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Initiation factors for translation of proteins in the rectus abdominis muscle from patients on overnight standard parenteral nutrition before surgery

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ABSTRACT

Previous studies have provided conflicting conclusions concerning the efficacy of improving protein balance in patients by standard intravenous nutrition [TPN (total parenteral nutrition)], which is either explained by suboptimal nutritional regimens or insensitive clinical methods. The aim of the present study was therefore to evaluate the effects on the initiation of translation of skeletal muscle proteins by standard overnight TPN. A total of 12 patients who underwent standard surgery were included. TPN was provided as an all-in-one treatment by constant infusion [0.16 gN · kg^{-1} of body weight · day^{-1} (30 kcal · kg^{-1} of body weight · day^{-1})]. Saline-infused patients served as controls. Rectus abdominis muscle biopsies were taken at the time of the operation. The phosphorylation state of the proteins for initiation of translation was quantified. Plasma glucose, and serum insulin, glycerol, triacylglycerols (triglycerides) and NEFAs (non-esterified fatty acids; 'free fatty acids') were not significantly altered during TPN infusion, whereas total plasma amino acids increased, as shown by increases in methionine, phenylalanine, threonine, alanine, arginine, aspartic acid, glycine and histidine ($P < 0.05$). Overnight TPN increased the formation of active eIF4G–eIF4E (where eIF is eukaryotic-initiation factor) complexes ($P < 0.05$), whereas the inhibitory complex 4E-BP1 (eIF4E-binding protein)–eIF4E was moderately decreased ($P < 0.06$). TPN increased the amount of the most phosphorylated form of 4E-BP1 ($P < 0.05$), and increased the amount ($P < 0.04$) and phosphorylation ($P < 0.01$) of $p70^{S6K}$ (70 kDa ribosomal protein S6 kinase). In conclusion, an overnight pre-operative constant infusion of standard TPN altered initiation factor complexes, indicating activation of the initiation of protein translation in rectus abdominis muscle in the presence of increased plasma amino acid levels, but without a concomitant increase in energy substrates and insulin. In contrast with our results from previous studies, the methodology used in the present study appears to be more sensitive in reflecting directional changes in human muscle protein synthesis compared with traditional methods, particularly based on measurements of amino acid flux.

Key words: amino acid, eukaryotic initiation factor, protein synthesis, rectus abdominis muscle, surgery, total parenteral nutrition (TPN).

Abbreviations: BCAA, branched-chain amino acid; eIF, eukaryotic initiation factor; 4E-BP1, eIF4E-binding protein 1; ICU, intensive care unit; IGF-1, insulin like growth factor-1; NEFA, non-esterified fatty acid; $p70^{S6K}$, 70 kDa ribosomal protein S6 kinase; TPN, total parenteral nutrition.

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INTRODUCTION

The mechanisms regulating protein balance in skeletal muscles have been evaluated extensively in human and animal models [1]. Our previous studies have indicated the rapid activation of protein synthesis in skeletal muscles during oral refeeding of mice, a phenomenon that is independent of muscle innervation and circulating insulin [2]. Intravenous infusion by stepwise increased loads of amino acids to unselected hospitalized patients and healthy volunteers have indicated that amino acids are involved in the process of initiation of muscle protein translation [3], as has also been shown by others in numerous studies, whereas carbohydrates and fat did not have such effects [4]. In addition, there was no indication that glutamine had any stimulatory effects *in vivo* in human muscles [5], whereas BCAAs (branched-chain amino acids) stimulate the incorporation of labelled amino acids into muscle proteins [6] and phosphorylate proteins involved in translation of mRNAs [7,8]. Accordingly, direct and indirect evidence have been reported in numerous studies on amino acid stimulation of muscle protein synthesis in a variety of clinical conditions [9–12]. However, we [13] and others [12] have also reported that classical experiments with the labelling of proteins in assessing protein synthesis at steady-state conditions may sometimes give erroneous results, even in the presence of flooding tracee conditions. Therefore there is a need for additional methods to be used to analyse the activation of protein synthesis *in vivo* [14–16].

The aim of the present study was, therefore, to apply recent methods of evaluating changes in the initiation of translation of skeletal muscle proteins following an overnight constant infusion of standard TPN (total parenteral nutrition).

Table 1 Clinical characteristics of patients randomized to receive pre-operative infusions with either TPN or saline

Values are means ± S.E.M. ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; SR, sedimentation rate.

	Patients receiving	
Characteristic	Saline	TPN
Cancer/non-cancer (*n*)	5/1	4/2
Male/female (*n*)	3/3	4/2
Age (years)	63 ± 8	65 ± 5
Nutritional status		
Weight (kg)	70.0 ± 5.4	67.8 ± 3.7
Weight loss (%)	5 ± 6	9 ± 3
Arm circumference (cm)	29.7 ± 0.7	27.6 ± 1.5
Triceps skinfold thickness (mm)	17.3 ± 5.3	11.6 ± 2.3
Arm muscle circumference (cm)	24.2 ± 1.7	23.9 ± 1.1
Blood and serum tests		
Sodium (mmol/l)	138 ± 1	138 ± 1
Potassium (mmol/l)	4.2 ± 0.1	4.2 ± 0.1
Calcium (mmol/l)	2.33 ± 0.05	2.38 ± 0.04
Protein (g/l)	71 ± 1	72 ± 2
Creatinine (μmol/l)	62 ± 7	67 ± 6
Albumin (g/l)	36 ± 1	37 ± 1
Hb (g/l)	130 ± 5	141 ± 5
SR (mm/h)	29 ± 11	17 ± 7
C-reactive protein (mg/l)	17 ± 10	6 ± 1
Serum IGF-1 (μg/l)	120 ± 15	115 ± 7
Liver function tests		
Bilirubin (μmol/l)	13 ± 4	14 ± 3
ASAT (μmol/l)	0.4 ± 0.02	0.5 ± 0.1
ALAT (μmol/l)	0.3 ± 0.1	0.5 ± 0.1
ALP (μmol/l)	1.8 ± 0.1	1.8 ± 0.6

MATERIAL AND METHODS

Patients and study design

A total of 12 patients were included in the study (Table 1). All patients underwent surgery of the upper gastrointestinal tract, with nine patients being operated upon to remove a local malignancy, and three patients to remove benign diseased tissue. On the day before operation, the patients were randomized to receive overnight infusions prior to surgery of either saline or TPN, which was supplied as an all-in-one bag [0.16 gN · kg^{-1} of body weight · day^{-1} (30 kcal · kg^{-1} of body weight · day^{-1}); Kabiven® Perifer; Fresenius Kabi; see http://www.clinsci.org/cs/114/cs1140603.htm for the composition of the Kabiven® TPN]. Measurements for nutritional status and baseline blood samples were taken before the start of infusions, which indicated manifest or incipient malnutrition. Infusions started between 16.00 and 17.00 hours on the day before the operation, and continued at a constant rate until muscle biopsies were taken from the rectus abdominis muscles directly after the induction of anaesthesia (15–16 h later) [17]. This procedure was chosen for practical reasons and due to the fact that artificial nutrition should provide protein anabolism within 12 h in order to be clinically efficacious. Blood samples were drawn at the same time for analysis of amino acids and substrate levels in the peripheral circulation. Inclusion criterion was patients who may be candidates for intravenous nutritional support post-surgery without having diabetes or steroid medications.

The study was approved by the Ethics Committee at the Medical Faculty of Göteborg University (no. S328-3), and all patients were provided written informed consent.

Analytical methods

Blood samples

Arterial blood samples were collected and immediately centrifuged at 4 °C. Serum and plasma was stored at −80 °C until analysis. For amino acid analysis, 0.5 ml of

plasma was deproteinized with 125 μl of sulfosalisylic acid (10 %). *p*-Fluorophenylalanine (20 μmol/ml of plasma) was added as an internal standard. After 30 min, precipitates were centrifuged, and the supernatants were used for amino acid analysis using a reverse-phase HPLC method [18]. All other plasma and serum factors were analysed using standard procedures at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital, Stockholm, Sweden.

Muscle biopsies

Muscle biopsies from the rectus abdominis muscle were removed with scissors with a minimum of handling and immediately put into RNA*later* solution (Ambion) [6,19,20]. The samples were kept at 4 °C for 24 h in RNA*later* and then stored at −20 °C until analysis; this procedure did not interfere with the phosphorylation state of protein as confirmed in pre-study experiments. Biopsies were homogenized in 7 vols of ice-cold buffer A {20 mmol/l Hepes (pH 7.4), 100 mol/l KCl, 0.2 mmol/l EDTA, 2 mmol/l EGTA, 1 mmol/l DTT (dithiothreitol), 50 mmol/l sodium fluoride, 50 mmol/l β-glycerophosphate, 0.1 mmol/l AEBSF [4-(2-aminoethyl)benzenesulfonyl fluoride], 1 mmol/l benzamidine and 0.5 mmol/l sodium vanadate}. The homogenate was centrifuged at 10 000 ***g*** for 10 min at 4 °C, and aliquots of the supernatant were used for analysis of protein phopshorylation and complex formation.

Analysis of eIF (eukaryotic-initiation factor) 4G–eIF4E and 4E-BP1 (eIF4E-binding protein 1)–eIF4E complexes

Aliquots of the supernatant were mixed with an agarose-conjugated mouse monoclonal anti-eIF4E antibody (sc-9976; Santa Cruz Biotechnology), and samples were rotated overnight at 4 °C. Next day, samples were centrifuged at 1000 ***g***, and the supernatant was removed. The pellets were washed with 1 ml of ice-cold buffer A. The washing procedure was repeated five times with a centrifugation step between each wash. After the final wash, pellets were resuspended in 2× SDS electrophoresis sample buffer and boiled for 3 min. Samples were centrifuged at 1000 ***g*** to pellet the agarose beads, and equal volumes of supernatant were thereafter used for immunoblotting. Samples were electrophoresed on either a 15 % (w/v) polyacrylamide gel (4E-BP1) or a 7.5 % (w/v) polyacrylamide gel (eIF4G). Proteins were transferred on to PVDF membranes. Membranes were incubated overnight at 4 °C with a rabbit anti-(rat 4E-BP1) antibody or for 90 min at 20 °C with a rabbit anti-(human eIF4G) antibody (sc-6936 and sc-11373 respectively; Santa Cruz Biotechnology) after blocking in 5 % (w/v) non-fat dry milk in TBS (Tris-buffered saline)/0.1 % Tween 20. The blots were then washed, incubated with secondary antibodies and developed using an ECL® Western Blotting kit (Amersham Biosciences), according to the manufacturer's instructions. For detection, membranes were exposed to Hyperfilm ECL (Amersham Biosciences). After detection of the signals, antibodies were removed by incubation for 30 min at 50 °C in stripping buffer [100 mmol/l 2-mercaptoethanol, 2 % (w/v) SDS and 62.5 mmol/l Tris/HCl (pH 7.6)], and the membranes were then reprobed to measure eIF4E by incubating the membranes overnight at 4 °C with a rabbit anti-(human eIF4E) antibody (sc-13963; Santa Cruz Biotechnology). Quantification of the signals was carried out with GS-710 imaging densitometer and Quantity One software (Bio-Rad Laboratories). The absorbance measured was expressed as arbitrary units in all analyses. All samples were analysed on two gels. Two lanes were loaded with MagicMark XP Western Protein Standards (Invitrogen) on each gel. The average absorbance for the standard bands, with similar molecular masses as the measured proteins, was used to normalize the signal intensity between immunoblots.

Analysis of the phosphorylation state of 4E-BP1

An aliquot of the homogenate supernatant was boiled for 5 min, cooled on ice and then centrifuged at 10 000 ***g*** for 30 min at 4 °C. A total of 4 vols of supernatant were mixed with 1 vol. of 5× SDS electrophoresis sample buffer. Samples were boiled for 3 min, and equal volumes of sample were electrophoresed on a 15 % (w/v) polyacrylamide gel. Western blots were performed as described above, except that membranes were incubated overnight at 4 °C with a rabbit anti (rat-4E-BP1) antibody (sc-6936; Santa Cruz Biotechnologies).

Analysis of the phosphorylation state of $p70^{S6K}$ (70 kDa ribosomal protein S6 kinase)

Aliquots of the homogenate supernatant were mixed with equal volumes of 2× SDS electrophoresis sample buffer. Samples were electrophoresed on a 7.5 % (w/v) polyacrylamide gel, and Western blots were performed as described above, except that membranes were incubated with rabbit anti-(rat $p70^{S6K}$) antibody (sc-230, Santa Cruz Biotechnologies) for 60 min at room temperature (20 °C).

Statistics

Results are presented as means ± S.E.M. Comparisons between groups were performed using a two-tailed Student's *t* test. $P \leqslant 0.05$ was considered statistically significant.

RESULTS

Levels of amino acids and substrates in peripheral blood

Total plasma amino acids increased significantly during TPN compared with saline infusions (15–16 h) (Table 2).

Table 2 Amino acid concentrations in peripheral blood from patients treated with either TPN or saline at a constant rate

Values are means ± S.E.M. NS, not significant.

	Patients receiving		
	Saline	TPN	*P* value
Essential amino acids (μmol/l)			
Isoleucine	64 ± 10	95 ± 8	< 0.05
Leucine	117 ± 14	129 ± 9	NS
Valine	234 ± 32	316 ± 28	NS
BCAAs	416 ± 54	541 ± 43	NS
Lysine	143 ± 12	163 ± 13	NS
Methionine	28 ± 3	72 ± 5	< 0.001
Phenylalanine	46 ± 4	86 ± 5	< 0.001
Threonine	116 ± 12	195 ± 17	< 0.01
Tryptophan	9 ± 1	12 ± 1	NS
Total essential amino acids	760 ± 72	1070 ± 67	< 0.05
Non-essential amino acids (μmol/l)			
Alanine	318 ± 47	481 ± 48	< 0.05
Arginine	77 ± 15	135 ± 14	< 0.05
Aspartic acid	4 ± 1	9 ± 1	< 0.001
Asparagine	30 ± 4	18 ± 2	NS
Citrulline	13 ± 3	16 ± 2	NS
Glutamic acid	103 ± 12	132 ± 23	NS
Glutamine	432 ± 38	466 ± 26	NS
Glycine	195 ± 21	326 ± 24	< 0.01
Histidine	70 ± 7	120 ± 6	< 0.01
Serine	121 ± 15	145 ± 16	NS
Taurine	40 ± 6	46 ± 7	NS
Tyrosine	51 ± 6	43 ± 3	NS
Ornithine	59 ± 7	78 ± 14	NS
α-Aba	9 ± 1	15 ± 2	< 0.05
Total amino acids (μmol/l)	2284 ± 160	3105 ± 173	< 0.01

Methionine, phenylalanine and threonine increased significantly in the group of essential amino acids, whereas tryptophan and lysine did not change. Only isoleucine increased among the BCAAs (Table 2). Of the non-essential amino acids, alanine, arginine, aspartic acid, glycine and histidine increased significantly.

Plasma glucose, and serum insulin, glycerol, triacylglycerols (triglycerides) and NEFAs (non-esterified fatty acids) did not change statistically significantly with TPN when compared with saline infusions (Table 3).

Table 3 Substrate levels in peripheral blood when muscle biopsies were taken during constant infusion of TPN or saline

Values are means ± S.E.M. No significant differences were seen between the two groups.

	Patients receiving	
	Saline	TPN
Plasma glucose (mmol/l)	6.4 ± 0.7	7.4 ± 0.7
Serum insulin (milli-units/l)	11 ± 9	19 ± 10
Serum glycerol (mmol/l)	0.17 ± 0.04	0.18 ± 0.01
Serum triacylglycerols (mmol/l)	1.11 ± 0.27	1.77 ± 0.37
Serum NEFAs (mmol/l)	0.73 ± 0.18	0.45 ± 0.09
Serum IGF-1 (μg/l)	73 ± 10	80 ± 7

Effects of TPN on the formation of 4E-BP1–eIF4E and eIF4G–eIF4E complexes

Provision of overnight TPN significantly increased the formation of the active eIF4G–eIF4E complex ($P < 0.05$) compared with saline-infused patients (Figure 1A and Figure 2). The inhibitory 4E-BP1–eIF4E complex was decreased in the TPN group compared with saline controls (Figure 1B and Figure 2), but this did not reach significance ($P < 0.06$).

Effects of TPN on 4E-BP1 phosphorylation

Phosphorylation of total 4E-BP1 protein was analysed as a complement to the analysis of the 4E-BP1–eIF4E complex, by determination of different isoforms following electrophoretic separation. The least phosphorylated forms migrate faster, whereas more extensively phosphorylated forms migrate at a slower rate through the gel. The least phosphorylated forms are attached to eIF4E. Increased phosphorylation makes the protein disassociate from eIF4E so that eIF4G binding can occur. Quantification of free and bound forms of the protein was performed by comparing the amount protein present in the most phosphorylated form with the total amount of protein. Provision of overnight nutrition increased the amount of 4E-BP1 in the most phosphorylated form to 72% compared with 60% in saline-treated patients ($P < 0.05$; Figure 1C and Figure 2).

Effects of TPN on p70^{S6K} phosphorylation

Provision of overnight TPN increased the total amount of p70^{S6K} and the phosphorylation of the protein (Figure 1D and Figure 2). Blots were quantified by measuring the total absorbance of all of the bands ($\alpha+\beta+\gamma$) and by determining the ratio between the least phosphorylated form (α) and the total ($\alpha+\beta+\gamma$) amount. In the group receiving TPN, total p70^{S6K} increased 30% compared with saline-treated patients ($P < 0.05$). The relative amount of the protein present in the least phosphorylated α band decreased from 63 to 49% ($P < 0.01$), which indicates the activation of the kinase by phosphorylation.

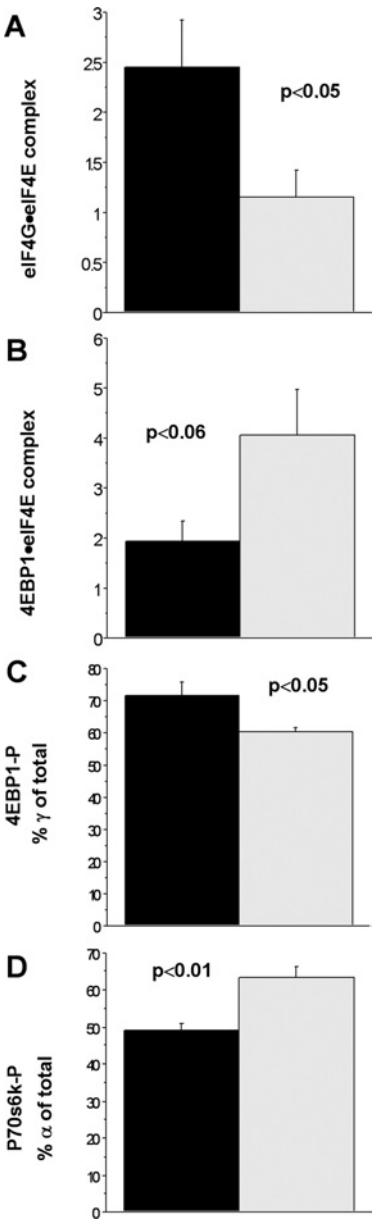

Figure 1 Quantification of (A) eIF4G–eIF4E complexes, (B) 4E-BP1–eIF4E complexes, (C) the phosphorylation state of 4E-BP1 and (D) the phosphorylation state of $p70^{S6K}$ in rectus abdominis muscle biopsies from patients who received overnight infusion of saline (grey bars) or TPN (black bars)

The amount of eIF4G was corrected for eIF4E recovered from immunprecipitation. No difference in eIF4E content was found between the groups. Antibodies recognizing multiple phosphorylation forms of 4E-BP1 and $p70^{S6K}$ were used, as described in the Materials and methods section. Values are means ± S.E.M., $n = 6$ patients in each group.

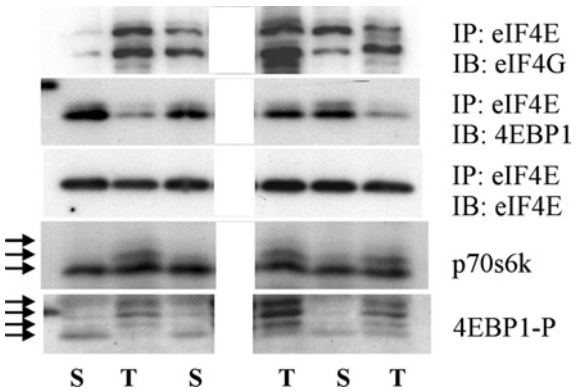

Figure 2 Western blot analysis of initiation factors in rectus abdominis muscle biopsies from patients who received overnight infusion of saline or TPN

S, saline; T, TPN. Proteins were immunoprecipitated (IP) with an anti-eIF4E antibody and were then immunoblotted (IB) with anti-eIF4G, anti-4E-BP1 or anti-eIF4E antibodies for the measurement of eIF4G–eIF4E and 4E-BP1–eIF4E complexes. Increased phosphorylation of $p70^{S6K}$ and total 4E-BP1 was measured as the relative change in protein mobility, as indicated by the arrows.

DISCUSSION

It is well established that malnutrition affects both muscle mass and function, with deteriorations that may explain reduced survival and increased morbidity in both patients and undernourished individuals [21,22]. Numerous experimental and clinical studies have been published illustrating the effects of exercise, hormones and nutrition on protein synthesis and the breakdown of skeletal muscles [1]. Unanimous findings are that reduced food intake causes protein imbalance, which can be explained by the decreased synthesis of mixed muscle protein and, sometimes, by elevated breakdown to support vital organs [23,24]. Thus refeeding initiates protein translation to support resynthesis of both globular and contractile proteins [2]. However, protein synthesis is not a simple activity, but a highly differentiated process with selective translation of specific proteins [25]. Thus the choice of methods to measure protein synthesis should be a matter under investigation. In clinical studies, it has usually been determined whether overall mixed protein synthesis is low, unchanged or increased [24]. Accordingly, most studies have relied on measurements of the net balance of amino acids across vascular beds or on labelling of mixed muscle proteins following a bolus or primed constant infusion of labelled amino acids [26,27], techniques that suffer from both advantages and limitations. Results from dynamic and static evaluations of protein synthesis should therefore be interpreted with caution, as required prerequisites are not always fulfilled [13]. Thus results may not always be indicative of what has been concluded, as emphasized by discrepancies between the incorporation rates of amino acids and phosphorylation/dephosphorylation of regulatory proteins [28]. Both under- and over-estimation of protein synthesis rates may occur when labelling techniques are used [13], and measurements of the net balance of amino acids across resting or exercising

muscles may not fully account for exchanges between various tissue components in organ compartments [3,29]. This situation may appear particularly uncertain if extracellular amino acids are controlling initiation of translation without prior equilibration with intracellular amino acids [30,31]. The present study took advantage of a technique measuring alterations in the phosphorylation of proteins, reflecting induction and inhibition of initiation of protein translation, independent of muscle steady-state conditions and tracer equilibration [8].

Alterations in protein synthesis is reflected by changes in the eIF4G–eIF4e complex and the binding of 4E-BP1 to eIF4E. A limiting step in the initiation of translation in protein synthesis involves the assembly of eIF4F. This complex is composed of eIF4E–eIF4G and eIF4A. During initiation of protein translation, the mRNA 5′-cap binds to eIF4E with subsequent binding of eIF4G and eIF4A to form a complex that allows translation to proceed. The binding of eIF4G to eIF4E is controlled, in part, by 4E-BP1. This binding protein can prevent the assembly of the eIF4F complex by binding to eIF4E at the site of eIF4G binding. The 4E-BP1 protein is controlled by phosphorylation at multiple sites. When 4E-BP1 becomes multiply phosphorylated it cannot bind to eIF4E. Free eIF4E for binding to eIF4G therefore becomes a limiting factor in the formation of eIF4F complexes [8,16]. Translation can also be regulated by the activation of $p70^{S6K}$ by phosphorylation, a kinase that is associated with increased translation of mRNAs containing a 5′-oligopyrimidine-tract-encoding components of the translational machinery [32]. This concept has recently been challenged, but activation of $p70^{S6K}$ was necessary for muscle fibres to achieve a normal size during development observed in cells null for the gene [7,33].

Studies by isotopic infusions in our laboratory have indicated that conventional TPN decreased net whole-body protein degradation, whereas stimulation of whole-body synthesis demanded considerably higher substrate infusion rates [34]. Amino acid balance across resting forearm and legs also indicated that high arterial concentrations of amino acids were required to switch net efflux of amino acids to influx [3], which should be seen in the light of observations in which the incorporation of labelled amino acids into mixed muscle proteins increased by 30 % [30], even when extracellular amino acids were only slightly increased compared with levels observed in the present study (Table 2). Therefore different techniques may result in varying results. Analyses of controlling factors, such as insulin, IGF-1 (insulin like growth factor-1) and exercise, have implied that both individual and groups of amino acids stimulate mixed human muscle protein synthesis both *in vitro* and *in vivo* [6,13,19,20,35,36]. A recent investigation on ICU (intensive care unit) patients on nutritional support suggested that conventional intravenous nutrition did not increase individual amino acids in arterial blood to the extent observed in orally fed healthy volunteers [37], although severely injured ICU patients were kept alive for a long period of time, despite a lack of indices of improved muscle protein balance [38]. Such discrepancies and uncertainties suggest that available clinical programmes for nutrition are still suboptimal or that methods applied for measurements of protein synthesis are insensitive or even inaccurate under some conditions [30]. By contrast, the present study implies that overall initiation of translation of muscle proteins in the rectus abdominis muscles in patients who received overnight standard TPN was activated and remained so as long as the infusion continued.

For practical reasons, previous studies have examined different muscles in humans, such as vastus lateralis and soleus muscles, as needle biopsies are usually undertaken. Such studies have, however, not provided consistent conclusions, as different muscle fibre compositions may be related to varying phosphoprotein status and mechanisms by which such proteins control protein dynamics. In the present study, we used the rectus abdominis muscle based on its significance in abdominal surgical procedures. This muscle is also sensitive to nutritional alterations due to its type I fibre composition [39,40]. A previous study examining the response to 3 h of infusion of amino acids in both soleus and vastus lateralis muscles from young healthy humans observed no effect on the phosphorylation of either 4E-BP1 at Thr^{37}/Thr^{46} or $p70^{S6K}$ at Thr^{789} compared with basal values, but observed both higher total amount and more phosphorylated 4E-BP1 in soleus muscle fibres [28]. The patients in our present study had variations in the total amount of 4E-BP1, whereas other measured proteins, such as $p70^{S6K}$ or eIF4E, did not show similar variations. These observations imply the importance of measuring initiation factor complexes, such as eIF4G–eIF4E, as it provides more direct information on the active complex formation. The present study is the first study in humans reporting eIF4G–eIF4E complex levels in skeletal muscles.

It may be questioned to what extent phosphoprotein status reflects protein synthesis activity. Results from rats, mice and pigs are available which show that changes in initiation factors with increasing eIF4G–eIF4E complex formation and decreasing 4E-BP1–eIF4E complexes are related to elevated protein synthesis. Exceptions to these reports occur where increased and decreased protein syntheses were not reflected by changes of initiation factors [41–43]. Similar studies on human skeletal muscles are limited, but infusion of BCAAs increased the amount of the γ form of 4E-BP1 and increased the phosphorylation of $p70^{S6K}$ without increased protein synthesis [44]. However, infusion of mixed amino acids caused similar changes in 4E-BP1 phosphorylation simultaneously with increased

protein synthesis [15]. Therefore it remains uncertain to what extent alterations in phosphoprotein status reflect dynamics in protein synthesis. The present study used a constant low dose amino acid infusion (approx. 43 mg · kg^{-1} of body weight · h^{-1}), which compares well with a low dose (43.5 mg · kg^{-1} of body weight · h^{-1}) used by Bohé et al. [30] inducing a 30 % increase in mixed muscle protein synthesis. However, another study by Bohé et al. [45] indicated that protein synthesis may became refractory despite the continuous availability of amino acids. By contrast, our present results have shown a sustained activation of proteins for initiation of translation during infusion of a low dose of amino acids for 16 h. This difference may be explained by amino acid overloading with a high dose infusion, indicated by the appearance of ureagenesis [45]. In addition, it is likely that activated phosphoproteins reflect continuously changing recruitment of various mRNAs for translation reflected by the sustained formation of initiation complexes. Therefore the selection of investigative methods should always be done with a specific aim in mind, where quantification of initiation complexes appears ideal for the determination of directional changes in protein synthesis in non-steady-state conditions.

In conclusion, the present study demonstrates that overnight pre-operative constant infusion of a standard TPN alters the phosphorylation status of proteins, indicating the activation of initiation of protein translation in the rectus abdominis muscle.

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