

Biochemical approaches for nutritional support of skeletal muscle protein metabolism during sepsis

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Sepsis initiates a unique series of modifications in the homeostasis of N metabolism and profoundly alters the integration of inter-organ cooperatively in the overall N and energy economy of the host. The net effect of these alterations is an overall N catabolic state, which seriously compromises recovery and is semi-refractory to treatment with current therapies. These alterations lead to a functional redistribution of N (amino acids and proteins) and substrate metabolism among injured tissues and major body organs. The redistribution of amino acids and proteins results in a quantitative reordering of the usual pathways of C and N flow within and among regions of the body with a resultant depletion of the required substrates and cofactors in important organs. The metabolic response to sepsis is a highly integrated, complex series of reactions. To understand the regulation of the response to sepsis, a comprehensive, integrated analysis of the fundamental physiological relationships of key metabolic pathways and mechanisms in sepsis is essential. The catabolism of skeletal muscles, which is manifested by an increase in protein degradation and a decrease in synthesis, persists despite state-of-the-art nutritional care. Much effort has focused on the modulation of the overall amount of nutrients given to septic patients in a hope to improve efficiencies in utilisation and N economies, rather than the support of specific end-organ targets. The present review examines current understanding of the processes affected by sepsis and testable means to circumvent the sepsis-induced defects in protein synthesis in skeletal muscle through increasing provision of amino acids (leucine, glutamine, or arginine) that in turn act as nutrient signals to regulate a number of cellular processes.

Muscle protein metabolism: Amino acid metabolism: Sepsis

Introduction

The host's response to systemic bacterial infection (sepsis) consists of multiple biological and pathological processes that depend upon the regulation of the metabolic response. One of the earliest metabolic manifestations of the host's response to sepsis is an accelerated and excessive excretion of N, indicative of a net loss of protein. The increased N excretion is a marker for profound alterations in whole-body protein metabolism following trauma or infection (Wilmore, 2000). N losses of up to 17 % of total body protein may be observed in septic patients despite aggressive nutritional support (for example, see Plank *et al.* 1998). Because skeletal muscle comprises approximately 45 % of body weight, whole-body N balance reflects changes in protein turnover in muscle during sepsis. As such, approximately 70 % of the septic-induced whole-body protein loss

comes from the erosion of skeletal muscle (Plank *et al.* 1998). The persistent loss of large amounts of protein in sepsis leads to organ-system dysfunction and, eventually, organ failure (Keys *et al.* 1950).

During the past two decades considerable advances in our fundamental understanding of the biological alterations induced by sepsis have provided dramatic therapies available to the health professionals caring for critically ill patients. Given the severe metabolic disturbances initiated by the septic episode, the nutritional requirements of the injured or septic patient differ from those of the starved individual. Critically ill patients have unique nutritional needs to combat not only the muscle wasting but provide adequate substrates for wound healing, a sustained acute-phase protein response, and support of the immune system.

The clinical implications of continued loss of skeletal muscle protein in septic patients include poor wound

Abbreviations: BCKD, branched-chain keto-acid dehydrogenase; CL, carbohydrate plus leucine; 4E-BP, eukaryotic initiation factor 4E binding protein; eIF, eukaryotic initiation factor; met-tRNA₁^{met}, initiator methionyl-tRNA; mTOR, mammalian target of rapamycin.

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healing (Cooney *et al.* 1997a), loss of muscle strength, diminished muscle activity (Brough *et al.* 1986; Boczkowski *et al.* 1988; Windsor & Hill, 1988), and, if severe enough, death (Keys *et al.* 1950). Muscle weakness in septic patients contributes to a continued dependence on mechanical respirators, an increased risk of pneumonia, and the complications associated with extended periods of bed rest. These complications prolong hospitalisation and convalescence, thereby increasing healthcare costs (Wilmore, 2000). An overall goal of nutritional support should be to maximise the retention and accretion of protein in skeletal muscle during sepsis.

Skeletal muscle protein metabolism during inflammation and sepsis

The protein wasting observed in skeletal muscle during sepsis results from an imbalance between the rates of protein synthesis and protein degradation. The imbalance in protein metabolism can be caused by an inhibition of protein synthesis and/or an acceleration of protein degradation leading to a loss of muscle protein during sepsis. The relative contribution of changes in protein synthesis and proteolysis to the overall net catabolic state in muscle varies depending upon the severity of the septic insult. Protein degradation varies, increasing as the septic episode worsens (Breuille *et al.* 1998) and appears independent of the fibre composition of the muscle. In contrast, sepsis causes a preferential reduction in protein synthesis in muscles composed of fast-twitch fibres, but not in those composed of slow-twitch fibres (Vary & Kimball, 1992; Cooney *et al.* 1994; Lang *et al.* 1996). In contrast to proteolysis, rates of protein synthesis are reduced to a similar extent regardless of the severity of the septic insult.

In muscle composed of fast-twitch fibres, the synthesis of both myofibrillar and sarcoplasmic proteins is diminished to the same extent, indicating that sepsis affects the overall process of protein synthesis (Vary *et al.* 1996a). As the septic process wanes, muscle protein mass and protein synthesis return toward control values (Jurasinski *et al.* 1995b).

Non-septic trauma also results in a decrease in protein synthesis and an increase in proteolysis in skeletal muscle. In contrast to infection, changes in proteolysis and protein synthesis are short-lived in non-septic trauma patients. With adequate nutritional support, restoration of positive N balance and lean body mass occurs within days of the injury. In agreement with studies in non-septic trauma patients, skeletal muscle protein synthesis is not decreased in rats with a sterile abscess (Vary & Kimball, 1992) or by the induction of experimental inflammation (Ballmer *et al.* 1993), provided adequate nutrient intake is maintained. The present review will focus on the molecular alterations in protein synthesis during sepsis and describe potential nutritional modifications that could potentially enhance lean body mass during infection.

Amino acids availability in the control of protein synthesis during sepsis

The response to sepsis includes a diminution in appetite, decreased nutrient intake, and an acute mobilisation of

endogenous glucose, fat, and protein stores. In terms of food intake, the effects of sepsis result in a semi-fasted state in the short term. Prolonged fasting (Garlick *et al.* 1975) lowers the protein content of muscles and reduces rates of protein synthesis. Therefore, a sepsis-induced anorexia may be responsible, in part, for the loss of protein. In our previous studies, control animals or rats with a sterile abscess were pair-fed to the septic rats and thus nutrient intake was the same in all groups (Vary *et al.* 1998a, 1999a; Svanberg *et al.* 2000), indicating that diminished rates of protein synthesis result from the septic response rather than prolonged food restriction.

Sepsis also decreases the plasma and skeletal muscle amino acid concentrations (Askanazi *et al.* 1980). Hence, decreases in skeletal muscle protein synthesis could also result from a generalised reduction in the plasma or muscle amino acid concentrations. Protein synthesis would be limited by diminished amino acid availability. However, two lines of evidence argue against this scenario. First, the K_m for amino acyl-tRNA transferase, the enzyme responsible for providing amino acids to the ribosome for protein synthesis, is some 100-fold less than the intracellular concentration of amino acids in muscle. Hence, the modest reduction in the plasma and muscle amino acid concentration during sepsis would be insufficient to limit the charging of the amino acyl-tRNA transferase. Second, we have previously reported that only minor decreases in plasma and skeletal muscle amino acid concentrations are observed in septic rats at a time when protein synthesis is impaired (Vary *et al.* 1988a, 1989a,b). Thus, it is improbable that the reduction in amino acid concentration alone is sufficient to cause the sepsis-induced inhibition in protein synthesis and other mechanisms must be explored to understand the diminished rates of skeletal muscle protein synthesis during sepsis.

Sepsis reduces mRNA translation initiation in the skeletal muscle

The synthesis of proteins is a complex process in mammalian cells (Kimball *et al.* 1994; Cooney *et al.* 1998). The process involves the association of the 40S and 60S ribosomal subunits, mRNA, initiator methionyl-tRNA ($\text{met-tRNA}_i^{\text{met}}$), other amino acyl-tRNA, cofactors (i.e. GTP, ATP), and protein factors, collectively known as eukaryotic initiation factors (eIF), elongation factors, and releasing factors, through a series of discrete reactions that lead to the translation of mRNA into proteins (Fig. 1). Translation of mRNA is comprised of three phases. These are: (a) initiation, whereby $\text{met-tRNA}_i^{\text{met}}$ and mRNA bind to 40S ribosomal subunits, followed by the subsequent binding of the 40S ribosomal subunit to the 60S subunit to form a ribosome complex capable of translation; (b) elongation, by which tRNA-bound amino acids are incorporated into growing polypeptide chains according to the mRNA template; (c) termination, where the completed protein is released from the ribosome.

Sepsis primarily affects the mRNA translation initiation step of protein synthesis (Table 1). The regulation of protein synthesis occurs predominately through changes in the abundance of ribosomes and/or translational efficiency.

Table 1. Effects of chronic sterile abdominal inflammation and chronic abdominal sepsis on mRNA content, peptide-chain initiation and elongation and termination in skeletal muscle compared with pair-fed controls

	Inflammation	Sepsis
mRNA content	↔	↔
Peptide-chain initiation	↔	↓
Elongation and termination	↔	↔

↔, No difference compared with controls; ↓, reduced compared with controls.

Alterations in the relative abundance of ribosomes are not responsible for the inhibition in skeletal muscle protein synthesis in septic rats (Vary & Kimball, 1992; Cooney *et al.* 1994; Jurasinski *et al.* 1995b). The efficiency of translation, calculated by dividing the protein synthesis rates by the total RNA (or mRNA) content, provides an index of how rapidly the existing ribosomes synthesise protein. Sepsis induces an inhibition of protein synthesis by reducing the translational efficiency by 50 % in the skeletal muscle (Vary & Kimball, 1992; Cooney *et al.* 1994; Jurasinski *et al.* 1995b; Svanberg *et al.* 2000). The decrease in translational efficiency does not result from a decreased amount of mRNA in muscles from septic rats (Vary *et al.* 1996a).

Reductions in translational efficiency can occur by inhibiting either the initiation of mRNA translation initiation (peptide-chain initiation) or peptide-chain elongation. Sepsis does not affect the rate of peptide-chain elongation

in skeletal muscle (Vary & Kimball, 1992). Therefore, the inhibitory effects of sepsis on protein synthesis are related to derangements in mRNA translation initiation.

Steps in translation initiation inhibited during sepsis

In the first step, the 80S ribosome dissociates into 40S and 60S ribosomal subunits (Fig. 1). In the second step, the binding of met-tRNA_i^{met} to the 40S subunit forms the 43S pre-initiation complex. In the third step, mRNA binds to the 43S pre-initiation complex and forms the 48S pre-initiation complex. In the fourth step, the 60S ribosomal subunit associates with the 48S pre-initiation complex leading to the formation of an 80S ribosome capable of carrying out the elongation phase of protein synthesis.

Two steps in translation initiation have been identified as the major regulatory points in the overall control of protein synthesis (Fig. 1). The first one is the binding of met-tRNA_i^{met} to the 40S ribosomal subunit to form the 43S pre-initiation complex. This reaction is mediated by eIF2 and is regulated by the activity of another eIF, eIF2B. The second regulatory step involves the binding of mRNA to the 43S pre-initiation complex, which is mediated by eIF4F. Sepsis inhibits both these regulatory steps involved in mRNA translation initiation (Vary & Kimball, 1992; Cooney *et al.* 1994; Jurasinski *et al.* 1995b; Vary *et al.* 1996b, 1998b; Voisin *et al.* 1996; Svanberg *et al.* 2000).

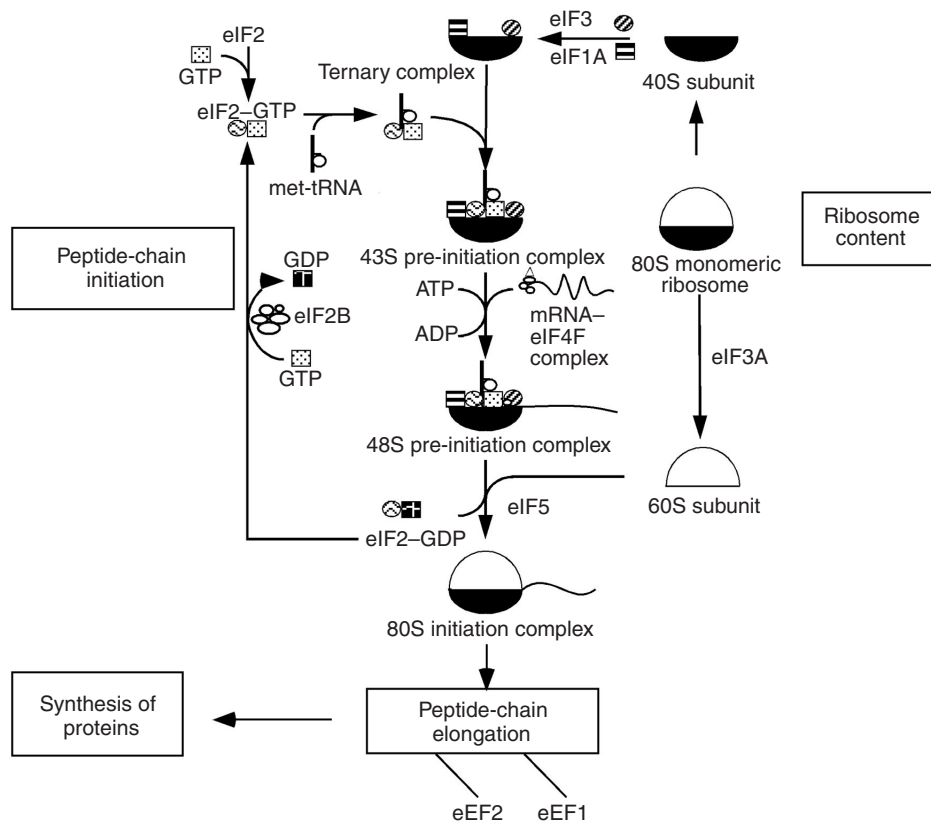


Fig. 1. Overview diagram of protein synthesis in skeletal muscle. eEF, eukaryotic elongation factor; eIF, eukaryotic initiation factor; met-tRNA, methionyl-tRNA. (Adapted from Kimball & Jefferson, 1991.)

Altered eukaryotic initiation factor 2B activity limits mRNA translation initiation in skeletal muscle during sepsis

Sepsis reduces the first regulated reaction in translation initiation, namely the assembly of the 43S pre-initiation complex (Vary *et al.* 1998b) (Fig. 1). Assembly of a 43S pre-initiation complex is mediated by eIF2 (Kimball & Jefferson, 1988; Karinch *et al.* 1993; Vary *et al.* 1994). Two mechanisms regulate this process. First, the amount of eIF2 in the cell may be altered. In this regard, the cellular content of eIF2 correlates with rates of protein synthesis in non-muscle tissues (Kimball *et al.* 1992; Vary *et al.* 1994). In contrast, the cellular content of eIF2 is not significantly decreased in skeletal muscle from septic rats (Vary *et al.* 1994, 1996b). Second, the activity of eIF2 is regulated through the eIF2B-mediated recycling of GTP for GDP on eIF2 (Kimball & Jefferson, 1988; Karinch *et al.* 1993; Vary *et al.* 1994). Inhibition of eIF2B activity prevents the recycling of guanine nucleotides and limits eIF2-GTP availability to form the ternary complex, thereby limiting translation initiation.

Sepsis decreases the activity of eIF2B in muscle (Vary *et al.* 1994, 1998b). The decrease in eIF2B activity is observed only in muscles composed of fast-twitch fibres (gastrocnemius and psoas) and not in muscles composed of slow-twitch fibres (soleus) (Vary *et al.* 1994), consistent with the inhibition of protein synthesis (Vary & Kimball, 1992). The mechanism responsible for the inhibition of eIF2B activity is not known. The best-characterised mechanism controlling eIF2B is through the phosphorylation of eIF2 on its α subunit (Levin *et al.* 1976). Phosphorylation of eIF2 α converts the protein from a substrate into a competitive inhibitor of eIF2B (Levin *et al.* 1976; Scorsone *et al.* 1987; Dholaka & Wabba, 1988), thereby limiting the ability of eIF2B to exchange GDP (Levin *et al.* 1976; Scorsone *et al.* 1987; Dholaka & Wabba, 1988). However, the extent of eIF2 α phosphorylation is not significantly altered in the skeletal muscle from septic rats (Vary *et al.* 1994) (Table 2). eIF2B is also subject to allosteric regulation (Dholaka & Wabba, 1988; Oldfield & Proud, 1992; Karinch *et al.* 1993; Singh *et al.* 1994). Inactivation of eIF2B by NAD⁺ and NADP⁺ is reversed by the addition of equimolar amounts of NADH or NADPH (Dholaka & Wabba, 1988; Karinch *et al.* 1993). However, we have shown that the NADPH:NADP⁺ concentration ratio is not significantly altered in the gastrocnemius of septic rats (Vary *et al.* 1998b). The diminished eIF2B activity does not relate to changes in the redox state during sepsis. Thus other mechanisms regulate eIF2B activity in skeletal muscle during sepsis.

Reducing the muscle eIF2B content could account for a reduced eIF2B activity during sepsis. The cellular content of eIF2B is reduced in chronic sepsis (Vary *et al.* 1996b; Voisin *et al.* 1996) (Table 2) and returns to control values as the animals recover from septic insult. However, eIF2B protein content is not increased in acute studies where protein synthesis in the gastrocnemius from septic rats is augmented (Jurasinski *et al.* 1995a; Jurasinski & Vary, 1995). Thus, reduced eIF2B protein content can be overridden and, thus, other mechanisms may be involved.

Table 2. Comparison of the changes in the eukaryotic initiation factor (eIF) 2 and eIF4F system in control of peptide-chain initiation in skeletal muscle during inflammation and sepsis

	Inflammation	Sepsis
eIF2 system		
eIF2 content	↔	↔
eIF2 phosphorylation	↔	↔
eIF2B activity	↔	↓
eIF2B content	↔	↓
eIF2B phosphorylation	↔	↑
eIF4F system		
eIF4E content	↔	↑
eIF4G content	↔	↔
eIF4E phosphorylation	↔	↔ or ↓*
4E-BP1 phosphorylation	↔	↓
4E-BP1 associated with eIF4E	↔	↑
eIF4G associated with eIF4E	↔	↓↓

4E-BP, eukaryotic initiation factor 4E binding protein; ↔, no difference compared with controls; ↓, reduced compared with controls; ↑, increased compared with controls.

* Depends upon the severity of the septic episode.

eIF2B undergoes reversible phosphorylation. An increased phosphorylation is associated with an inhibition of eIF2B activity. In this regard, sepsis increases eIF2B kinase activity by 175 % (Table 2). As a result of the increased eIF2B kinase activity, the phosphorylation state of the epsilon subunit of eIF2B (eIF2B ϵ) is increased during sepsis.

mRNA recruitment to ribosomes by eukaryotic initiation factor 4E is impaired in skeletal muscle during sepsis

Another rate-controlling step in the process of peptide-chain initiation involves the recognition, unwinding and binding of mRNA to the 40S ribosomal subunit (Fig. 1). This step is catalysed by the multi-subunit complex of eukaryotic factors eIF4F (Rhoads, 1993; Rhoads *et al.* 1994; Sonenberg, 1994). eIF4F is composed of (1) eIF4A (an RNA helicase that functions with eIF4B to unwind secondary structures in the 5' untranslated region of mRNA), (2) eIF4E (a protein that binds directly to the m⁷GTP cap structure present at the 5' end of most eukaryotic mRNA), and (3) eIF4G (a protein that functions as a scaffold for eIF4E, eIF4A and the mRNA and the ribosome). eIF4G appears to be the nucleus around which the initiation complex forms, because it has binding sites not only for eIF4E but also for eIF4A and eIF3 (Lamphear *et al.* 1995). eIF4E is the least abundant initiation factor in most cells. eIF4E activity plays a critical role in determining global rates of mRNA translation, because essentially all mammalian mRNA contain the m⁷GTP cap structure at their 5' ends. eIF4E is regulated by alterations in either its availability or phosphorylation (Morley & Traugh, 1989, 1990; Rhoads, 1993; Sonenberg, 1994). Decreasing eIF4E content through transfection with anti-sense RNA causes an inhibition of protein synthesis at the level of translation initiation. However, the cellular content of eIF4E is not decreased in the gastrocnemius of septic rats (Vary & Kimball, 2000) (Table 2). eIF4E is also regulated by

reversible phosphorylation (Morley & Traugh, 1989, 1990; Rhoads, 1993; Sonenberg, 1994). eIF4E is phosphorylated following activation of the MNK1 kinase. Phosphorylation of eIF4E enhances the affinity of the factor for m⁷GTP cap on mRNA and for eIF4G and eIF4A and correlates with enhanced rates of protein synthesis in cells in culture stimulated with mitogens, growth factors or serum (Morley & Traugh, 1990, 1993), or transformed with ras or src oncogenes (Morley & Traugh, 1989, 1990, 1993). Conversely, reduced phosphorylation of eIF4E correlates with an inhibition of protein synthesis with serum depletion (Duncan *et al.* 1987). eIF4E phosphorylation is either unchanged or decreased during sepsis.

Translation initiation may also be regulated through the formation of the eIF4E–eIF4G complex (Fig. 2). A positive linear relationship between rates of protein synthesis and the amount of eIF4G associated with eIF4E in muscle is observed *in vivo* (Vary *et al.* 2001). Although this correlation does not prove cause and effect, the relationship between protein synthesis and the amount of eIF4G associated with eIF4E is consistent with the proposed role of the eIF4G–eIF4E complex in the overall regulation of protein synthesis. The assembly of the eIF4E–eIF4G complex is significantly diminished in skeletal muscle from septic rats (Vary & Kimball, 2000; Vary *et al.* 2001) (Table 2). The diminished assembly of the eIF4E–eIF4G complex is not the result of a reduced amount of eIF4G in the muscles from septic rats (Vary *et al.* 2001). Reduced amounts of eIF4E associated with eIF4G following chronic sepsis would be expected to diminish the association of mRNA with the ribosome, and hence limit protein synthesis.

The availability of eIF4E for binding to eIF4G is regulated, in part, through the association of eIF4E with a family of translational repressor proteins (eIF4E binding proteins; 4E-BP) (Lin *et al.* 1994, 1995; Pause *et al.* 1994; Bhandari *et al.* 2001) (Fig. 2). In skeletal muscle, 4E-BP1 is the predominant form of 4E-BP. When eIF4E is bound to

4E-BP1, eIF4E cannot bind to eIF4G. Consequently, the mRNA cannot bind to the ribosome (Haghihat *et al.* 1995), thereby inhibiting the cap-dependent translation of mRNA by physically sequestering eIF4E into an inactive 4E-BP1–eIF4E complex. In muscles from control rats, eIF4E binding to 4E-BP1 is reduced following the phosphorylation of 4E-BP by insulin through a phosphoinositol-3-phosphate kinase-dependent pathway involving signalling through protein kinase B (PKB, AKT) and mammalian target of rapamycin (mTOR) (Kimball *et al.* 1996, 1997, 1998; Fig. 3). Phosphorylation of 4E-BP releases eIF4E from the 4E-BP1–eIF4E complex and allows the eIF4E–mRNA complex to bind to eIF4G and, then, to the 40S ribosome (Lin *et al.* 1994). Unlike muscles from controls, increased phosphorylation of 4E-BP1 and decreased eIF4E–4E-BP1 complex formation is not associated with an enhanced formation of the eIF4E–eIF4G complex in muscles from septic rats (Vary *et al.* 2001; Table 2). Hence sepsis alters the formation of the eIF4E–eIF4G complex through mechanisms other than eIF4E availability.

Effect of elevating amino acid concentrations on protein synthesis during sepsis

The provision of increased protein has been shown to be beneficial in several critically ill patient populations including those with burns, head injury, trauma and sepsis. Nutritional intervention is the standard of care in the USA to attenuate the sepsis-induced catabolism of muscle proteins (Stene & Vary, 1999; Streat *et al.* 2000). Nutritional support generally contains a source of amino acids in the range of 1.2–1.5 g/kg. New nutritional formulations, including individually modified nutrients, represent a major advance in the nutritional support of septic patients which may have a substantial influence on the clinical management of these patients. Clinicians and nutritional scientists have focused not only on the quantity of nutrients administered, but also on the composition of the nutrient mix provided to septic patients.

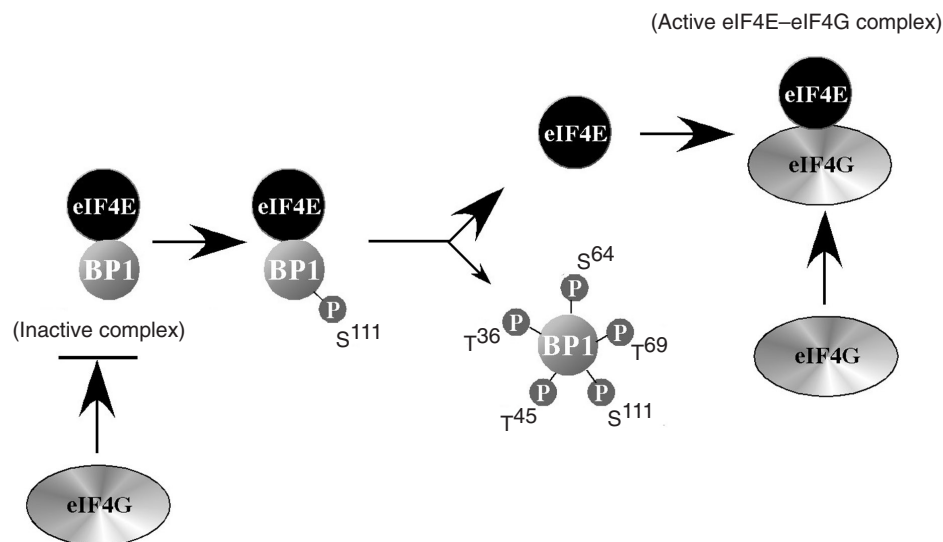


Fig. 2. Regulation of eukaryotic initiation factor (eIF) 4F assembly through phosphorylation of eIF4E binding protein 1 (BP1).

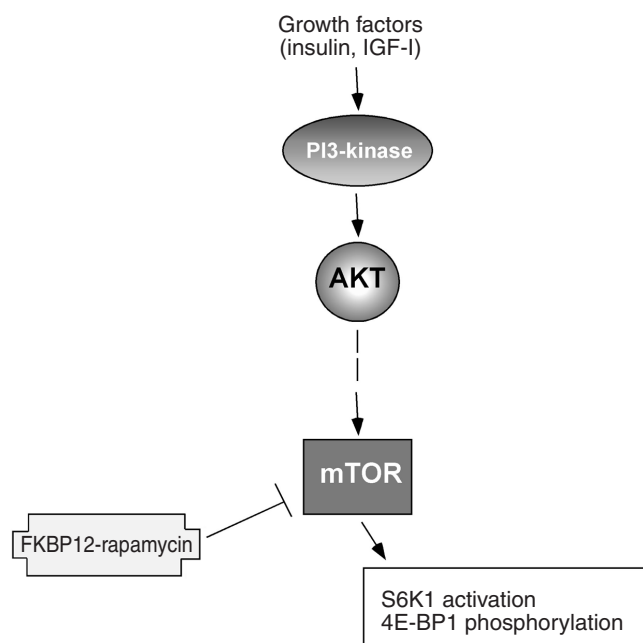


Fig. 3. Altered control of mammalian target of rapamycin (mTOR) by signal transduction pathways stimulated by hormones in phosphorylation of eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and 70 kDa ribosomal protein S6 kinase (S6K1). IGF, insulin-like growth factor; PI3-kinase, phosphoinositol-3-phosphate kinase; AKT, protein kinase B; FKBP, FK 506 binding protein.

Hypoaminoacidaemia is observed in both the intracellular and extracellular fluid compartments during sepsis (Askanazi *et al.* 1980; Jeevanandam *et al.* 1995; Cooney *et al.* 1997b). Increasing amino acid concentrations may have a stimulatory effect on protein anabolism in skeletal muscle (Vary *et al.* 1999b; Anthony *et al.* 2000a,b; Balage *et al.* 2001). However, information is limited regarding the ability of amino acids to stimulate protein synthesis in skeletal muscle during sepsis. Elevating the amino acid concentration in a perfused hindlimb preparation stimulated protein synthesis more than 4-fold in the gastrocnemius of septic rats and corrected the sepsis-induced defect in protein synthesis (Jurasinski *et al.* 1995a). In critically ill patients, elevating protein intake lessens protein losses over a 10 d period (Ishibashi *et al.* 1998). Infusion of IL-1ra into septic patients elevates the plasma concentration of eleven amino acids and total amino acid concentration was increased by 50 % (Cooney *et al.* 1997b). Concentrations of several amino acids, including leucine, were elevated by up to 2-fold by the infusion of IL-1ra. This was associated with a decreased N excretion. Therefore, augmenting amino acid availability may limit the extent of protein catabolism in septic patients.

Amino acids augment assembly of eukaryotic initiation factor 4E–eukaryotic initiation factor 4G in skeletal muscle

The infusion of a mixture of branched-chain amino acids improves N balance following trauma, an effect not mediated by alanine (Freund *et al.* 1979, 1980). These changes

in N balance could result from a stimulation of protein synthesis or from an inhibition of protein degradation. In this regard, Sherwin (1978) has suggested that leucine acts through an acceleration of protein synthesis because the excretion of 3-methylhistidine (a surrogate for the breakdown of myofibrillar proteins) is unaffected by leucine, inferring that the degradation of muscle protein is not affected by leucine. Hence the effects of leucine appear to be mediated through an acceleration of protein synthesis.

The synthesis of skeletal muscle proteins is rapidly stimulated after the oral intake of nutrients. Of the nutrients provided by a complete meal, amino acids are important for enhancing protein accretion in human skeletal muscle. The concept that amino acids *per se* have a stimulatory effect on protein synthesis in muscle is supported by investigations utilising muscles incubated *in vitro* (Buse & Reid, 1975), as well as the perfused hindlimb (Li & Jefferson, 1978; Jurasinski *et al.* 1995a; Vary *et al.* 1999b). In perfused muscles, elevating the amino acid concentrations above those of normal rat plasma stimulates protein synthesis (Li & Jefferson, 1978; Jurasinski *et al.* 1995a; Vary *et al.* 1999b). Likewise, infusing amino acids *in vivo* enhances muscle protein synthesis independent of any stimulatory effect induced by changes in plasma anabolic hormone concentrations (Tessari *et al.* 1987; Fukagawa *et al.* 1988; Bennet *et al.* 1989, 1990; Giordano *et al.* 1996; Svanberg *et al.* 1996).

Three lines of evidence suggest that amino acids are probably not augmenting protein synthesis merely by increasing substrate availability. First, the tRNA involved in protein synthesis are essentially fully charged at the intracellular amino acid concentrations found in skeletal muscle from control or septic rats. Thus, increasing the amino acid concentration above that found in control rats would not further charge the tRNA. Second, increasing substrate availability should accelerate protein synthesis by enhancing peptide-chain elongation. However, amino acids accelerate peptide-chain initiation to a greater extent than elongation (Li & Jefferson, 1978; Jurasinski *et al.* 1995a; Vary *et al.* 1999b; Anthony *et al.* 2000a, 2001). Third, the removal of leucine can prevent the overall stimulation of protein synthesis by amino acids; yet the removal of methionine is without effect (Vary *et al.* 1998b). Thus, amino acids affect protein synthesis in skeletal muscle independent of augmenting substrate availability.

Instead, amino acids appear to modulate the functions of various components of the protein synthetic machinery. Acute modulation of protein synthesis by amino acid availability occurs via changes in the activity of eIF4E rather than eIF2B. In perfused hindlimb, the acceleration of protein synthesis with increasing amino acid concentrations is associated with increases in the amount of eIF4G bound to eIF4E, and a corresponding decrease in the amount of eIF4E in the inactive eIF4E–4E-BP1 complex (Vary *et al.* 1999b). Likewise, feeding rats a meal composed of 20 or 25 % protein stimulated protein synthesis and inhibited protein degradation (Yoshizawa *et al.* 1998; Balage *et al.* 2001). The stimulation of protein synthesis is also associated with an increased assembly of the active eIF4E–eIF4G complex.

Unique role of leucine in improving protein balance in skeletal muscle

Of the amino acids, much attention has focused on the use of branched-chain amino acids for nutritional support. Branched-chain amino acids can rise to very high concentrations without adverse consequences in septic patients (Jimenez-Jimenez *et al.* 1991). The addition of branched-chain amino acids to total parenteral nutrition has been reported to improve N balance and the preservation of plasma protein concentrations. Branched-chain amino acid administration improves N balance during sepsis (Cerra *et al.* 1987; Garcia-de-Lorenzo *et al.* 1997) and results in a faster recovery of muscle protein (Jimenez-Jimenez *et al.* 1991). The parenteral administration of branched-chain amino acid-enriched (45 %; 586 kJ/g N) solutions improves mortality in septic patients (Garcia-de-Lorenzo *et al.* 1997). Branched-chain amino acids and leucine in particular intensify protein accretion in skeletal muscle. The ability of branched-chain amino acids to spare protein is not mimicked by the transamination product of leucine, α -keto-isocaproic acid, indicating it is the amino acid rather than the metabolism of leucine that is important in modulating protein turnover (Hasselgren *et al.* 1988). Elevating the leucine concentration 10-fold in perfused hindlimb preparations accelerates protein synthesis by up to 2-fold (Li & Jefferson, 1978; Vary *et al.* 1999b), through stimulating mRNA translation initiation.

Of the branched-chain amino acids, leucine is well suited as a nutrient signal for several reasons. First, leucine is the most potent of the branched-chain amino acids with regard to regulating protein metabolism. Second, it cannot be synthesised *de novo* by mammals. Third, it is the most abundant essential amino acid in dietary protein and thus is a crucial nutrient following the ingestion of a protein meal. Fourth, once taken up by the gut, leucine cannot be directly metabolised by the liver, which lacks the branched-chain amino transferase, the enzyme responsible for removal of the N. This is in contrast to the liver's capacity to initiate the catabolism of other amino acids. In contrast to liver, most other peripheral tissues have excess capacity to catalyse this first step. This may facilitate postprandial rises in plasma leucine concentrations, as leucine would not be cleared in the first pass of the liver.

The mechanism by which leucine stimulates protein synthesis is only beginning to be elucidated, and there is no information available in experimental models of sepsis. The effect of an orally administered dose of leucine fed alone or in combination with carbohydrate on protein synthesis in the gastrocnemius was examined to better understand the role of leucine in stimulating protein synthesis *in vivo* (Anthony *et al.* 2000a). Rats were either freely fed or food deprived for 18 h. Food-deprived rats were administered either saline, carbohydrate, leucine, or a combination of carbohydrate plus leucine (CL). Carbohydrate and CL meals were isoenergetic and provided 15 % of the daily energy requirements. Leucine and CL meals each delivered 270 mg leucine. Muscle protein synthesis was reduced by 35 % in the rats administered saline compared with the freely fed rats. Lower rates of protein synthesis in the saline-administered rats were associated with a decreased

hyperphosphorylation of 4E-BP1 leading to a greater association of 4E-BP1 with eIF4E and a concomitant reduction in eIF4E associated with eIF4G. The oral administration of leucine (leucine or CL), but not carbohydrate, restored protein synthesis to the values observed in the freely fed rats and resulted in a 3-fold enhanced phosphorylation of 4E-BP1. Consequently, the amount of the eIF4E–4E-BP1 complex fell and the binding of eIF4E to eIF4G increased. No differences in the phosphorylation state of eIF2 α or the activity of eIF2B were observed among the different experimental groups.

Leucine may stimulate protein synthesis *in vivo* by enhancing the binding of eIF4E to eIF4G independently of increases in serum insulin. As sepsis is associated with a reduced formation of the active eIF4E–eIF4G complex, the possibility exists that leucine may increase protein synthesis during sepsis by increasing the formation of the active eIF4E–eIF4G complex.

Role of mammalian target of rapamycin in leucine-induced stimulation of protein synthesis

Leucine is a direct-acting nutrient signal that regulates protein synthesis. The effects on protein synthesis are brought about, at least in part, by the activation of a cell-signalling pathway involving the serine threonine protein kinase, mTOR (Fig. 4). In skeletal muscle, the mTOR signalling pathway appears to play a role in the stimulation of protein synthesis by growth-promoting anabolic hormones (Fig. 3). Two well-known substrates of mTOR, namely 70 kDa ribosomal protein S6 kinase and the translational repressor, 4E-BP1, are implicated in the regulation of protein synthesis. At least two signalling pathways appear to be important in leucine's acute regulation of protein synthesis, a rapamycin-sensitive pathway involving mTOR and an unknown rapamycin-insensitive pathway that may or may not involve mTOR. It is presently unclear exactly how leucine activates mTOR aside from the fact that the mechanism differs from that used by insulin. For example, no leucine receptor has been yet identified. In fact, it has not been unequivocally established whether the effects of leucine are mediated by leucine or by a metabolite derived from leucine transamination, such as α -keto-isocaproate, or by leucine oxidation (Fig. 4).

Mitochondrial metabolism may be required for the activation of mTOR by leucine. Specifically, a metabolically linked signal arising from the activation of leucine metabolism in the mitochondria results in mTOR activation (for a review, see McDaniel *et al.* 2002). A potential target cited is the branched-chain keto-acid dehydrogenase (BCKD) complex. The BCKD multi-enzyme complex catalyses the rate-limiting and first irreversible step in leucine oxidation. The reaction converts branched-chain α -keto-acids (such as α -keto-isocaproate) to CoA derivatives (such as isovaleryl CoA). This conversion is catalysed by BCKD. There are several compelling arguments in favour of the notion that BCKD kinase activities might provide a link between leucine signalling and the activation of mTOR. The first is that α -keto-isocaproate is a physiological inhibitor of BCKD kinase. The second

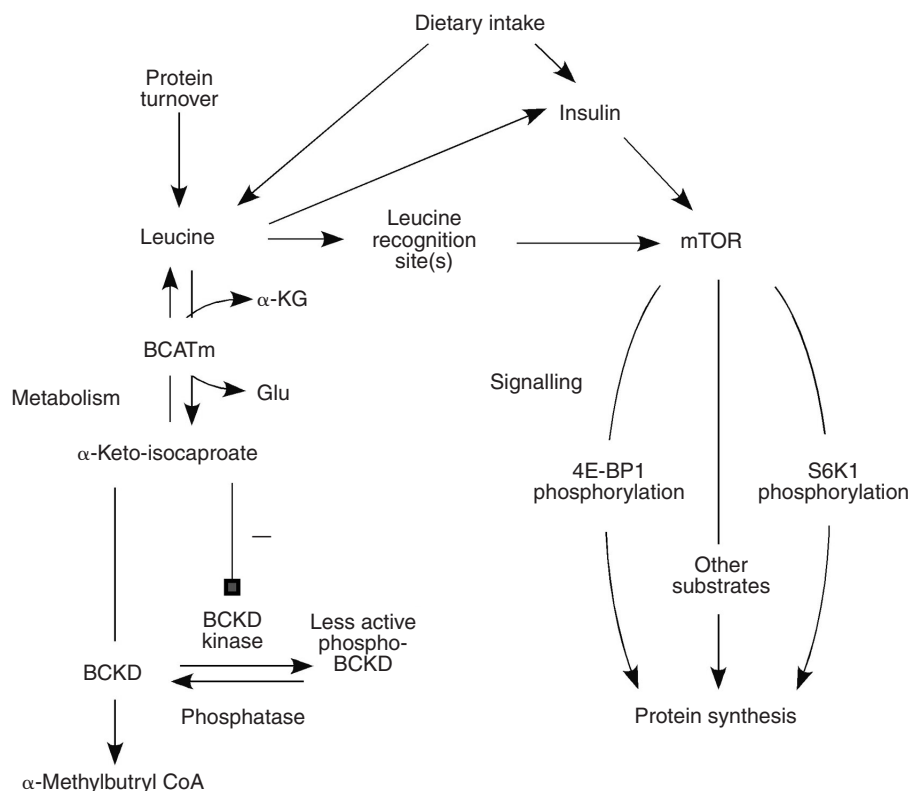


Fig. 4. Leucine metabolism in skeletal muscle: potential signalling to mammalian target of rapamycin (mTOR). α -KG, α -ketoglutarate; BCATm, mitochondrial branched-chain aminotransferase; Glu, glutamate; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; S6K1, 70 kDa ribosomal protein S6 kinase; BCKD, branched-chain keto-acid dehydrogenase; —, inhibition of BCKD kinase by α -keto-isocaproate.

is that the order of potency of leucine > isoleucine > valine and the importance of leucine in the activation of mTOR signalling (Lynch *et al.* 2000) is similar to that of their respective keto-acids' ability to activate the BCKD complex via inhibition of the BCKD kinase. A third observation that supports this potential mechanism is that mTOR appears to be associated with mitochondria membrane *in situ*. However, leucine metabolism may only be important in regulating the magnitude and duration of the signal for mTOR activation (Lynch *et al.* 2003). The molecular mechanism responsible for the activation of mTOR and protein synthesis by leucine remains unknown.

Glutamine supplementation during sepsis

Glutamine is the most abundant amino acid in the body, constituting upwards of 60 % of the total amino acid pool. Glutamine has many diverse roles including acid-base balance, protein synthesis, N transport, and as an energy substrate. Glutamine functions as a carrier of NH_3^+ groups from the skeletal muscle to the liver and kidneys for the production of urea. The C backbone of glutamine is a source for synthesis of the key antioxidant glutathione by the liver. In addition, small-intestine mucosal cells, macrophages, fibroblasts, and lymphocytes preferentially utilise glutamine as a primary energy source. Enterocytes

constitute the largest group of rapidly dividing cells in the body and hence glutamine oxidation by gut mucosal cells is substantial.

The glutamine content in proteins represents only about 15 % of the amino acids, yet it accounts for over 30 % of the amino acids released by muscle, indicating that substantial *de novo* synthesis of glutamine occurs in skeletal muscle. Almost all tissues in the body synthesise glutamine. Glutamine is synthesised from glutamate and NH_3^+ by the enzyme glutamine synthase. Glutamate is not taken up appreciably by skeletal muscle and is produced within the muscle. Glutamate arises through the transamination of α -ketoglutarate by amino acids. NH_3^+ may be produced from the glutamate dehydrogenase reaction or by the deamination of AMP to inosine monophosphate catalysed by the enzyme AMP deaminase. Both these enzymes are sufficiently active to account for glutamine synthesis in skeletal muscle.

A fall in the plasma and muscle glutamine concentration is a characteristic feature of the metabolic response to trauma and sepsis (Askanazi *et al.* 1980; Vary *et al.* 1988c, 1989a). Glutamine release by muscle (Vary & Murphy, 1989; Vary *et al.* 1989b) and utilisation by other cells of the body accelerates, making glutamine a conditionally essential amino acid during catabolic conditions (Lacey & Wilmore, 1990). As such, the use of glutamine in nutritional support has been advanced (Furst *et al.* 1989). The

catabolism and loss of intramuscular glutamine is attenuated by providing glutamine (20 g/d) as a supplement to parenteral nutritional support in post-surgical patients (Stehe *et al.* 1989; Vinnars & Hammervist, 1990). Initial attempts to show a similar effect with enteral feeding proved problematic as enterocytes metabolised the glutamine provided to the gut. To increase body glutamine concentrations, higher doses (0.5 g/kg per d) are required (Wellbourne, 1995). Glutamine supplementation in patients undergoing elective surgery improves N balance, restores the plasma glutamine concentration and enhances protein synthesis. Six randomised blind studies show a decreased length of stay in post-surgical patients (for a review, see Wilmore, 2001). After blunt trauma, glutamine supplementation increased plasma glutamine concentrations, attenuated the immunosuppression, and decreased the rate of infection (for a review, see Wilmore, 2001). While a benefit of glutamine is clearly indicated in surgical or trauma patients without infection, there are several studies that fail to show an improvement in sepsis (Khan *et al.* 1991; Wustemann *et al.* 1993).

Furthermore, it is unclear that a fall in glutamine in skeletal muscle has an adverse regulatory function on protein synthesis. In rats with a sterile abscess, muscle glutamine concentrations are reduced but rates of protein synthesis are maintained (Vary *et al.* 1988*b,c*). Pharmacological reductions in skeletal muscle glutamine are not associated with changes in either whole-body or skeletal-muscle protein kinetics (OldeDiamink *et al.* 1999). The addition of glutamine to incubated muscle preparations does not enhance rates of protein synthesis in either control or septic rats (Fang *et al.* 1995; TC Vary, unpublished results). Likewise, the addition of glutamine to nutritional support offered no additional benefit on protein synthesis in skeletal muscle than amino acids alone. These observations cast doubt for a direct role of glutamine as a regulator of protein synthesis in skeletal muscle. Therefore it remains unclear whether or not intracellular glutamine concentrations are an important factor regulating protein synthesis in skeletal muscle.

Instead, a major benefit of glutamine supplementation may lie in its ability to preserve intestinal mucosal cells (Klimberg & Souba, 1990). Glutamine administration increases villous height, stimulates small-intestine mucosa cellular proliferation and maintains small-intestine integrity (Yoshida *et al.* 1992; Naka *et al.* 1997). The reasons for these observations remain unknown. One possible explanation is that glutamine represents a major source for energy production by the cells of the small intestine. Normally, most of the glutamine is obtained from glutamine present in the lumen. However with decreased feeding associated with injury or sepsis, the amount of amino acids available to the enterocytes via the lumen is diminished. Under these conditions, most of the glutamine is obtained from the blood thereby increasing the demand for glutamine.

The loss of integrity of the intestinal mucosa has been implicated as a major factor driving bacterial translocation in sepsis. Bacterial translocation represents a pathological process by which bacteria in the gastrointestinal tract pass through the epithelial mucosa to infect the mesenteric lymph nodes and the systemic circulation. Indeed, glutamine supplementation attenuates the immunosuppression

commonly observed following blunt trauma and decreases the rate of subsequent infection (Wilmore, 2001). By preserving the intestinal mucosa, glutamine may protect the host from bacterial translocation, limiting a potential second source of bacterial infiltration rather than directly regulating the protein synthetic machinery in skeletal muscle.

Arginine supplementation during sepsis

Like glutamine, arginine may become a conditionally essential amino acid during sepsis. Arginine has been shown to possess a potentially wide range of beneficial effects in injured or critically ill patients (Elsair *et al.* 1978; Barbul *et al.* 1990). Arginine appears to be important for tissue repair and immune-cell function. As such, the administration of arginine has been associated with maintaining T-cell function after trauma, reducing the cellular immune suppression observed after injury and enhancing collagen synthesis at the wound site. Arginine is converted to ornithine and this may be important in promoting the proliferation of enterocytes and immune cells through the subsequent metabolism of ornithine to polyamines. The conversion of arginine to ornithine may also be the mechanism by which arginine promotes wound healing. In this regard, the provision of ornithine α -ketoglutarate in nutritional support improves gut function and prevents bacterial translocation and dissemination. These compounds may be useful in the prevention of gut-derived bacterial translocation in critically ill patients. A potential complication of arginine administration may involve the generation of NO that has both beneficial and deleterious effects during sepsis. This issue remains unresolved rather than directly regulating the protein synthetic machinery in skeletal muscle.

Conclusion

Pronounced catabolism with the loss of lean body mass is a major cause of morbidity and mortality following severe injury and sepsis. The loss of lean body mass prolongs convalescence thereby extending the duration of the illness. Optimising cardiopulmonary, immunological and nutritional support will ultimately improve survival and hopefully accelerate recovery from the septic episode. Anti-catabolic and anabolic strategies show significant clinical benefit, and the incorporation of these modalities may well improve outcomes and rates of recovery in septic patients. One aspect of this approach, namely the provision of specific amino acids in nutritional support, seems promising. The provision of amino acids with specific effects on protein synthesis may be more beneficial than simply providing a protein intake sufficient to meet protein needs. In this regard, the present review has focused on the possible beneficial role of leucine, glutamine and arginine in meeting these nutritional requirements during sepsis.

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