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Bolus versus Continuous Feeding to Optimize Anabolism in Neonates

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Abstract

Purpose of review—Neonates with feeding difficulties can be fed by orogastric tube, using either continuous or bolus delivery. This review reports on recent findings that bolus is advantageous compared to continuous feeding in supporting optimal protein anabolism.

Recent findings—Whether bolus or continuous feeding is more beneficial has been controversial, largely due to limitations inherent in clinical studies such as the presence of confounding variables and the inability to use invasive approaches. Recent studies using the piglet as a model of the human neonate showed that, compared to continuous feeding, bolus feeding enhances protein synthesis and promotes greater protein deposition. The increase in protein synthesis occurs in muscles of varying fiber type and in visceral tissues whereas muscle protein degradation is largely insensitive to feeding pattern. This higher protein synthesis rate is enabled by the rapid and profound increases in circulating amino acids and insulin that occur following a bolus feed, which activate the intracellular signaling pathways leading to mRNA translation.

Summary—Recent findings indicate that bolus feeding enhances protein synthesis more than continuous feeding, and promotes greater protein anabolism. The difference in response is attributable to the pulsatile pattern of amino acid- and insulin-induced translation initiation induced only by bolus feeding.

Keywords

amino acids; protein synthesis; translation initiation; skeletal muscle; infant

Introduction

Approximately 8% of newborns are of low birth weight (LBW; less than 2.5 kg or 5.5 pounds; 1). These infants face uncertain futures, ranging from insufficient postnatal growth to compromised neurodevelopmental outcomes (2). Thus, optimization of their nutritional management is crucial for achievement of their long-term health and well-being. Nonetheless most of these infants are discharged weighing less than the tenth percentile for age despite improvements in their nutritional management (3). Some remain small to adulthood and exhibit adverse long-term developmental outcomes including learning

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impairments and reduced work capacity (4,5). Because growth failure of LBW infants has been attributed, in part, to the provision of inadequate levels of protein and energy, more aggressive nutritional support is now being advocated (6). Evidence suggests that this approach is justified because early provision of parenteral amino acids to extremely LBW infants is associated with improved growth (7) and provision of moderate vs. low amino acid levels by parenteral infusion increases whole body protein synthesis and accretion rates in LBW infants (8). Parenteral feeding allows rapid nutrition when enteral nutrition is not possible due to respiratory problems, limited gastric capacity, reduced intestinal mobility, and a perceived risk for necrotizing enterocolitis (6). When an infant is medically stable, minimal enteral feeding is provided to “prime” the intestine and the percentage of enteral to parental feeding is advanced until full enteral feedings can be achieved.

Due to the lack of ability of the majority of preterm infants to coordinate suckling, swallowing, and breathing, neonatologists prescribe suitable tube feeding methods to ensure sufficient feeding tolerance and to support optimal growth (9). Therefore, feeding by orogastric or nasogastric tube using either continuous or intermittent bolus delivery of formula and human milk is common practice for these infants (10). Intermittent bolus feeding simulates the feeding pattern of infants when they are breast or bottle fed and has been advocated to promote more physiological feeding-fasting hormonal levels than continuous feedings (11). Several studies have shown that intermittent bolus feedings reduces the time to achieve full enteral feeds, decreases feeding intolerance and increases weight gain (12) but contrary results have also been published (13). A recent Cochrane review found no differences in growth or time to reach full enteral feeds but cites small sample size, methodological limitations, and difficulties in controlling variables that can affect outcomes as limitations to discerning the effectiveness of the tube feeding methods (14). More detailed experimental evidence from studies using neonatal pigs, a well-recognized animal model for human infants, demonstrated that intermittent bolus compared to continuous orogastric feeding promotes better weight gain, intestinal growth and development (15). Until recently, no other information was available on the impact of these different feeding modalities on the growth and maturation of other organ systems including skeletal muscle.

During the neonatal period, all tissues undergo rapid growth. Skeletal muscle is the fastest growing and largest protein mass in neonates and a major determinant of their amino acid requirements (16). Protein accretion is dependent on the rate of protein synthesis being higher than the rate of protein degradation. The fractional rate of protein synthesis in the majority of tissues, especially skeletal muscle, is very high immediately after birth and decreases with development. Additionally, protein degradation is moderately elevated in early life, and thus, the high rate of neonatal muscle growth is mainly the result of the high rate of protein synthesis. Our work in rats and pigs suggests that neonatal animals use dietary amino acids efficiently for growth because they can increase protein synthesis in response to ingestion of a meal (16). Several studies indicate that feeding stimulates protein synthesis in newborn humans and growing animals (17, 18), but the response is much smaller in adults (19). Until recently, no information was available regarding the effect of intermittent bolus feeding vs. continuous feeding on tissue protein anabolism. In this review,

we will describe current understanding of the mechanisms that govern the anabolic effect of different feeding modalities on neonatal growth.

Feeding stimulates neonatal growth

Feeding stimulates whole body protein synthesis in the human neonate (17). This feeding-induced stimulation of protein synthesis is crucial to support the rapid growth rate of early postnatal life (20). Due to ethical considerations, invasive approaches cannot be used in the human infant to investigate the intracellular mechanisms by which feeding regulates protein synthesis and growth. Therefore, animal models have been utilized, particularly the neonatal pig which is similar to the human infant in its anatomy, developmental physiology, and metabolism. Our studies in the neonatal pig have shown that ingestion of a meal stimulates protein synthesis in all tissues with the greatest response occurring in skeletal muscle (20). Following ingestion of a meal that provides one-sixth of the daily dietary requirements, the stimulation of muscle protein synthesis is rapid and reaches peak activation within 30 min (21). The response is more sustained in glycolytic compared to oxidative muscles but returns to baseline by 4 h (22). The stimulation is more profound, although not sustained, in pancreatic tissue than in other visceral tissues while it is more long-lasting in the liver (22).

In vivo studies in the neonatal pig have delineated two anabolic agents that play major roles in protein synthesis regulation after a meal – insulin and amino acids. Using the pancreatic-substrate clamp technique to independently control the effects of insulin, glucagon, glucose, and amino acids, these studies have shown that the post-prandial rise in the circulating levels of both insulin and amino acids facilitates the feeding-induced increase in protein synthesis in skeletal muscle of the neonate (16). The dose-response effect of both amino acids and insulin on muscle protein synthesis is additive until maximal rates of protein synthesis are achieved, thus ensuring that after a meal amino acids are rapidly and efficiently channeled towards protein synthesis, rather than being catabolized. The increase in visceral tissue protein synthesis is primarily regulated by amino acids (18). This ability of insulin and amino acids to stimulate protein synthesis decreases with development, in parallel with the decline in the response of protein synthesis to feeding (18).

Insulin and amino acids mediate the feeding-induced activation of signaling components leading to protein synthesis

A major outcome of feeding-induced anabolism is protein deposition, supported by an enhanced rate of protein synthesis with no change in protein degradation (17). Since protein degradation plays a minor part in this context, we have focused our discussion on the role of insulin and amino acids in the regulation of protein synthesis. The major steps of the insulin and amino acid signaling cascades have been reviewed elsewhere (23–27) and are summarized in Figure 1. Whereas the insulin signaling pathway that regulates protein synthesis is well-characterized, the molecular mechanism by which amino acids affect protein synthesis is still not well-understood (27). Much of the work on the regulation of protein synthesis by amino acids has been generated from cell culture studies, however, these results cannot be generalized to all cells or tissue types, or to physiological conditions that occur in the whole animal. Although studies on the effects of amino acids and insulin on

protein synthesis have been performed by other laboratories in adults (e.g., 28,29), in this review we will focus on work performed in neonates.

Studies in the neonatal pig suggest that the rise in insulin after a meal leads to the activation of the insulin signaling pathway (16,18), including the insulin receptor, insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI 3-kinase), phosphoinositide-dependent kinase-1 (PDK-1), and protein kinase B (PKB). The activated PKB then deactivates the inhibitory complex, tuberous sclerosis protein 1 and 2 (TSC1/2), thereby activating the master protein kinase, mammalian target of rapamycin complex 1 (mTORC1) (18). These responses decrease with development in parallel with the decline in protein synthesis. Our findings in the neonatal piglet model (16,18) support other work (30) demonstrating that amino acids stimulate mTORC1 but not PKB or TSC1/2. Examination of purported amino acid signaling components leading to mTORC1 activation, including FK506-binding protein 383 (FKB38), the vacuolar protein sorting 34 (Vps34), proline-rich Akt substrate of 40 kDa (PRAS40), phospholipase D1 (PLD1), the small GTPase Ras homolog enriched in brain (Rheb), the RAS-related GTP-binding proteins (Rag), the G protein beta subunit-like (GβL), and the regulatory-associated protein of mTOR (raptor), have yielded little evidence for their involvement in the rapid stimulation of protein synthesis that occurs after a meal, albeit PLD1, the Rag proteins, and raptor were shown to be more abundant in neonatal muscle (16,18,31). Nonetheless, the post-prandial rise in both amino acids and insulin, independently and dose-dependently, mediate the feeding-induced increase in the phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor (eIF) 4EBP1, direct down-stream targets of mTORC1. The resulting detachment of 4EBP1 from an inactive 4EBP1•eIF4E complex allows eIF4E to form an active complex with eIF4G, thereby activating translation initiation (18).

An evaluation of the time course of the changes in protein synthesis regulators after ingestion of a meal that provides one-sixth of the daily dietary requirements has shown that there is a sharp post-prandial increase in circulating insulin concentrations which then rapidly falls toward baseline whereas the increase in amino acids is more modest but maintained for most of the 4 h feeding period (21). The rise in PKB activation in skeletal muscle after a meal is rapid but not sustained, mirroring the changes in circulating insulin concentrations (21). In contrast, the phosphorylation and activation of mTOR (22), 4EBP1, ribosomal S6 (rpS6), and eIF4G and the formation of the active eIF4E•eIF4G complex in muscle is maximized by 30 min and is sustained for at least 2 h, falling to baseline by 4 h, similar to the circulating amino acids levels. These changes in mTORC1 signaling parallel the changes in the number of ribosomes associated with polysomes, indicating upregulation of translation initiation in muscle (21). Furthermore, ingestion of a meal elicit a similar time-dependent pattern of mTORC1 signaling leading to protein synthesis in muscles of different fiber types as well as in visceral tissues (22). Thus, mTORC1 signaling to protein synthesis is activated for a limited period following ingestion of a meal and hence, frequent meals are essential for sustaining optimal rates of protein synthesis and consequently, growth in the neonate.

Differential effects of bolus and continuous feeding on protein anabolism

Because our previous studies in neonatal pigs had shown that the stimulation of muscle translation initiation and protein synthesis after a meal is mediated by the postprandial increase in insulin and amino acids, we hypothesized that it is the resulting surge in these anabolic agents that is required to activate the signaling components involved in the regulation of protein synthesis. Conversely, we hypothesized that the low and constant hormone-substrate pattern incurred with continuous feeding attenuates the muscle protein synthetic response. To test these hypotheses, neonatal pigs received a complete formula for 24 h by orogastric tube either as an intermittent bolus feed every 4 h, or they were fed the same amount of food continuously. Because the growth rate of piglets is greater than that for infants, the protein and energy requirements are higher. The milk replacer contained 5% whey protein concentrate, 1% lactose, and 6.5% fat, providing 192 kcal, 10 g protein and 13 g fat per kg of body weight per day. By performing the study in the neonatal piglet model, we could eliminate confounding factors that are common to human studies such as differences in pathological and clinical complications, selection bias, protocol violations, food composition, and mode of nutritional support.

The results of this work demonstrated that circulating insulin and amino acid levels increase minimally and remain constant in continuously fed pigs compared to fasted pigs, whereas the intermittent bolus feeds elicit a pulsatile insulin and amino acid pattern (Figure 2; 32). These results are supported by previous studies showing that preterm infants who receive bolus feeding exhibit marked cyclical surges in hormone levels (33). Furthermore, we showed that continuous feeding increases muscle protein synthesis compared to fasting (Figure 3; 32), consistent with the demonstrated ability of continuous feeding to promote growth (14), but the increase in protein synthesis is greater after an intermittent bolus meal. In all tissues examined (gastrocnemius, masseter and soleus muscles, heart, liver, pancreas, jejunum, and kidney), both feeding modalities enhance protein synthesis, but the greatest increase occurs after a bolus feed (34). Using stable isotope tracer methodology in a multi-catheterized piglet model which allows for the dynamic measurements of both protein synthesis and breakdown, we showed that the increase in protein synthesis leads to an increase in protein deposition whereas protein degradation is insensitive to feeding frequency (Figure 4; 35). Consistent with the protein synthesis and degradation data, phosphorylation of PKB, S6K1, and 4EBP1 and formation of the active eIF4E/eIF4G complex are higher in the intermittent bolus fed compared to the continuously fed piglets (Figure 3; 32). The abundance of the E3-ligases MURF-1 and Atrogin-1 is unchanged, albeit the LC3-II to total LC3 ratio is reduced (35). These results implicate mTORC1-dependent translation initiation and perhaps the autophagy-lysosome system, but not the ubiquitin-proteasome degradation system, in the enhanced protein deposition with intermittent bolus feeding.

Although continuous feeding blunts protein synthesis compared to intermittent bolus feeding in piglets (32, 34, 35), continuous feeding is still indicated in some infants due to feeding intolerance (12). Thus, new strategies are needed to optimize the nutritional management of continuously fed infants. Previous studies from our laboratory have demonstrated that the stimulatory effect of amino acids on neonatal protein synthesis can largely be reproduced by

leucine (16) and that supplementation with leucine, either parenterally or enterally, simulates protein synthesis provided an adequate supply of amino acids is available (36–39). To determine whether leucine can be used as a nutritional supplement to promote protein anabolism in neonates who are continuously fed, the effect of leucine pulses delivered parenterally every 4 h for 24 h in neonatal pigs fed continuously by orogastric tube was determined (40). The results showed that this feeding regimen increases protein synthesis in muscles of different fiber types by stimulating translation initiation and may reduce protein degradation via the autophagy-lysosome, but not the ubiquitin-proteasome pathway. Although leucine pulses increase protein synthesis beyond that achieved by continuous feeding alone, the rates attained in most muscles are not as great as those obtained following intermittent bolus feeding.

Conclusions

The benefits of bolus versus continuous feeding in LBW infants have been debated for some time. Meta-analyses of randomized trials (14) have been unable to discern the clinical benefits due to confounding factors and methodological limitations. Thus animal studies provide critical information on the mechanism by which feeding modalities regulate protein anabolism. Data from our studies in neonatal pigs suggest that intermittent bolus feedings induce greater rates of protein synthesis in most tissues compared to continuous feedings. Mechanistically, the higher rate of tissue protein synthesis is associated with enhanced activation of signaling components that regulate translation initiation. Furthermore, using tracer techniques, we showed that the rate of protein degradation in both feeding modalities is similar suggesting that higher protein deposition in intermittent bolus-fed pigs is mainly due to the higher rate of protein synthesis. Further studies are required to determine whether this increase in protein deposition rate, when sustained chronically, will translate into an increase in lean body mass and growth in the neonatal piglet model. Nonetheless, the results of our studies provide unequivocal evidence that intermittent bolus feeding is the preferable feeding method to enhance protein deposition in orogastric tube-fed infants.

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Keypoints

- Limited studies of the effectiveness of intermittent bolus versus continuous feeding in the neonate have not discerned their clinical benefit on protein metabolism.
- Studies in the neonatal piglet model have shown that intermittent bolus feeding enhances protein synthesis more than continuous feeding.
- The greater effect of intermittent bolus compared to continuous feeding on protein synthesis is due to the more rapid and profound changes in circulating amino and insulin concentrations.
- This pulsatile pattern of circulating amino acids and insulin after a meal activates the insulin and amino acid signaling pathways leading to an increase in mTORC1-dependent mRNA translation.

Figure 1.

Recent concepts of the insulin and amino acid signaling pathways that regulate the activation of mTOR1 towards protein synthesis (23–27). 4EBP1, 4E-binding protein 1; eIF4E, eukaryotic translation initiation factor 4E; eIF4G, eukaryotic translation initiation factor 4G; FKBP12, the 12-kDa FK506-binding protein; GβL, G protein beta subunit-like; IRS-1, insulin receptor substrate-1; mTORC1, mammalian target of rapamycin complex 1; PI 3-Kinase, phosphoinositide 3-kinase (PI 3-kinase); PDK1, phosphoinositide-dependent kinase-1; PKB, protein kinase B; Rag, RAS-related GTP-binding proteins; Ragulator, a complex encoded by the MAPKSP1, ROBLD3, and c11orf59 genes; Raptor, regulatory-associated protein of mTOR; Rheb, the small GTPase Ras homolog enriched in brain; rpS6, ribosomal S6; S6K1, ribosomal protein S6 kinase 1; TSC1/2, tuberous sclerosis protein 1 and 2; vacuolar H(+)-ATPases (v-ATPase).

Figure 2.

Plasma insulin (A) and branched-chain amino acid (BCAA; B) concentrations in intermittent bolus fed (closed circles) and continuous fed (open circles) of neonatal pigs. Arrows indicate time of feeding (32). Values are means $\pm$ SEM, $n = 5-7$. Repeated-measures analysis showed an effect of time, treatment, and their interaction on plasma glucose, insulin, and BCAA concentrations, $P < 0.05$.

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Figure 3.

Protein synthesis rate (A) and eIF4G•eIF4E abundance (B) in the longissimus dorsi muscle of intermittent bolus fed, continuous fed, and feed-deprived neonatal pigs (32). Values are means $\pm$ SEM, $n = 5-7$. ANOVA showed an effect of treatment, $P < 0.05$. Means without a common letter differ, $P < 0.05$.

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Figure 4.

Hindquarter rates of protein synthesis (A), degradation (B), and deposition (C) in neonatal pigs that were fasted (FAS), continuous fed (CON), or intermittent bolus fed (INT; 7×4 h meals) for 29 h. Values are means $\pm$ SE; $n = 6$. Data are derived from the study reported in reference 35.

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