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BRIEF COMMUNICATION

Carnitine Supplementation Accelerates Normalization of Food Intake Depressed During TPN

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LAVIANO, A., M. M. MEGUID, T. RENVYLE, Z.-J. YANG AND J. L. BEVERLY. *Carnitine supplementation accelerates normalization of food intake depressed during TPN.* PHYSIOL BEHAV **60**(1) 317–320, 1996.—When total parenteral nutrition (TPN; containing glucose, fat, and amino acids; caloric ratio 50:30:20) providing 100% of the rat's daily caloric intake is given for 3–4 days, food intake rapidly decreases by approximately 85%. After stopping TPN, there is a lag period of 3–4 days before food intake returns to previous level, which appears to be related to fatty acid oxidation and fat deposition. Carnitine plays a key role in the oxidation of fatty acids, and was demonstrated to reduce fat deposition in rats receiving TPN, by increasing beta oxidation. We therefore investigated whether rats receiving TPN supplemented with carnitine may prevent either the decrease or speed up the resumption or normalization of food intake, after TPN is stopped. Fourteen adult Fischer-344 rats had a central venous catheter inserted. After 10 recovery days, controls ($n = 7$) were infused with TPN providing 100% of rat's daily caloric intake for 3 consecutive days, followed by 4 more days of normal saline. The carnitine group ($n = 7$) received the same solution, but which provided 100 mg/kg/day carnitine. Daily food intake was measured and data were analyzed using ANOVA and Student's *t*-test. Both parenteral solutions depressed food intake maximally by almost 90% by day 3. Carnitine accelerated the normalization of food intake by decreasing the lag period by 1 day. We conclude that the addition of carnitine enhanced the normalization of post-TPN food intake and argue that this may be on the basis of enhanced fatty acid oxidation, a substrate known to play a significant role in the anorexia induced by TPN.

Feeding Carnitine Total parenteral nutrition Food intake Rat Liver fat deposition Liver fatty acid oxidation

TOTAL parenteral nutrition (TPN), providing a mixture of glucose, fat, and amino acids, normally in the caloric ratio of 50:30:20, plus electrolytes and micronutrients, is a safe and effective method to deliver adequate caloric and nutrient needs in patients who will not, should not, or cannot eat (10). However, the IV infusion of nutrients is also known to suppress food intake in both animals and humans (9,20). Moreover, when TPN is stopped, there is a delay of several days before food intake returns to normal pre-TPN levels. We therefore developed a rat model to better investigate the causes for this phenomenon and to attempt to understand the mechanisms involved in inducing anorexia and the delays in food intake normalization. In this model, rats typically received a constant infusion of a parenteral nutrition solution. This solution provides 100% of their daily caloric needs (TPN-100). It reduces their food intake by approximately 80–85% (9). When TPN-100 is stopped, there is a 3–4-day period before food intake returns to preinfusion levels, during which food intake is low (9). Because the TPN provides 100% of the daily mean caloric requirements eaten by the rats during the pre-TPN period, the calories thus derived from the 15–20% of food ingested during parenteral nutrition infusion constitute surfeit calories in excess of 100%. These surfeit calo-

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ries are stored as fat and therefore may account for the delay in normalization of food intake after TPN is stopped.

Using our rat TPN model, we have demonstrated that surfeit calories during TPN-100 enhance brain glycogen storage levels (12) and increase fat deposition in fat depots and in the liver (3,15). Thus, it is conceivable that TPN-induced changes in hepatic metabolism, particularly in fatty acid partitioning between oxidation and deposition, may contribute to the reduction of food intake during and after TPN-100.

Carnitine [3-hydroxy-4-(trimethylammonio)butanoate, $C_7H_{15}NO_3$; MW-161] is an essential cofactor of fatty acid metabolism that is normally found in animal tissues and is critical for the oxidation of free fatty acids. It facilitates their transport across the mitochondrial membrane to the site of oxidation (6). Carnitine supplementation in food-restricted rats receiving a TPN solution providing 120% of rat's daily caloric requirements was demonstrated to significantly enhance nitrogen balance and ameliorate fat deposition, by increasing free fatty acid oxidation (21). Unfortunately, the effects of carnitine supplementation on food intake were not studied by the investigators.

We thus studied in rats whether supplementation of carnitine, at a dosage known to increase liver beta oxidation and reduce fat deposition (21), may influence the level to which food intake decreases during TPN-100 or enhances the rate of food intake normalization during the post-TPN-100 period. Data obtained show that carnitine supplementation did not affect the development of TPN-induced anorexia, but it significantly accelerated the normalization of food intake after stopping TPN-100. The conclusion, based on our data, suggests that fatty acid oxidation (and fat deposition) likely have a significant role in the delay of food intake recovery after parenteral infusion of nutrient solutions.

METHOD

Animals

The study was approved by the Committee for the Humane Use of Animals at the SUNY Health Science Center, Syracuse, and was in accordance with the guidelines established by the National Institutes of Health.

Adult male Fischer-344 rats (Charles River, Inc., Wilmington, MA) weighing 250 g were housed in holding wire cages. Room temperature was 26 ± 1°C, humidity 45%, with 12-h light/dark cycle (light off at 1800 h). Fresh water and coarsely ground rat chow (Diet #5008; Ralston Purina, St. Louis, MO) were available ad lib. This diet contains 70% carbohydrates, 23.5% protein, and 6.5% fat.

Placement of Jugular Catheter

After a 2-week acclimation period, an IV catheter was inserted into the right jugular vein of all rats, using the procedure previously described in detail (9). Briefly, while anesthetized (ketamine 100 mg/ml, 1.5 ml + acetopromazine 10 mg/ml, 0.5 ml + xylazine 20 mg/ml, 1.5 ml; 0.5 ml/kg SC) 2 cm of a silastic catheter (0.025 in. i.d. × 0.047 in. o.d.) was inserted toward the heart via the jugular vein. The catheter was exteriorized at the nape of the neck via a SC tunnel, then protected by a weighted spring and swivel device (Instech, Plymouth Meeting, PA) sutured to the skin. The catheter was fitted to volumetric infusion pumps (IMED, San Diego, CA), and 0.9% normal saline was infused at 3 ml/h to maintain catheter patency.

TPN Infusion and Measuring Food Intake

After a 10-day recovery period, rats were randomly assigned to the infusion of a parenteral solution, providing 100% of rat's daily caloric intake (control group; $n = 7$), or of the same solution supplemented with 100 mg/kg/day of carnitine (carnitine group; $n = 7$). The given dose of carnitine is based on a previous report, which shows that it is effective in increasing beta oxidation and ameliorating fat deposition in food-restricted rats receiving a TPN solution providing 120% of rat's daily caloric requirements (21). Carnitine was purchased from Sigma Chemical (St. Louis, MO). On day 14, in both control and carnitine groups, solutions were switched back to normal saline for 4 more days.

The composition of the sterile pyrogen-free TPN-100 solution was that which is commonly used in clinical practice. The caloric ratio of glucose to fat (Intralipid 20% stock solution containing 20% soy bean oil, 1.2% egg yolk phospholipid, 2.25% glycerine, and water; KabiVitrum, Stockholm, Sweden) to a well-balanced amino acid solution (Novamine; KabiVitrum) was 50:30:20, and the ratio was kept constant throughout the study. The infusion rate was 3 ml/h throughout the experiment. Because rats were eating throughout the TPN-100 test period and because previous studies showed no difference in food intake during 3 days of TPN-100 whether or not electrolytes, vitamins, or trace elements were added to the TPN-100 (9), it was considered unlikely that acute micronutrient deficiencies would occur and thereby influence food intake. These costly additives were therefore not included in the TPN-100 solutions. Daily food intake was measured gravimetrically.

Statistical Analysis

Food intake (grams) was analyzed by repeated-measures one-way analysis of variance (ANOVA) for each of the two groups. Contrast *t*-test were used to compare the mean at each of days 11–17 (the test and posttest periods) with the mean of days 8–10 (the pretest or baseline periods). The figure plots food intake as means ± SE at each study day.

RESULTS

As shown in Fig. 1, in the pre-TPN-100 infusion period, food intake was similar in the two groups. On day 10, mean food

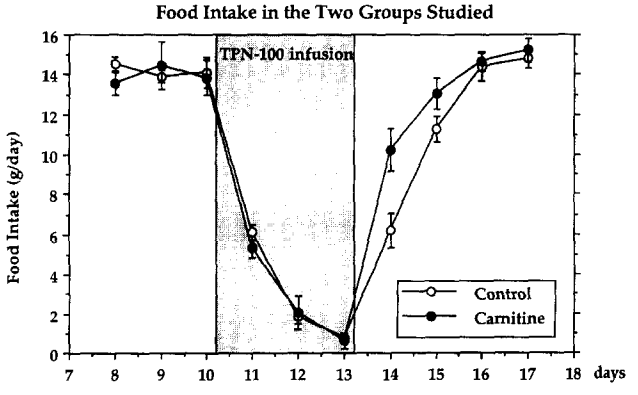

FIG. 1. Food intake in the two groups of rats studied before, during, and after the infusion of total parenteral solutions supplemented (carnitine 100 mg/kg/day) or not supplemented (control). Data represents means ± SEM. Food intake significantly decreased in response to TPN-100 infusion in both groups. When TPN-100 infusion was stopped, food intake reverted to normal faster in the carnitine group relative to the control group.

intake in the control and carnitine groups was 14.1 ± 0.7 g and 13.8 ± 0.9 g, respectively. On starting TPN-100 infusion, food intake immediately and significantly declined in both control and carnitine rats, and on day 13 it averaged 0.8 ± 0.2 g and 0.6 ± 0.4 g, respectively ($p < 0.01$ vs. pre-TPN-100 period, both groups).

After stopping TPN-100 infusion, food intake increased in both groups, being significantly greater in carnitine group than in control group on day 14 (10.2 ± 1.0 g vs. 6.2 ± 0.8 g, respectively; $p < 0.05$). On day 15, food intake in the two groups was not significantly different (11.3 ± 0.6 g in control rats, and 13.0 ± 0.8 g in carnitine rats; $p = 0.08$). But in the carnitine group it had completely normalized to the pre-TPN-100 levels (p = NS), whereas in the control group it was still significantly less ($p < 0.02$) (Fig. 1). On days 16 and 17, no differences were found in food intake between the two groups of rats or with regard to the pre-TPN-100 levels (Fig. 1).

DISCUSSION

The major findings of this study are: 1) both carnitine- and noncarnitine-supplemented TPN-100 reduced food intake maximally by day 3 and to the same extent in normal rats; and 2) carnitine-supplemented TPN-100 relative to standard TPN-100 accelerated the normalization of food intake.

Because a TPN-100 solution does not eliminate the ingestion of food but reduces food intake by only 85–90%, a surfeit of caloric intake occurs during the infusion period, which is the likely cause for the delay in food intake normalization in the post-TPN-100 period. We showed that TPN-100 increases whole-brain glycogen content, but that this is not causally related to the decrease of food intake during and after the infusion (12). More recently, we demonstrated that surfeit calories during TPN-100 result in increased carcass adiposity (15). This appears to be associated to the delayed food intake resumption, because when the caloric intake is limited to that provided only by TPN-100, carcass adiposity was not increased and food intake normalized during the first post-TPN-100 24 h (15). It is therefore conceivable that the partitioning of fatty acids between oxidation and deposition controls food intake during the post-TPN period and that this mechanism mainly occurs in the liver.

The mechanisms by which the liver controls food intake are extremely complex. Data suggest that innervation of the liver plays a contributory role (14,17,19,23). It has been proposed that selective sensors for different nutrients located in the porto–hepatic area sense the presence of nutrients in the bloodstream (18) and then transmit this information to specific hypothalamic areas participating in the regulation of feeding, including the lateral hypothalamic area (LHA) (11,13). Consistent with this, we recently observed that LHA–dopamine response to eating and TPN is increased in liver-denervated rats (22), and that this is associated with changes in meal size (14). However, the hepatic–vagal–hypothalamic axis is not the only mechanism controlling food intake, because we observed that total liver denervation modified the determinants of food intake (i.e., meal number and meal size), but had no effect on overall caloric intake (19).

Besides its role as a sensor for circulating nutrients, the liver is also known to control food intake via the metabolic processing of nutrients. In particular, it has been proposed by Friedman that feeding activity is governed by a signal originating from oxidation of metabolic fuels (4). Accordingly, the storage and mobilization of fat affect food intake indirectly by altering fuel oxidation. Consequently, the signal for feeding may originate in the liver, when both fatty acids and glucose are unavailable for oxidation. Supporting data have been generated, indicating that the inhibition of liver fatty acid oxidation by the block of carnitine palmitoyltransferase I (CPT I) increases food intake of rats maintained on a diet high in long-chain fatty acids, which require CPT I for mitochondrial uptake and oxidation (5). More recently, the role of CPT I in controlling food intake was confirmed by Moir and Zammit (16), who proposed that changes in liver cell pH and volume may inhibit CPT I, thus stimulating food intake. Data from our laboratory further support the role of hepatic oxidation of fatty acids in controlling food intake, by showing that during TPN-100: 1) feeding is stimulated when the metabolic utilization of fat is inhibited by mercaptoacetate, but 2) not when the utilization of glucose is simultaneously inhibited by 2-deoxy-D-glucose; and 3) lipoprivic-induced feeding is abolished by hepatic vagotomy (1,2). When these data are taken together, they strongly suggest that mitochondrial transport of fatty acids, controlled by carnitine, plays a role in the control of food intake, and that CPT I participates in that control by regulating the partitioning of long-chain fatty acids between pathways of storage and intramitochondrial oxidation.

In this light, our present results are readily understood. During TPN-100 infusion, food intake was depressed because of caloric provision in TPN-100. Carnitine supplementation increased fatty acid oxidation in the liver (6), as well as in other tissues (7,8). From this we deduce that reduced fat deposition occurs in carnitine-supplemented rats when compared to rats receiving nonsupplemented TPN-100 (21). Consequently, when TPN-100 was stopped, lower concentrations of fatty acids were available for hepatic oxidation, thus generating a stronger feeding signal as hypothesized by Friedman (4), and leading to increased food intake during the recovery period after TPN-100.

In summary, our study indicates that carnitine supplementation accelerates the normalization of food intake suppressed by TPN-100, and by inference suggests that liver fat oxidation may play a role in this effect.

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