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INVITED REVIEW

# Leucine is a major regulator of muscle protein synthesis in neonates

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**Abstract** Approximately 10 % of infants born in the United States are of low birth weight. Growth failure during the neonatal period is a common occurrence in low birth weight infants due to their inability to tolerate full feeds, concerns about advancing protein supply, and high nutrient requirements for growth. An improved understanding of the nutritional regulation of growth during this critical period of postnatal growth is vital for the development of strategies to improve lean gain. Past studies with animal models have demonstrated that muscle protein synthesis is increased substantially following a meal and that this increase is due to the postprandial rise in amino acids as well as insulin. Both amino acids and insulin act independently to stimulate protein synthesis in a mammalian target of rapamycin-dependent manner. Further studies have elucidated that leucine, in particular, and its metabolites, α-ketoisocaproic acid and β-hydroxy-β-methylbutyrate, have unique anabolic properties. Supplementation with leucine, provided either parenterally or enterally, has been shown to enhance muscle protein synthesis in neonatal pigs, making it an ideal candidate for stimulating growth of low birth weight infants.

**Keywords** Amino acids · Leucine · Low birth weight · mTOR · Neonatal · Protein synthesis

**Abbreviations**

| | |
|---|---|
| 4EBP1 | 4E-binding protein 1 |
| AA | Amino acids |
| Akt/PKB | Protein kinase B |
| BCAA | Branched-chain amino acids |
| BOL | Bolus fed |
| CON | Continuously fed |
| CON+LEU | Continuously fed and pulsed with leucine |
| eIF4E | Eukaryotic initiation factor 4E |
| eIF4G | Eukaryotic initiation factor 4G |
| HMB | β-Hydroxy-β-methylbutyrate |
| KIC | α-Ketoisocaproic acid |
| mTOR | Mammalian target of rapamycin |
| S6 | Ribosomal protein S6 |
| S6K1 | Ribosomal protein S6 kinase 1 |

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## Introduction

The rate of growth during the neonatal period is higher than at any other stage of postnatal development (Davis et al. 1996). The majority of this growth is comprised of skeletal muscle (Davis and Fiorotto 2009) and, therefore, muscle is a significant determinant of amino acid and energy requirements at this stage of development. Approximately 10 % of newborn infants in the United States are of low birth weight and many remain small at hospital discharge and are of short stature as adults (Ehrenkranz 2007). Poor growth in the immediate neonatal period is due to a number of factors including reduced nutrient intake as a result of overall feeding intolerance or inability to handle full protein feeding. An understanding of the mechanisms underlying growth in the neonatal period, especially with respect to muscle growth, is crucial for the development of strategies to improve survival and long-term health of low birth weight infants. Previously, we demonstrated that the increase in muscle protein synthesis following a meal is due to the postprandial rise in amino acids as well as insulin (Wray-Cahen et al. 1998; Davis et al. 2002; O'Connor

et al. 2003). In particular, the branched-chain amino acid, leucine, and its metabolites, α-ketoisocaproic acid and β-hydroxy-β-methylbutyrate, have unique anabolic properties and the potential to enhance protein synthesis and growth during the neonatal period. In this review, we will discuss the regulation of skeletal muscle growth during the neonatal period, through mTOR-related pathways, and how nutrient ingestion, particularly of leucine and its metabolites, can be used to modulate the protein synthetic machinery in muscle.

## Muscle growth and development in the neonatal period

The neonatal period is characterized by a rapid rate of growth and is, therefore, a critical stage of development (Senterre and Rigo 2013). Growth faltering during this time often leads to both short- and long-term consequences including short stature, neuronal deficits (Ford et al. 2000; Hay 2008), and increased risk of cardiovascular disease and metabolic syndrome, including obesity, in later life (Barker 2004; Yajnik 2004; Senterre and Rigo 2013; Brown 2014). For the low birth weight infant (≤2,500 g; Ehrenkranz 2007), the risk of poor growth and adverse developmental outcomes is especially high (Brown 2014). Regardless of technological advances and improvements in the nutritional management of these infants, many are discharged weighing less than the 10th percentile of intrauterine growth standards (Ehrenkranz et al. 1999). Due to concerns regarding the ability of low birth weight infants to tolerate full feeds or to metabolize nutrients and the potential for hyperammonemia, metabolic acidosis, and necrotizing enterocolitis (Johnson et al. 1972; Thureen and Hay 2001; Hay 2008; Abdelhamid et al. 2011), the move to feed with higher protein content is often delayed, resulting in reduced nutrient consumption and a failure to meet the infant's requirements (Senterre and Rigo 2013). This is despite evidence of improved growth with greater parenteral infusion of amino acids (Thureen et al. 2003; Ibrahim et al. 2004) or dietary protein intake (Kashyap et al. 1986, 1988; Cooke et al. 2006; Premji et al. 2006). A more complete understanding of nutrient metabolism in infants, as well as the physiology of muscle growth, will aid in the development of nutritional interventions aimed at optimization of lean growth during the neonatal period.

In the neonate, skeletal muscle represents 30 % of body mass (Davis and Fiorotto 2009) and is the most rapidly growing body compartment (Davis et al. 1989, 1996). The muscle protein pool, therefore, is an important determinant of overall body protein metabolism and amino acid requirements in the young, growing animal (Liu and Barrett 2002; Lobley 2003). It has been demonstrated previously that feeding stimulates protein synthesis in all tissues in the young rat (Davis et al. 1989), pig (Burrin et al. 1992; Davis et al. 1993) and human infant (Denne et al. 1991) and that this rise is most pronounced for skeletal muscle (Davis and Fiorotto 2009). The response in protein synthesis to a meal is rapid, reaching peak activation within 30 min and remaining elevated for a minimum of 2 h post-meal (Wilson et al. 2009).

## Role of amino acids and insulin in the postprandial stimulation of muscle protein synthesis

A number of potential positive regulators of muscle protein synthesis have been discussed previously (Lobley 1998; Liu and Barrett 2002), including IGF-1, insulin, glucose, growth hormone, and amino acids. Thus, the rise in plasma levels of insulin, glucose, and amino acids in response to a meal is of potential importance for the regulation of protein synthesis. Indeed, the circulating levels of these plasma metabolites were shown by Wilson et al. (2009) to parallel the changes in protein synthesis in muscle tissue. The importance of the rise in plasma metabolites in response to a meal with respect to initiation of protein synthesis was also demonstrated by El-Kadi et al. (2012) who examined the effect of bolus versus continuous feeding in young pigs. It was shown that bolus feeding, which elicits a rapid rise in plasma insulin, glucose, and amino acids, resulted in greater rates of muscle protein synthesis than in those pigs fed an equivalent amount of the same diet continuously, which produced steady and low plasma levels of the same metabolites (El-Kadi et al. 2012). The importance of the postprandial rise in plasma metabolites was also demonstrated in studies which showed that a twofold increase in plasma aminoacidemia is required to increase protein synthesis (Tessari et al. 1987; Giordano et al. 1996; Dangin et al. 2001).

Elucidating which dietary component or hormonal response to dietary intake is responsible for the postprandial rise in protein synthesis is confounded by the concurrent changes in a number of plasma metabolites and hormones in response to feeding. This is especially difficult to determine when several potential anabolic signals are interrelated, as is the case with plasma insulin, glucose, and amino acids. The pancreatic–substrate clamp technique allows for independent assessment of the effects of insulin, glucose, and amino acids by blocking pancreatic insulin secretion, maintaining constant glucose, and manipulating plasma levels of insulin and other hormones and nutrients (Wray-Cahen et al. 1998). Using this technique, we demonstrated that the feeding-induced stimulation of muscle protein synthesis is independently regulated by the postprandial rise in amino acids and insulin (Fig. 1; Wray-Cahen et al. 1998; Davis et al. 2002; O'Connor et al. 2003) and the ability of both amino acids and insulin to stimulate protein synthesis is unique to skeletal muscle (Davis et al. 2002). Raising plasma insulin to

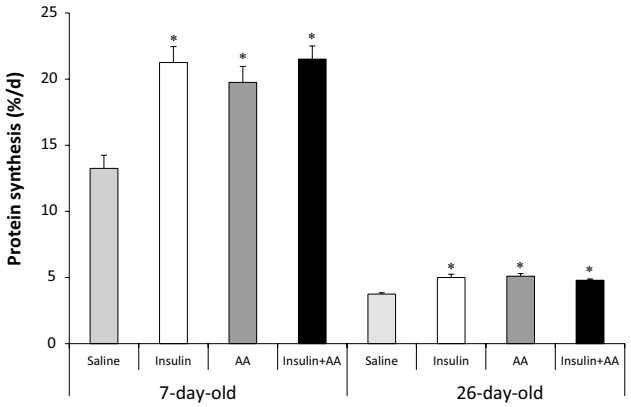


**Fig. 1** Fractional protein synthesis rates in longissimus dorsi muscle of 7- and 26-day-old neonatal pigs in which saline, insulin, amino acids (AA), or both amino acids and insulin were infused during a pancreatic clamp. Euglycemia was maintained at the fasting level. *Significantly different than control within an age group ($P < 0.05$). Adapted from Davis et al. (2002) with permission

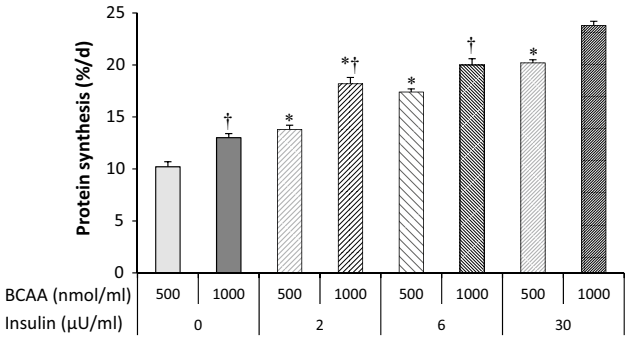


**Fig. 2** Fractional protein synthesis rates in longissimus dorsi muscle of neonatal pigs at different levels of insulin and amino acids. *Significantly different than previous insulin level within same amino acid level ($P < 0.05$). †Significantly different than 500 nmol/ml branched-chain amino acids (BCAA) within same insulin level ($P < 0.05$). Adapted from O'Connor et al. (2003) with permission

levels similar to those observed in the fed state, while maintaining amino acids at fasting levels, resulted in maximum stimulation of protein synthesis, with no further stimulation of protein synthesis at insulin levels above fed values (Wray-Cahen et al. 1998). Similarly, raising plasma amino acid levels to the fed state resulted in an increase in protein synthesis (Fig. 2; O'Connor et al. 2003), although it is not known whether higher levels of amino acids would have continued to increase muscle protein synthesis.

Although these results are in agreement with previous studies that suggest amino acids alone can stimulate protein synthesis (Bennet et al. 1989, 1990; Watt et al. 1992), this has not been a consistent finding. For example, Preedy and Garlick (1986) and Garlick and Grant (1988) suggested that amino acids only act to enhance insulin-stimulated protein synthesis in skeletal muscle and do not act independently of insulin. One possible explanation for this discrepancy is the plasma insulin level at which the effect of amino acids was examined in the different studies. O'Connor et al. (2003) demonstrated a dose–response of protein synthesis to both insulin and amino acids (Fig. 2), with the effect of amino acids and insulin being additive up to plasma insulin concentrations equivalent to those observed in the fed state. Davis et al. (2002) also demonstrated that there was no difference in protein synthesis when insulin and amino acids were independently or concurrently increased to fed levels (Fig. 1).

In addition, when comparing results from different studies, the age at which the anabolic effects of insulin and amino acids are determined must be considered. The response of protein synthesis to a meal has been shown to rapidly decline with age in the rat (Baille and Garlick 1991), human (Davis et al. 1989; McNurlan et al. 1993), and pig (Fig. 1; Davis et al. 1996) and this decline is more evident for skeletal muscle than for visceral tissue protein synthesis (Davis et al. 1996). Moreover, the developmental decline in the response of protein synthesis to insulin and amino acids parallels the decline in protein synthesis observed with feeding (Suryawan et al. 2007). Those studies in which amino acids were shown to have little or no effect on protein synthesis may have been at an age in which the anabolic response is reduced, making it more difficult to detect differences across treatments, or in tissues that respond differently to insulin and amino acid signaling.

## Regulation of protein synthesis through the mTOR pathway

The postprandial increase in protein synthesis is regulated through the effects of amino acids, growth factors, and hormones, such as insulin, on the mammalian target of rapamycin (mTOR) signaling pathway (Fig. 3; Burrin and Davis 2013; Kimball 2013). There are two distinct mTOR complexes, mTOR complex 1 (mTORC1) and complex 2 (mTORC2), with mTORC1 clearly being associated with regulation of protein turnover (Kimball 2013). This review will focus on the regulatory importance of mTORC1 and the use of mTOR will refer solely to this complex. The pathway through which insulin activates mTOR is well characterized and has been presented in a number of reviews (Wullschleger et al. 2006; Hietakangas and Cohen 2009). In brief, insulin binds to and activates its receptor which in turn initiates a signaling cascade, activating phosphoinositide 3-kinase and phosphoinositide-dependent kinase 1. This in turn leads to activation of Akt (also known as protein kinase B) which phosphorylates and inactivates tuberous sclerosis complex 1 and 2, a repressor of mTOR activity (Kimball 2013). The mechanism by which amino

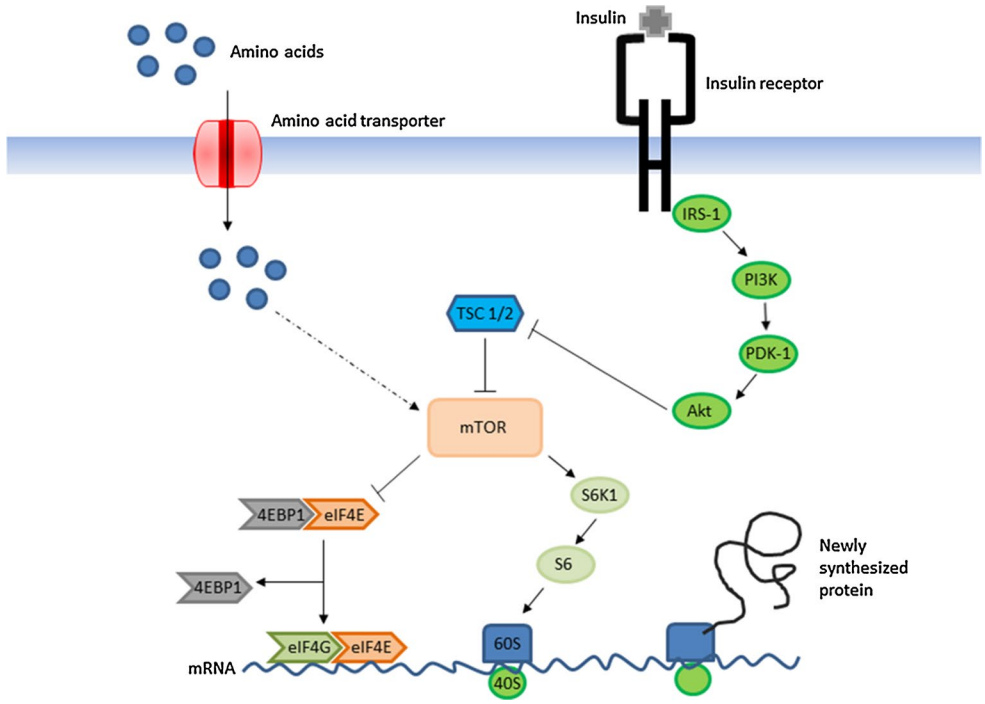


**Fig. 3** Regulation of protein synthesis through the mTOR signaling pathway by amino acids and insulin. Insulin binds to its receptor and activates insulin receptor substrate-1 (IRS-1) which in turn activates phosphoinositide-3 kinase (PI3K), phosphoinositide-dependent kinase (PDK), and Akt. Activation of Akt removes the inhibition of mTOR by the tuberous sclerosis complex 1 and 2 (TSC 1/2). Both amino acids and insulin activate mTOR, which results in inactivation of the eukaryotic initiation factor repressor 4E-binding protein 1 (4EBP1), release of eukaryotic initiation factor (eIF) 4E, and formation of the active eIF4E·eIF4G complex. mTOR also activates ribosomal protein S6 kinase 1 (S6K1) which in turn activates ribosomal protein S6 (S6) allowing recruitment of the 60S and 40S ribosomal subunits. Phosphorylation of S6 and formation of the active eIF4E·eIF4G complex stimulates translation initiation and protein synthesis

acids act to stimulate protein synthesis through mTOR is less well understood, however, it is widely accepted that amino acids stimulate protein synthesis through a pathway independent of the insulin signaling cascade (Efeyan et al. 2012). Evidence for this is provided by observations that amino acid infusion alone, without insulin, fails to result in the activation of Akt (Liu et al. 2002; Suryawan et al. 2012) indicating mTOR regulation by amino acids occurs through a distinct pathway. Moreover, the in vivo infusion of rapamycin, an inhibitor of mTOR activity, is capable of eliminating the stimulatory effect of amino acids on protein synthesis (Suryawan et al. 2008; Dickinson et al. 2011), indicating that amino acids do act to regulate mTOR activity. Regardless of upstream events, stimulation by both insulin and amino acids results in activation of mTOR. Activation of mTOR results in phosphorylation and activation of the 70-kDa ribosomal protein S6 kinase 1 (S6K1) which, in turn, phosphorylates ribosomal protein S6 (S6). mTOR also phosphorylates the eukaryotic initiation factor (eIF) repressor 4E-binding protein 1 (4EBP1), releasing eukaryotic initiation factor-4E (eIF4E) which can then bind to eIF4G (Kimball 2013). Phosphorylation of ribosomal protein S6 and the formation of the active eIF4E·eIF4G complex stimulates translation initiation and protein synthesis.

The rapid increase in protein synthesis following a meal was shown by Wilson et al. (2009) to be highly correlated with phosphorylation of key signals in the mTOR signaling pathway, such as Akt, mTOR, S6, and 4EBP1 (Fig. 4). Given the central role of mTOR signaling for translation initiation, mTOR activation has been utilized to indicate an increase in protein synthesis rate, however, it has been demonstrated that the phosphorylation status of targets of mTOR (i.e., 4EBP1 and S6K1) does not always correlate with measured rates of fractional protein synthesis (Escobar et al. 2005; Greenhaff et al. 2008; Atherton et al. 2010a). There are several possible reasons for this, including the timing of muscle tissue sampling for determination of translation initiation signaling versus the period over which protein synthesis is determined as well as the stimulation of the protein synthetic machinery versus the availability of protein precursors. It is, therefore, important to validate observations of mTOR activation with actual rates of protein synthesis and caution should be used when interpreting results of studies in which the latter is not reported.

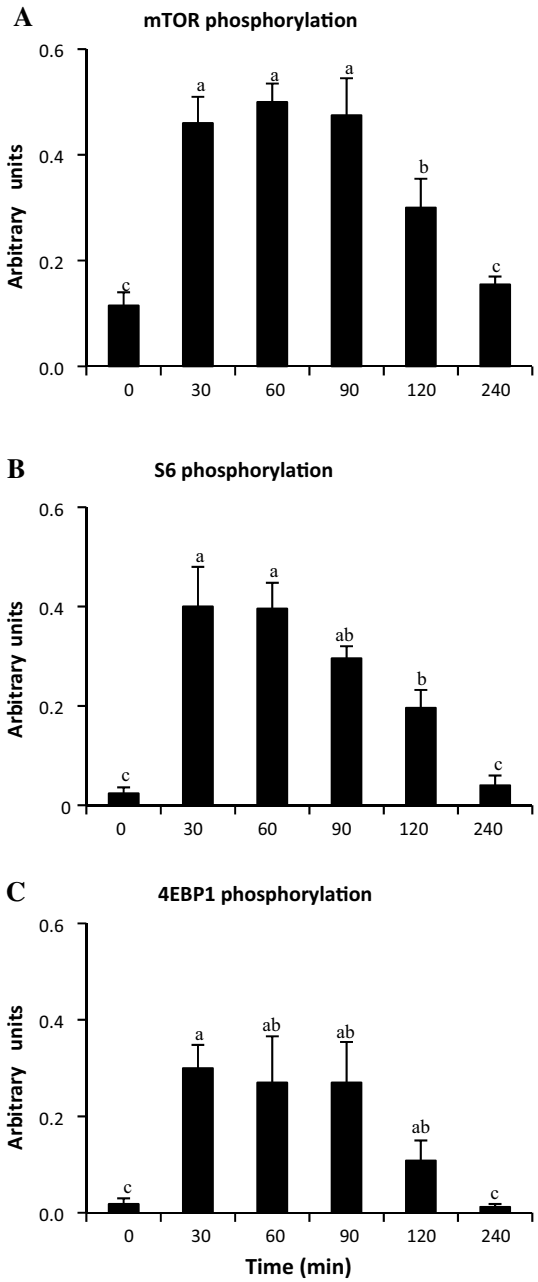


**Fig. 4** Changes in phosphorylation of mTOR$^{Ser2448}$ (**a**), ribosomal protein S6$^{Ser235/236}$ (**b**), and 4EBP1$^{Thr70}$ (**c**) over 240 min in longissimus dorsi muscle of neonatal pigs following a meal providing one-sixth of their daily requirements. Means without a common letter differ ($P < 0.05$). Adapted from Wilson et al. (2009) with permission

## Anabolic effect of dietary amino acids on muscle protein synthesis

The dose–response of protein synthesis to increasing intake of dietary protein has been clearly demonstrated in human adults (Moore et al. 2009; Churchward-Venne et al. 2014; Witard et al. 2014), infants (Kashyap et al. 1986, 1988; Thureen et al. 2003), and young animals (O'Connor et al. 2003; Frank et al. 2006). Furthermore, it has been demonstrated that muscle protein synthesis will continue to increase with additional dietary protein but eventually reaches a plateau beyond which no further increase is achieved (Frank et al. 2006). The lack of a growth response to further increases in dietary protein indicates that the protein requirement for maximal muscle growth has been reached or another nutrient, such as energy, has become limiting (NRC 2012). In addition, Frank et al. (2006) demonstrated that there is no further increase in activation of mTOR signaling components in piglets at levels of dietary protein above the requirement (NRC 1998), indicating a molecular basis for this plateau as well.

Due to the importance of protein and amino acids in stimulating muscle protein synthesis, the amino acids responsible for this effect have been the subject of a number of investigations. The amino acid composition of dietary protein sources can differ drastically (FAO 1970; NRC 2012), resulting in very different rates of protein synthesis when equivalent amounts of different protein sources are fed (Norton et al. 2012). It is generally assumed that indispensable amino acids are primarily responsible for the anabolic response of muscle protein synthesis to dietary protein (Borsheim et al. 2002; Liu et al. 2002; Volpi et al. 2003) with little impact of dispensable amino acids. However, regardless of their impact on intracellular signaling of protein synthesis, dispensable amino acids may play an important role in maintaining the increased rates of synthesis (Davis et al. 2002), most likely due to their presence in muscle protein and role as precursors for a number of other protein metabolites (Wu et al. 2013). In adult male humans, approximately 10 g of total indispensable amino acids appears to be required to maximize the synthetic response to ingestion of a protein meal (Cuthbertson et al. 2005; Moore et al. 2009), which is equivalent to about 20–25 g of high-quality protein such as whey, casein, egg white, or soy. The variability in the maximum protein synthetic rate observed with differing protein sources (Norton et al. 2012) is likely due to the total indispensable amino acid content but may also be due to the ability of individual amino acids, such as leucine, to stimulate protein synthesis.

### Regulation of muscle protein synthesis by leucine

The stimulatory ability of individual amino acids is still a topic of debate, with studies demonstrating that indispensable amino acids are capable of enhancing protein synthesis through mTOR-dependent pathways in muscle (Atherton et al. 2010b). Of all the indispensable amino acids, it is clear that leucine, a branched-chain amino acid, has anabolic properties distinct from its function as a component of proteins. When comparing the anabolic response of

C2C12 skeletal muscle cells to indispensable amino acids, Atherton et al. (2010b) demonstrated that while phosphorylation of S6K1 was increased with all indispensable amino acids, this response was greatest with leucine and leucine was the only amino acid also capable of activation of mTOR and deactivation of 4EBP1. Nonetheless, some studies have demonstrated that the combined anabolic response to a mixture of indispensable amino acids may be sufficient to stimulate protein synthesis. Churchward-Venne et al. (2012), for example, demonstrated that supplementing a low-protein meal with a mix of indispensable amino acids without leucine resulted in the same degree of increase in protein synthesis as supplementing with leucine alone. However, it is possible that the leucine content of the low-protein meal was sufficient to activate protein synthesis and the amino acid mix simply provided more substrate to enable synthesis to occur.

Evidence that leucine is an important anabolic factor was first provided by Buse and Reid (1975) who observed that in vitro incubation of rat hemidiaphragms with leucine increased muscle protein synthesis. The importance of leucine was further demonstrated in vivo by Anthony et al. (2000) and Lynch et al. (2002) who observed that an oral gavage of leucine stimulated protein synthesis in rats in an mTOR-dependent manner. However, these studies used a pharmacological dose of leucine that would never be achieved with normal feeding (Lynch et al. 2002). To determine the efficacy of physiological increases in leucine for stimulating muscle protein synthesis, Escobar et al. (2005) infused leucine intravenously in overnight fasted neonatal pigs at rates that achieved plasma leucine levels similar to those observed following a meal, (i.e., approximately two- to threefold increase above postabsorptive levels). Leucine infusion increased protein synthesis in skeletal muscle and activated key targets of translation initiation including S6K1 and 4EBP1, indicating that the effect of leucine was mTOR dependent (Escobar et al. 2005). Escobar et al. (2005) further demonstrated that a minimum twofold increase in plasma leucine levels was required to maximize protein synthesis, a finding supported by other studies (Escobar et al. 2006, 2007; Boutry et al. 2013). In a follow-up study, Escobar et al. (2006) confirmed that, among the branched-chain amino acids, the response of muscle protein synthesis is unique to leucine and that valine and isoleucine failed to stimulate activation of the mTOR pathway or protein synthesis (Fig. 5).

Another key finding of these leucine infusion studies was that although leucine stimulated muscle protein synthesis this stimulation was maintained only for a short period of time (1 h) and protein synthesis rates returned to baseline values within 2 h of infusion despite continued activation of the mTOR pathway (Escobar et al. 2005). This response is most likely due to the utilization of amino

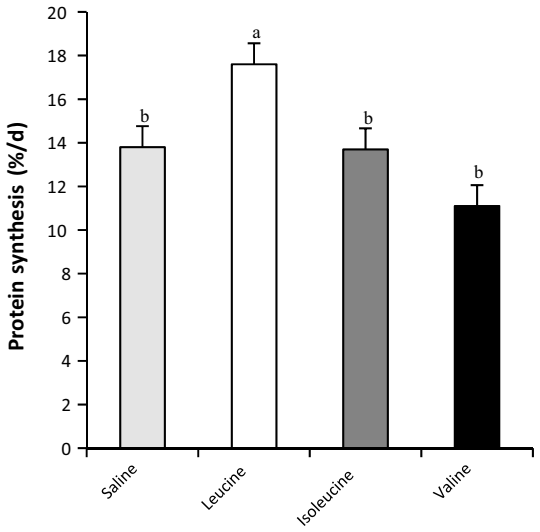


**Fig. 5** Fractional protein synthesis rates in longissimus dorsi muscle of fasted neonatal pigs intravenously infused with saline, leucine, isoleucine, or valine. Means without a common letter differ ($P < 0.05$). Adapted from Escobar et al. (2006) with permission

acids for protein synthesis to the point that there is insufficient substrate for synthesis to continue, regardless of the continued activation of translation initiation signals. Thus, although leucine alone can enhance muscle protein synthesis, this increase cannot be maintained indefinitely without provision of a source of amino acids to maintain plasma aminoacidemia. This conclusion was substantiated by the observation that after 2 h of leucine infusion, the plasma levels of other amino acids had decreased from basal levels (Escobar et al. 2005). To test whether the reduction in protein synthesis after long-term leucine infusion was dependent on substrate availability, Escobar et al. (2007) infused leucine to achieve fed levels in overnight fasted pigs with or without an infusion of an amino acid mixture to keep all other amino acids at the fasted level. Indeed, when plasma amino acid concentrations were maintained, protein synthesis continued to be enhanced beyond 2 h. This enhancement of protein synthesis by leucine could be extended up to 24 h so long as the decline in plasma amino acid concentrations was prevented (Wilson et al. 2010). Therefore, the provision of sufficient substrate is critical for the leucine-induced increase in muscle protein synthesis. Among the essential amino acids, isoleucine and valine have shown a more dramatic decline in plasma levels with leucine supplementation in some studies (Yin et al. 2010; Suryawan et al. 2012). Although it has been suggested that all branched-chain amino acids may need to be supplied in diets with supplemental leucine, few studies have been performed to date to address this hypothesis. Churchward-Venne et al. (2014) reported that while supplementation of a low-protein meal resulted in a reduction in plasma valine

and isoleucine, intracellular levels of the branched-chain amino acids were not affected, suggesting that the drop in plasma branched-chain amino acids may be due to uptake to maintain intracellular levels. Moreover, in a study by Yin et al. (2010), the fall in isoleucine and valine did not appear to limit protein synthesis as supplementation with leucine still resulted in an increase in weight gain.

## Leucine metabolites and muscle protein synthesis

Although the anabolic effects of leucine have been well documented, the exact mechanism by which leucine exerts its anabolic effects is not clear. Moreover, it is not known whether leucine specifically or a molecular characteristic or metabolite unique to leucine is responsible for the increase in protein synthesis. The use of leucine metabolites, such as α-ketoisocaproic acid (KIC) and β-hydroxy-β-methylbutyrate (HMB), may also be useful in therapeutic programs in which muscle growth is desirable but nitrogen intake must be restricted, as in patients with kidney disease. The first step in leucine breakdown is the deamination by branched-chain aminotransferase to produce glutamate and KIC (Suryawan et al. 1998). When Escobar et al. (2010) infused either leucine or KIC into neonatal pigs it was found that leucine and KIC increased muscle protein synthesis to the same extent. The infusion of both KIC and leucine also increased the phosphorylation of 4EBP1 and formation of the active eIF4E·eIF4G complex. It should be noted, however, that the deamination of leucine by branched-chain aminotransferase is a reversible step in the catabolism of leucine. Indeed, infusion of KIC resulted in an increase in plasma leucine concentration (Escobar et al. 2010), indicating that a significant portion of the KIC infused was transaminated to leucine and, therefore, may not have been directly responsible for the increase in protein synthesis. In an early study, Tischler et al. (1982) demonstrated that incubation of muscle tissue with leucine in the presence or absence of an inhibitor of leucine transaminase resulted in the same increase in protein synthesis, indicating that breakdown of leucine to KIC is not required for the stimulatory ability of leucine. The use of KIC to enhance protein synthesis is still a viable alternative in those situations in which reduced dietary protein intake is desirable as no additional nitrogen is provided with KIC.

Another leucine metabolite that has been shown to have potential anabolic properties in adults is HMB (Nissen et al. 1996; Flakoll et al. 2004). HMB is a breakdown product of leucine metabolism produced by the action of α-ketoisocaproatedioxygenase on KIC (Van Koevering and Nissen 1992), with approximately 5 % of daily leucine metabolism being channeled to this fate (Nissen et al. 2000). The ability of HMB to stimulate lean gain in the young or neonatal animal has been largely ignored but, as with KIC, has the potential to be used a therapeutic alternative to promote protein anabolism when reduced dietary protein is required. Unlike KIC, however, the breakdown of leucine to HMB is irreversible and, therefore, any observed responses to HMB cannot be attributed to production of leucine. One study has examined the ability of HMB to stimulate protein synthesis in the neonatal animal. In this study, intravenous infusion of increasing doses of HMB into fasted neonatal piglets for 1 h resulted in activation of the mTOR pathway and an increase in muscle protein synthesis (Wheatley et al. 2014; Fig. 6). Interestingly, it was reported that the greatest response was observed with the dose that resulted in plasma HMB levels of 90 μU/ml, whereas higher doses showed either a reduced anabolic effect or failed to stimulate protein synthesis above the fasting baseline levels. The most effective doses of HMB produced plasma levels of HMB similar to those seen in HMB-supplemented adults (Zanchi et al. 2010).

## Application of leucine to increase lean gain in neonates

Many low birth weight infants experience extrauterine growth failure due to our inability to provide an ideal diet which is sufficiently high in protein and other nutrients to meet their needs (Hay 2008; Berseth 2001). The limitations can be attributed to feeding intolerance and other complications associated with the escalation of oral feeds. In addition, there are concerns of amino acid toxicity, uremia, and acidosis (Hay 2008) when advancing the amount of protein fed to premature infants. Although it is not known whether the ability of low birth weight infants to efficiently increase protein synthesis in response to feeding is impaired, growth is increased with feeding a high-protein diet (Kashyap et al. 1986, 1988). Thus, the reduction in growth during this period is likely due, at least in part, to inadequate nutrition to meet requirements for growth (Hay 2008). Therefore, nutritional therapies that can optimize growth without substantial increases in dietary protein or feeding would be beneficial. Given its anabolic effects, leucine has the potential to improve lean gain in neonates where growth has been restricted. The supplementation of protein-restricted diets with leucine may enhance the efficiency of utilization of amino acids for growth through activation of mTOR signaling. To determine if leucine can improve muscle protein synthesis in protein-restricted neonates, Torrazza et al. (2010) fed neonatal pigs a low-protein meal, a high-protein meal, or a low-protein meal supplemented with leucine. Supplementation of the low-protein meal with leucine resulted in an increase in protein synthesis in both muscle and visceral tissues, (Torrazza et al. 2010). This same effect was maintained when the leucine supplemented

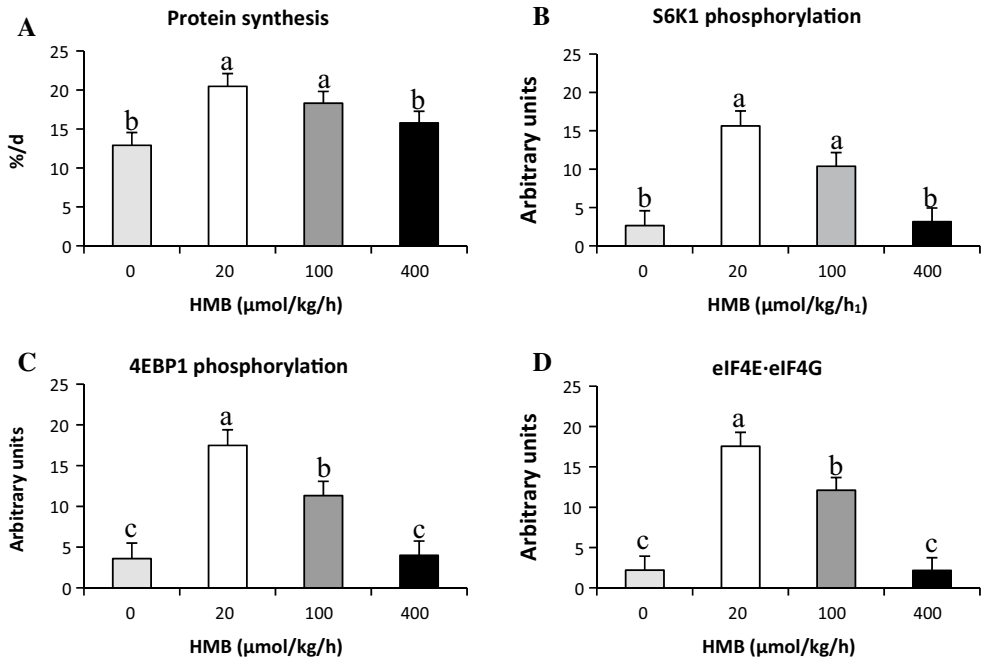


**Fig. 6** Fractional protein synthesis rates (**a**), phosphorylation of $S6K1^{Thr389}$ (**b**) and $4EBP1^{Thr46}$ (**c**), and formation of the active eIF4E·eIF4G complex (**d**) in longissimus dorsi muscle of fasted neonatal pigs intravenously infused with 0, 20, 100, or 400 µmol/kg/hHMB for 1 h. Means without a common letter differ ($P < 0.05$). Adapted from Wheatley et al. (2014) with permission

diet was fed for 24 h, although the extent of this increase was not to the same level as the increase seen with a high-protein meal (Suryawan et al. 2012). It is likely that amino acid supply may have begun to limit protein synthesis in the low-protein-fed pigs after 24 h or that the higher level of insulin produced in the high-protein-fed pigs independently enhanced protein synthesis. Leucine supplementation of a low-protein diet has also been shown to increase muscle protein synthesis in weaned pigs (Yin et al. 2010) and adults (Churchward-Venne et al. 2012, 2014), indicating that the effect of leucine to improve anabolism under dietary restriction is not lost with age.

Many neonates are incapable of feeding properly, with orogastric tube feeding commonly used in these situations to deliver feed. Under normal conditions, intermittent bolus delivery of food is the ideal feeding modality for optimizing growth (Gazzaneo et al. 2011; El-Kadi et al. 2012), however, the use of continuous feeding is necessary in many infants incapable of tolerating full feeds (Dollberg et al. 2000). It was hypothesized by Boutry et al. (2013) that a pulse of leucine could mimic the effect of bolus feeding in neonatal pigs fed continuously by orogastric tube. Over a 24-h period, pigs were fed either by intermittent bolus every 4 h or were fed continuously, with or without a leucine pulse administered every 4 h. Rates of protein synthesis in muscle were greater in continuously fed pigs administered leucine pulses than in those fed continuously with no leucine pulse, but were lower than in those fed by intermittent bolus fed (Fig. 7). This was despite leucine stimulating 4EBP1 phosphorylation and formation of the active eIF4E·eIF4G complex to the same extent as bolus feeding (Fig. 7; Boutry et al. 2013) suggesting that other factors may be involved.

Clearly, leucine has the potential to improve lean tissue growth in low birth weight infants where protein intake is restricted or normal meal feeding is not possible. However, there have been no studies to date on the efficacy of the long-term use of supplemental leucine. Therefore, it is unknown if the increase in muscle protein synthesis observed in these studies will translate into an increase in overall body weight and lean body composition. Future studies should attempt to determine the long-term efficacy of leucine supplementation to improve growth in low birth weight infants in the immediate neonatal period.

## Conclusions

The increase in protein synthesis following a meal is due to the postprandial rise in insulin and amino acids, which can act independently to increase muscle protein synthesis in an mTOR-dependent manner. Leucine specifically has been shown to have unique anabolic properties and the supplementation of leucine or its metabolites, α-ketoisocaproic acid and β-hydroxy-β-methylbutyrate, has been shown to enhance muscle protein synthesis in neonates. Leucine in

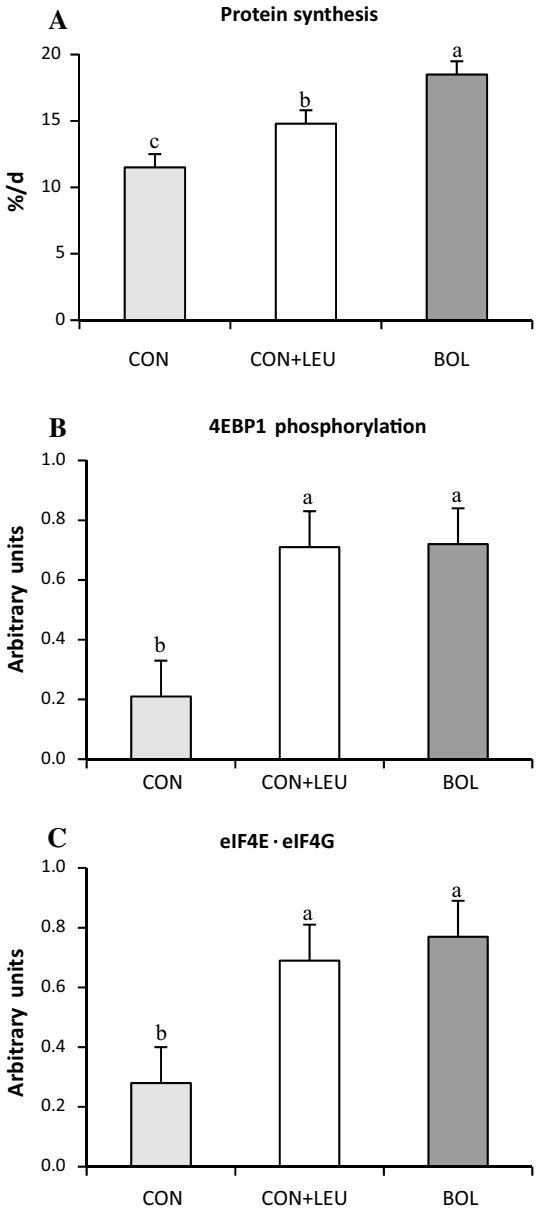


**Fig. 7** Fractional protein synthesis rates (**a**), phosphorylation of S6K1$^{Thr389}$ (**b**) and 4EBP1$^{Thr46}$ (**c**), and formation of the active eIF4E·eIF4G complex (**d**) in longissimus dorsi muscle of neonatal pigs continuously fed (CON), continuously fed and pulsed with leucine (CON+LEU), or bolus fed (BOL). Means without a common letter differ ($P < 0.05$). Adapted from Boutry et al. (2013) with permission

particular has been evaluated extensively for its ability to enhance muscle protein synthesis with low-protein diets and its potential to improve growth in low birth weight infants whose growth has been compromised due to insufficient feed or protein intake merits further investigation.

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**Conflict of interest** The authors have no conflicts of interests.

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