Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 60 (2011) 195–205
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Effects of glucose or fat calories in total parenteral nutrition on fat metabolism and systemic inflammation in rats

Pei-Ra Ling[a], Charlotte Andersson[a], Robert Strijbosch[b], Sang Lee[b], Anthony Silvestri[a], Kathleen M. Gura[b], Mark Puder[b], Bruce R. Bistrian[a,*]

[a]*Laboratory of Nutrition/Infection, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA*
[b]*Department of Surgery and The Vascular Biology Program, Children's Hospital Boston, Harvard Medical School, Boston, MA 02215, USA*

Received 9 September 2009; accepted 14 December 2009

Abstract

This study compared the effects of total parenteral nutrition (TPN) *by central vein* with or without fat provided at maintenance energy requirement on fatty acid metabolism, de novo lipogenesis, and the risk of hepatic and systemic inflammation in rats. Study 1 was conducted in 2 groups: high glucose (HG), where fat-free TPN was given at maintenance levels of 180 kcal/(kg d), and low glucose (LG), where fat-free TPN containing 30% fewer calories at 126 kcal/(kg d) was provided by reducing 54 kcal/(kg d) from parenteral glucose. Study 2 contained 3 TPN groups: 1 LG group at 126 kcal/(kg d) and 2 groups at 180 kcal/(kg d) with 30% of total calories (54 kcal/[kg d]) either from soybean or fish oil emulsion. In both studies, animals fed a chow diet ad libitum were included. Plasma and hepatic triglyceride and phospholipid fatty acid profiles, enzymes indicating hepatic injury, and C-reactive protein levels (CRP) reflecting systemic injury were measured. In study 1, evidence of de novo lipogenesis was noted in LG and was more prominent in HG with elevation of CRP in HG. In study 2, de novo lipogenesis was reduced by adding either fat to LG to achieve maintenance energy levels. Moreover, adding fat as soybean oil but not fish oil significantly increased plasma and hepatic triglyceride and also elevated aspartate aminotransferase and CRP levels, reflecting inflammation. Thus, in rats, either hypocaloric feeding as glucose-based TPN or TPN provided at maintenance energy levels with the addition of fish oil limits hepatic lipid accumulation and prevents the evidence of hepatic and systemic injury found with maintenance level TPN as glucose only or glucose plus soybean oil.

1. Introduction

Total parenteral nutrition (TPN) has been demonstrated to be a major advance in medical therapy allowing for the provision of energy and other essential nutrients to critically ill patients who are unable to eat [1]. Total parenteral nutrition feeding reduces both morbidity and mortality in critically ill medical or surgical patients [2,3], although TPN feeding often increases the risk of infectious complications compared with enteral nutrition [2]. However, in the present era of tight glucose control and early adequate feeding, it appears that both infectious morbidity and overall mortality can be improved when these 2 therapeutic goals are achieved without distinction between the outcomes related to route of feeding [2-5]. Early studies with poor glycemic control did find an increased infection risk without a survival benefit in well-nourished patients receiving TPN [6] in part related to the parenteral energy intake. In that study, patients received approximately 44 kcal/kg from the TPN plus oral diet [6], where their estimated energy expenditure would have been 20 to 25 kcal/kg [7], reflecting substantial overfeeding. In a more recent study, patients receiving 36 vs 31 kcal/(kg d) as parenteral energy intake also developed more bloodstream infections [8]. Bypassing the gastrointestinal tract and liver, TPN produces a much greater insulin response than enteral feeding [9] and leads to a greater likelihood of hyperglycemia in the critically ill [5]. This propensity for the development of

Conflicts: Children's Hospital Boston has submitted a patent for Omegaven on behalf of Drs Gura and Puder. Dr Bistrian receives patent royalties through Beth Israel Deaconess Medical Center from Abbott and Nestle.

* Corresponding author. Tel.: +1 617 632 8545; fax: +1 617 632 0204.
E-mail address: bbistria@bidmc.harvard.edu (B.R. Bistrian).

0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.12.014

hyperglycemia can be further compounded when excessive energy is provided by glucose calories, leading to a much greater risk for the development of infection [10]. Excessive glucose administration also fosters accelerated hepatic triglyceride synthesis that can be seen as lipid droplets accumulating in the liver, especially in neonates and infants [11]. This process is further enhanced by fat-free feeding, particularly due to the absence of polyunsaturated fatty acids of the omega 3 and omega 6 series because these fatty acids inhibit de novo lipogenesis [12-14]. Patients and animals given fat-free TPN can develop severe fatty liver, often with hyperglycemia, hyperinsulinemia, and hypotriglyceridemia [9,15]; and these metabolic derangements are clearly improved by supplementing TPN with fat calories, particularly when combined with a reduction in glucose calories [16-18]. Therefore, an optimal TPN energy intake may have several components: total calories provided, the use of both glucose and fat, and the type of fat.

Fat in approximate amounts of 20% up to one third of total calories significantly improves the metabolic consequences of fat-free TPN [19]. The parenteral fat can be provided discontinuously as a piggyback infusion or as a 3-component emulsion containing amino acids, glucose, and fat, which has been found to be a safe, stable, and economical alternative. The soybean oil emulsion provides essential fatty acids of the omega 3 and 6 series to reduce de novo lipogenesis for patients receiving glucose-only TPN, whereas fish oil emulsion has a high content of very long chain omega 3 fatty acids that are particularly effective for inhibiting de novo lipogenesis. Furthermore, fish oil emulsions have substantial anti-inflammatory effects as well [20]. Recently, the concept of hypocaloric or underfeeding with TPN has been considered as a reasonable alternative approach in critically ill patients, especially for those who are overweight or obese [21]. In this therapy, the greater reduction in glucose calories and the availability of endogenous fat to meet the energy gap combine to lower de novo lipogenesis and to curtail the other metabolic derangements. As a clinical counterpart to these findings, in intensive care unit medical patients, those receiving only 35% to 67% of their recommended daily caloric intake had a higher survival rate and spent less time on ventilation than those receiving either greater or lesser energy intake [22]. However, the relative contribution of caloric vs carbohydrate reduction to these improvements in outcome is uncertain.

2. Materials and methods

2.1. TPN solutions

All the TPN solutions contained 2 g of nitrogen per kilogram per day, and the additional energy was from either glucose alone or a combination of glucose and fat emulsion according to the experimental designs. When present, fat provided 30% of total TPN calories. Two different fat emulsions—Intralipid (Fresenius Kabi, Uppsala, Sweden), a 20% soybean oil emulsion, and Omegaven (Fresenius Kabi, Graz, Austria), a 10% fish oil emulsion—were used. Table 1 lists the fatty acid compositions of these 2 emulsions as measured in our laboratory. The TPN solutions were freshly prepared by the pharmacy of Beth Israel Deaconess Medical Center. The necessary vitamins and minerals were also provided in the TPN solutions. The TPN solutions were intravenously infused at 2.3 mL/h using a syringe infusion pump (Harvard Apparatus, South Natick, MA). The syringe was changed every day. Chow diet was a traditional F-6 rodent diet (7964; Harlan Teklad, Madison, WI) that contained 18% energy from fat with more than 50% from soybean oil.

2.2. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center. Male Sprague-Dawley rats (Taconic Farm, Germantown, NY), weighing 250 to 270 g, were housed in individual cages and maintained under a 12:12-hour light-dark photoperiod and ambient temperature at 24°C and 26°C for 4 days before the experiments. Standard rat chow diet (Harlan Teklad) and tap water were provided ad libitum. After 4 days of accommodation, intravenous catheters were inserted into the right jugular vein under anesthesia for animals planned to receive saline infusion or TPN feeding; and these rats were then placed in individual metabolic cages, given water, and maintained on regular chow diet ad libitum overnight to recover from surgery. On the experimental day, chow diet was removed; and TPN was given for 4 days.

Based on the measured energy expenditure determined in an open system with similar, awake animals in a previous

Table 1
Fatty acid compositions in different fat emulsions

Fatty acids (%nmol)	Intralipid[a]	Omegaven[b]
C14:0	0.03 ± 0.05	6.9 ± 0.2
C16:0	12.4 ± 0.2	14.2 ± 0.4
C16:1	0.18 ± 0.05	10.1 ± 0.5
C18:0	3.3 ± 0.1	2.1 ± 0.1
C18:1	26.0 ± 0.2	17.2 ± 0.1
C18:2w6	51.3 ± 0.2	3.7 ± 0.1
C18:3w3	6.3 ± 0.2	1.6 ± 0.0
C20:4w6	0.21 ± 0.01	1.6 ± 0.1
C20:5w3	0.05 ± 0.01	20.1 ± 0.6
C22:4w6	0.02 ± 0.00	0.14 ± 0.02
C22:5w6	0.02 ± 0.01	0.5 ± 0.0
C22:5w3	0.01 ± 0.00	1.8 ± 0.0
C22:6w3	0.18 ± 0.01	20.0 ± 0.2
Total	100.0	100.0

Mean ± SEM from 3 samples.

[a] Intralipid with 20% of soybean oil emulsion (Fresenius Kabi, Uppsala, Sweden).

[b] Omegaven with 10% of highly refined fish oil emulsion (Fresenius Kabi, Graz, Austria).

study [23], the required caloric intake was estimated at 200 kcal/(kg d). To avoid overfeeding, the level for adequate energy intake was set at 180 kcal/(kg d). In addition, 30% below the predicted adequate caloric intake (126 kcal/[kg d]) was provided as an underfeeding level that has been shown to be optimal in one large clinical study [22].

2.3. Experimental design

2.3.1. Study 1

This study was designed to examine whether increasing glucose calories in TPN *by central vein* led to fat accumulation in the liver with associated liver injury patterns and the development of systemic inflammation. Total parenteral nutrition was given as a fat-free solution with 2 g of nitrogen per kilogram per day at a total energy intake of 180 kcal/(kg d), with 130 kcal/(kg d) provided by glucose, representing the high–glucose intake group (HG, n = 7), and at a total energy intake of 126 kcal/(kg d), with 76 kcal/(kg d) provided by glucose, representing the low–glucose intake group (LG, n = 7). In addition, one group consuming a chow diet ad libitum was included as a control (Chow, n = 7) (Table 2).

After 4 days of TPN feeding, all animals were killed by decapitation. Blood was collected for measurements of plasma levels of glucose, C-reactive protein (CRP), and total triglycerides. Liver enzyme tests, including measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK), and glutathione *S*-transferase (GST), were performed. Livers were removed and weighed. Portions of liver were taken for the determination of triglyceride and phospholipid fatty acid profiles. Plasma triglyceride and phospholipid fatty acid profiles were also determined.

All samples were stored at −80°C for later determinations.

2.3.2. Study 2

This study examined the effects of added calories from fat emulsion to replace glucose in the HG TPN on fat accumulation in the liver and on hepatic and systemic injury. To allow comparison with results from study 1, this study also included a Chow group that consumed chow diet ad libitum (Chow, n = 10) and an LG group, as in study 1, which received TPN feeding at total intake of 126 kcal/(kg d) with 76 kcal/(kg d) provided by glucose (LG, n = 10). Two additional TPN feeding regimens were given at total energy intakes of 180 kcal/(kg d) similar to the HG group in study 1 by adding fat at 30% of total caloric intakes (54 kcal/[kg d]) provided by fat emulsion, either as Intralipid (Fresenius Kabi, Uppsala, Sweden) (LCT, n = 7) or Omegaven (Fresenius Kabi, Graz, Austria) (Fish oil, n = 7). Thus, similar to the LG group in study 1, 126 kcal/(kg d) was provided by glucose and 2 g of nitrogen per kilogram per day such that all 3 groups receiving TPN had a basal glucose intake at this level to which 54 kcal/(kg d) was added as one of the 2 lipids to the other 2 TPN groups.

At the end of 4 days of feeding, animals were killed. Blood and liver tissue were collected for the same measurements listed in study 1. All of the samples were stored at 80°C for later determinations.

2.4. Assays

Plasma glucose concentrations were determined by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman, Brea, CA). Plasma total triglyceride concentrations were determined by triglyceride determination kit (Sigma, St Louis, MO). The liver function tests, including measurement of AST, ALT, and ALK, were performed using individual reagent sets (Pointe Scientific, Canton, MI). The plasma levels of GST were determined by Biotrin rat α GST enzyme immunoassay kit (Biotrin International, Dublin, Ireland). The levels of CRP were determined using a rat CRP kit (Helica Biosystem, Fullerton, CA).

Lipids from the fat emulsions, plasma, and liver tissue were extracted by liquid-liquid extraction (ie, solvent extraction) with 6 vol of chloroform-methanol (2:1, vol/vol) and centrifuged at 800*g* for 10 minutes; and the resulting lower phase was aspirated. Before the extraction, 30 μL of a 1-mg/mL solution of diheptadecanoyl phosphatidylcholine and triheptadecanoyl glycerol (17:0) (Nu-Check Prep, Elysian, MN) in chloroform-methanol (1:1, vol/vol) was

Table 2
Experimental design

Study	Groups	n	Feeding		Nitrogen	Glucose kcal/(kg d)	Fat kcal/(kg d)
Study 1							
	Chow	7	Chow	Ad libitum			
	LG	7	TPN	126 kcal/(kg d)	2 g/(kg d)	126	0
	HG	7	TPN	180 kcal/(kg d)	2 g/(kg d)	180[a]	0
Study 2							
	Chow	10	Chow	Ad libitum			
	LG	10	TPN	126 kcal/(kg d)	2 g/(kg d)	126	0
	LCT	9	TPN	180 kcal/(kg d)	2 g/(kg d)	180	54[b]
	Fish oil	9	TPN	180 kcal/(kg d)	2 g/(kg d)	180	54[c]

[a] HG group received additional 54 kcal/(kg d) from glucose compared with LG group.
[b] Provided by Intralipid as LCT group.
[c] Provided by Omegaven as Fish oil group.

added as an internal standard to all samples. The chloroform-methanol extracts were evaporated to dryness under nitrogen. The samples were then redissolved in 200 μL of chloroform and further fractionated into triglycerides and phospholipids by aminopropyl columns (Sigma) using chloroform-propanol (2:1, vol/vol) and methanol, respectively. The triglycerides and phospholipids fraction were again evaporated to dryness under nitrogen. Afterward, 0.5 mL of sodium-methoxide (Sigma) was added and mixed; and the samples were placed in a heating block at 100°C for 3 minutes and removed from the heating block and allowed to cool down to room temperature. Afterward, 0.5 mL of methanol base–boron trifluoride reagent (Sigma) was added, mixed, and incubated at 100°C for 1 minute. After cooling down the tubes, 0.5 mL of hexane was added and mixed; then 6.5 mL of a saturated NaCl solution was added and mixed. The mixture was centrifuged at 800*g* for 3 minutes. The upper hexane layer was aspirated.

Fatty acid methyl esters were analyzed by gas chromatography with a Hewlett Packard 5890a gas chromatograph (Hewlett Packard, Palo Alto, CA), using a Supelcowax-10 0.25-mm–internal diameter column (Sigma). Fatty acid methyl ester peaks were identified by comparison of retention times of a standard mixture and quantified using the internal standard. The triglyceride concentration in the liver was calculated as nanomoles per milligram. The fatty acid profiles were expressed as mole percentage of total fatty acid content in the samples.

Because of the limited amount of blood from each animal, the numbers of each measurement listed in tables or figures might not be the same as the numbers of animals studied in each group. In addition, blood samples were taken at a fed state.

2.5. Statistical analysis

Results are presented as mean ± standard error of mean (SEM). To assess the statistical significance of differences in mean values among groups, 1-way analysis of variance (ANOVA) with Fisher least significant test was used in each study. Significance for all analyses was defined as $P \leq .05$.

3. Results

3.1. Study 1

3.1.1. Food intake and body weight changes

The caloric intakes in LG and HG groups were well controlled at 126 and 180 kcal/(kg d), respectively. With TPN feeding, the LG group had a total weight loss of 17.7 ± 4.4 g, whereas the HG group lost 15.9 ± 1.9 g. There were no differences in body weight changes between LG and HG groups even though the total caloric intake was 30% (54 kcal/[kg d]) more in the HG group compared with the LG group. Animals in the oral feeding group (Chow) consumed 300.9 ± 20.1 kcal/(kg d), or almost 2 times as many calories as those provided by TPN feeding. The animals in Chow group had an average weight gain of 14.8 g.

3.1.2. Triglyceride concentrations and triglyceride fatty acid profiles in plasma and liver

The triglyceride concentrations in plasma and liver are shown in Fig. 1. The highest plasma level of triglyceride was found in the Chow group. No differences in plasma triglycerides were found between the LG and HG groups. In the liver, the content of triglyceride was increased by TPN feeding (LG and HG), more in HG, compared with chow diet feeding (Chow); but the increases did not reach statistical significance.

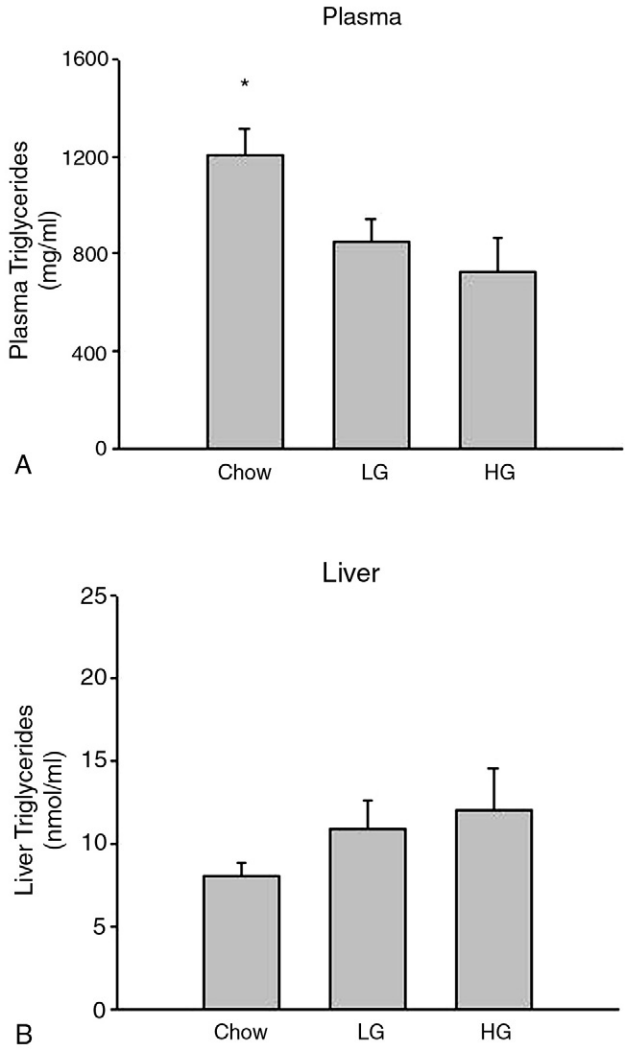

Fig. 1. The effects of feeding with oral chow diet ad libitum (~300 kcal/[kg d]) or fat-free TPN with 2 g of nitrogen per kilogram per day at lower (126 kcal/[kg d]) or adequate energy intake (180 kcal/[kg d]) on (A) plasma levels of triglycerides (in milligrams per milliliter) and on (B) triglyceride concentration in the liver (in nanomoles per milligram) in rats. Chow: oral feeding with chow diet ad libitum. LG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at 126 kcal/(kg d). HG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at adequate energy intake (180 kcal/(kg d). *$P < 05$ vs others.

Table 3
Triglyceride profiles in plasma (percentage nanomole per milliliter) and in the liver (percentage nanomole per milligram)

Fatty acid	Plasma			Liver		
	Chow (n = 6)	LG (n = 7)	HG (n = 7)	Chow (n = 7)	LG (n = 7)	HG (n = 7)
C16:0	19.5 ± 1.0	19.1 ± 1.4	23.5 ± 1.9§	25.2 ± 0.7	31.4 ± 1.8†	30.3 ± 0.7†
C16:1w7	0.6 ± 0.1	2.5 ± 0.5*	3.7 ± 0.6*	0.4 ± 0.1	1.7 ± 0.9	1.2 ± 0.2
C18:0	8.7 ± 1.6	4.0 ± 0.4	7.1 ± 1.6§	10.7 ± 1.1	10.7 ± 1.6	9.2 ± 1.5
C18:1w9	12.5 ± 0.8	19.7 ± 0.9†	18.6 ± 1.9†	20.2 ± 1.3	19.4 ± 2.2	25.6 ± 2.4
C18:2w6	33.8 ± 1.0	21.4 ± 1.4*	19.2 ± 0.7*	28.2 ± 1.1	19.2 ± 1.3*	19.2 ± 0.8*
C18:3w3	1.0 ± 0.1	0.3 ± 0.1*	0.2 ± 0.1*	1.0 ± 0.1	0.6 ± 0.1†	0.6 ± 0.1†
C20:3w9	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
C20:3w6	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0
C20:4w6	20.8 ± 1.9	27.2 ± 2.4	23.5 ± 2.6	8.8 ± 1.3	10.6 ± 1.9	8.1 ± 1.4
C20:5w3	0.8 ± 0.2	2.6 ± 0.7‡	1.3 ± 0.3‡	1.9 ± 1.2	0.5 ± 0.1	0.6 ± 0.3
C22:4w6	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
C22:5w6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.8 ± 0.5	0.7 ± 0.2
C22:5w3	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
C22:6:3	1.8 ± 0.2	2.9 ± 0.4‡	3.4 ± 0.4‡	2.4 ± 0.1	3.5 ± 0.3‡	3.0 ± 0.3‡

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.
* $P < .001$, LG and HG vs Chow.
† $P < .005$, LG and HG vs Chow.
‡ $P < .05$, LG and HG vs Chow.
§ $P < .05$, LG vs HG.

Table 3 lists the triglyceride fatty acid profiles in plasma and liver. Although neither LG and HG received fat from their TPN solution, TPN feeding significantly increased plasma levels of palmitoleic acid (C16:1w7, $P < .001$), oleic acid (C18:1w9, $P < .005$), eicosapentaenoic acid (C20:5w3, $P < .05$), and docosahexaenoic acid (C22:6w3, $P < .05$) but significantly reduced levels of linoleic acid (C18:2w6, $p< 0.001$) and α linolenic acid (C18:3w3, $P < .001$) in plasma triglycerides as compared with chow diet feeding. The significant differences between the LG and HG groups were higher plasma levels of palmitic acid (16:0, $P < .05$) and stearic acid (C18:0, $P< .05$) in the HG group. In the liver triglycerides, significantly higher levels of palmitic acid (C16:0, $P < .005$) and docosahexaenoic acid (C22:6w3, $P < .05$) but significantly lower levels of linoleic acid (C18:2w6, $P < .001$) and α linolenic acid (C18:3w3, $P< .005$) were found in the LG and HG group as compared with the Chow group. No differences in any fatty acid levels were found in the liver triglycerides between the LG and HG groups.

Table 4
Phospholipid profiles in plasma (percentage nanomole per milliliter) and in the liver (percentage nanomole per milligram)

Fatty acid	Plasma			Liver		
	Chow (n = 6)	LG (n = 5)	HG (n = 6)	Chow (n = 7)	LG (n = 7)	HG (n = 8)
C16:0	27.3 ± 0.8	36.8 ± 3.0*	31.1 ± 2.2*	23.3 ± 0.3	25.8 ± 0.8†	27.3 ± 0.4†
C16:1w7	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.1‖	0.1 ± 0.1	0.3 ± 0.0†	0.4 ± 0.1†
C18:0	20.8 ± 1.0	18.1 ± 0.8	22.6 ± 3.6	23.7 ± 0.7	20.4 ± 0.3§	20.5 ± 0.9§
C18:1w9	8.2 ± 0.6	9.6 ± 1.0	12.7 ± 1.5¶	6.7 ± 0.3	9.6 ± 1.7	8.4 ± 0.3
C18:2w6	27.6 ± 0.7	16.1 ± 1.2†	16.2 ± 1.8†	17.1 ± 0.5	14.9 ± 0.6†	12.9 ± 0.3†
C18:3w3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
C20:3w9	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C20:3w6	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1
C20:4w6	10.9 ± 1.4	15.2 ± 1.8	12.3 ± 1.9	20.8 ± 0.4	19.7 ± 0.8	20.7 ± 0.6
C20:5w3	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.9 ± 0.7	0.6 ± 0.1	0.5 ± 0.1
C22:4w6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
C22:5w6	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
C22:5w3	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.0
C22:6:3	2.1 ± 0.2	3.6 ± 0.2‡	3.9 ± 0.5‡	5.3 ± 0.2	6.8 ± 0.4†	7.2 ± 0.3†

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.
* $P < .05$, LG and HG vs Chow.
† $P < .001$, LG and HG vs Chow.
‡ $P < .0$, LG and HG vs Chow.
§ $P < .05$, LG and HG vs Chow.
‖ $P < .01$, HG vs Chow and LG.
¶ $P < .05$, HG vs Chow and LG.

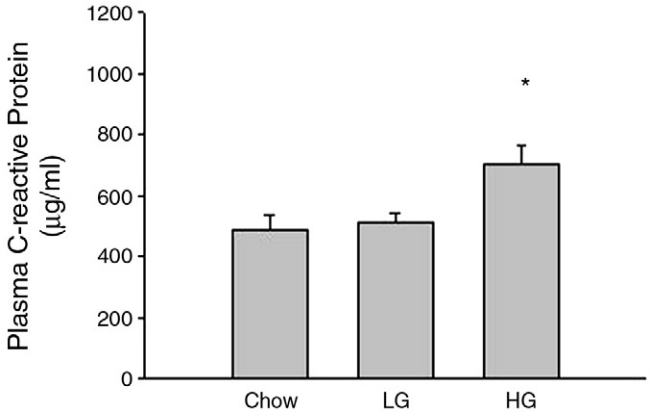

Fig. 2. The effects of feeding with oral chow diet ad libitum (~300 kcal/[kg d]) or fat-free TPN with 2 g of nitrogen per kilogram per day at lower (126 kcal/[kg d]) or adequate energy intake (180 kcal/[kg d]) on plasma levels of CRP (in micrograms per milliliter) in rats. Chow: oral feeding with chow diet ad libitum. LG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at 126 kcal/(kg d). HG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at adequate energy intake (180 kcal/[kg d]). *$P < 05$ vs others.

3.1.3. Phospholipid concentrations and phospholipid fatty acid profiles in plasma and liver

The phospholipid concentration was not significantly different in plasma or the liver among Chow, LG, and HG groups (data not shown).

Table 4 lists the phospholipid fatty acid profiles in plasma and in the liver tissue. In plasma, LG and HG groups had higher levels of palmitic acid (C16:0, $P < .05$) and docosahexaenoic acid (C22:6w3, $P < .01$) but lower levels of linoleic acid (C18:2w6, $P < .001$) as compared with the Chow group. The plasma levels of palmitoleic acid (C16:1w7, $P < .01$) and oleic acid (C18:1w9, $P < .05$) were significantly higher in the HG group compared with those in both LG and Chow groups. In the liver, the levels of palmitic acid (C16:0, $P < .001$), palmitoleic acid (C16:1w7, $P < .001$), and docosahexaenoic acid (C22:6w3, $P < .001$) were significantly higher in the LG and HG compared with the chow group. The levels of stearic acid (C18:0, $P < .005$) and linoleic acid (C18:2w6, $P < .001$) were significantly lower in the LG and HG groups compared with the Chow group. There were no differences in any phospholipid fatty acid in the liver between the LG and HG groups.

3.1.4. Plasma glucose concentrations, liver enzyme tests, and plasma CRP levels

Plasma glucose concentrations were not different among groups, with 143 ± 3, 145 ± 16 ,and 144 ± 18 mg/dL in the Chow, LG, and HG group, respectively. In addition, all the measured liver enzymes, including ALT, AST, ALK, GST activity, and total amount of GST, were not different among these 3 groups (data not shown). The AST values were 156.1 ± 5.3 in Chow, 154.2 + 13.6 in LG, and 174.2 ± 9.9 IU/L in HG, respectively. However, plasma CRP concentrations were significantly elevated in the HG group compared with the LG and Chow groups ($P < .05$). There were no differences in CRP between the Chow and LG groups (Fig. 2).

3.2. Study 2

3.2.1. Food intake and body weight changes

Animals consuming the chow diet consumed an average of 316 ± 16 kcal/(kg d). These animals also gained an

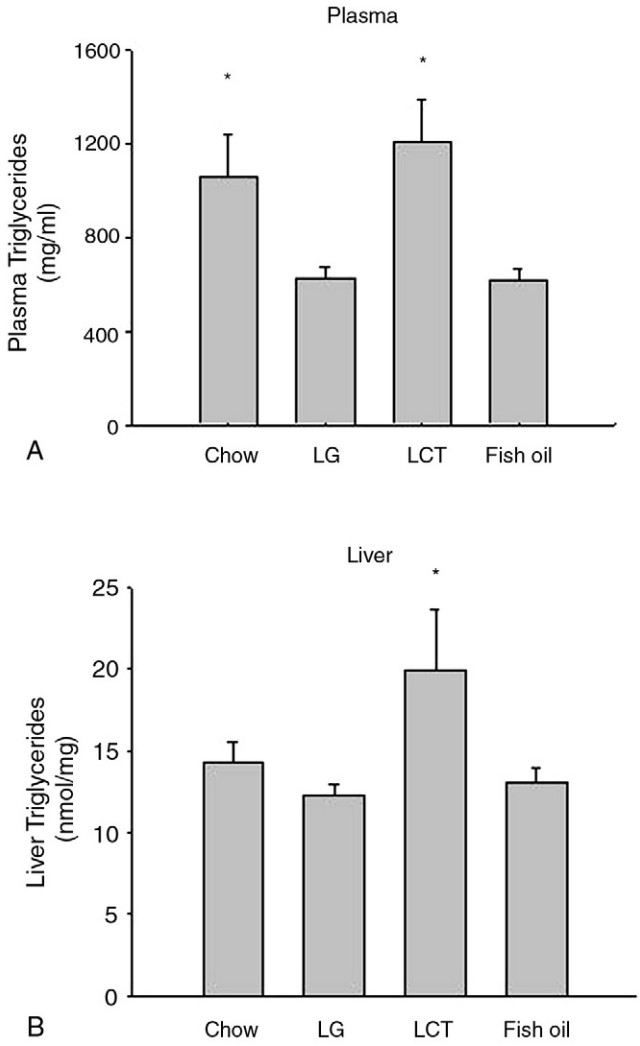

Fig. 3. The effects of feeding with oral chow diet ad libitum (~300 kcal/[kg d]) or fat-free TPN with 2 g of nitrogen per kilogram per day at lower (126 kcal/[kg d]) or adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total energy from fat, either Intralipid (LCT) or Omegaven (Fish oil), on (A) plasma levels of triglycerides (in milligrams per milliliter) and on (B) triglyceride concentration in the liver (in nanomoles per milligram) in rats. Chow: oral feeding with chow diet ad libitum. LG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at 126 kcal/(kg d). LCT: TPN feeding at adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total calories from Intralipid. Fish oil: TPN feeding at adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total calories from Omegaven. *$P < 01$ Chow and LCT vs LG and Fish oil; **$P < .05$ vs others.

average of 17.8 ± 2.6 g in weight. Total parenteral nutrition groups received lower caloric intakes, that is, 126 kcal/(kg d) in the LG group and 180 kcal/(kg d) in the LCT and Fish oil groups, respectively. All the TPN feeding groups lost body weight. The weight loss was 21 ± 4 g in the LG group, 16 ± 3 g in the LCT group, and 16 ± 6 g in the Fish oil group, respectively. The weight changes in LCT and Fish oil groups were not different from LG, although LG received 30% less caloric intake compared with both LCT and Fish oil. The weight changes were also not different between LCT and Fish oil groups.

3.2.2. Triglyceride concentrations in plasma and in the liver

Fig. 3 shows the concentrations of triglyceride in plasma and liver. Chow and LCT groups had significantly higher levels of plasma triglyceride than LG and Fish oil groups ($P < .01$). No differences in plasma triglyceride were found between Chow and LCT groups, or between LG and Fish oil groups. In the liver, the highest level of triglyceride concentration was in the LCT group, which was significantly higher than Chow, LG, and Fish oil groups ($P < .05$). No differences in triglyceride concentration were found in the liver among Chow, LG, and Fish oil groups.

3.2.3. Triglyceride fatty acid profiles in plasma and liver

Tables 5 and 6 show the triglyceride fatty acid profiles in plasma and in the liver, respectively. Similar to the results found in study 1, fat-free TPN feeding (LG) resulted in higher levels of palmitoleic acid (C16:1w7, $P < .01$) and stearic acid (C18:0, $P < .01$) (indicating de novo lipogenesis), arachidonic acid (C20:4w6, $P < .005$), eicosapentaenoic acid (C20:5w3, $P < .005$), and docosahexaenoic acid (C22:6w3, $P < .005$) and lower levels of linoleic acid (C18:2w6, $P < .001$) and α linolenic acid (C18:3w3, $P < .001$) in plasma as compared with Chow feeding. Adding soybean oil to the TPN solution (LCT), the values of palmitoleic acid

Table 5
Triglyceride profiles in Plasma (percentage nanomole per milliliter)

Fatty acid	Plasma			
	Chow (n = 8)	LG (n = 8)	LCT (n = 7)	Fish oil (n = 7)
C16:0	15.1 ± 0.4	16.5 ± 0.7	14.6 ± 1.5	15.8 ± 0.5
C16:1w7	1.1 ± 0.1	2.4 ± 0.3*	0.9 ± 0.1	4.2 ± 0.6†
C18:0	2.3 ± 0.1	3.8 ± 0.3‡	5.8 ± 1.2§	4.6 ± 0.1§
C18:1w9	17.7 ± 0.6	17.4 ± 0.6	19.1 ± 1.8	19.2 ± 1.1
C18:2w6	37.4 ± 0.7	20.9 ± 0.9‡	29.5 ± 2.7‖	13.7 ± 1.3†
C18:3w3	1.7 ± 0.2	0.3 ± 0.1‡	1.6 ± 0.3	0.7 ± 0.1¶
C20:3w9	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
C20:3w6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
C20:4w6	21.6 ± 1.4	32.7 ± 2.3‡	25.5 ± 6.1	14.1 ± 1.1†
C20:5w3	1.5 ± 0.4	2.2 ± 0.9	0.9 ± 0.2	16.2 ± 1.6†
C22:4w6	0.2 ± 0.4	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
C22:5w6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C22:5w3	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.5 ± 0.5†
C22:6:3	1.5 ± 0.2	3.5 ± 0.5#	2.0 ± 0.2	10.8 ± 1.3†

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.
* $P < .001$, LG vs LCT and Chow.
† $P < .001$, Fish oil vs others.
‡ $P < .05$, LG vs LCT and Chow.
§ $P < .001$, LCT and Fish oil vs Chow.
‖ $P < .001$, LCT vs Chow.
¶ $P < .005$, Fish oil vs LCT and Chow.
# $P < .05$, LG vs Chow.

Table 6
Triglyceride profiles in the liver (percentage nanomole per milligram)

Fatty acid	Liver			
	Chow (n = 10)	LG (n = 10)	LCT (n = 10)	Fish oil (n = 10)
C16:0	25.0 ± 0.5	28.9 ± 1.2*	25.9 ± 1.2	25.6 ± 0.5
C16:1w7	0.4 ± 0.0	1.5 ± 0.6†	0.5 ± 0.0	0.7 ± 0.1
C18:0	12.7 ± 1.3	11.3 ± 0.7	9.8 ± 1.1	11.7 ± 0.7
C18:1w9	18.4 ± 0.8	20.1 ± 1.0	20.8 ± 1.5	17.5 ± 1.2
C18:2w6	25.6 ± 0.8	20.6 ± 1.1§	28.3 ± 1.1	17.8 ± 1.2‡
C18:3w3	0.9 ± 0.1	0.4 ± 0.1	1.2 ± 0.1‖	0.7 ± 0.1¶
C20:3w9	0.0 ± 0.0	0.0± 0.0	0.0 ± 0.0	0.0 ± 0.0
C20:3w6	0.7 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
C20:4w6	11.0 ± 0.6	9.7 ± 0.7	8.4 ± 1.1#	8.2 ± 0.7#
C20:5w3	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.0	6.1 ± 0.5**
C22:4w6	0.5± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.1 ± 0.0
C22:5w6	0.2 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
C22:5w3	0.9 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.9 ± 0.2**
C22:6:3	3.1 ± 0.2	3.3 ± 0.2	3.0 ± 0.2	9.1 ± 0.8**

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.
* $P < .05$, LG vs others.
† $P < .05$, LG vs LCT and Chow.
‡ $P < .001$, Fish oil vs LCT and Chow.
§ $P < .001$, LG vs LCT and Chow.
‖ $P < .05$, LCT vs LG.
¶ $P < .05$, Fish oil vs others.
# $P < .05$, Fish oil and LCT vs Chow.
** $P < .001$ Fish oil vs others.

Table 7
Phospholipid profiles in plasma (percentage nanomole per milliliter)

Fatty acid	Plasma			
	Chow (n = 7)	LG (n = 8)	LCT (n = 7)	Fish oil (n = 7)
C16:0	29.2 ± 1.0*	34.3 ± 1.8	36.6 ± 2.5	35.4 ± 1.5
C16:1w7	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.1
C18:0	22.5 ± 0.5	23.0 ± 1.4	14.4 ± 1.9†	16.8 ± 1.7†
C18:1w9	6.1 ± 0.4	5.9 ± 0.7	11.3 ± 1.3†	20.0 ± 2.7†,‡
C18:2w6	27.9 ± 0.8*	16.0 ± 0.8	17.9 ± 1.9	13.6 ± 0.6§
C18:3w3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C20:3w9	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C20:3w6	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2‡
C20:4w6	11.3 ± 1.5*	15.5 ± 0.9	15.1 ± 1.5	6.6 ± 0.8‡
C20:5w3	0.5 ± 0.2	0.2 ± 0.1	0.4 ± 0.2	2.9 ± 0.6‡
C22:4w6	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C22:5w6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
C22:5w3	0.3 ± 0.1	0.2 ± 0.0	0.6 ± 0.3	0.7 ± 0.2
C22:6:3	2.0 ± 0.3*	4.7 ± 0.5‖	3.6 ± 0.1	3.1 ± 0.5

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.
* $P < .05$, Chow vs others.
† $P < .001$, LCT and Fish oil vs Chow and LG.
‡ $P < .001$, Fish oil vs others.
§ $P < .05$, Fish oil vs LCT.
‖ $P < .01$, LG vs Fish oil.

(C16:1w7), α linolenic acid (C18:3w3), arachidonic acid (C20:4w6), eicosapentaenoic acid (C20:5w3), and docosahexaenoic acid (C22:6w3) were back to levels near those found in the Chow group. Although the value of linoleic acid (C18:2w6) was higher in LCT than in LG, it was still lower than in Chow ($P < .01$). Adding fish oil to the TPN solution (Fish oil) led to higher levels of palmitoleic acid (C16:1w7), eicosapentaenoic acid (C20:5w3), docosapentaenoic acid (C22:5w3), and docosahexaenoic acid (C22:6w3) and lower levels of linoleic acid (C18:2w6) and arachidonic acid (C20:4w6) ($P < .001$) compared with other groups. The values of α linolenic acid (C18:3w3) in the Fish oil group were higher than in the LG group but lower than in the Chow and the LCT groups. In the liver (Table 6), some similar changes reflecting de novo lipogenesis to those observed in plasma included significantly higher levels of palmitic acid (C16:0, $P < .05$) and palmitoleic acid (C16:1w7, $P < .05$) in LG compared with Chow. There were no differences in stearic acid (C18:0) among groups. However, the Chow group had the highest values of arachidonic acid (20:4w6, $P < .001$), whereas the Fish oil group had the highest levels of eicosapentaenoic acid (20:5w3, $P < .001$), docosapentaenoic acid (22:6w6, $P < .001$), and docosahexaenoic acid (22:6w3, $P < .001$) and lowest levels of arachidonic acid (20:4w6, $P < .001$) among groups.

3.2.4. Phospholipid fatty acid profiles in plasma and liver

As shown in Table 7, fat-free TPN feeding (LG) significantly increased the levels of palmitic acid (C16:0, $P < .05$), arachidonic acid (C20:4w6, $P < .05$), eicosapentaenoic acid (C20:5w3, $P < .001$), and docosahexaenoic acid (C22:6w3, $P < .001$) in the phospholipid fraction, but significantly reduced the levels of linoleic acid (C18:2w6, $P < .001$) as compared with the chow diet feeding. Few fatty acids were different among the LG, LCT, and Fish oil groups. However, LG did have higher levels of palmitoleic acid (C16:1w7, $P = .05$) and stearic acid (C18:0, $P < .001$) and lower levels of oleic acid (C18:1w9, $P < .001$) compared with LCT and Fish oil, whereas the Fish oil group had lower levels of linoleic acid (C18:2w6, $P < .001$) and arachidonic acid (C20:4w6, $P < .001$) but higher levels of oleic acid (C18:1w9, $P < .001$), dihomogammalinolenic acid (C20:3w6, $P < .001$), eicosapentaenoic acid (C20:5w3, $P < .001$), and docosapentaenoic acid (C22:5w3, $P < .001$) compared with other groups. In the liver (Table 8), unlike the changes in plasma, the values of palmitic acid (C16:0, $P <$

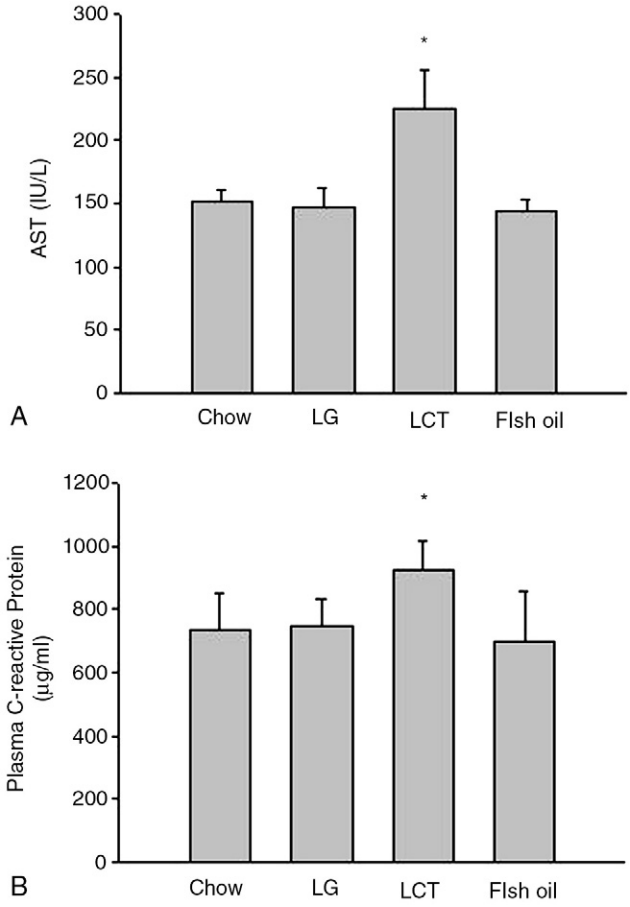

Fig. 4. The effects of feeding with oral chow diet ad libitum (~300 kcal/[kg d]) or fat-free TPN with 2 g of nitrogen per kilogram per day at lower (126 kcal/[kg d]) or adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total energy from fat, either Intralipid (LCT) or Omegaven (Fish oil), on (A) plasma levels of AST (in international units per liter) and (B) CRP (in micrograms per milliliter) in rats. Chow: oral feeding with chow diet ad libitum. LG: fat-free TPN feeding with 2 g of nitrogen per kilogram per day at 126 kcal/(kg d). LCT: TPN feeding at adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total calories from Intralipid. Fish oil: TPN feeding at adequate energy intake (180 kcal/[kg d]) with 2 g of nitrogen per kilogram per day and 30% of total calories from Omegaven. *$P = .01$ vs others; $P = .05$ vs others.

Table 8
Phospholipid profiles in the liver (percentage nanomole per milligram)

Fatty acid	Liver			
	Chow (n = 10)	LG (n = 10)	LCT (n = 9)	Fish oil (n = 9)
C16:0	24.4 ± 0.8	26.1 ± 0.5	22.7 ± 0.3*	25.1 ± 0.5
C16:1w7	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
C18:0	23.6 ± 0.7	22.0 ± 0.7	23.8 ± 0.4	20.8 ± 0.6†
C18:1w9	7.6 ± 0.4	7.2 ± 0.7	6.4 ± 0.4‡	8.0 ± 0.3
C18:2w6	15.3 ± 1.7	14.3 ± 0.5	16.4 ± 0.6	10.9 ± 0.5†
C18:3w3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C20:3w9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C20:3w6	0.8 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
C20:4w6	20.7 ± 1.7	20.4 ± 0.5	21.7 ± 0.8	15.2 ± 0.3†
C20:5w3	0.3 ± 0.1	0.7 ± 0.1§	0.4 ± 0.1	67.0 ± 0.4†
C22:4w6	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
C22:5w6	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C22:5w3	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 0.1†
C22:6:3	5.5 ± 0.6‖	7.3 ± 0.2	6.6 ± 0.3	10.5 ± 0.3†

Mean ± SEM. Statistical differences are made by 1-way ANOVA with least significant test as post hoc test.

* $P < .01$, LCT vs others.

† $P < .001$, Fish oil vs others.

‡ $P < .05$, LCT vs Fish oil and Chow.

§ $P < .05$ LG vs Chow.

‖ $P < .05$, Chow vs LG and LCT.

.01) and oleic acid (C18:1w9, $P < .005$) were significantly lower in LCT compared with other groups. In addition, the values of linoleic acid (C18:2w6, $P < .001$) and arachidonic acid (C20:4w6, $P < .001$) were significantly lower in the Fish oil group compared with other groups. In contrast, significantly higher levels of eicosapentaenoic acid (C20:5w3, $P < .001$), docosapentaenoic acid (C22:5w3, $P < .001$), and docosahexaenoic acid (C22:6w3, $P < .001$) were observed in the Fish oil group compared with the other groups. The level of docosahexaenoic acid (C22:6w3, $P < .05$) in the LG group was significantly higher than in the chow group but significantly lower than in the Fish oil group.

3.2.5. Plasma glucose concentrations, liver enzyme tests, and plasma CRP levels

Plasma glucose concentrations were in the range of 139 ± 3 to 146 ± 14 mg/dL and not different among groups. Of the liver enzyme tests, only AST was significantly higher in the LCT group compared with the others ($P < .05$) (Fig. 4A). Plasma CRP was also significantly higher in the LCT group ($P = .05$), without differences in plasma CRP among Chow, LG, and Fish oil groups (Fig. 4B).

4. Discussion

De novo lipogenesis is the endogenous physiologic process of converting carbohydrate to fat that is generally a very minor component of total energy expenditure in man during meal feeding [24]. However, parenteral glucose provided in a continuous fashion leads to substantial rates of lipogenesis even during hypocaloric feeding [25]. Accelerated lipogenesis from parenteral feeding is at least in part related to the higher levels of peripheral insulin found with parenteral vs enteral feeding of a glucose-based TPN formula [9]. One disadvantage of the resultant de novo lipogenesis found with glucose-based feeding is the caloric inefficiency because about 20% of the energy contained in the carbohydrate is used in this conversion [17]. A more serious consequence is the increase in hepatic triglyceride storage with some evidence for hepatic injury reflected in elevated liver enzymes [26].

Feeding a parenteral fat-free diet in study 1 with the LG and HG groups led to an increase in hepatic triglyceride compared with the chow diet, although the increases did not reach statistical significance. The higher level of plasma triglyceride in the Chow group is presumably due to the greater caloric intake of fat calories consumed as well as the impact of a glucose-based TPN to reduce hepatic triglyceride secretion [15]. This was associated with a significant increase in the levels of palmitic acid (C16:0) in hepatic triglycerides and palmitoleic acid (C16:1w7) and oleic acid (C18:1w9) in plasma triglycerides. Phospholipid fatty acid profiles showed similar changes with increased palmitic acid in plasma and palmitic and palmitoleic acids in hepatic phospholipids. Palmitic acid and stearic acids are products of de novo lipogenesis, and palmitoleic acid and oleic acid reflect stearyl coenzyme A reductase or δ 9 desaturase activity that also indicates de novo lipogenesis [28]. Oleic acid can be endogenously synthesized and/or released from fat stores, and all 4 of these fatty acids can be found in the diet. These findings suggest there is substantial de novo lipogenesis with fat-free feeding even when glucose is provided at hypocaloric levels. The elevated palmitic and stearic acids in plasma triglycerides and palmitoleic acid in plasma phospholipids in HG indicated even greater de novo lipogenesis with the higher glucose intake. The significant reduction in the levels of linoleic acid and α linolenic acid in plasma and hepatic triglycerides and the levels of linoleic acid in plasma and hepatic phospholipids as compared with fat-containing chow diet and TPN feeding occurs because these 2 fatty acids are essential fatty acids and only can be obtained from the diet. Thus, the levels of these 2 fatty acids in plasma and tissues are directly influenced by their dietary intakes, absent in the case of the LG and HG groups and adequately present in the Chow group that consumed more than twice the amount of calories of the LG and HG groups.

In contrast to the changes in linoleic and α linolenic acids, fat-free feeding significantly increased eicosapentaenoic acid and/or docosahexaenoic acid in plasma and liver in both LG and HG groups compared with the Chow group (Tables 3-4). The progression from the 18-carbon fatty acid precursors, α linolenic acid and linoleic acid, the 2 essential fatty acids, and oleic acid to their 20-carbon counterparts eicosapentaenoic acid, arachidonic acid, and Mead acid, respectively, is controlled through 2 shared control enzymes, δ 6 and δ 5 desaturase. When there is lower intake of α linolenic or linoleic acid, these enzymes are up-regulated in an attempt to maintain eicosapentaenoic and particularly arachidonic acid levels. In rats, for instance, it has been demonstrated that the messenger RNA amount and activity levels of δ 6 and δ 5 desaturases and associated elongases are up-regulated in the liver with essential fatty acid deficiency [27,28].

In study 2, the added fat as soybean oil or fish oil to the LG TPN increased the levels of certain fatty acids in the plasma and the liver, related to the dietary content with soybean oil containing substantial linoleic and α linolenic acids, and fish oil containing eicosapentaenoic and docosahexaenoic acids as well as considerable palmitic, palmitoleic, and oleic acid (Tables 5-8). In plasma phospholipids, the ratio of linoleic acid to arachidonic acid (C18:2w6/C20:4w6) was significantly increased in Fish oil as compared with LCT and LG groups (both Ps $< .05$). In addition, the ratio of eicosapentaenoic acid to docosahexaenoic acid (C20:5w3/C22:6w3) was also significantly increased in both LCT and Fish oil groups (both Ps $< .001$) compared with the LG group. These results do support the ability of both types of fat, soybean oil with high amounts of w6 fatty acids and fish oil with markedly elevated w3 fatty acids, to down-regulate desaturase activity, with the greater impact of fish oil on arachidonic acid levels. Furthermore, fatty acid markers of de novo lipogenesis (palmitic,

palmitoleic, stearic, and oleic acids) were reduced by either soybean oil or fish oil. Adding a fat source to the fat-free TPN solution, LG, to match the energy level of HG as in study 1 also had substantially different effects on serum and hepatic triglyceride levels. Whereas plasma triglycerides were elevated both in chow feeding and with soybean oil, only the latter was associated with excessive hepatic fat accumulation. The markers of dietary fat intake, linoleic acid and α linolenic acid, suggest as well that the source of this hepatic fat was at least in part the administered soybean oil.

In the fed state in this study, an increase in glucose calories (HG group) up to the level of the estimated energy expenditure (180 kcal/[kg d]) did not produce any evidence of liver injury (study 1). In contrast, adding soybean oil to the TPN solution (LCT) to this same energy level significantly increased AST in study 2 (Fig. 4A), suggesting liver injury. It has been shown previously that overfeeding with fat-free TPN causes hepatic dysfunction and that the addition of fat to the TPN regimen improves this hepatic dysfunction [29]. However, in this study, both HG and LCT were given at 180 kcal/(kg d) of calories and would not be considered overfeeding in relation to the size of rats used. Thus, the present results indicate that the fat component as soybean oil itself may induce hepatic injury during TPN feeding. Interestingly, fish oil as the fat source in the TPN solution counters both the hepatic fat accumulation and evidence for hepatic and systemic injury induced by soybean oil (Fig. 4A).

Fat-free TPN with added soybean oil did increase plasma CRP concentration (Fig. 2) about 35% higher compared with added fish oil, ad libitum feeding with a chow diet, or hypocaloric feeding (LG group, with 70% of the estimated energy expenditure) (Fig. 2). C-reactive protein is an acute-phase protein widely appreciated as a biomarker for inflammation among many species [30,31]. The percentage elevation of CRP with added soybean oil in study 2 was similar to that seen with added glucose in study 1, although it is likely that the mechanism is different. Although the threshold is not well defined in rats, an increase in plasma CRP in the HG group may imply that glucose infusion alone can also induce a low-grade inflammation compared with oral food feeding (Chow) or feeding with glucose at 30% lower caloric intake as with LG. In study 1, the mechanism of injury is likely to be related to altered glucose metabolism because it has been shown that parenteral glucose infusion that produces hyperglycemia initiates a systemic inflammatory response [32]. Although plasma glucose levels were not significantly different among these 3 groups and maintained at 145 mg/dL, the extra glucose infused in HG would still cause enhanced oxidation of the extra glucose that could increase the production of reactive oxygen species [32,33]. Increased reactive oxygen species do induce expression of various inflammatory genes, leading to an increase in the release of inflammatory cytokines.

In summary, hypocaloric feeding with glucose-based TPN or added fat as fish oil to provide eucaloric feeding appears to minimize de novo lipogenesis and the likelihood of hepatic triglyceride accumulation with some evidence for reduction in hepatic and systemic injury in rats.

Acknowledgment

PRL and BRB designed and conducted the study, measured all the markers, analyzed data, and had primary responsibility for writing the paper. CA performed fatty acid analysis with gas chromatography. AS prepared the TPN solutions. RS and SL helped with tissue collections. KG and MP provided Omegaven and participated in study design. All authors reviewed and approved the manuscript for final content.

References

[1] Blackburn GL, Wollner S, Bistrian BR. Nutrition support in the ICU: an evolving science. Arch Surg 2009 (in press).
[2] Simpson F, Doig GS. Parenteral vs. enteral nutrition in the critically ill patient: a meta-analysis of trials using the intention to treat principle. Intensive Care Med 2005;31:12-23.
[3] Bistrian BR. Parenteral vs. enteral nutrition? Crit Care Med 2008;36: 3133.
[4] van den Berghe G, Wouters P, Weckers F, et al. Intensive insulin therapy in the critically ill patients. N Engl J Med 2001;345:1359-67.
[5] van den Berghe G, Wouters PJ, Bouillon R, et al. Outcome benefit of intensive insulin therapy in the critically ill: insulin dose versus glycemic control. Crit Care Med 2003;31:359-66.
[6] Perioperative total parenteral nutrition in surgical patients. The Veterans Affairs Total Parenteral Nutrition Cooperative Study Group. N Engl J Med 1991;325:525-32.
[7] Hunter DC, Jaksic T, Lewis D, et al. Resting energy expenditure in the critically ill: estimations versus measurement. Br J Surg 1988;75: 875-8.
[8] Dissanaike S, Shelton M, Warner K, et al. The risk of blood stream infectious is associated with increased parenteral caloric intake in patient receiving parenteral nutrition. Crit Care 2007;11:R114.
[9] DenBesten L, Reyna RH, Connor WE, et al. The different effects on the serum lipids and fecal steroids of high carbohydrate diets given orally or intravenously. J Clin Invest 1973;52:1384-93.
[10] McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. Crit Care Clin 2001;17:107-24.
[11] Cohen C, Olsen MM. Pediatric total parenteral nutrition: liver histopathology. Arch Pathol Lab Med 1981;105:152-6.
[12] Wilson MD, Blake WL, Salati LM, et al. Potency of polyunsaturated and saturated fats as short-term inhibitors of hepatic lipogenesis in rats. J Nutr 1990;120:544-52.
[13] Clarke SD, Armstrong MK, Jump DB. Dietary polyunsaturated fats uniquely suppress rat liver fatty acid synthase and S14 mRNA content. J Nutr 1990;120:225-31.
[14] Herzberg GR, Rogerson M. Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow or marine oil. J Nutr 1988;118:1061-7.
[15] Hall RI, Grant JP, Ross LH, et al. Pathogenesis of hepatic steatosis in the parenterally fed rat. J Clin Invest 1984;74:1658-68.
[16] Meguid MM, Schimmel E, Johnson WC, et al. Reduced metabolic complication in total perateral nutrition pilot study using fat to replace one-third of glucose calories. JPEN J Parenteral Enteral Nutr 1982;6: 304-7.
[17] Nagayama M, Takai T, Okuno M, et al. Fat emulsion in surgical patients with liver disorders. J Surg Res 1989;47:59-64.

[18] Ohkawa H, Fukuwa C, Matsuzawa-Nagata N, et al. Soybean fat supplementation controls insulin resistance caused by fat-free total parenteral nutrition. J Pharm Pharmacol 2008;60:461-5.
[19] Acheson KJ, Schutz Y, Bessard T, et al. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. Am J Clin Nutr 1988;48:240-7.
[20] Mayer K, Seeger W. Fish oil in critical illness. Curr Opin Clin Nutr Metab Care 2008;11:121-7.
[21] McCowen KC, Friel C, Sternberg J, et al. Hypocaloric total parenteral nutrition: effectiveness in prevention of hyperglycemia and infectious complications—a randomized clinical trial. Crit Care Med 2000;28: 3606-11.
[22] Krisnan JA, Parce PB, Martinez A, et al. Caloric intake in medical ICU patients: consistency of care with guidelines and relationship to clinical outcomes. Chest 2003;124:297-305.
[23] Maiz A, Yamazki K, Sobrado J, et al. Protein metabolism during total parenteral nutrition (TPN) in injured rats using medium-chain triglycerides. Metabolism 1984;33:901-9.
[24] Hellerstein MK, Christiansen M, Kaempfer S, et al. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. J Clin Invest 1991;87:1841-52.
[25] Wolfe RR, Allsop JR, Burke JF. Glucose metabolism in man: responses to intravenous glucose infusion. Metabolism 1997;28:210-20.
[26] Javid PJ, Greene AK, Garza J, et al. The route of lipid administration affects parenteral nutrition–induced hepatic steatosis in a mouse model. J Pediatr Surg 2005;40:1446-53.
[27] Kramer JA, LeDeaux J, Butteiger D, et al. Transcription profiling in rat liver in response to dietary docosahexaenoic acid implicates stearoyl–coenzyme A desaturase as a nutritional target for lipid lowering. J Nutr 2003;133:57-66.
[28] Melin T, Nilsson A. Delta-6-desaturase and delta-5-desaturase in human Hep G2 cells are both fatty acid interconversion rate limiting and are upregulated under essential fatty acid deficient conditions. Prostaglandins Leukot Essent Fatty Acids 1997;56:437-42.
[29] Nishimura M, Yamauchi A, Yamaguchi M, et al. Major role of soybean oil in preventing decreases in CYP4A1 mRNA content relative to PPARalpha mRNA down-regulation in infant rats. Biol Bull Pharm 2005;28:1517-20.
[30] Ho KM, Lipman J. An update on C-reactive protein for intensivists. Anaesth Intensive Care 2009;37:234-41.
[31] Ridker PM. Inflammatory biomarkers and risks of myocardial infarction, stroke, diabetes, and total mortality: implications for longevity. Nutr Rev 2007;65(12-pat 2):S253-9.
[32] Ling PR, Mueller C, Smith RJ, et al. Hyperglycemia induced by glucose infusion causes hepatic oxidative stress and systemic inflammation, but nor STAT3 or MAP kinase activation in liver in rats. Metabolism 2003;52:868-74.
[33] Aljada A, Friedman J, Ghanim H, et al. Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumor necrosis factor alpha messenger RNA by mononuclear cells in healthy human subjects. Metabolism 2006;55:1177-85.