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Effect of Different MCT/LCT Ratios on Protein Synthesis in Injured Rats Fed Parenterally

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The effect of the lipidic composition of the diet on the proteic synthesis of the male Sprague-Dawley rats fed parenterally for four days has been studied. All animals received identical nitrogen and caloric intake, but the ratio of medium to long-chain triglycerides (MCT/LCT) varied: group 1, control (0/100); group 2 (30/70); group 3 (50/50) and group 4 (70/30). Hepatic and jejunal protein synthesis were determined with L-(1-^{14}C)-leucine. In groups 1, 3 and 4 muscle protein synthesis was measured with L-(3,4-^{3}H)-phenylalanine. In liver, there were no significant differences in the fractional synthesis rate among the groups. In jejunum, the control group showed a higher fractional synthesis rate with statistically significant differences among the groups. In muscle, group 3 presented the highest fractional synthesis rate with statistically significant differences ($p < 0.05$).

Key words: Protein synthesis, Stress, Fats.

Since BACH and BABAYAN (1) developed the use of triglycerides from coconut oil, there has been increased interest in medium-chain triglycerides (MCT) as a calorie source. Studies involving administration of MCT in animals and man have indicated that MCT have a metabolic and physiologic pathway other than that of long-chain triglycerides (LCT). As a result of differences in intracellular metabolism, MCT are more easily utilized for caloric energy than LCT, but are less effectively incorporated into adipose tissue. Medium chain free fatty acids have been shown to be poor substrates for energy stores and for synthesizing new free fatty acids in tissues. However, MCT calories are more

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readily available than LCT, since MCT-derived fatty acids are able to enter mitochondria directly without undergoing biochemical transformation, while LCT-derived fatty acids must use the carnitine transport pathway.

When administered intravenously triglycerides are hydrolyzed within the capillaries by lipoprotein-lipase. Analysis of MCT and LCT kinetics and elimination processes indicate different removal sites for the two emulsion particles (2). The use of different proportions of MCT and LCT could provide a rapid source of calories to cover essential fatty acid requirements. Although the MCT, or structured, lipid infusion seems to improve nitrogen balance and decrease leucine oxidation in burned rats (6), there are few reports which determine the effect that different triglyceride ratios in total parenteral nutrition (TPN) may have on protein metabolism. In a previous study we suggested that the composition of the lipid caloric source administered could influence the level of hepatic and jejunal mucosa protein synthesis in injury (9).

The aim of this work was to study the effects of four lipid emulsions on the use of available amino acids for hepatic, jejunal and muscle protein synthesis in rats submitted to surgical stress. Nutrition solutions were given parenterally for four days and were identical for all groups except in the ratio of MCT to LCT.

Materials and Methods

Animals. — Ninety-three male Sprague-Dawley rats ranging in weight from 148 to 177 g were used.

The trauma experimental model involved production of a femoral fracture followed by Kirschner pin insertion into the medullary canal of both fragments as reduction (3). To provide a constant flow of TPN solution, a catheter was placed under sterile conditions into the vena cava, via the jugular vein (11). After ligation, the catheter was drawn subcutaneously to the back and through a hole in the harness and connected to a syringe pump (Perfusor, Secura, B. Braun).

The rats were divided into four groups. TPN was given for four days, and on the fifth protein synthesis was determined. During parenteral feeding, weight gain, urea and creatinine excretion were measured.

Parenteral solutions. — The nutritional solutions were prepared under a laminar air flow in individual syringes and were changed every day. All groups received 1.8 g of N/kg/day (Aminoplasmal P.O., Braun supplemented with glycine), and 350 kcal/kg/day, with a carbohydrate/lipid proportion of 75/25. The MCT/LCT ratio given was: group 1 (control), 0/100; group 2, 30/70; group 3, 50/50, and group 4, 70/30. The lipid emulsion used for 0/100 MCT/LCT was from Intralipid (Kabi-Pfrimmer), and the emulsions with different MCT/LCT ratios were supplied by Braun, Melsungen. Nutrition mixtures were supplemented with 0.75 ml/kg • day of polyvitaminic solution (M.I.V. Lyophilized Amour Pharmaceutical Company) and 0.04 IU/kg • day of vitamin K (Konakion, Roche). The volume administered daily was 330 ml/kg.

Protein synthesis. — Hepatic and jejunal protein synthesis were determined in all the groups with L-1-^{14}C-leucine (7) (330 mCi/mmol) in a flooding dose technique. The isotopic solution was prepared with labelled and unlabelled leucine and had a final concentration of 100 µmol/ml and 15 µCi/ml. The dose administered was 1 ml/100 g b.w. In all groups, 5 animals were killed 2 minutes after isotope injection and the remaining, after 10 minutes.

Muscle protein synthesis was measured with L-3,4-^{3}H-phenylalanine (4) (28 Ci/mmol) in flooding doses. The solution was prepared with labelled and unlabelled phenylalanine and had a final concentration of 150 µmol/ml and 50 µCi/ml. The dose administered was 1 ml of phenylalanine solution per 100 g b.w. In groups 1, 3 and 4 the rats were killed after 10 minutes of isotope injection. Liver, jejunal mucosa and muscle (quadriceps) were removed as quickly as possible, weighed and frozen in liquid nitrogen. Samples were stored at −30 °C until protein synthesis was measured (3).

The calculation of the rate of protein synthesis (the proportion of the protein pool synthesized daily) was from the equation: $Ks = S_b\ 100/S_a{\cdot}t$, where Ks is the fractional synthesis rate, S_b is the specific radioactivity of the amino acid in protein, S_a is the mean specific radioactivity of tissue free amino acid, and t is the time expressed in days.

All diferences were statistically evaluated by the Student's *t* and Mann-Whitney U tests.

Results

Since the caloric and nitrogen intake in the four groups studied was fixed beforehand and all rats received the same quantity of nutrients, no statistically significant differences in weight gain expressed as percentage (4.7 % to 6.6 %) were found among the groups. No statistical differences in daily urea (257 ± 52 mg to 311 ± 36 mg) and creatinine excretion (3.4 ± 0.7 mg to 4.02 ± 0.63 mg) were seen.

In liver, no statistically significant differences in the fractional protein synthesis, expressed as percentage were observed: group 1; 62.0 ± 3.0 %; group 2, 65.0 ± 7.1 %; group 3, 61.5 ± 3.7 % and group 4, 58.4 ± 9.3 % (fig. 1). In jejunum, group 1 (100 % LCT) showed the best fractional synthesis rate (K_s: 193 ± 28 %) with statistical differences vs the other groups ($p < 0.05$); group 2, 161 ± 11 %; group 3, 132 ± 23 %, and group 4, 166 ± 19 % (fig. 2). The highest frac-

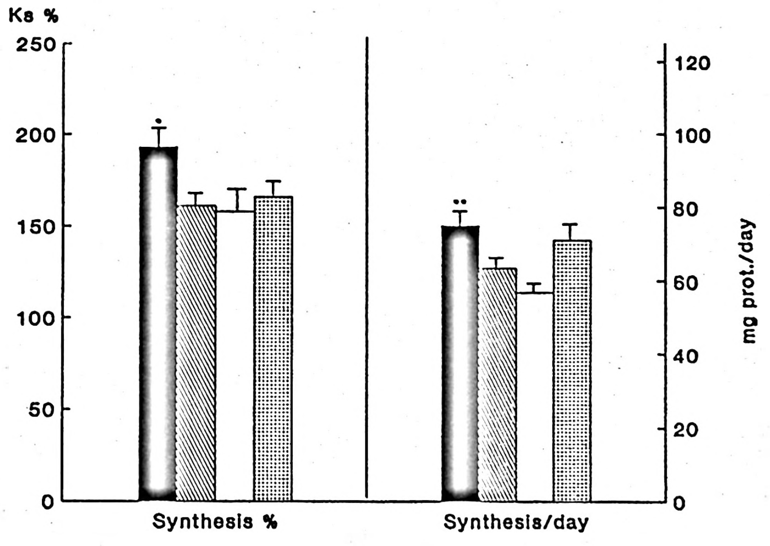

Fig. 1. *Hepatic protein synthesis.*
Black: group 1 (0/100); striped: group 2 (30/70); open: group 3 (50/50); dotted: group 4 (70/30).
* $p < 0.025$ vs 0/100.

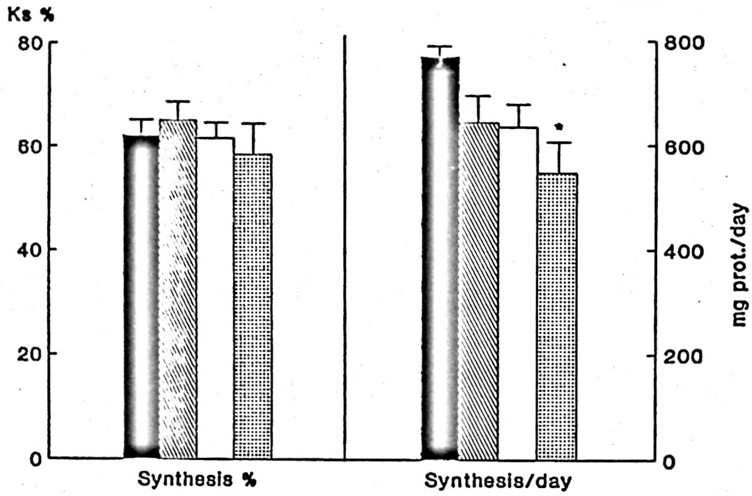

Fig. 2. *Jejunal protein synthesis.*
Black: group 1 (0/100); striped: group 2 (30/70); open: group 3 (50/50); dotted: group 4 (70/30).
* $p < 0.05$ vs all groups. ** $p < 0.05$ vs 2 and 3.

tional synthesis rate in muscle was found in group 3 fed 50/50 MCT/LCT (K_s: 4.85 ± 0.75 %) with statistical differences ($p < 0.05$) among the groups: 1 and 4 of 3.8 ± 0.9 % and 2.98 ± 1.03 %, respectively (fig. 3).

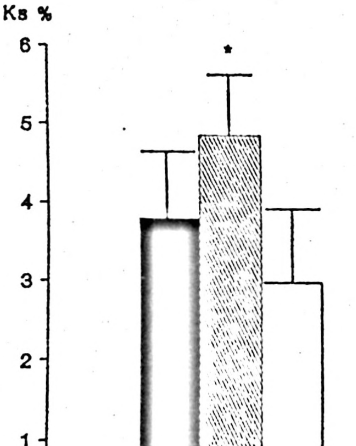

Fig. 3. *Muscle protein synthesis.*
Black: group 1 (0/100); striped: group 3 (50/50); open: group 4 (70/30). * $p < 0.05$ vs all groups.

Discussion

Nutritional support has been generally accepted to play an important role in the evolution of surgical stress. An exogenous amino acid supply is necessary to minimize catabolism in the flow phase. The nitrogen sparing effect of glucose and LCT emulsions has been well established (8), but the effects of MCT on protein metabolism are still undefined. Studies in this field have used a flooding dose technique based on the theory that high concentrations of free amino acids result in a rapid balance between the intra and extra cellular pools, thus allowing the rates of protein synthesis to be calculated with little error (5).

After four days of parenteral nutrition, no statistically significant differences in body weight increase, urea and creatinine excretion were found among the studied groups, indicating an apparently similar evolution in all animals.

No statistical differences in the fractional synthesis rate, expressed as percentage were found, while the absolute synthesis in liver expressed as mg/day was lower in the group fed 70 % MCT. This result suggests a possible negative effect on this organ when MCT is administered at high levels.

Protein synthesis in the jejunum, as indicated in both the fractional and absolute synthesis data, was higher in the group fed 100 % LCT, suggesting efficient metabolic utilization of the long chain triglycerides as a source of calories. These results differ from those previously found in rats fed different MCT/LCT ratios orally *ad libitum* where 40/60 was the best proportion for the jejunum (9).

With intravenous feeding the luminal surface of the gut receives no nutritional stimulus. If this fact is taken into consideration, the route of administration could be extremely important in lipid metabolism and the enterocytes might be considered as principal target sites. The fact that in this work 100 % LCT stimulated jejunal, but not hepatic protein synthesis, could support the theory that the lipid composition of nutritional solutions can modify the response of the small intestine.

Muscle protein is an important component of whole-body nitrogen losses in starvation and post-surgical stress. Protein losses in muscle are regulated through alterations of the synthesis rate as a basic response. An important decrease in the release of muscle amino acids under MCT/LCT infusion has been reported in healthy post-absorptive volunteers (10). Our results show a significant improvement in muscle protein synthesis when a 50/50 MCT/LCT ratio was used.

Our data indicate that the administration of nutrients produces varying effects on protein synthesis in several tissues. Thus, organ specific nutritional solutions for injury could provide optimum support in organ damage and might be a future goal.

Resumen

Se estudia el efecto de la composición lipídica de la dieta sobre la síntesis proteica de ratas Dawley sometidas a estrés quirúrgico. Los animales se someten a un régimen de nutrición parenteral durante 4 días, recibiendo idéndico aporte calórico y nitrogenado y diferente proporción de triglicéridos de cadena media y larga: grupo 1, control, (0/100), grupo 2 (30/70), grupo 3 (50/50) y grupo 4 (70/30). La síntesis proteica hepática y yeyunal se mide con L-(1-C^{14})-leucina. En los grupos 1, 3 y 4 se determina la síntesis proteica muscular con L-(3,4-^{3}H)-fenilalanina. En hígado no se hallan diferencias estadísticamente significativas en la fracción sintetizada de proteínas por día (K_s), expresada como porcentaje, entre los grupos. En yeyuno, el grupo que recibe una relación de triglicéridos 0/100, muestra una mayor síntesis, con diferencias estadísticamente significativas, con respecto a los otros grupos. En músculo, la síntesis más elevada corresponde al grupo que recibe una relación 50/50, con diferencias significativas con respecto a los otros dos grupos.

Palabras clave: Síntesis proteica, Estrés, Lípidos.

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