High-Calorie Total Parenteral Nutrition Reduces Hepatic Insulin-like Growth Factor-I mRNA and Alters Serum Levels of Insulin-like Growth Factor–Binding Protein-1, -3, -5, and -6 in the Rat

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High-calorie total parenteral nutrition (TPN) is associated with hepatic dysfunction and steatosis. Because TPN-induced steatosis might compromise hepatic expression of insulin-like growth factor-I (IGF-I) and thereby limit its potential nutritional benefit, we examined hormonal and IGF-I responses in male Sprague-Dawley rats (270 to 300 g) fed by continuous intravenous infusion with high-calorie, high-dextrose (350 kcal/kg) TPN solutions for 0 (control), 2, 4, and 8 days. Since IGF-binding proteins (IGFBPs) are thought to modulate the biological effects of IGFs in target tissues, we also determined serum levels of IGFBPs. Animals developed hepatic steatosis after 2 to 8 days of TPN, as reflected by a sevenfold to 15-fold increase in hepatic triacylglycerol content ($P < .001$ *v* control on each day). Serum corticosterone and insulin levels were significantly higher after 2 and 4 days of TPN, whereas serum growth hormone levels were reduced after 4 and 8 days. Serum IGF-I levels were not significantly different during TPN. However, there was a coordinate reduction in the three major hepatic IGF-I transcripts (7.0, 1.9, and 1.0 kb) after 2, 4, or 8 days of TPN, and IGF-I transcripts corresponding to multiple initiation sites within exons 1 and 2 were coordinately downregulated with TPN. Western ligand blotting indicated that serum levels of 38K to 43K, 30K to 34K, and 24K IGFBPs were increased approximately twofold after 4 and 8 days of TPN as compared with control values. Immunoprecipitation with specific antisera revealed that these changes reflect increased levels of immunoreactive IGFBP-3 (38K to 43K), IGFBP-5 (32K), and IGFBP-6 (24K to 26K). In contrast, serum levels of 32K and 34K forms of IGFBP-1 were reduced and levels of IGFBP-2 and -4 were not changed. In summary, high-calorie TPN results in hepatic steatosis and decreased hepatic abundance of IGF-I mRNA, suggesting that hepatic synthesis of IGF-I may be compromised during high-calorie TPN. Changes in circulating levels of IGFBP-1, -3, -5, and -6 emphasize that high-calorie TPN exerts complex effects on the IGF/IGFBP system, which may have important implications for understanding the biological impact of parenteral nutritional therapy.

TOTAL PARENTERAL NUTRITION (TPN) is a widely used and often vital feeding technique for individuals who are unable to absorb sufficient nutrients from the gastrointestinal tract. Direct infusion of nutrients into the systemic circulation during TPN induces metabolic alterations in part because the liver does not receive nutrients directly from the portal vein, but instead receives nutrients in proportion to the flux in the hepatic artery.[1] Complications of TPN include hepatic dysfunction, insulin resistance, and impaired anabolism or replenishment of lean body mass.[2] TPN-associated hepatic dysfunction, one of the most serious and frequent complications of TPN, is characterized by hepatic steatosis,[3] cholestasis,[4] and abnormal liver function tests.[2,5] Insulin resistance during TPN has been attributed to continuous infusion of solutions containing high concentrations of dextrose or lipid in both human[6] and animal studies.[7,8]

Insulin-like growth factor-I (IGF-I) is an important mediator of growth and development.[9] IGF-I circulates bound to specific binding proteins (IGF-binding proteins [IGFBPs]) that protect it from degradation and influence access to tissue receptors.[10] To date, six distinct IGFBPs have been isolated and their cDNAs cloned.[11] Although the functions of these IGFBPs are an area of active investigation, in vitro studies indicate that they may either inhibit or enhance the biological effects of IGFs depending on specific experimental conditions.[10,12-14]

The regulation of IGF-I and IGFBPs is complex and includes input from growth hormone, insulin, and nutrition.[10,15] Because the liver is an important synthetic site for circulating IGF-I,[15] hepatic dysfunction may alter the synthesis and circulating levels of IGF-I and affect anabolism. Lower serum IGF-I levels have been observed in patients who have received long-term TPN for gastrointestinal dysfunction[2] and have abnormal results on tests of liver function. Previously, we demonstrated that growing rats administered normocaloric TPN for 14 days without evidence of hepatic dysfunction or steatosis showed no difference in the hepatic abundance of IGF-I mRNA or serum IGF-I levels as compared with enterally fed controls.[16] One objective of this experiment was to test the hypothesis that hepatic steatosis alters hepatic abundance of IGF-I mRNA during high-calorie TPN. To gain a better understanding of how parenteral nutrition affects the IGF system, we also examined the effects of high-calorie TPN on serum levels of IGFBP-1 through -6.

MATERIALS AND METHODS

Animals and Experimental Design

We produced steatosis in rats by maintaining them exclusively with continuous parenteral infusion of high-calorie, high-dextrose (350 kcal/kg/d, 77% of nonprotein energy from dextrose), nutritionally complete TPN solutions for 0, 2, 4, and 8 days. High-calorie,

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high-dextrose TPN was used because the development of hepatic steatosis and dysfunction in the parenterally fed rat[3] and in humans[17] has been demonstrated to be a function of the total amount of energy infused and the proportion of energy provided by dextrose.

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) weighing 270 to 300 g were housed in individual stainless steel cages with free access to water in a room maintained at 27°C on a 12-hour light/dark cycle (lights on 8 AM to 8 PM). Animals were adapted to the animal facilities for 5 days before surgery. After anesthesia (50 mg ketamine and 10 mg xylazine per kg by intramuscular injection), animals underwent surgical placement of catheters in the superior vena cava via the external jugular vein.[8] Surgery was followed by a 5-day recovery period and consumption of Purina rat chow ad libitum (Ralston-Purina, St Louis, MO). During the last 2 days of the recovery period, a small volume (25 to 35 mL) of TPN was administered in addition to the rat chow. Animals were then maintained exclusively by continuous infusion of TPN solution for 0, 2, 4, or 8 days (n = 6 to 8). Animals remained free-moving throughout the infusion period by means of a 22-gauge swivel-infusion assembly (Instech Laboratories, Horsham, PA).

Age-matched reference animals were fed Purina rat chow ad libitum for 1 week and then killed (n = 6). Two other control groups (n = 6 each) were killed after recovery from surgery, including six freely feeding rats (day 0) and six rats that were fasted for 24 hours, and in some cases 30 hours, after recovering from surgery. These control groups were treated the same as the TPN groups, including receiving the infusion of TPN during the last 2 days of the recovery period. Both 24- and 30-hour periods of fasting were used to ensure the availability of serum to serve as a positive control for the IGFBP assays.

Animals were killed between 9 and 11 AM by overexposure to CO_2. Blood was collected by cardiac puncture and allowed to clot at 0°C. Serum was isolated by centrifugation at 4°C for 20 minutes at 1,200 × *g*. Livers were excised immediately, weighed, frozen in liquid nitrogen, and stored at −70°C until analysis of lipid contents and extraction of RNA. Animal facilities and protocols were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Composition of TPN Solution

TPN solutions were prepared aseptically using commercial preparations of crystalline amino acids (Freamine III 8.5%, Kendall McGaw, Irvine, CA), dextrose, 20% lipid emulsion (Intralipid, KabiVitrum, Alameda, CA), vitamins, trace elements, and electrolytes, as previously reported.[8] TPN solutions contained 30 g amino acids, 285 g dextrose, and 30 g lipid per liter, which provided 23% of nonprotein energy from fat and 77% of nonprotein energy from dextrose. Animals received 75 to 80 mL TPN solution per day, which provided approximately 350 total or 320 nonprotein kcal/kg body weight/d and 1.3 g N/kg body weight/d. The TPN solution was nutritionally adequate for the rat and provided an energy intake that was approximately 40% in excess of that demonstrated to promote growth in rats maintained with TPN.[8]

Evaluation of Liver and Serum Indices of Liver Function

Hepatic tissue was evaluated by determination of liver weight and lipid content and by histological evaluation. Lipids were extracted from liver samples with chloroform-methanol (2:1, vol/vol) and assayed for cholesterol and triacylglycerol concentrations; the total liver lipid concentration was determined gravimetrically.[18] A portion of liver was fixed in buffered Formalin solution and embedded in paraffin, and sections were stained with hematoxylin and eosin. Histological evaluation was performed by a veterinary pathologist who was unaware of the treatment groups for individual rats. Serum concentrations of glucose, cholesterol, electrolytes, total protein, and albumin and serum enzyme activity including alkaline phosphatase, aspartate amino transferase, and lactate dehydrogenase were determined by automated analysis in the University of Wisconsin Hospital Clinical Laboratory.

Radioimmunoassays for Serum IGF-I, Insulin, Growth Hormone, and Corticosterone

Serum IGF-I was quantified by radioimmunoassay (RIA)[19] after IGFBPs were removed by high-performance liquid chromatograph under acidic conditions.[16,20] Serum samples (75 μL) were acidified with 3 μL 2N HCl to a final pH of 2.8 and then centrifuged at 4°C for 10 minutes at 1,600 × *g* and 250 μL supernatant was injected onto a 7.8 × 300-mm Waters protein-pak column with a separation range of 2,000 to 80,000 molecular weight ([MW] Milford, MA). IGFs were eluted with 0.2 mol/L acetic acid and 0.1 mol/L trimethylamine hydrochloride at a flow rate of 1.7 mL/min, and 1.2-mL fractions were collected and pooled based on the elution of an IGF-I standard. Recovery of [^{125}I]IGF-I from the eluates was 85% to 90%. Direct measurement of IGFBP levels was not made in the fractions assayed for IGF-I because of the clear separation of proteins with such large differences in MW as IGF-I (~7,000) and IGFBP-3 (~50,000). Materials for the RIA included recombinant human IGF-I as a standard (Eli Lilly & Co., Indianapolis, IN), [^{125}I]IGF-I (Amersham, Arlington Heights, IL), polyclonal antibody to human IGF-I (National Hormone and Pituitary Program, Baltimore, MD), and goat antirabbit IgG and normal rabbit serum (Antibodies, Davis, CA). Serum extracts were assayed in duplicate in a single assay with an interassay coefficient of variation of 6%.

Commercial RIA kits were used to measure serum levels of insulin (Ventrex Laboratories, Portland, ME), rat growth hormone (Amersham), and rat corticosterone (ICN Biomedicals, Costa Mesa, CA), with intraassay coefficients of variation of 7% to 10%.

Western Analysis and Immunoprecipitation of Serum IGFBPs

Serum levels of IGFBPs were estimated by Western ligand blotting using the method of Hossenlopp et al.[21,22] Aliquots of serum (2 μL) were mixed with 20 μL Laemmli sample buffer without β-mercaptoethanol, heated to 60°C for 10 minutes, and then loaded onto a 7.5% to 15% sodium dodecyl sulfate (SDS)–polyacrylamide gradient gel for electrophoresis. Proteins were transferred onto 0.2-μm nitrocellulose filter paper, and membranes were blocked and then probed for IGFBPs with [^{125}I]IGF-I. IGFBPs were visualized by autoradiography with Kodak X-Omat film (Eastman Kodak, Rochester, NY) and intensifying screens (Dupont, Wilmington, DE) at −70°C for 2 to 4 days. MWs were determined by comparison to prestained high-MW protein standards (Gibco, Grand Island, NY). The band intensity for 38K to 43K, 30K to 34K, and 24K IGFBPs was quantified by laser densitometry (Discovery Series densitometer with Quantity One software, PDI, Huntington Station, NY), and the results were expressed as OD units (0 to 1.5) times millimeters.

Immunoprecipitation studies were performed by the method of Unterman et al[23] and Lamson et al[24] using specific antisera against rat IGFBP-1,[23] -3, -5, and -6 (generously provided by S. Shimasaki, The Whittier Institute, La Jolla, CA)[11] and commercially available antisera developed against bovine IGFBP-2 and human IGFBP-4 (Upstate Biotechnology, Lake Placid, NY). Each of these antisera

is highly specific, except that the antiserum against IGFBP-4 also recognizes IGFBP-2.

In brief, washed staphylococcal protein A (Calbiochem, La Jolla, CA) was incubated with antiserum against IGFBP-1, -2, -3, -4, -5, or -6 or nonimmune rabbit serum. Immunoprecipitation studies were performed with representative sera from 0-day TPN (control) and 8-day TPN groups. Test serum was preincubated with fresh protein A to reduce the amount of nonspecific binding of 38K to 43K, 30K to 34K, and 24K IGFBPs to protein A. Samples were rinsed, heated in sample buffer, and then microfuged, and supernatants were loaded for 13% nonreduced SDS–polyacrylamide gel electrophoresis (PAGE). Proteins were transferred onto nitrocellulose for ligand blotting as before. Membranes were probed with [^{125}I]IGF-I in studies of IGFBP-1, -2, -3, -4, and -5. [^{125}I]IGF-II[23] was used to probe membranes for IGFBP-6, since IGFBP-6 binds IGF-II with approximately 100-fold greater affinity than IGF-I.[11]

Northern and RNase Protection Assays of IGF-I

Total cellular hepatic RNA was extracted from 0.5-g aliquots of liver[25] and quantified by UV absorbance. Ribosomal RNAs (18S and 28S) were visualized under UV light with ethidium bromide to confirm the integrity of RNA and ensure equal loading.

Northern blot analysis[16,20] was used to characterize the hepatic transcripts using cDNA probes for IGF-I (~1.0-kb mouse IGF-I insert) and β-actin (2.0-kb, full-length cDNA fragment from human fibroblast). Briefly, 20 μg total RNA was applied to a 1.2% agarose gel containing 2.2 mol/L formaldehyde, electrophoresed, and then transferred to nitrocellulose filter paper. IGF-I (cDNA insert) and β-actin (linearized plasmid) were radiolabeled by random primer extension (Promega, Madison, WI) using [α-^{32}P]dCTP (Amersham), and hybridization was performed in 50% formamide for 48 hours at 42°C. The filters were then washed and exposed to X-Omat film at 70°C for 12 to 48 hours with intensifying screens. The autoradiographs were developed, and the density of each transcript was quantified by laser densitometry as described earlier.

An RNase protection assay[26] was also used to measure hepatic IGF-I mRNA abundance using an antisense RNA probe.[27] The cDNA containing the exon-1 coding region of rat IGF-I (514 bp, *Sau* 3A-NIaIV) was a gift from C. Roberts (National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD). The riboprobe vector was linearized with *Eco*RI before synthesis of antisense RNA with T7 RNA polymerase using [α-^{32}P]rUTP (10 mCi/mL, New England Nuclear, Boston, MA). Total RNA (20 μg) was hybridized overnight with 2 × 10^5 cpm of the IGF-I probe at 50°C in 50% (vol/vol) formamide, 40 mmol/L PIPES (pH 6.4), 0.4 mol/L NaCl, and 1 mmol/L EDTA. After hybridization, 40 μg RNase A/mL (Sigma Chemical, St Louis, MO) was added and incubated at room temperature for 15 minutes. Protected RNA-RNA hybrids were resolved on denaturing 6% polyacrylamide, 8-mol/L urea gels. A ^{35}S-end-labeled *Hin*fI digest of pBR322 DNA was used as the MW marker. Autoradiography was performed at −70°C with Kodak X-Omat film and two intensifying screens.

Statistical Analysis

Differences among treatment groups were assessed by one-way ANOVA using the SAS general linear models program.[28] Group means were considered significantly different at P less than .05 as determined by the least-significant difference technique. Values are expressed as the mean ± SEM.

RESULTS

Effects of High-Calorie TPN on Body Weight Gain, Liver Weight, and Liver Lipid Concentrations

Animal weights were not significantly different among treatment groups at surgery (290 to 310 g), and animals regained their presurgical weights by 5 days after surgery. Body weight gain averaged 7 g/d in animals maintained for 4 or 8 days with TPN (Table 1). Relative liver weight (grams wet weight per 100 grams body weight) decreased significantly between 2 and 8 days of TPN. The liver total lipid concentration increased approximately 1.5-fold and the triacylglycerol concentration increased sevenfold to 15-fold with 2, 4, or 8 days of TPN as compared with either the 0-day TPN or reference groups. The liver total cholesterol content also was significantly greater after 4 or 8 days of TPN.

Liver Histology and Serum Indices of Liver Function

Histological evaluation revealed that none of the livers had changes outside the range of physiologic variability, although hepatic steatosis was noted after 2 days of TPN. Also, livers from animals maintained for 4 or 8 days with TPN showed more vacuolation than livers from the 0- and 2-day TPN groups. Vacuolated hepatocytes were present in all levels of the lobule, but tended to be most marked around the central vein.

Results of the automated chemistry panel showed little change in levels of total bilirubin, aspartate amino transferase, and lactate dehydrogenase. Alkaline phosphatase activity declined with a longer duration of TPN, as previously noted in rats.[8] Serum total protein and albumin concentrations were significantly greater with 4 or 8 days of TPN as compared with the 0- and 2-day TPN groups (data from the automated chemistry panel are not shown).

Table 1. Effects of High-Calorie TPN on Body Weight Gain, Liver Weight, and Liver Lipid Concentrations

Determinations	Reference	TPN (d)			
		0	2	4	8
Weight gain (g/d)	6.7 ± 0.4	3.8 ± 0.8	3.4 ± 0.8	7.2 ± 1.7*	7.1 ± 0.6*
Liver weight (g/100 g weight)	4.7 ± 0.3	4.4 ± 0.1	4.7 ± 0.1	4.5 ± 0.2	4.2 ± 0.2†
Total lipids (mg/g)	67 ± 10	64 ± 3	108 ± 10‡	111 ± 10‡	93 ± 10‡
Triacylglycerol (μmol/g)	5.4 ± 0.3	4.5 ± 1.1	77.2 ± 6.4‡	56.9 ± 14.2‡	38.3 ± 8.1‡
Cholesterol (μmol/g)	7.9 ± 0.7	8.1 ± 0.4	9.9 ± 1.2	13.0 ± 1.2‡	11.3 ± 0.8‡

NOTE. Values are the mean ± SEM; n = 6 to 8 in each group.

*P < .05 v 0- and 2-day TPN.

†P < .05 v 2-day TPN and reference.

‡P < .001 v 0-day TPN and reference.

RIA of Serum IGF-I, Insulin, Growth Hormone, and Corticosterone

Serum concentrations of IGF-I were not significantly different among the TPN and control groups (Fig 1). Serum insulin levels increased twofold to threefold after 2, 4, or 8 days of TPN as compared with both the reference and 0-day TPN groups ($P < .001$). Serum insulin levels were positively correlated with hepatic total lipid, triacylglycerol, and cholesterol concentrations ($r = .47$ to $.51, P < .01$). Serum growth hormone levels were significantly lower after 4 and 8 days of TPN as compared with the 0-day TPN group. There was no significant correlation between serum growth hormone and IGF-I levels, although serum growth hormone levels were negatively correlated with hepatic total lipid and triacylglycerol concentrations ($r = .37$ to $-.40, P < .01$). Serum corticosterone levels were significantly higher after 2 and 4 days of TPN as compared with the 0-day and reference groups.

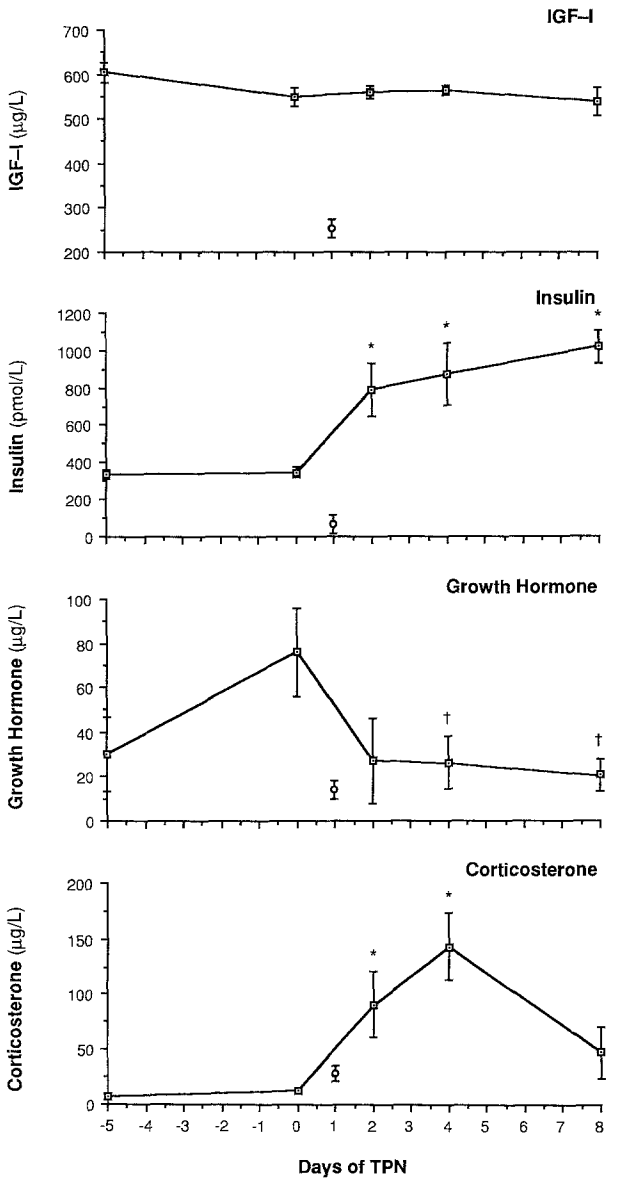

Fig 1. Serum IGF-I, insulin, growth hormone, and corticosterone levels in rats maintained with high-calorie TPN for 0, 2, 4, or 8 days. Levels for a nonsurgical reference group of animals are shown at −5 days. (–○–) Levels for animals who recovered from surgery and were then fasted for 24 hours. *$P < .01$ *v* 0-day TPN and reference; †$P < .05$ *v* 0-day TPN.

Serum IGFBPs

Western ligand blotting and densitometric analysis revealed that levels of IGFBPs increased twofold in rat serum after 4 or 8 days of TPN as compared with reference, 0- and 2-day TPN groups (Figs 2 and 3). Increased levels of 38K to 43K, and 30K to 34K IGFBPs were especially apparent, but 24K IGFBPs also were increased significantly after 4 or 8 days of TPN.

We used specific antisera against IGFBP-1, -2, -3, -4, -5, and -6 to determine if circulating levels of these IGFBPs were altered during TPN. As shown in Fig 4A through F, immunoprecipitation studies demonstrated that circulating levels of IGFBP-1, -3, -5, and -6 were differentially affected by high-calorie TPN, whereas levels of immunoreactive IGFBP-2 and -4 were not altered during TPN. As shown in Fig 4C, circulating levels of immunoreactive IGFBP-3 were high after 8 days of TPN, accounting for the increase in 38K to 43K IGFBPs detected on ligand blots (Figs 2 and 3). Levels of IGFBP-5 (32K) also were increased during TPN (Fig 4E) and appeared to account for the increase in 30K to 34K IGFBPs observed on ligand blots, since levels of IGFBP-1 (32K and 34K forms) were reduced (Fig 4A) and levels of 32K IGFBP-2 were not altered by high-calorie TPN. Finally, levels of 24K to 26K forms of IGFBP-6 also were increased in TPN serum (Fig 4F) and appeared to account for the increase in 24K IGFBPs noted by densitometric analysis of ligand blots, since levels of IGFBP-4 (24K) were not changed during TPN (Fig 4D). (Note that this antiserum against IGFBP-4 also recognizes IGFBP-2, accounting for both 32K (IGFBP-2 and glycosylated BP-4) and 24K bands in these immunoprecipitation studies of IGFBP-4.)

Hepatic Abundance of IGF-I mRNA

Northern blot analysis demonstrated a coordinate reduction in hepatic abundance of the three major IGF-I transcripts (7.0, 1.9, and 1.0 kb) with 2, 4, or 8 days of high-calorie TPN as compared with control levels (Figs 5 and 6). Fasting also resulted in a coordinate reduction in hepatic IGF-I transcripts.

To examine more specifically the effects of high-calorie TPN on hepatic abundance of IGF-I transcripts, we performed a RNase protection assay using an antisense RNA probe[27] that can distinguish between the five transcription start sites within exon 1 and the transcription start site within exon 2 of the rat IGF-I gene (Fig 7). The RNase protection assay confirmed that the activity of all IGF-I transcription start sites was coordinately reduced by high-calorie TPN as suggested by Northern analysis. We observed start site 2 (520 bp), 3 (420 bp), 4 (210 bp), and a start site with alternative splicing in exon 1 (258 bp). Start site 1 has a low frequency of usage and was not detected in

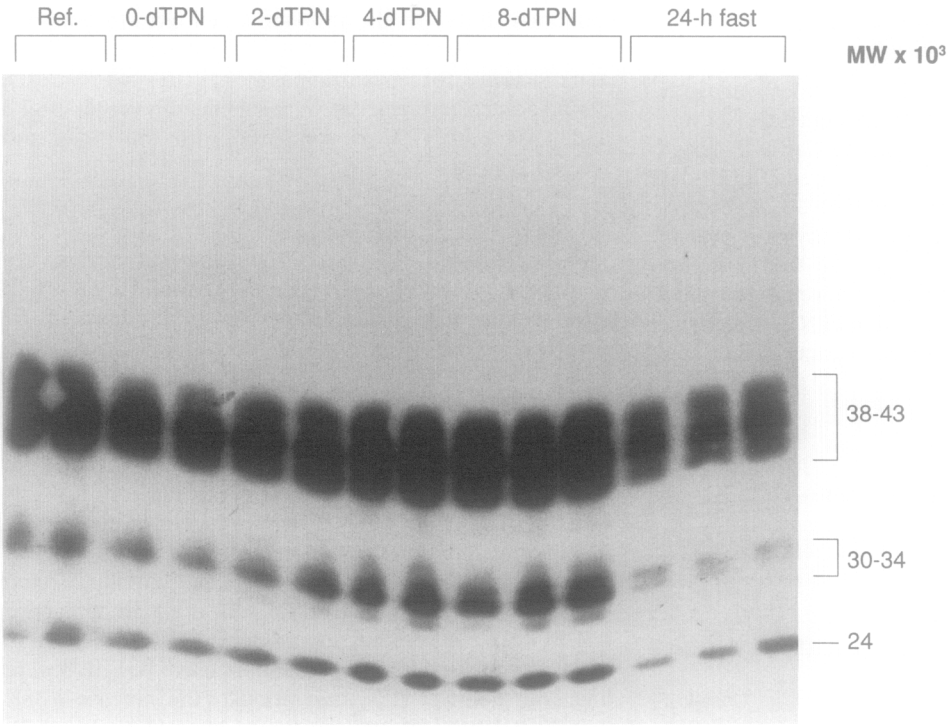

Fig 2. Western ligand blotting of serum IGFBPs. Serum proteins were separated by nonreduced 7.5% to 15% gradient SDS-PAGE and transferred to nitrocellulose. Membranes were probed with [^{125}I]IGF-I, and IGFBPs were identified by autoradiography. Treatment groups indicated across the horizontal axis, and MWs of bands along vertical axis based on migration of prestained, high-MW protein markers.

the exposure times of this assay, as previously noted.[27] Also detected was the 176-bp fragment corresponding to the start site within the 3′ region of exon 2.

DISCUSSION

Nutrition is an important regulator of IGF-I synthesis in the liver, yet little is known about the molecular mechanisms involved in this nutritional regulation. Studies in humans indicate that serum IGF-I levels are a sensitive indicator of malnutrition and that they increase during nutritional repletion with TPN.[29-31] However, little is known about how the metabolic complications associated with TPN, especially hepatic dysfunction and insulin resistance, affect IGF-I responses. To our knowledge, this is the first demonstration that high-calorie TPN disrupts hepatic IGF-I gene expression and increases circulating levels of specific IGFBPs, including IGFBP-3, -5, and -6.

In the present study, we observed a striking reduction in

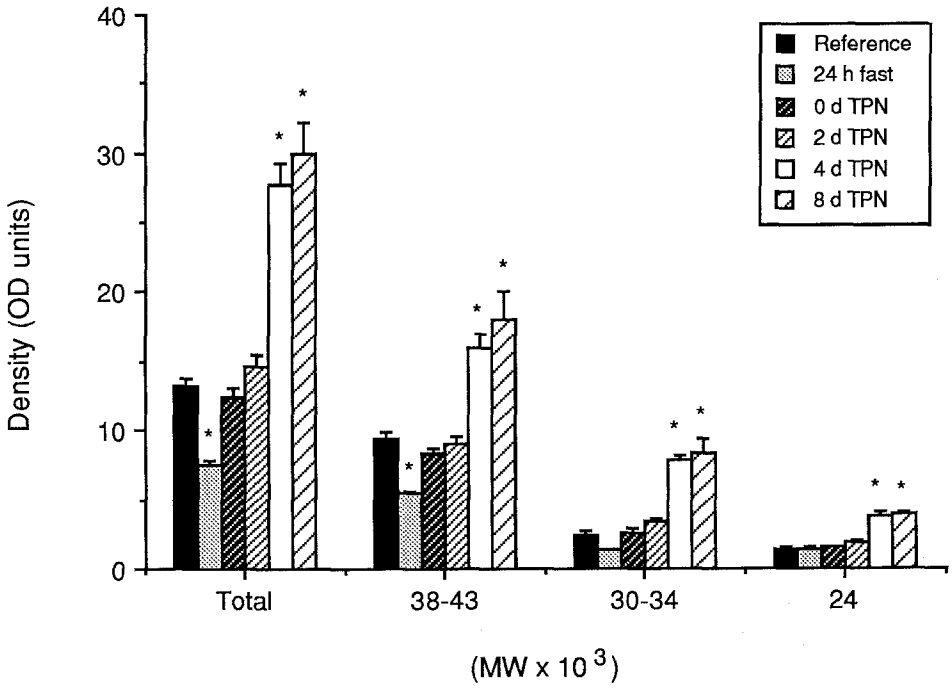

Fig 3. Laser densitometry of ligand blot autoradiographs. Relative changes in the band density are shown for the total sample and for the 38 to 43, 30 to 34, and 24 × 10^3 MW bands of specific IGF binding. Data for each band are expressed as mean scanning density units (OD × mm) ± SEM; n = 6 to 8. *P <.001 v all other groups.

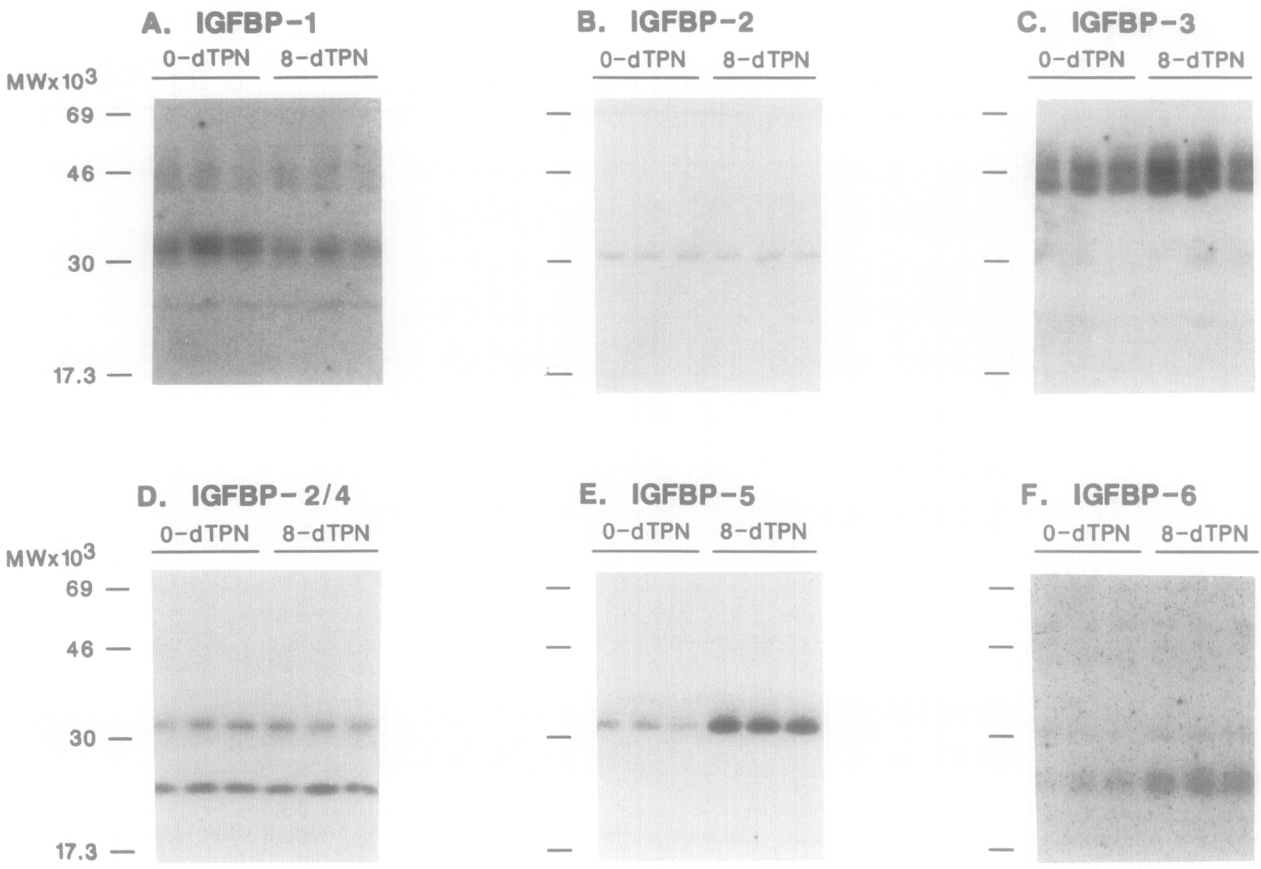

Fig 4. Immunoprecipitation and ligand blotting of IGFBPs in serum from control (0-d TPN) rats and from rats maintained with high-calorie TPN for 8 days (8-d TPN). IGFBPs in 10 μL serum were precipitated with specific antiserum against IGFBP-1, -2, -3, -5, or -6, or antiserum that recognizes both IGFBP-2 and -4 (¾), and then prepared for nonreduced SDS-PAGE and transfer. Nitrocellulose membranes were probed with [^{125}I]IGF-I (IGFBP-1 through -5) or [^{125}I]IGF-II (IGFBP-6) before autoradiography, as in Fig 2.

all species of hepatic IGF-I mRNAs in growing rats administered high-calorie TPN for 2, 4, or 8 days. IGF-I gene transcription was coordinately downregulated at each alternative start site within exons 1 and 2 (Fig 7). Stress induced by the continuous infusion system may contribute to decreased hepatic IGF-I gene expression during high-calorie TPN. Elevated serum corticosterone levels after 2 and 4 days of TPN are consistent with TPN-induced stress. Glucocorticoid excess has previously been associated with decreased IGF-I mRNA in liver,[32] skeletal,[33] neuronal, and glial cells.[34] Luo and Murphy[32] observed that glucocorticoids were particularly effective at preventing the induction of IGF-I mRNA by growth hormone in hypophysectomized rats. Of note, stress also is associated with reduced serum growth hormone levels in the rat.[20] Further, since both insulin and glucocorticoids are thought to inhibit growth hormone secretion,[35,36] it would appear that the combined effects of hyperinsulinemia, elevated corticosterone levels, and decreased secretion of growth hormone may interact to reduce hepatic IGF-I gene expression in rats administered high-calorie TPN.

Hepatic steatosis may also contribute to decreased hepatic IGF-I gene expression during high-calorie TPN. The pathogenesis of TPN-induced hepatic steatosis has been previously reviewed.[3] Our observations of steatosis and greater vacuolation of hepatocytes due to high-calorie TPN are consistent with histological changes associated with TPN-induced hepatic dysfunction.[4] Of note, we recently reported that growing rats administered TPN with an adequate but not excessive energy intake developed hyperinsulinemia without hepatic steatosis[18] and did not show a reduction in hepatic IGF-I mRNA.[16] This suggests that the dramatic decrease in hepatic expression of IGF-I noted in the present study is associated with hepatic steatosis and is not due to hyperinsulinemia.

Interestingly, we did not observe a significant decrease in serum IGF-I levels, despite a dramatic decrease in the abundance of hepatic IGF-I transcripts in rats receiving TPN. Early time-course studies of hypophysectomized rats treated with growth hormone indicated that increased hepatic production of IGF-I may account for approximately 50% of the increase in circulating levels of IGF-I following a single dose of growth hormone.[37] In addition, studies of nutritional deficiency states in rats, including fasting[38] and ingestion of diets restricted in energy[39,40] or protein,[41] suggest that changes in hepatic production of IGF-I may contribute to reduced circulating levels of IGF-I in these states. Based on the results of the present study, it is interesting to speculate that extrahepatic tissues also may make an important contribution to serum levels of IGF-I

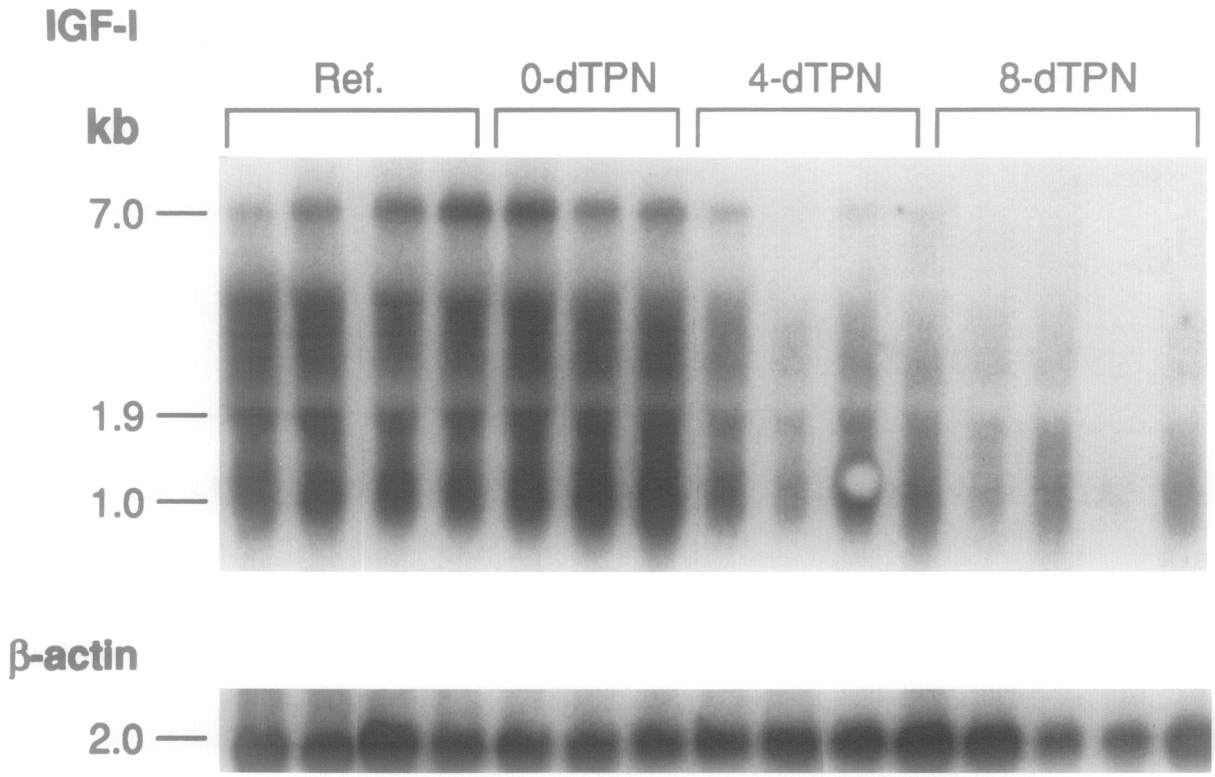

Fig 5. Autoradiograph of a representative Northern blot of rat liver IGF-I mRNA from animals maintained with TPN for 0, 4, or 8 days. Twenty micrograms of total RNA sample was hybridized with a random-primed mouse IGF-I cDNA fragment or linearized β-actin plasmid as described. The IGF-I probe detected a major transcript at 7.0 kb and two minor ones at 1.9 and 1.0 kb and other multiple forms in rat liver. All IGF-I transcripts were coordinately reduced by 2 (data not shown), 4, or 8 days of high-calorie TPN. The exposure time was 12 to 24 hours at −70°C with two intensifying screens.

during high-calorie TPN, when serum levels of growth hormone are low but levels of insulin and the availability of nutrients are increased relative to control.

In addition, it is important to note that the changes we observed in circulating levels of IGFBPs may contribute to differences in the clearance and/or tissue distribution of

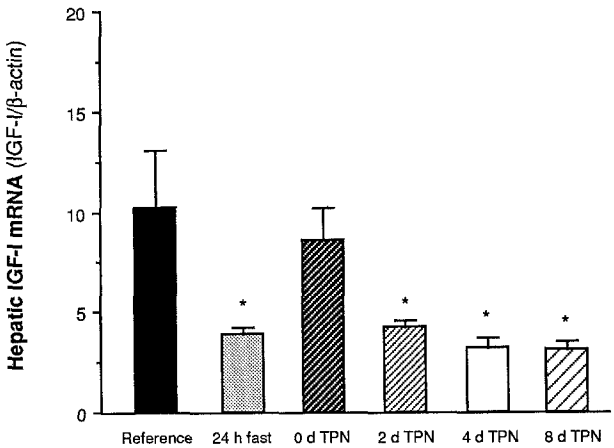

Fig 6. Laser densitometry of Northern blot autoradiographs of rat liver IGF-I mRNA from animals maintained with TPN for 0, 2, 4, or 8 days or fasted for 24 hours. Data are expressed as the ratio of band intensity (OD × mm) for the sum of the three IGF-I transcripts to β-actin. *$P < .001$ *v* 0-d TPN and reference.

IGF-I during high-calorie TPN. In particular, IGFBP-3 is the predominant IGFBP in serum from control animals, and levels of IGFBP-3 are increased in high-calorie TPN animals. Since most IGFs circulate in association with IGFBP-3 as part of a high-MW complex,[42] it is reasonable to speculate that increased levels of IGFBP-3 may lead to increased formation of these complexes and thereby expand the pool and increase the concentration of circulating IGFs. Additional studies will be required to examine each of these possibilities.

Immunoprecipitation studies revealed that serum levels of several specific IGFBPs are altered during high-calorie TPN, including IGFBP-1, -3, -5, and -6. Previous studies have shown that multiple factors contribute to the regulation of circulating levels of IGFBP-3. Growth hormone and IGF-I both increase serum levels of IGFBP-3 in hypophysectomized animals.[43] Interestingly, serum levels of IGFBP-3 and the hepatic abundance of IGFBP-3 mRNA are increased in animals treated with high doses of glucocorticoids,[44] whereas levels of IGFBP-3 are reduced in insulin-deficient animals.[43] In the present study, serum levels of IGF-I and growth hormone were not elevated. On the other hand, levels of corticosterone and insulin were high and may have contributed to increased levels of IGFBP-3 during high-calorie TPN. Of note, we have also observed greater levels of insulin and IGFBP-3 during TPN containing medium-chain triglyceride as compared with long-chain

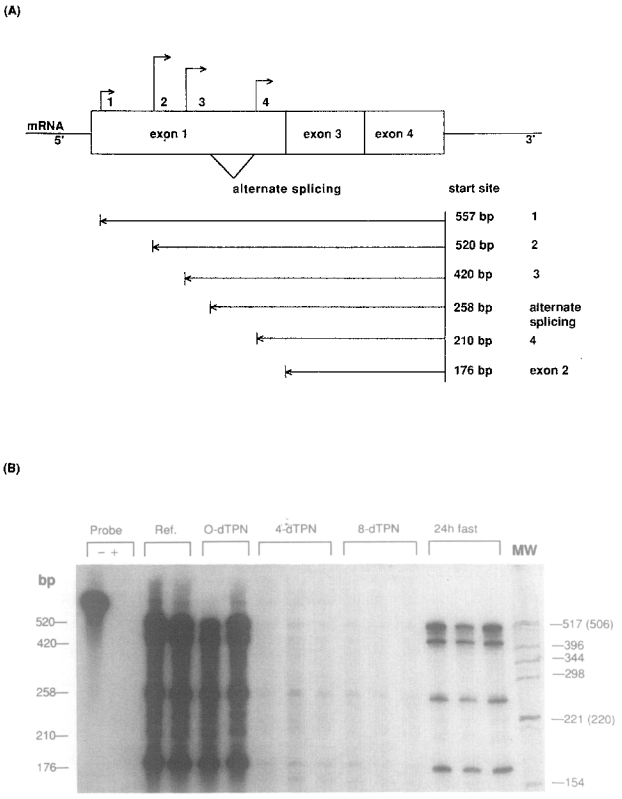

Fig 7. RNase protection assay that distinguishes between the different transcription start sites of rat IGF-I mRNAs. (A) General structure of a class I IGF-I mRNA; the multiple transcription start sites are indicated by arrows above exon 1. Indicated are the different mRNA fragments and their size protected by antisense riboprobe spanning exons 1, 3, and 4. (B) Autoradiograph of mRNA fragments protected by the above riboprobe from animals fed Purina rat chow ad libitum and killed before surgery (Ref.), maintained with TPN for 0, 4, or 8 days, or fasted for 24 hours after recovery from surgery. Twenty micrograms of total RNA was studied. Numbers to the right show the migration of the MW marker as bp. The lines on the left indicate size (bp) of the protected mRNA bands. The probe was digested with (probe +) or without (probe −) RNase.

triglyceride emulsions,[16] and the possibility that alterations in fat metabolism may contribute to increased levels of IGFBP-3 during high-calorie TPN also must be considered.

Insulin potently inhibits hepatic expression and decreases circulating levels of IGFBP-1.[45] Whereas glucocorticoids appear to stimulate hepatic production of IGFBP-1, the inhibitory effects of insulin appear to be dominant in combination with glucocorticoids.[45] Thus, it is likely that hyperinsulinemia accounts for the reduction in circulating levels of IGFBP-1 during high-calorie TPN. In contrast, little is known regarding the regulation of circulating levels of either IGFBP-5[14] or -6, and additional studies will be required to determine the relative roles that insulin, glucocorticoids, and growth hormone play in the regulation of IGFBP-5 and -6 in vivo. Indeed, to our knowledge, this report represents the first demonstration that circulating levels of immunoreactive IGFBP-5 and -6 are detectable in the rat and are altered in response to changes in nutrition. It also is important to note that the apparent MW of immunoreactive IGFBP-6 we detected in the circulation (24K to 26K) is consistent with the predicted MW based on amino acid content.[11] In contrast, IGFBP-6 purified from cerebrospinal fluid has a higher MW, reflecting the fact that this form of IGFBP-6 is *O*-glycosylated.[46] Taken together, these findings suggest that multiple forms of IGFBP-6 are found in body fluids, and that alterations in glycosylation and perhaps other posttranslational modifications may determine the distribution and/or function of IGFBP-6 in vivo.

In summary, our results indicate that high-calorie TPN causes hepatic steatosis and reduces hepatic abundance of IGF-I mRNA in association with higher circulating levels of IGFBP-3, -5, and -6, insulin, and corticosterone, lower serum levels of growth hormone and IGFBP-1, and little change in serum IGF-I levels. These data suggest that hepatic steatosis and altered hormonal responses during TPN contribute to decreased hepatic IGF-I gene expression. Further studies are needed to define better how TPN affects the synthesis of IGF-I and IGFBPs and how changes in IGF-I/IGFBP metabolism influence anabolic processes such as tissue regeneration, repair, and growth during TPN.

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