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Modification of Organ Protein Synthesis After Surgical Stress by Low Energy Diets with Different Supplements

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Summary

We have studied the effects of hypocaloric diets with different supplements on liver and jejunal mucosa protein synthesis. The supplements assayed were medium chain triglycerides (diet MCT, with 50 % carbohydrates: 25 % long chain triglycerides (LCT): 25 % medium chain triglycerides (MCT), standard amino acids), branched-chain amino acids (diet BCA, identical to control diet L50, with 15.3 % of nitrogen replaced by branched-chain amino acids) and glutamine (diet GLN, identical to diet L50, with 15.3 % of nitrogen replaced by glutamine). The control diet (L50) had 50 % carbohydrates: 50 % LCT and standard amino acids. The diets were assayed on 86 rats with femoral fracture immobilized by Kirschner pin insertion. Nutrition was administered for 4 days. On the fifth day, liver and jejunal mucosa protein synthesis was determined. A branched-chain amino acid supply in a proportion higher than 21.2 % of amino acid nitrogen significantly decreased liver and jejunal mucosa protein synthesis, while the same amount of glutamine did not modify it. MCT had no effect on jejunal mucosa protein synthesis, while it was decreased significantly in the liver.

Key words

Hypocaloric parenteral nutrition – Glutamine – Branched-chain amino acids – Medium chain triglycerides – Protein synthesis – Liver – Jejunal mucosa

Introduction

For surgical patients who have lost their enteral feeding ability, total parenteral nutrition (TPN) provides the most complete nutritional support. However, if the patient's inability to accept enteral nutrition is anticipated to be short-term (one week or less) and his nutritional status is well-preserved, support is generally limited to peripheral parenteral infusion of 5 % glucose (Garcia *et al.* 1991, Schwartz *et al.* 1984). In this situation there is a reluctance to use TPN because it requires catheter placement in a central vein, increasing the risk of infection and involving time-consuming maintenance procedures (Pennington 1991, Matuchansky *et al.* 1992). Thus, in short-term feeding, the disadvantages of TPN are generally considered to outweigh the potential benefits.

An alternative to the administration of glucose alone is low energy parenteral nutrition (HPN), which provides the same energy as conventional glucose-saline infusion plus amino acids. The osmolality of these solutions permits infusion of HPN into a peripheral vein, avoiding the main drawback of TPN administration.

Amino acid intake supplied by HPN is more beneficial than glucose alone, as has been demonstrated previously (Blackburn *et al.* 1973, Askanazi *et al.* 1978, Elwin *et al.* 1979, Skillman *et al.* 1987, Langran *et al.* 1992). One of the strongest points in its favour is the decrease in whole body protein breakdown observed with a mixture of glucose plus amino acids when compared to glucose infusion alone (Schwartz *et al.* 1984). In the light of these results, HPN utilization is becoming more prevalent as a

clinical support tool for postoperative patients (Stock *et al.* 1980, Elwin *et al.* 1978).

To date, there is a paucity of published data concerning the composition of HPN, usually formulated as low-energy, lipid-free TPN (e.g. Isoplasmal G: 4.8 g nitrogen/l, 1340 J/l (320 kcal/l) and Periplasmal G: 5.6 g nitrogen/l, 1423 J/l (340 kcal/l), from B. Braun, Germany; Periamin-G: 3.85 g nitrogen/l, 1256 J/l (300 kcal/l), from Kabi-Pharmacia, Sweden; Periphramine: 4.8 g nitrogen/l, 544 J/l (130 kcal/l), from Farmiberia S.A., Spain). With this support, patients are in a state of semi-starvation induced by the low energy intake, typically half that needed. This condition of semi-starvation modifies the metabolic dynamics (Fu and Hornick 1992) and should require a nutritional formula different from current HPN solutions, which reproduce TPN composition without considering that the latter ensures a correct energy intake.

It is not known what influence the addition of medium chain triglycerides to HPN might have on the efficacy of the nutrition. The effect of glutamine or branched-chain amino acids has also not been investigated yet. The influence of all these variables on the rate of protein synthesis has been extensively documented for TPN support (Sueiras *et al.* 1993, Schwartz *et al.* 1991, Cerra *et al.* 1982, Kirvela *et al.* 1986, Abumrad *et al.* 1989, Hammarqvist *et al.* 1989), but not for HPN.

The aim of the present work was to study the effects of different composition of hypocaloric nutrition, i.e. of separate amino acid supplements and addition of medium chain triglycerides, on liver and jejunal mucosa protein synthesis of rats subjected to surgical stress.

Material and Methods

Animals and surgical procedures

Open femoral fracture, followed by Kirschner pin insertion was performed under anaesthesia on 86 male Sprague-Dawley rats, with an average weight of 160.6±9.6 g (S.D.). The animals were randomized into 4 groups each of which received a qualitatively different nutritional formula.

A catheter was inserted into the cava vein via the jugular vein under sterile conditions. The catheter consisted of a PE-20 polyethylene tube (0.38 mm inner diameter) which was welded to the end of a PE-10 tube (0.28 mm inner diameter), and the other end was connected to a silicone tube. The silicone part of the catheter was then inserted in the cava vein.

The rats were placed in individual cages which allowed free movement, in a room with controlled temperature, humidity and noise and a 12-hour light-dark cycle. Procedures involving rats were carried out according to the guidelines of the Research Committee of the Vall d'Hebron Hospital.

Nutrition

Immediately after surgery, nutrition was started at a rate of 330 ml/kg.day. Each group of rats received one of the solutions shown in Table 1.

Table 1
Energy intake and body weight loss on day 5 compared to day 1

Group	Energy intake kcal/(kg.day)	Weight loss %	Statistical significance vs day 1
L50	168.8±13.5	−15.7± 4.0	$p<0.0001$
MCT	163.8±7.9	−13.4±3.8	$p<0.0001$
BCA	167.1±7.0	−9.5±3.2*	$p<0.0001$
GLN	168.4±5.9	−12.6±5.3	$p<0.0001$

*Data expressed as mean (±S.D.). Statistical significance of body weight on day 5 compared to day 0 (paired Student's t-test). Diets: L50 (control), 50 % carbohydrates: 50 % LCT and standard amino acids. MCT, with 50 % carbohydrates: 25 % LCT: 25 % MC and, standard amino acids. BCA, identical to control diet L50, with 15.3 % of nitrogen replaced by branched-chain amino acids. GLN, identical to diet L50 with 15.3 % of nitrogen replaced by glutamine. * significant difference versus control group L50 in body weight variation.*

All nutrients were pooled in an all-in-one mixture. The characteristics common to all the formulas were as follows: 6.667 g nitrogen/l, at an infusion rate of 2.2 g nitrogen/kg.day; and 2093 J/l (500 kcal/l), at an infusion rate of 733 J/(kg.day) (175 kcal/(kg.day)) and 691 J/(kg.day) (165 kcal/(kg.day)) of non-protein energy. The amino acid composition of the formula (relative amounts of amino acids) was: ile 2.48 %, leu 3.36 %, lys-glu.$2H_2O$ 3.80 %, met 3.18 % phe 2.95 %, thr 3.18 %, trp 1.24 %, val 2.71 %, arg

8.26 %, his 2.06 %, ala 15.34 %, N-ac-cis 0.64 %, glu 11.29 %, gly 22.39 %, pro 8.26 %, ser 8.26 %, N-ac-tyr 0.58 %. Glycine, equivalent to 1.022 g nitrogen/l (15.3 % of total nitrogen), was replaced in diet BCA by the same nitrogen amount of an equimolar mixture of branched-chain amino acids, and in diet GLN by glutamine.

Experimental design

After surgery, nutrition was infused for four days. On the fifth day, rats of Group L50, with the 50/50 lipid to carbohydrate ratio usually utilized in TPN and the standard amino acid formula, served as the control group.

Protein synthesis determination

The rates of protein synthesis in the liver and jejunal mucosa were measured using L-[1-^{14}C]leucine by the flooding dose method adapted from McNurlan *et al.* (1979) as described by Schwartz *et al.* (1987). Briefly, rats were injected through the catheter of a massive dose of leucine with specific activity of 111 mCi/mol and a leucine concentration of 135 mmol/l, in a dose of 1 ml per 100 g of animal body weight. In each group, 5 animals were decapitated 2 min after the injection. The remaining animals were killed 10 min after injection.

A 20 cm segment of intestine was taken after discarding the first 30 cm from the pylorus, and measuring the intestine vertically with a 3.5 g weight attached to the end. The lumen was exposed by cutting the segment longitudinally with a scalpel and the jejunal mucosa was scraped with a glass slide. Jejunal mucosa and liver were weighed and immediately frozen in liquid nitrogen and stored at −30 °C until analysis.

Tissues were ground in a precooled aluminum mortar until cell breakage was complete. Amino acids were extracted with cold 20 g/l perchloric acid and ultrafiltered to remove protein contamination by centrifugation through Centriflo CF-25 conical membranes with a molecular weight cut-off of 25 000 (Amicon Div., W.R. Grace & Co., Danvers, MA, USA). The specific activity of tissue free leucine (Sa) was calculated from this fraction by measuring: 1) the leucine concentration with HPLC (PicoTag system, Millipore Corp., Waters Chromatographic Division, MA, USA), and 2) the leucine radioactivity: 0.5 ml of sample was added to 5 ml of scintillation cocktail (Cocktail-22 Normascint, Scharlau S.A., Barcelona, Spain) and measured in a beta-counter (TriCarb liquid scintillation counter, Canberra Packard International S.A., Zurich, Switzerland).

The protein pellet obtained by perchloric acid precipitation was washed three times with the same 2 % perchloric acid cold solution and solubilized in 10 ml 0.3 mol/l NaOH (1 hour at 37 °C). Specific activity of the protein-bound leucine (Sb) was determined in this solution by measuring leucine radioactivity. The protein-bound leucine concentration was obtained after acidic hydrolysis of the protein. The error of the protein synthesis rate caused by radioactive ketoisocaproate (KIC) contamination was assessed as described by López-Hellin *et al.* (1993). In brief, KIC is separated from leucine after tissue perchloric acid extraction using cation exchange chromatography. Radioactive KIC and leucine are measured, allowing a correction of the protein synthesis rate determinations by using the true Sa value.

Calculations

Protein synthesis is expressed as the fractional synthesis rate (Ks %), i.e. the ratio between the amount of newly synthesized proteins per day and the organ total protein content, expressed as percentage. It can be calculated from

$$Ks\ (\%) = (100\ Sb)/(Sa\ .\ t)$$

where t is the time (in days) between isotope infusion and organ freezing. Protein synthesis is expressed in mg protein/(g tissue. day), i.e. as the synthesis per organ weight (SOW), to compensate the differences in jejunal mucosa weight found among the groups.

Statistics

Group mean comparisons were performed using the one-way ANOVA test. Individual group comparisons were carried out with the least significant differences (LSD) method. When differences in variance (assessed by the Snedecor F test) barred the use of this test, the Kruskal-Wallis non-parametric procedure was used. The paired Student t-test was used to assess the weight loss on day 5 with respect to day 1 in each group. The Kolmogorov-Smirnov test was used to test the normality of the sample distribution.

Results

In our samples, all the variables studied showed a normal distribution. The final number of animals per group are shown in Table 1. No significant differences were found in the energy/nitrogen intake among the 7 groups (mean intake = 698±37.2 (S.D.) J/(kg.day)), ensuring their comparability. All groups showed a statistically significant weight loss with respect to day 0 (Table 1).

KIC contamination error

The error produced by radioactive KIC contamination was constant in all groups, and did not influence the inter-group Ks comparisons. The mean values found to correct Ks determinations were 5.3 % (S.D. = 2.1) for liver samples and 2.1 % (S.D. = 0.7) for jejunal mucosa samples.

Table 2
Liver and jejunal mucosa protein synthesis rates for different diet compositions

	Liver			jejunal mucosa		
Diet	n	SOW ± S.E.M.	Differences vs control	n	SOW ± S.E.M.	Differences vs control
L50 (control)	17	130.4 ± 7.9	–	17	202.7 ± 8.9	–
MCT	11	82.7 ± 6.9	$p<0.05$	11	190.7 ± 19.9	NS
BCA	16	80.8 ± 6.4	$p<0.05$	17	149.8 ± 11.9	$p<0.05$
GLN	13	108.0 ± 7.0	NS	13	202.8 ± 14.3	NS

Protein synthesis is expressed per organ weight as mg of protein per g of tissue and day (SOW). Diets: L50 (control), 50 % carbohydrates: 50 % LCT and standard amino acids. MCT, with 50 % carbohydrates: 25 % LCT: 25 % MCT and standard amino acids. BCA, identical to control diet L50, with 15.3 % of nitrogen replaced by branched-chain amino acids. GLN, identical to diet L50 with 15.3 % of nitrogen replaced by glutamine.

Protein synthesis

The results on protein synthesis were calculated after excluding animals with problems during isotope infusion (injection time longer than 30 s, isotope loss during injection) or sample processing. The number of animals remaining in each group is shown in Table 2.

MCT supplementation

The 50 % MCT/50 % LCT infusion significantly decreased liver protein synthesis when compared to the control group L50 with LCT alone (Table 2), while it had no effect on jejunal mucosa protein synthesis.

Amino acid supplementation

Branched-chain amino acid supplementation at a rate of 0.336 g nitrogen/kg.day (21.2 % of the amino acidic nitrogen) reduced a liver and jejunal mucosa protein synthesis rate which was significantly lower ($p<0.05$) than that found in the control group with glycine alone. Glutamine supplementation did not cause any significant improvement with respect to the control group L50 (Table 2).

Considerable intestinal atrophy was observed in the experimental animals, the organ weight being, less than one third of that in normal chow-fed rats. The mucosa weights obtained in each group from a 20 cm segment of the jejunum were (mean ± S.E.M.): L50 = 0.237 ± 0.010 g, GLN = 0.293 ± 0.016 g, BCA = 0.260 ± 0.022 g and MCT = 0.253 ± 0.015 g, with significant differences among the groups ($p<0.004$). In comparison, the mean mucosa weight obtained in a previous experiment (Schwartz *et al.* 1993) for the same segment from rats with similar body-weight fed standard diet was 0.803 ± 0.033 g.

Discussion

We have used L50 diet in the control group because is similar in qualitative composition to the standard TPN, and makes it possible to introduce MCT without modifications in the lipid:carbohydrate ratio. The use of fasting or glucose-saline fed rats as the control group would be incorrect since these groups would not receive amino acids, adding a differential factor and perturbing the statistical comparison among the groups.

The main difficulty encountered in this work was to obtain jejunal mucosa samples (due to intestinal atrophy). This was caused by the use of parenteral nutrition, as has been previously reported by Illig *et al.* (1992), and was perhaps enhanced by the low energy supply to the animals. Intestinal atrophy made sample withdrawal remarkably difficult and when the sample were obtained they were too small to be used for the determinations. We did not include the results from group B2 in the final data consideration because of the small number of usable samples obtained. With HPN the rate of protein synthesis was reduced by MCT, while with TPN maximum Ks was obtained using a 50 % LCT/50 % MCT ratio (Schwartz *et al.* 1991). These contrasting results may be due to the low energy intake of HPN compared with TPN.

Previous reports have demonstrated the benefits of providing branched-chain amino acids in TPN after surgical stress, trauma and sepsis (Morrison 1968, Mori *et al.* 1988, Farriol *et al.* 1990). Their purpose is to enhance liver and protein synthesis, although doubts have been cast as to their effectiveness (Iapichino *et al.* 1985). It can be concluded from our data that, in the situation of non-hypercatabolic stress studied, BCA supplementation of HPN formulas does not improve liver protein synthesis.

Glutamine is regarded as a major fuel for the small intestine and lymphocyte function and a promoter of muscle protein synthesis (Smith *et al.* 1982, Abumrad *et al.* 1989, Hammarqvist *et al.* 1989). Nevertheless, in the present study, there was no enhancement of liver protein synthesis in the glutamine-supplemented diet. Because of the difficulty in obtaining samples, jejunal mucosa protein synthesis was not determined in the glutamine group. We observed a striking difference in the pattern of protein synthesis in the liver and jejunum between hypocaloric and normocaloric nutritions of varying compositions. In previous experiments with enteral and parenteral normocaloric nutrition (Sueiras *et al.* 1993, Schwartz *et al.* 1991), we saw that the liver and jejunum responded differently to different diet compositions. These results led us to postulate organ-specific nutrition which would selectively enhance the protein synthesis rate in a particular organ. However, in this study we found parallel, interrelated protein synthesis patterns in the two organs with different hypocaloric nutrition formulas. This might also be explained by the state of semi-starvation produced by the hypocaloric diet.

Our data show that the addition of glutamine does not improve the organ synthesis rates, and amounts of BCA equivalent to 1.37 g nitrogen/l actually decrease nutritional efficacy. MCT shows an organ specific action: while it does not affect jejunal mucosa protein synthesis, it decreases it in the liver.

These data cannot be extrapolated directly to human beings, but show clearly that there exists a metabolic response to changes in the HPN nutrition formula different from that found with TPN. Thus, the best HPN composition is not that found for TPN under similar condition. Further studies on patients must be performed in order to establish optimum HPN composition.

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