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Influence of Fat Emulsions in Parenteral Nutrition on Visceral Protein Synthesis: Study in Hepatectomized Rats

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The aim of this study was to evaluate the effect of total parenteral nutrition (TPN) with 100 % long-chain triglyceride (LCT) (group A) and 50/50 % medium/long-chain triglyceride (MCT/LCT) (group B) fat emulsions on visceral protein synthesis in 70 % hepatectomized rats. The rats were fed TPN continuously for 7 days posthepatectomy. Protein synthesis was measured in the liver and jejunal mucosa with the flooding dose method. All rats received the same caloric and nitrogen intake, without statistically significant differences. The only difference between the two groups was the proportion of MCT to LCT in the diets. Hepatic and jejunal mucosa protein synthesis were significantly higher in group B, fed 50/50 % MCT/LCT. These results suggest that fat emulsions with 50/50 % MCT/LCT significantly enhance visceral protein synthesis after partial hepatectomy.

Key Words: Protein synthesis, Partial hepatectomy, Fat emulsion.

The influence of diet on protein synthesis after partial hepatectomy has not been extensively studied.

After partial hepatectomy protein metabolism has specific characteristics. Within the first 24 hours post-surgery a 23 % increase in protein content has been found in the remnant liver. This increase arises from both a reduction in protein catabolism (19) and a significant increase in protein synthesis (7, 15).

Changes in the liver tissue which remains after partial hepatectomy have been observed, and appear even before increased mitotic activity. An infiltration of fat occurs in the liver, and energy metabolism shifts from a predominant utilization of glucose to an increased utilization of lipids (9, 20).

However, no studies have indicated an

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influence of lipids on liver protein synthesis, taking into account the changes in energy metabolism after partial hepatectomy. An increase of plasma insulin and a reduced liberation of free fatty acids from adipose tissue is associated with the infusion of glucose. These metabolic and hormonal changes delay considerably the onset of DNA synthesis (10, 20).

The majority of studies in this area are focused on proliferative response and DNA synthesis, and in many cases are limited to the effects of starvation. STIRLING *et al.* (21) emphasized the dependence of protein synthesis on adequate administration of amino acids and energy, the absence of one of them delaying the proliferative response after partial hepatectomy. CHIBA *et al.* (3) stated that exogenous amino acids are more important than the energy source (glucose) for hepatic regeneration. Experimental studies (12, 14) suggested that liver regeneration can be accelerated by administering a parenteral nutrition tailored to normalize the deranged pattern of plasma amino acids characteristic of liver insufficiency.

In previous studies with enteral nutrition in normal and post-surgically stressed rats, the qualitative intake of energy substrates influenced the levels of hepatic and jejunal mucosa protein synthesis. This organ-dependent influence provides the possibility for organ-specific nutrition while preserving an overall level of protein synthesis appropriate to the metabolic state (16, 18).

Studies with parenteral nutrition also support the hypothesis that the stimulation of protein synthesis in an organ and specific tissue may be influenced by the qualitative composition of the solution (4, 17). It is not known if this hypothesis may be applied to the post-hepatectomy phase.

The aim of this work was to study the influence of the medium/long chain triglyceride (MCT/LCT) ratio on hepatic and jejunal mucosa protein synthesis in rats after partial hepatectomy.

Materials and Methods

Animals.— Experiments were carried out on 28 male Sprague-Dawley rats (Biocentre, Barcelona, Spain), with an initial body weight of 150 ± 5 g. The rats were provided with a harness, and a period of adaptation followed. The animals were housed individually in metabolic cages in a room with constant humidity and temperature (21 ± 2 °C), and a 12 hour light-dark cycle. Oral food and water were allowed ad libitum until the adaptation period was over.

After surgery rats were divided into two groups: A (n = 14) and B (n = 14), and given different total parenteral solutions (TPN).

Surgical procedure.— On the fifth day after arrival, a 70 % hepatectomy was performed under light ether anesthesia according to methods described by HIGGINS and ANDERSON (5). The median and left lateral lobes were removed and weighed.

After liver resection, a catheter was placed under sterile conditions into the vena cava via the jugular vein, using the operative technique described by WEEKS (22). The catheter was drawn subcutaneously to the back of the rat and through a hole in the harness.

The catheter was made as described by ROOS *et al.* (13): a PE-20 polyethylene tube (0.38 mm inner diameter) was welded to the end of a PE-10 tube (0.28 inner diameter), and the other end connected to a silicone tube. The silicone part of the catheter was inserted into the superior vena cava via the jugular vein.

Parenteral solutions.— The nutritional substrates were mixed under a laminar air flow, and the final solutions kept in individual sterilized vials which were changed every day.

The individual perfusion lines were formed from silicone tubing and a pump chamber connected to a Holter-Roller

Table I. *Composition per liter (g) of total parenteral solution.*

Amino acid solution[a]	4.45
Glycine	1.01
Glucose[b]	218.73
LCT emulsion (Group A)[c]	31.7
MCT/LCT emulsion (Group B)[c]	32.86

[a] Aminoplasmal P.O. (Braun)
[b] 50 % Glucose solution (Pfrimmer)
[c] Intralipid 20 % (KabiVitrum)
[d] MCT/LCT Emulsion 20 % (Braun)

pump, type 904 (Extracorporeal Medical Specialties Inc., USA). Sterile air cannulas were connected to the vial.

The infusion was performed continuously to provide a constant amount of solution per kg of body weight and day (330 ml/kg · d). The TPN solutions were administered for 7 days, beginning at 3 p.m. on day 1 and ending at 11 a.m. on day 8.

The nutritional solutions had the same amount of nitrogen and were supplemented with glycine, giving a final concentration of 5.46 grams of nitrogen per liter. Both solutions had the same total calories: 75 % carbohydrates and 25 % lipids. Lipid composition of solution A was 100 % LCT while solution B had 50/50 % MCT/LCT (table I). Mineral composition of the commercial solutions is shown in table II. Trace elements were supplied by Pfrimmer and the multiple-vitamin solution by Amour Pharmaceutical Company.

Table II. *Mineral composition per liter of TPN (nmol).*

NaCl	35.91
KCl	15.98
$CaCl_2$	4.46
Ca Gluconate	3.37
KH_2PO_4	6.13
$MgSO_4$	5.98

Isotope.— L-(1-^{14}C)leucine (50 Ci/mol or 1850 KBq/mol) was combined with unlabelled leucine to give 15 μCi and 135 μmol/ml. One milliliter of this solution was administered per 100 g animal body weight via TPN catheter to unanesthetized animals. Isotope was injected just after stopping parenteral nutrition.

Samples.— The method used was described by McNURLAN *et al.* (8). In each group, 5 animals were killed by decapitation without anesthesia 2 min after isotope injection and the remaining animals at 10 min.

Liver and jejunal mucosa were removed as soon as possible, weighed, and frozen in liquid nitrogen. The average time for taking liver samples was 1.5 min.

Jejunal mucosa was obtained by removal of the proximal small intestine. The first 30 cm from the pylorus were discarded and the followimg 20 cm were taken after being stretched with a 3.5 g weight. Mucosa was separated from serosa by slitting the intestine longitudinally and scraping the luminal side with a microscope slide; the sample was then transferred to a polypropylene tube and stored at −30 °C until analysis.

Tissue analysis.— Protein content was determined by the method of Lowry. All measurements of radioactivity were taken in a Beta Scintillation Counter (Intertechnique).

Leucine was analyzed by single column ion-exchange chromatography (Chromaspeck: Rank-Hilger) under the following conditions: Resin: Rank-Hilger MK1; column length: 50 cm; temperature: 40 to 60 °C; column flow: 0.17 ml/min; pressure: 2-4 MN/m^2; sample injection time: 60 s; sample volume: 200 μl; buffer Li^+: acid (pH 2.2) and basic (pH 11.5); colour reagent: ninhydrin; incubation temperature: 98 °C; absorbance at 570 nm; analysis time: 120 min. The internal standard used was L-norleucine: 100 nmol/ml final concentration.

The samples were homogenized in a metallic mortar pre-cooled with solid CO_2 to obtain a fine powder. Portions of 0.1-0.5 g were then precipitated in cold 2 % (w/v) $HClO_4$ and centrifuged. Two milliliters of the supernatant were ultrafiltered by centrifugation with conical membranes (Centriflo CF-25, Amicon, 95% retention of molecules above 25,000 MW) for 30 min at 2,000 rpm. This ultrafiltrate was used to measure the specific activity of free leucine. The precipitate was washed three times with cold 2 % $HClO_4$ and the pellet was resuspended in 10 ml of 0.3 M NaOH and incubated at 37 °C for 1 hour. In this solution, specific radioactivity of protein-bound leucine was determined. In addition, another fraction was processed and used for correcting radioactivity contamination due to free leucine. Protein was hydrolyzed with HCl 6 N, incubated at 110 °C for 18 hours, and ultrafiltered for 1 hour at 2000 rpm.

Calculations.— Calculation of the fractional synthesis rate, Ks, in percentage per day was from the equation: $Ks = Sb \times 100 / Sa \times t$ where Sb is the specific radioactivity of leucine in protein, Sa is the mean specific radioactivity of tissue free leucine (using groups of animals killed at 2 and 10 min to assess the change in specific radioactivity over 0-10 min) and t is the time expressed in days (17). The value of Ks was determined for each animal in the 10 min group, and its own Sa value was calculated. In liver it was proved that no differences were obtained from 90 sec after killing; thus the time used in all calculations was 11.5 min.

Absolute protein synthesis was calculated by multiplying the Ks by the protein content of the organ and results were expressed in milligrams of protein synthesized per day ($mg \cdot d^{-1}$).

Statistics.— Distribution frecuency of samples was normal. To evaluate statistical differences the Student's t test was used.

Results

After seven days of parenteral nutrition, both groups had received the same amount of calories and nitrogen. No significant differences in per cent of weight increase were observed (table III).

There were no significant differences in hepatic and jejunal mucosa masses (table IV), both when the results were expressed as absolute (g) and relative (g/100 g B.W.).

No significant differences were observed in liver protein content. However, significant differences ($p < 0.005$ and $p < 0.025$) were found in jejunal mucosa protein content when the results were expressed as relative (mg/g organ) and absolute (mg/organ) (table V).

Protein synthesis results (table VI) in liver show statistically significant differences ($p < 0.001$) according to the fat emulsion used. The better group was B, both when results were expressed in frac-

Table III. *Quantitative intake and % weight increase (% W).*
Values are expressed as mean ± S.D. Statistically non significant differences.

	A (LCT)	B (MCT/LCT)
Kcal/Kg/d	350.9 ± 12	352.5 ± 9
g N/Kg/d	1.805 ± 0.06	1.813 ± 0.05
% W	13.46 ± 4.4	15.07 ± 3.4

Table IV. *Hepatic and jejunal masses.*
Values are expressed as mean ± S.D. Statistically non significant differences.

Organ	Group	O.W.	O.W./100 g B.W.
Liver	A	7.51 ± 1.06	4.16 ± 0.59
	B	7.82 ± 0.93	4.19 ± 0.47
Jejunum	A	0.293 ± 0.04	0.162 ± 0.02
	B	0.301 ± 0.04	0.161 ± 0.02

Group A: 100 % LCT; Group B: 50/50 % MCT/LCT; O.W. Organ weight (g)

Table V. *Protein content in liver and jejunum.* Values are expressed as mean ± S.D. Statistically significant differences in jejunum: * $p < 0.005$, ** $p < 0.025$

Organ	Group	O.W.	O.W./100 g B.W.
Liver	A	141.4 ± 15.5	1061.5 ± 195
	B	137.5 ± 16.5	1076.1 ± 174
Jejunum	A	102.0 ± 13.7	29.79 ± 5.49
	B	116.2 ± 8.7*	34.98 ± 5.76**

Table VI. *Fractional synthesis rate and absolute protein synthesis.* Values are expressed as mean ± S.D. Statistically significant differences: * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$.

Organ	Group	Ks %	A.S. $mg \cdot d^{-1}$
Liver	A	70.5 ± 11.4	719.9 ± 162.3
	B	99.2 ± 6.1***	1108.8 ± 81.6***
Jejunum	A	132.5 ± 18.6	41.5 ± 5.9
	B	176.4 ± 15**	60.1 ± 11.5*

Group A: 100 % LCT; Group B: 50/50 % MCT/LCT; Ks: Fractional synthesis rate (%); A.S.: Absolute synthesis (mg of protein synthesized per day).

tional synthesis rate (99.2 ± 6.1 %) and absolute protein synthesis (1108 ± 81.6 $mg \cdot d^{-1}$).

In jejunal mucosa (table VI) higher values of protein synthesis were again obtained with solution B, in comparison with solution A. The differences were statistically significant, $p < 0.005$ and $p < 0.01$, respectively.

Discussion

It is well established that exogenous amino acids are more important for hepatic regeneration than calories supplied as glucose (3, 6, 12, 14, 21). Some authors have advised against the infusion of hypertonic glucose in this situation because of the effect of diminishing the release of fatty acids from adipose tissue, and the changes observed in the energy metabolism in the remnant liver, with an increased utilization of lipids (9, 10, 20). The effect of fat has not been extensively studied after partial hepatectomy, and its use is generally not recommended in patients suffering from liver disease, but POMPOSELLI *et al.* (11) reported that fat as MCT/LCT emulsion was beneficial as an energy source in experimental hepatic insufficiency. The different liver metabolism of MCT and LCT suggests that MCT may be a beneficial substrate after partial hepatectomy.

The two TPN solutions studied differed only in the proportion of MCT to LCT. As each group of rats received the same caloric and nitrogen intake (table III), the varying results obtained should be attributed to this qualitative difference.

Both groups showed a similar weight increase (table IV). The smaller weight gain reported in rats fed MCT in comparison with rats fed LCT in enteral nutrition (1) was not observed.

Nor did the hepatic and jejunal masses (table IV) show significant differences. The rats at the end of seven days of parenteral nutrition showed higher liver weight/100 g b.w. than reported by other authors (2, 5) after partial hepatectomy, but values were similar to those found in normal rats (8, 15).

Whereas no differences were found in protein content in liver (table V), statistically significant differences were found in jejunal mucosa. In this case better utilization of MCT than LCT by jejunal mucosa may be assumed.

Protein synthesis in liver (table VI) and in jejunal mucosa showed statistically significant differences with superior results in group B. Although an increase in the hepatic mass was produced during the period of study, the differences in protein synthesis cannot be attributed to this increase, since no significant differences or correlation were found for either absolute

or relative masses in the groups. Therefore, the varying results in liver synthesis seem to be due to the different parenteral solutions used.

The results of protein synthesis in liver are lower than those found by other authors (15, 19) at 36 and 48 hours after partial hepatectomy, but higher than in normal rat liver (8) and in postsurgical stress (16).

These results suggest that considering organ-specific nutrition for liver and for jejunum, and taking the liver and jejunal mucosa as a unit, higher stimulation of protein synthesis after partial hepatectomy is obtained with a total parenteral solution containing 50/50 % MCT/LCT.

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Resumen

Se valora el efecto de una nutrición parenteral total con un 100 % de triglicéridos de cadena larga (LCT) (grupo A) y una solución conteniendo 50 % de triglicéridos de cadena larga y 50 % de triglicéridos de cadena media (MCT) (grupo B), sobre la síntesis proteica hepática y en mucosa yeyunal en ratas con hepatectomía del 70 %. Las ratas alimentadas con nutrición parenteral total por vía central mediante un cateter colocado en la vena yugular durante 7 días post-cirugía, reciben el mismo aporte calórico y nitrogenado. La única variable en las fórmulas nutricionales es la proporción de ambos tipos de triglicéridos. La síntesis proteica se mide por el método de dosis masiva. La síntesis hepática y yeyunal es significativamente mayor en el grupo B, que recibe una proporción 50/50 % de MCT y LCT. Estos resultados sugieren que emulsiones con este porcentaje de ambos tipos de triglicéridos suponen una mejora de la síntesis hepática y yeyunal después de una hepatectomía parcial.

Palabras clave: Síntesis proteica, Hepatectomía parcial, Emulsiones lipídicas.

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