

Modification of Organ Protein Synthesis After Surgical Stress by Low Energy Diets with Different Lipid/Glucose Ratios

J. LÓPEZ-HELLIN, E. GARCIA-ARUMI, M. FARRIOL, M.A. ARBOS, A.L. ANDREU, S. SCHWARTZ

Centre for Research in Biochemistry and Molecular Biology, Hospitals Vall d'Hebron, Barcelona, Spain

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Summary

The aim of this work was to study the effects of low energy parenteral diets with different lipid/glucose ratios on rat liver and jejunal mucosa protein synthesis. The studied diets were: L0 (100 % glucose, control diet), L25 (25 % lipids: 75 % glucose), L50 (50 % lipids: 50 % glucose) and L75 (75 % lipids: 25 % glucose). All diets were isoenergetic and isonitrogenated, with a standard amino acid content. The diets were assayed in 93 rats with open femoral fracture immobilized by Kirschner pin insertion. The diets were administered for 4 days. On the fifth day, liver and jejunal mucosa protein synthesis were determined. Highest liver protein synthesis rates were obtained with the diet compositions: lipid/carbohydrate ratio: 25 % lipids and 75 % carbohydrates (expressed as energy ratio). A higher proportion of lipids significantly decreases liver protein synthesis ($p < 0.05$). Jejunal mucosa protein synthesis followed the same pattern, with the same statistical differences.

Key words

Hypocaloric nutrition – Parenteral nutrition – Protein synthesis – Liver – Jejunal mucosa

Introduction

After surgery, if the enteral feeding ability is lost for a short period and the nutritional status is well preserved, low energy parenteral nutrition (HPN) is a matter of choice as nutritional support (Jimenez *et al.* 1992, Jimenez *et al.* 1994). HPN provides amino acids in a similar amounts as total parenteral nutrition (TPN), but the energy component is reduced, matching that of conventional 50 g/l glucose infusions. The low osmolality of HPN solutions permits their infusion through a peripheral vein. TPN is not recommended in this situation because it increases the risk of complications, both metabolic and infectious, outweighing its potential benefits (Pennington 1991, Matuchansky *et al.* 1992). Conventional 50 g/l glucose-saline infusions, although widely used, are very poor as nutritional support. Amino acid intake supplied by HPN is more beneficial than glucose alone, as has been demonstrated previously (Askanazi *et al.* 1978, Blackburn *et al.* 1973, Elwyn *et al.* 1979, Skillman *et al.*

1987, Langram *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 (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, Elwyn *et al.* 1978).

Although the effect of qualitative changes in TPN composition on metabolism have been extensively studied, there is a paucity of published data concerning the effects of HPN composition on metabolic parameters. The low energy intake provided by HPN, only half that needed, induces a state of semi-starvation in the patients. This condition modifies the metabolic dynamics (Fu and Hornick 1992) and could modify the response to qualitative differences in the nutrition, compared to those observed with TPN, which ensures sufficient energy support.

Protein synthesis is one of the key metabolic parameters which is altered by pathophysiological

processes such as surgical stress, sepsis, food deprivation, etc. It is also well known that nutrition can modify protein synthesis. From this point of view, protein synthesis can be considered a convenient parameter for studying the effects of different nutritional components in surgical stress. The liver and jejunal mucosa were the tissues chosen for the study due to this important metabolic role.

The aim of the present report was to study the effects of low energy diets with different lipid to carbohydrate ratios on the rates of protein synthesis in the liver and jejunal mucosa of rats submitted to surgical stress.

Material and Methods

Open femoral fracture, followed by Kirschner pin insertion, was performed under anaesthesia: 2 ml/kg of a mixture composed of ketamine (10 ml Ketolar 50 mg/ml, Parke-Davis SA, Barcelona, Spain) and xilacene (1 ml Rompun 2 %, Bayer, Viladecans, Spain) on 93 male Sprague-Dawley rats (Letica, Hospitalet, Spain), with an average weight of 164.1 ± 15.2 g (S.D.).

A catheter was placed under sterile conditions into the cava vein *via* the jugular vein. The catheter was made of a PE-20 polyethylene tube (0.38 mm ID, Portex, Hythe, England) which was welded to the end of a PE-10 tube (0.28 mm ID, Portex, Hythe, England), and the other end connected to a silicone tube (0.12 mm ID, Silastic, Dow-Corning, Midland, USA). The silicone part of the catheter was then inserted into 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. All procedures involving rats were carried out according to the guidelines of the Research Committee of the Vall d'Hebron Hospitals.

Nutrition

The animals were randomized into 4 groups (L0, $n = 24$; L25, $n = 23$; L50, $n = 25$; L75, $n = 21$) and each received simultaneously a qualitatively different nutritional formula, starting immediately after surgery. The characteristics common to all the nutritional formulas were: 6.667 g nitrogen/l, at an infusion rate of 2.2 g nitrogen/(kg.day); and 2093 J/l, at an infusion rate of 733 J/(kg.day) and 691 J/(kg.day) of non-protein energy. The amino acid composition of the formula per kg of amino acids was: ile 24.8 g/kg, leu 33.6 g/kg, lys-glu.2H₂O 38.0 g/kg, met 31.8 g/kg, phe 29.5 g/kg, thr 31.8 g/kg, trp 12.4 g/kg, val 27.1 g/kg, arg 82.6 g/kg, his 20.6 g/kg, ala 153.4 g/kg, N-ac-cis 6.4 g/kg, glu 112.9 g/kg, gly 223.9 g/kg, pro 82.6 g/kg, ser 82.6 g/kg, N-ac-tyr 5.8 g/kg. Each group of rats received one of the following lipid/glucose ratios: group L0 (control) = 0 % lipids, 100 % glucose;

group L25 = 25 % lipids, 75 % glucose; group L50 = 50 % lipids, 50 % glucose and group L75 = 75 % lipids, 25 % glucose. The percentages calculated as energy ratios. The lipids administered were long-chain triglycerides. Glucose (Glucosa 50 %, Grifols, Barcelona, Spain) and amino acid solutions (Traumafusin, Kabi Pfrimmer, SA, Argentina, Spain plus glycine, Merck, Darmstadt, Germany) were sterilized by filtration. A lipid solution (Intralipid 20 %, Pharmacia AB, Sweden) was added under sterile conditions. Nutrition was infused during four days using a syringe pump (SE 400 Bm, Becton-Dickinson, Brezins, France) at a rate of 330 ml/(kg.day). On the fifth day, the rats were killed by decapitation and liver and jejunal mucosa protein synthesis was determined.

Protein synthesis determination

The liver and jejunal mucosa protein synthesis rates were measured using L-[1-¹⁴C]leucine (Amersham, Little Chalfon, England) with the flooding dose method adapted from McNurlan *et al.* (1979) as described by Schwartz *et al.* (1987). Briefly, rats received a massive injection of leucine through the catheter (specific activity of 4.10×10^9 Bq/mol in a concentration of 135 mmol/l) in a dose of 1 ml per 100 g body weight. In each group, 5 animals were killed by decapitation 2 min after the injection and the remaining animals 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 longitudinally the segment 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 analysed.

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 at 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 the 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 h 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 is obtained after acidic hydrolysis of the protein with HCl 6 mol/l, 12 h at 110 °C.

The error concerning the protein synthesis rate caused by radioactive ketoisocaproate (KIC) contamination was assessed as described by López-Hellin *et al.* (1993). Briefly, 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 calculated as the fractional synthesis rate (Ks %), i.e. as the ratio between the amount of newly synthesized proteins per day and the organ total protein content, expressed in percentages. It can be calculated from

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

where t is the time between isotope infusion and organ freezing, in days. Protein synthesis is expressed as synthesis per organ weight (SOW), mg of protein/(g tissue. day), to compensate the differences in jejunal mucosa weight found among the various 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's t-test was used to study the weight loss on day 5 with respect to day 1 in each group. The Pearson *r* coefficient was used to assess the correlation between liver and jejunal mucosa Ks values. The Kolmogorov-Smirnov test was used to test the normality of the sample distribution.

Table 2

Liver and jejunal mucosa protein synthesis rate per organ weight, SOW, expressed as mg protein/(g tissue. day) for different diet compositions.

Group	Liver			Jejunal mucosa		
	SOW ± SEM	(n)	Differences vs L0	SOW ± SEM	(n)	Differences vs L0
L0	163.9 ± 4.3	(21)	—	245.0 ± 14.8	(14)	—
L25	172.1 ± 15.0	(17)	N.S.	239.0 ± 14.2	(12)	N.S.
L50	130.4 ± 7.9	(17)	p < 0.05	202.7 ± 8.9	(17)	p < 0.05
L75	128.8 ± 12.5	(14)	p < 0.05	158.0 ± 16.1	(14)	p < 0.05

Groups: L0 (control group) = 0 % lipids, 100 % glucose; L25 = 25 % lipids, 75 % glucose; group L50 = 50 % lipids, 50 % glucose and L75 = 75 % lipids, 25 % glucose. The percentage are expressed as energy ratios.

Results

In our samples, the distribution of all the variables studied was normal. No significant differences were found in the energy/nitrogen intake among the 4 groups (mean intake = 697 ± 71.1 J/(kg.day), S.D. = 71.1), ensuring their comparability. All groups showed a statistically significant of body loss weight with respect to day 0 (Table 1).

KIC contamination error

The error produced by radioactive KIC contamination was constant in all the groups and did not influence inter-group comparisons. The mean values found to correct Ks determinations were 5.65 % (S.D. = 2.0) for liver samples and 2.1 % (S.D. = 0.75) for jejunal mucosa samples.

Table 1

Body weight variation of day 5 with respect to day 1.

Group	Weight variation, %	Statistical significance
L0	-10.9 ± 5.2	p < 0.0001
L25	-7.9 ± 6.2	p < 0.0001
L50	-15.7 ± 4.0	p < 0.0001
L75	-7.3 ± 4.1	p < 0.0001

Data expressed as mean (±S.D.). Statistical significance of the weight on day 5 with respect to the weight on day 0 (paired Student' t-test). No significant differences were found in body weight variations vs the control group L0. Groups: L0 (controls) = 0 % lipids, 100 % glucose; L25 = 25 % lipids, 75 % glucose; group L50 = 50 % lipids, 50 % glucose and L75 = 75 % lipids, 25 % glucose. The percentages are expressed as energy ratios.

Protein synthesis

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

In the liver, the highest protein synthesis rates were found (Table 2) with diets containing the highest carbohydrate content, L0 and L25, but no significant differences between these two groups. Protein synthesis in groups L50 and L75 was significantly lower than in the control group L0 ($p < 0.05$).

As far as the jejunal mucosa was concerned, considerable intestine atrophy was observed, intestinal being less than one third of that of normal chow-fed rats. The mucosa weight of 20 cm segment of jejunum obtained in each group was (mean \pm S.E.M.): L0 = 0.2567 ± 0.07 g, L2 = 0.231 ± 0.020 g, L50 = 0.237 ± 0.010 g and L75 = 0.306 ± 0.017 g, the differences between the groups being significant for $p < 0.004$. In comparison, the mean mucosa weight of the same segment obtained in a previous report (Schwartz *et al.* 1993) in rats of similar body weight fed a standard diet was 0.803 ± 0.033 g, (S.D.), which is significantly higher. The protein synthesis values in jejunal mucosa followed a pattern parallel to that observed in the liver, as can be seen in Table 2. There was positive correlation ($p < 0.02$) between liver and jejunal mucosa protein synthesis, with a correlation coefficient $r = 0.953$.

Discussion

Low energy parenteral nutrition represents one of the main potential applications in postoperative patients who are unable to receive enteral feeding for a short period of time. The postoperative rat model is well-suited for HPN composition studies, permitting matched diet intake and reproducible stress and environmental conditions for all the animals. We have preferred young growing rats for this work because of their higher sensitivity to food intake compared to adult animals (Baillie and Garlick 1991). Some points of controversy concerning this model do, however, exist: the differences in metabolic rate and life span make it uncertain what "physiological" periods are comparable in rats and humans. It is generally accepted that a specific time period in the rat is equivalent to a much longer period in man. For this reason, the effect caused in rats by the change of diet in four days could only be expected to occur in man after more prolonged periods. A second point of controversy is the amount of energy supplied to the rats which could be considered as equivalent to a hypocaloric diet in human beings. The energy component supplied to the animals in this study was chosen to be half that previously given to TPN experimental rat models (Farriol *et al.* 1990),

which in turn was based on the mean energy intake of a group of rats fed oral diets *ad libitum* (Schwartz *et al.* 1993). Although some authors contend that this energy intake is not hypocaloric for the rat (Morrison 1968, Mori *et al.* 1988), the weight loss which occurred in all the groups between days 1 and 5 supports our figures.

It was very difficult to obtain jejunal mucosa samples, due to intestinal atrophy. This was provoked by the use of parenteral nutrition, as has previously been reported (Illig *et al.* 1992), and was perhaps enhanced by the low energy content of the nutrients. Intestinal atrophy made sample withdrawal remarkably difficult and sometimes the samples obtained were too small to be used for determinations of protein synthesis.

The lipid/carbohydrate variations in the composition of nutrients induced significant modifications of protein synthesis. The highest protein synthesis values were found with low or no lipid intake. A lipid intake higher than 25 % reduced protein synthesis. Our results are not in agreement with data obtained in similar experiments, in which, however, adequate energy intake was provided by TPN (Sueiras *et al.* 1993). In those experiments, higher protein synthesis rates were obtained with a 50 % lipid : 50 % carbohydrate ratio. The contrasting results found in this study may be due to the semi-starvation state of our animals under low energy intake. In this condition, glucose is probably more effectively utilized by the organism than lipids.

We observed in previous experiments with enteral and parenteral normocaloric nutrition (Schwartz *et al.* 1991) 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 the present study we found parallel, significantly correlated protein synthesis patterns in the two organs with different low energy nutrition formulas (Table 2). This might also be explained by the state of semi-starvation produced by the low energy diet.

The data obtained in this report cannot be extrapolated directly to human beings, but show clearly that a metabolic response to changes in the HPN nutrition formula is different from that found with TPN. Thus, the best HPN composition must not be assumed to be that found for TPN under similar conditions, and studies with patients should be performed in order to establish an optimum HPN composition.

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References

- ASKANAZI J., ELWYN D.H., KINNEY J.M.: Muscle and plasma amino acids after injury: the role of inactivity. *Ann. Surg.* **188**: 797–802, 1978.
- BAILLIE A.G.S., GARLICK P.J.: Attenuated responses of muscle protein synthesis to fasting and insulin in adult female rats. *Am. J. Physiol.* **262**: E1–E5, 1991.
- BLACKBURN G.L., FLATT J.P., CLOWES G.H.A., O'DONNELL T.F., HENSLE T.E.: Protein sparing therapy during periods of starvation with sepsis or trauma. *Ann. Surg.* **177**: 588, 1973.
- ELWYN D.H., GUMP F.E., ILES M., LONG C.L., KINNEY J.M.: Protein and energy sparing of glucose added in hypocaloric amounts to peripheral infusions of amino acids. *Metabolism* **27**: 325–331, 1978.
- ELWYN D.H., GUMP F.E., MUNRO H.N., ILES M., KINNEY J.M.: Changes in nitrogen balance of depleted patients with increasing infusions of glucose. *Am. J. Clin. Nutr.* **32**: 1597–1602, 1979.
- FARRIOL M., BALSELLS J., SCHWARTZ S., MURIO J.E., GARCÍA E., BONNIN J.: Influence of fat emulsions in parenteral nutrition on visceral protein synthesis: study in hepatectomized rats. *Rev. Esp. Fisiol.* **46**: 297–302, 1990.
- FU D., HORNICK C.A.: Alterations in lipolytic activity at hepatic subcellular sites of fed and fasted rats. *Am. J. Physiol.* **262**: C1102–C1108, 1992.
- ILLIG K.A., RYAN C.K., HARDY D.J., RHODES J., LOCKE W., SAX H.C.: Total parenteral nutrition-induced changes in gut mucosal function: atrophy alone is not the issue. *Surgery* **112**: 631–637, 1992.
- JIMENEZ F.J., ORTIZ LEYBA C.: Nutrición parenteral periférica hipocalórica en pacientes postquirúrgicos (Proyecto EUROPLAN). *Nutr. Hosp.* **7**: 245–252, 1992.
- JIMENEZ F.J., ORTIZ LEYBA C., JIMENEZ L., GARCÍA M.S.: Estudio de la nutrición parenteral periférica hipocalórica en pacientes postquirúrgicos (Proyecto EUROPLAN) II. *Nutr. Hosp.* **9**: 139–154, 1994.
- LANGRAN M., MORAN B.J., MURPHY J.L., JACKSON A.A.: Adaptation to a diet low in protein: effect of complex carbohydrate upon urea kinetics in normal man. *Clin. Sci.* **82**: 191–198, 1992.
- LÓPEZ-HELLIN J., SCHWARTZ S., GARLICK P.J., ROSSELL M., ARBOS M.A., ANDREU A.L., GARCIA E.: Ketoisocaproate contamination errors in protein synthesis determinations using L-[1-¹⁴C]leucine. *J. Biochem. Biophys. Meth.* **27**: 57–63, 1993.
- MATUCHANSKY C., MESSING B., JEEJEEBHOY K., BEAU P., BELIAH M., ALLARD J.P.: Cyclical parenteral nutrition. *Lancet* **340**: 588–592, 1992.
- McnURLAN M.A., TOMKINS A.M., GARLICK P.J.: The effect of starvation on the rate of protein synthesis in rat liver and small intestine. *Biochem. J.* **178**: 373–379, 1979.
- MORI E., HASEBE M., KOBAYASHY K.: Effect of total parenteral nutrition enriched in branched-chain amino acids on metabolite levels in septic rats. *Metabolism* **37**: 824–830, 1988.
- MORRISON S.D.: The constancy of the energy expended by rats on spontaneous activity and the distribution of activity between feeding and non-feeding. *J. Physiol. Lond.* **197**: 305, 1968.
- PENNINGTON C.R.: Parenteral nutrition: the management of complications. *Clin. Nutr.* **10**: 133–137, 1991.
- SCHWARTZ S., FARRIOL M., PADRÓ J.B., PIÉ J., SABÍN P, SAGÜES L.: Evaluation of protein metabolism and albumin in patients submitted to peripheral parenteral nutrition. *Infusionstherapie* **11**: 137–140, 1984.
- SCHWARTZ S., FARRIOL M., GARCÍA E, AFONSO J.J., RODRIGUEZ R.: Influence of the MCT/LCT ratio in enteral nutrition on liver and jejunal mucosa protein synthesis. *J. Clin. Nutr. Gastroenter.* **2**: 31–35, 1987.
- SCHWARTZ S., FARRIOL M., BALCELLS J., MURIO J.E., GARCÍA E., GEMAR E., LÓPEZ-HELLIN J., ANDREU A.L.: Effect of different MCT/LCT ratios on protein synthesis in injured rats fed parenterally. *Rev. Esp. Fisiol.* **47**: 81–86, 1991.
- SCHWARTZ S., ANDREU A.L., GARCÍA E., FARRIOL M., LÓPEZ J., ARBÓS M.A.: Inhibition of visceral protein synthesis by certain amino acids supplements. *Physiol. Res.* **42**: 347–350, 1993.
- SKILLMAN J.J., ROSENOER V.M., SMITH P.C., FANG S.: Improved albumin synthesis in postoperative patients by amino acid infusion. *Infusionstherapie* **14**: 202–208, 1987.
- STOCK S.E., CLAGUE M.B., JOHNSTON D.A.: Postoperative fatigue: a real phenomenon attributable to the metabolic effects of surgery on body nutritional stores. *Clin. Nutr.* **192**: 251–257, 1980.
- SUEIRAS A., MURIO J.E., GEMAR E., FARRIOL M., SCHWARTZ S.: Influence of the lipid/carbohydrate ratio in parenteral nutrition on the visceral protein synthesis in a surgical stress experimental model. (Abstract in Spanish), *Nutrición Hospitalaria* **8**: 30, 1993.

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S. Schwartz, Centre d'Investigacions en Bioquímica i Biologia Molecular, Hospitals Vall d'Hebron, Pº Vall d'Hebron, s/n. 08035 Barcelona, Spain.