillar proteins (Davis et al., 2001).

The stimulatory effects of insulin on whole body amino acid disposal and skeletal muscle protein synthesis in the early postnatal pig are consistent with the ability of insulin to stimulate whole body amino acid utilization (Thureen et al., 2000) and protein synthesis in the fetal sheep (Liechty et al., 1992), hindlimb of the young lamb (Wester et al., 2000), and skeletal muscle of the weaned rat (Garlick et al., 1983). In marked contrast to studies conducted during growth and development, most studies in adult animals and humans show little, if any, response of muscle protein synthesis to an increase in insulin within the physiological range (Baille and Garlick, 1992; Gelfand and Barrett, 1987; Heslin et al., 1992; Louard et al., 1992; McNulty et al., 1993). Collectively, these results suggest that insulin mediates the feeding-induced stimulation of protein synthesis in skeletal muscle of young mammals and that this
response decreases with development.

Interestingly, insulin also stimulates protein synthesis in another peripheral tissue, the skin, and in cardiac muscle of the neonatal pig (Davis et al., 2001; McNulty et al., 1993; Pain et al., 1983). The insulin-stimulated responses in these tissues, like that in skeletal muscle, also decrease with development. By contrast, insulin infusion, even in the presence of near-fasting levels of glucose and amino acids, does not stimulate protein synthesis in visceral tissues including the liver, intestine, pancreas, and kidney (Davis et al., 1999, 2001), even though feeding stimulates protein synthesis in these tissues (Burrin et al., 1995, 1997; Davis et al., 1996, 1997). This suggests that the feeding-induced stimulation of visceral tissue protein synthesis in the young pig is not mediated by insulin and that different mechanisms regulate the synthesis of muscle and visceral tissue proteins in the neonatal animal.

**Role of Amino Acids in the Developmental Regulation of Protein Metabolism**

The postprandial rise in amino acids plays an important role in the feeding-induced stimulation of protein synthesis. Our recent studies have shown that the infusion of a balanced amino acid mixture, in the presence of either euinsulinemia or hyperinsulinemia, stimulates protein synthesis in skeletal muscle of the neonatal pig, similar to the stimulatory effect of feeding and insulin infusion (Davis et al., 1998 and unpublished data). The response to hyperaminoacidemia decreases with development in parallel with the developmental decline in the feeding-induced stimulation of skeletal muscle protein synthesis. The stimulation of skeletal muscle protein synthesis by amino acids is greater in those muscles that contain predominately fast-twitch muscle fibers. In contrast, amino acid infusion has little effect on other insulin-responsive tissue, i.e., cardiac muscle and skin. Hyperaminoacidemia, in the presence of either euinsulinemia or hyperinsulinemia, increases protein synthesis in insulin-unresponsive tissues, i.e., liver, kidney, and pancreas. The ability of amino acids to stimulate protein synthesis in liver appears to wane with development, consistent with the ability of amino acids to stimulate liver protein synthesis in growing (Flaim et al, 1982; Shah et al, 1999) but not in adult (Mosoni et al, 1993) rats. Interestingly, amino
acid infusion, like insulin infusion, has no effect on protein synthesis in the intestine.

The results suggest that the feeding-induced stimulation of protein synthesis in skeletal muscle is mediated by both amino acids and insulin. The postprandial rise in amino acids also stimulates protein synthesis in the liver, kidney, and pancreas, whereas the postprandial rise in insulin also stimulates protein synthesis in the heart and skin. Because amino acids were infused into the peripheral circulation, we postulate that amino acid supply to the mucosal rather than the serosal side of the enterocyte may be the important modulator of the feeding-induced stimulation of intestinal protein synthesis. Thus, it appears that the stimulation of protein synthesis by feeding is mediated by either insulin or amino acids in most tissues of the neonatal pig, but in skeletal muscle, the feeding-induced stimulation of protein synthesis is regulated by both insulin and amino acids. The ability of skeletal muscle to respond to two anabolic stimuli likely contributes to the more rapid gain in protein mass in the skeletal muscle of the neonate than in the body as a whole.

Intracellular Mechanisms Regulating the Developmental Changes in Muscle Protein Synthesis

Recent studies are revealing the cellular mechanisms that regulate the rapid growth of skeletal muscle during early postnatal life. The high rate of skeletal muscle protein synthesis in the neonate and its decline with development are largely driven by an elevated number of ribosomes at birth and a developmental decline in ribosome abundance as the musculature matures (Davis et al., 1989, 2001). The feeding-induced stimulation of protein synthesis can be attributed to an increase in the efficiency of the translation process, i.e., the amount of protein synthesized per ribosome (Davis et al., 1993, 1996). Because acute regulation of protein synthesis is achieved in part through changes in the rate of translation of mRNA via alterations in peptide-chain initiation (Harmon et al., 1984; Kimball and Jefferson, 1998; Kimball et al., 1994), recent studies have examined the developmental changes in the expression and activation of eukaryotic initiation factors (eIF) which regulate translation initiation in skeletal muscle. Because the postprandial elevation in skeletal muscle protein synthesis in neonatal pigs
is mediated, in part, by insulin (Davis et al., 2001; Wray-Cahen et al., 1998), the developmental changes in the insulin signaling pathway that regulate protein synthesis have also been examined.

Our studies have shown that insulin receptor abundance in skeletal muscle decreases with development in parallel with the overall developmental decline in muscle protein synthesis (Suryawan et al., 2001). The activation of the insulin receptor in response to feeding decreases with development, even when expressed relative to insulin receptor abundance. This developmental decline in the feeding-induced activation of the insulin receptor is propagated down the signaling pathway to insulin receptor substrate (IRS)-1 and -2, phosphatidylinositol 3-kinase (PI 3-kinase), and protein kinase B (PKB). The phosphorylation of subsequent signaling components that regulate the protein synthetic apparatus, ribosomal protein S6 kinase (S6K1) and 4E-BP1, also increases after a meal, a response that decreases with development (Davis et al., 2000). The feeding-induced increase in the phosphorylation of 4E-BP1 results in the dissociation of the inactive 4E-BP1-eIF4E complex, an increased association of eIF4E-eIF4G, and thus an enhanced assembly of the active eIF4F complex which mediates the binding of mRNA to the 40S ribosomal subunit. The feeding-induced activation of S6K1, assembly of the active eIF4F complex, and stimulation of protein synthesis in neonatal pig muscle can be attenuated, but not completely blocked, by the drug rapamycin, which inhibits the protein kinase mTOR (mammalian target of rapamycin), a component of the insulin signal transduction pathway that is downstream of PKB (Kimball et al., 2000)

Together, these results suggest that the developmental decline in the response of skeletal muscle protein synthesis to food consumption results from a reduction in the capacity of the intracellular signaling pathway to transduce to the translational apparatus the stimulus provided by the postprandial rise in circulating insulin and/or amino acid concentration. The results further suggest that an mTOR-dependent process involving enhanced assembly of the active eIF4F complex accounts for part of the postprandial stimulation of muscle protein synthesis. However, additional steps may be involved in the regulation of this process, and warrant further study.
Implications

Neonatal animals deposit protein at very high rates and efficiently utilize dietary amino acids for protein deposition. This high efficiency is associated with an elevated stimulation of tissue protein synthesis by feeding which is particularly profound in skeletal muscle. While the rapid rate of protein synthesis in skeletal muscle of the immature animal is due, in part, to a high ribosome concentration, the enhanced sensitivity of skeletal muscle protein synthesis to both insulin and amino acids also contributes to this phenomenon. The capacity of protein synthesis to respond to the post-prandial rise in both insulin and amino acids is specific to skeletal muscle in the neonatal animal; i.e., the feeding-induced stimulation of protein synthesis in other tissues is mediated by only one of these anabolic stimuli. Recent studies have shown that an enhanced activation of the insulin signaling pathway leading to translation initiation in skeletal muscle of the neonatal pig following food consumption plays a crucial role in determining the high rate of protein deposition in skeletal muscle during early life. Further study of the fundamental mechanisms which regulate the high rate of protein deposition in early life may have important implications with respect to the improvement of neonatal survival and the enhancement of lean tissue growth in pigs.


or with insulin on muscle and liver protein synthesis in adult and old rats. Am. J. Physiol. 264:E614-0E620.


