Roles of Insulin and Amino Acids in the Regulation of Protein Synthesis in the Neonate\textsuperscript{1,2}

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ABSTRACT Neonates deposit protein at a very high rate and efficiently utilize dietary amino acids for protein deposition. This high efficiency is associated with an elevated stimulation of tissue protein synthesis by feeding. Our recent studies have focused on identification of the factors that mediate this response in the neonate. A positive curvilinear relationship between skeletal muscle protein synthesis and plasma insulin concentration was identified in fasted and fed suckling pigs; the relationship changes with development. To test the specific effects of insulin on protein metabolism in the neonate, a procedure to clamp amino acids, under hyperinsulinemic conditions, was developed. By using this technique, we showed that insulin-stimulated whole-body amino acid disposal is elevated in the neonate, and this response may account for the efficient use of dietary amino acids for protein accretion. More recent studies suggest that the enhanced stimulation of skeletal muscle protein synthesis by feeding in the neonate is primarily insulin mediated; however, the stimulation of liver protein synthesis by feeding seems to be largely a function of amino acid concentration. J. Nutr. 128: 347S–350S, 1998

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The neonatal period is characterized by high rates of protein turnover that support the rapid deposition of body protein (Davis et al. 1989, Denne and Kalhan 1987, Goldspink and Kelly 1984). Neonates are also very efficient at utilizing dietary amino acids for protein deposition (Davis et al. 1993b, Fiorotto et al. 1991, McCracken et al. 1980). This efficiency declines markedly with development. Our early work in rats and pigs suggested that neonates are able to utilize their dietary amino acids more efficiently for growth because they are capable of a much greater increase in protein synthesis in response to feeding than older animals (Burrin et al. 1991 and 1992, Davis et al. 1991, 1993a and 1996). In the fed state, the fractional rates of tissue protein synthesis, particularly in skeletal muscle, are elevated at birth and decline markedly during the suckling period. Protein synthesis rates are higher in the fed state than in the fasted state. The younger the animal, the greater is this stimulatory effect of nutrient intake on tissue protein synthesis. These results in neonatal rats and pigs are consistent with those reported by Denne et al. (1991), who found that fed, newborn humans had higher rates of whole-body protein synthesis than fasted newborns and that this response to feeding was greater than that of adults.

IDENTIFICATION OF POTENTIAL MEDIATORS OF THE STIMULATION OF PROTEIN SYNTHESIS BY FEEDING IN THE NEONATE

Our recent work has focused on identification of the mechanism responsible for the enhanced stimulation of protein synthesis by nutrients in the neonate and the decline in this response with development. As candidates for this role, four hormones or growth factors were considered: insulin-like growth factor-I (IGF-I), growth hormone, glucagon and insulin. Three of the four candidates were rejected because of their inability to both stimulate protein synthesis and increase rapidly after food ingestion. Although IGF-I has been demonstrated to stimulate protein synthesis (Douglas et al. 1991, Fryburg 1994, Tomas et al. 1991), circulating IGF-I does not increase rapidly with feeding (Davis et al. 1996). Moreover, our recent studies suggest that skeletal muscle protein synthesis rates and circulating IGF-I concentrations are not correlated in neonatal animals (Burrin et al. 1997, Davis et al. 1997). Although growth hormone has been demonstrated to stimulate

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FIGURE 1  Skeletal muscle protein synthesis rate vs. plasma insulin concentration in 7- (○) and 26- (●) d-old pigs. The relationships between protein synthesis and insulin were exponential and the $r^2$ values were 50% and 36% for 7- and 26-d-old pigs, respectively.

protein synthesis (Boisclair et al. 1995, Fryburg and Barrett 1993, Seve et al. 1993), it does not rise with feeding (Ross and Buchanan 1990). Glucagon increases in response to feeding, particularly when high protein diets are fed, but it has little effect on protein synthesis (Flakoll et al. 1994). Of the four potential mediators considered, only insulin increases rapidly in response to feeding and has been demonstrated to stimulate muscle protein synthesis in weaned, but still growing rats (Garlick et al. 1983).

Examination of the relationship between skeletal muscle protein synthesis and plasma insulin concentration in fed and fasted suckling pigs suggests that insulin may mediate the developmental changes in the stimulation of protein synthesis by feeding. As shown in Figure 1, there are positive curvilinear relationships between skeletal muscle protein synthesis and plasma insulin concentrations in fasted and fed suckling pigs. The $r^2$ values were 50% at 7 d of age and 36% at 26 d of age. These data suggest that the relationship between protein synthesis and insulin is also influenced by other variables, and that their influence becomes of greater quantitative importance as the animals develop. However, because of the lack of control for the potential effects of the rise in plasma amino acid and glucose concentrations that occurs with feeding, limited conclusions can be drawn from these data with regard to a primary role of insulin in the feeding response. Therefore, to determine the effects of insulin, independent of changes in circulating glucose and amino acid concentrations, on protein synthesis during early postnatal life, studies are required in which the rate of protein synthesis is determined in fasted pigs infused with insulin while circulating amino acid and glucose concentrations are maintained at the fasting level.

DEVELOPMENT OF AN AMINO ACID CLAMP TECHNIQUE

In our initial studies to examine the effects of insulin on protein metabolism, we used the hyperinsulinemic-euglycemic clamp technique, developed by DeFronzo et al. (1979), to maintain blood glucose concentrations at the fasting level during the infusion of insulin. We found that circulating amino acids fell markedly with increasing insulin concentrations, and that this response changed with development (Wray-Cahen et al. 1997a). Only low physiologic concentrations of insulin were needed to reduce the circulating amino acid concentrations to a minimum. These results in neonatal pigs contrast with those in adult humans in which the nadir of the plasma amino acid concentrations occurred only at pharmacologic insulin concentrations (Flakoll et al. 1989).

Because a low circulating amino acid concentration could limit the anabolic response to insulin by reducing substrate supply, we developed a method to maintain circulating amino acids during the hyperinsulinemic-euglycemic clamp (Wray-Cahen et al. 1997a). Along with glucose, the concentration of a “representative” essential amino acid is monitored frequently during the infusion of insulin, and the infusion rate of an amino acid mixture is adjusted to maintain circulating amino acids at the fasting level during the hyperinsulinemic-euglycemic clamp. To monitor “representative” amino acids, rapid enzyme assays were developed for both plasma lysine concentrations and the total branched-chain amino acid concentrations (Beckett et al. 1996a and 1996b). We found that, by using Trophamine (McGaw, Irvine, CA) as the amino acid source, branched-chain and essential amino acids were held constant at the fasting level, but nonessential amino acids were maintained only partially.

INSULIN-STIMULATED AMINO ACID DISPOSAL DECREASES WITH DEVELOPMENT

We first used this amino acid clamp method to determine the effect of development on insulin-stimulated amino acid disposal (Wray-Cahen et al. 1997a). Amino acid disposal was measured as the rate of amino acid infusion required to maintain the circulating amino acid concentrations at the fasting level as the circulating insulin concentration was increased. The results, shown in Figure 2, demonstrated that the maximal rate of amino acid disposal was higher in 7- than in 26-d-old pigs. The maximal amino acid disposal rate was achieved in both age groups at $\sim 150 \mu \text{U/mL}$ of insulin. However, the insulin concentration required to achieve the half-maximal amino acid disposal rate, i.e., the $ED_{50}$, was lower in 7- than in 26-d-old pigs (21 vs. 61 $\mu \text{U/mL}$), indicating that whole-body amino acid disposal is more sensitive to insulin in young than in older suckling animals. Furthermore, in 7- but not 26-d-old pigs, the threshold of insulin-stimulated amino acid disposal rate was within the range of postprandial insulin con-
centrations. During the clamps, plasma urea nitrogen concentrations did not increase in 7-d-old pigs and even decreased in 26-d-old pigs, indicating that the infused amino acids were not catabolized. These results suggest that the enhanced response of whole-body amino acid disposal to insulin during early postnatal life may be an underlying mechanism for the more efficient use of dietary amino acids for protein accretion in the neonate and reflect the inherent anabolic drive and growth potential of the young animal.

**STIMULATION OF SKELETAL MUSCLE PROTEIN SYNTHESIS BY INSULIN DECREASES WITH DEVELOPMENT**

To determine whether insulin per se mediates the stimulation of tissue protein synthesis by feeding in the neonate and whether this response changes with development, hyperinsulinemic-euglycemic–amino acid clamps were performed in 7- and 26-d-old fasted pigs (Wray-Cahen et al. 1997b). Pigs were infused with insulin at rates that resulted in plasma insulin concentrations that are normally seen in suckling pigs in the fasted state (5 μU/mL), the fed steady state (10 μU/mL) and the refed state shortly after a meal feed (30 μU/mL). Plasma amino acid and glucose concentrations were maintained at the fasting level so that the independent effects of insulin on tissue protein synthesis could be determined.

The results showed that insulin stimulated skeletal muscle protein synthesis in both 7- and 26-d-old pigs, but the response was greater in the younger animals. At both ages, the maximum stimulation of muscle protein synthesis rate was achieved at the concentration of insulin typically seen in the fed steady state (10 μU/mL). These results contrast with the lack of (or moderate) response of whole-body amino acid disposal to 10 μU/mL of insulin in 26- or 7-d-old pigs, indicating that the protein synthetic system in skeletal muscle is more sensitive to insulin than that of other tissues. Furthermore, the response of skeletal muscle protein synthesis to insulin infusion, when circulating amino acids and glucose concentrations were maintained at the fasting level, was similar to the response of muscle protein synthesis to feeding in our previous study (Davis et al. 1996). These observations are consistent with the hypothesis that insulin mediates the stimulation of skeletal muscle protein synthesis by feeding during early postnatal life and that the response to insulin decreases with development. On the other hand, the hyperinsulinemic-euglycemic–amino acid clamp studies suggest that insulin does not stimulate liver protein synthesis in 7-d-old pigs and decreases liver protein synthesis in 26-d-old pigs. In view of these results, it is unlikely that the stimulation of visceral tissue protein synthesis by feeding is attributable to insulin.

**SKELETAL MUSCLE AND LIVER RESPOND DIFFERENTLY TO AMINO ACIDS**

Because the amino acid infusate (Trophamine) used in the hyperinsulinemic-euglycemic–amino acid clamp studies is enriched in essential amino acids, there is a decrease in the concentration of circulating nonessential amino acids despite clamping of the essential amino acids. Therefore, we designed a new amino acid infusate based on the amino acid composition of body protein. When this new amino acid solution was infused, branched-chain, other essential and nonessential amino acids were maintained at the fasting level during hyperinsulinemic-euglycemic–amino acid clamps. Preliminary studies in 7-d-old pigs suggested that rates of liver protein synthesis were similar when amino acids were clamped at the fasting level with the use of either Trophamine or the new amino acid infusate during hyperinsulinemic-euglycemic clamps. Thus, the lack of stimulation of liver protein synthesis by insulin infusion was not due to an amino acid imbalance in the infusate. The stimulation of skeletal muscle protein synthesis by insulin was also similar when either Trophamine or the new amino acid infusate was used to clamp amino acids at the fasting level, suggesting that a reduction in circulating concentrations of nonessential amino acids did not limit the synthetic response to insulin.

Studies have been initiated recently to determine whether the increase in circulating amino acids that occurs with feeding can stimulate tissue protein synthesis. Preliminary evidence from such studies in 7-d-old pigs indicated that a twofold increase in circulating amino acids had only a modest effect on skeletal muscle protein synthesis but markedly increased liver protein synthesis. This ability of amino acids to stimulate liver protein synthesis is supported by the findings of other studies in newborn pigs (Burrin et al. 1995) and suggests that nutrient intake, particularly that of amino acids, is the primary factor.
stimulus for liver protein synthesis in response to feeding in newborn pigs.

In conclusion, our studies have shown that the enhanced stimulation of skeletal muscle protein synthesis by feeding in the neonate is primarily insulin-mediated. The enhanced stimulation of liver protein synthesis by feeding in the neonate appears to be dependent primarily on amino acid supply. Further research is required to determine the fundamental mechanisms that underlie the developmental changes in the response of protein synthesis to insulin and amino acids, and the different responses of various body tissues to them.

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LITERATURE CITED


