Determinants of Plasma Concentrations of Insulin-Like Growth Factor-I and Albumin and Their Hepatic mRNAs: The Role of Dietary Protein Content and Tumor Necrosis Factor in Malnourished Rats

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Protein restriction decreases plasma concentrations of albumin and insulin-like growth factor-I (IGF-I) by reducing their hepatic mRNA levels, whereas protein restriction increases IGF-I binding protein-2 (IGFBP-2) gene expression in the liver. Tumor necrosis factor (TNF), as an inducer of the injury response, decreases plasma albumin concentration and albumin mRNA in the liver. The present study was designed to evaluate the effects of protein repletion and TNF on plasma albumin and IGF-I and their mRNAs and IGFBP-2 mRNA in the liver of protein-restricted rats. After 2 weeks of feeding a 2% casein diet, rats were assigned to four groups according to either being refed with a 2% or 20% casein diet or receiving saline or TNF by intraperitoneal injection (50 μg/kg · d) for 4 days. Plasma IGF-I and albumin were assayed. Hepatic mRNAs of IGF-I, albumin, and IGFBP-2 were determined. Protein repletion increased plasma concentrations of IGF-I and albumin and their mRNA content in the liver, but decreased IGFBP-2 mRNA. TNF did not alter plasma IGF-I concentration but did increase hepatic IGF-I mRNA in protein-repleted animals, and plasma albumin concentration was significantly decreased with unaltered hepatic albumin mRNA. Thus, protein repletion of malnourished rats increased plasma IGF-I and albumin concentrations in association with increased expression of their mRNAs in the liver. However, plasma albumin but not IGF-I decreased following TNF in protein-restricted rats, whereas TNF increased hepatic IGF-I mRNA in protein-repleted rats. Thus, only plasma albumin concentration responds to both principal determinants, diet and injury, in the development of malnutrition.

SERUM ALBUMIN concentrations are greatly decreased in patients with the kwashiorkor form of malnutrition.[1,2] Dietary protein deprivation with adequate dietary energy in rats produces the distinctive clinical features of kwashiorkor, including hypoalbuminemia and edema, since plasma albumin is principally regulated by dietary protein rather than by energy intake.[3,4] For this reason, serum albumin concentration is widely used clinically as an indicator of nutritional status, as well as a marker of the inflammatory response and an indicator of prognosis.[5-7]

Similar to albumin, circulating insulin-like growth factor-I (IGF-I) is principally produced by the liver.[8,9] Plasma IGF-I has also been described as a precise and reliable indicator for evaluating nutritional status during dietary protein deficiency.[10-12] However, unlike albumin, plasma IGF-I is an important regulator of postnatal somatic growth and has many other anabolic effects.[9,13,14] Plasma IGF-I is principally complexed with high-affinity IGF-binding proteins (IGFBPs), which are important components in the regulation of IGF-I actions.[15-17] At least six distinct IGFBPs have been characterized.[16] IGFBP-3 generally responds in parallel with IGF-I, whereas hepatic IGFBP-2 mRNA reacts reciprocally, increasing with fasting and dietary protein restriction.[18,19]

It has been demonstrated that dietary protein restriction significantly decreases plasma concentrations of albumin and IGF-I by decreasing their mRNA abundance in the liver.[20-22] However, the response of hepatic albumin, IGF-I, and IGFBP-2 mRNAs to dietary protein repletion in protein-restricted animals is largely unknown.

Tumor necrosis factor (TNF) is generally accepted to be the principal proximal cytokine underlying the inflammatory response that is an important contributing factor in the development of protein-calorie malnutrition.[23] Infusion of TNF into experimental animals has been shown to cause weight loss, muscle wasting, and net body protein loss while increasing the weight and protein content of visceral organs such as the liver, heart, and lungs.[24] TNF infusion also invariably decreases plasma albumin concentration.[25,26] The decrease in serum albumin by TNF is considered the result of impaired gene expression of albumin mRNA in the liver.[27] Thus, both inadequate feeding and injury produce hypoalbuminemia, but the individual and combined effects of nutrition and TNF on hepatic albumin and IGF-I mRNA concentration in a malnourished condition has not been reported to date.

The purpose of the present study was to examine the effects of protein malnutrition, dietary protein repletion, and TNF administration on plasma levels of albumin and IGF-I and their hepatic mRNAs and IGFBP-2 mRNA in the liver of malnourished rats.

MATERIALS AND METHODS

Animal Procedures and Experimental Design

Male Sprague-Dawley rats (N = 23; Taconic Farms, Germantown, NY) weighing 180 to 200 g were acclimated upon arrival in an environment controlled with respect to light (12-hour light/dark cycle) and temperature (24°C) for 5 days. Rats were housed individually in wire-bottomed cages and given free access to a standard laboratory diet (Prolab; Agway Country Foods, Syracuse, NY) and water. The animals were then fed an AIN 76 diet with 2% casein ad libitum for 14 days. On the afternoon of day 15 (2 PM), the rats were placed on two diets of different protein content (Table 1) and received a daily intraperitoneal injection of saline or TNF (50

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Table 1. Diet Compositions (g/kg)

Ingredient	2% Casein	20% Casein
Casein	20	200
DL-Methionine	0.3	3
Cornstarch	192.2	150
Sucrose	640.5	500
Cellulose	50	50
Corn oil	50	50
Salt Mix #200000	35	35
Vitamin Mix #300050	10	10
Choline bitartrate	2	2
Energy (J/g)	16.16	16.16

NOTE. Salt Mix #200000 includes the following (mg/kg diet): calcium 5,200, phosphorus 4,000, potassium 3,600, sodium 1,020, chloride 1,560, sulfur 337, magnesium 507, iron 35, copper 6.0, manganese 54.0, zinc 30.0, chromium 2.0, iodine 0.2, and selenium 0.1. Vitamin Mix #300050 includes the following (mg/kg diet): thiamine 6, riboflavin 6, pyridoxine HCL 7, niacin 30, calcium pantothenate 16, folic acid 2, biotin 0.2, cyanocobalamin 10, and menadione sodium bisulfite 0.8. It also contains vitamin A 4,000 IU, vitamin E 175 IU, and vitamin D_3 1,000 IU.

μg/kg · d; Genentech, San Francisco, CA) until day 19. The dosage of TNF chosen for this study was based on our previous experience. A total of four groups were included: two continued on the 2% casein diet and received saline (2% casein + saline, n = 5) or TNF (2% casein + TNF, n = 5) injection, respectively. The remaining two groups were repleted with the AIN 76 diet with 20% casein and received saline (20% casein + saline, n = 6) or TNF (20% casein + TNF, n = 7) injection, respectively. All animals were pair-fed to the 2% casein + TNF group. Body weight was recorded on days 15 and 19.

On day 19, the rats were killed by decapitation at 10 AM. Blood was collected into chilled sodium EDTA tubes and centrifuged, and the plasma was stored at −20°C for determination of plasma albumin and IGF-I. The liver was quickly removed and weighed. One piece of the left lobe of the liver was dried for determination of nitrogen content. The right lobe of the liver was frozen in liquid nitrogen and stored at −80°C for subsequent RNA isolation and analysis.

Analytical Procedures

Plasma albumin was determined by a colorimetric method using an albumin kit with human albumin standards (Sigma, St Louis, MO). Plasma IGF-I level was measured by a radioimmunoassay kit using an acid-ethanol extraction method to separate IGF-I from binding proteins (Nichols Institute, San Juan Capistrano, CA).

Liver nitrogen content was determined after micro-Kjeldahl digestion.[28]

RNA Extraction and Dot-Blotting

Total liver RNA was extracted by TRI REAGENT according to the manufacturer's protocol (Molecular Research Center, Cincinnati, OH). Optimal hybridization and washing conditions for respective cDNA probes were previously established by Northern analysis, and dot blots were used in this study. Ten micrograms of total RNA per sample was denatured and applied to a nylon membrane (Gene Screen Plus; Dupont-NEN Products, Boston, MA) using a dot-blot vacuum manifold apparatus (Schleicher and Schuell, Keene, NH). After RNA immobilization by UV cross-linking, the blots were hybridized with ^{32}P-labeled rat IGF-I (Peter Rotwein, Washington University, St Louis, MO), IGFBP-2 (Dr S. Shimisaki, Whittier Institute, La Jolla, CA), and mouse albumin cDNA probes.[29] A murine 18s ribosomal cDNA probe was used as a control for total RNA loading. Blots were hybridized overnight at 42°C and washed according to methods described by the manufacturer of the nylon membranes. Blots were exposed in a phosphorimager cassette, and relative intensities were quantified by a phosphorimager system (Molecular Dynamics, Sunnyvale, CA). Hepatic mRNA concentrations of IGF-I, albumin, and IGFBP-2 are presented as arbitrary units relative to the 20% casein + saline group after correcting for ribosomal RNA.

Statistical Analysis

Results are presented as the mean ± SEM. Group means were compared by two-way ANOVA (20% *v* 2% casein and saline *v* TNF) using the SYSTAT statistical software package (SYSTAT, Evanston, IL). The correlations of plasma albumin versus liver albumin mRNA and plasma IGF-I versus liver IGF-I mRNA were examined. Significance was defined as P less than .05. Comparisons among groups were determined by least-significant difference (SYSTAT) when ANOVA was found to be significant at the 95% confidence level.

RESULTS

Body Weight, Energy Intake, and Liver Protein Content

The animals lost 16% of starting body weight (238.4 ± 2.2 *v* 199.7 ± 1.7 g) over the 2-week feeding with the 2% casein diet. Mean body weights for all groups at day 15 were not different. Energy intake was not different from day 15 through day 19 among any animals (Table 2). Rats on the 20% casein diet refeeding had a significantly increased body weight, liver weight, and liver protein content in comparison to rats kept on the 2% casein diet. TNF administration did not have additional effects on body weight, liver weight, or liver protein content in either the rats kept on the 2% casein diet or rats refed the 20% casein diet (Table 3).

Plasma IGF-I and Albumin Concentrations

Compared with the 2% casein diet refeeding, 20% casein dietary repletion significantly increased plasma IGF-I and albumin by 87% and 41%, respectively ($P < .01$; Figs 1 and

Table 2. Energy and Nitrogen Intake Over the 4-Day Refeeding Period

Group	Treatment	No.	Energy		Nitrogen	
			J/d	J/kg · d	g/d*	g/kg · d*
2% Casein	Saline	5	258.2 ± 8.2	1,240.3 ± 84.1	0.05 ± 0.00	0.25 ± 0.01
	TNF	5	252.9 ± 19.3	1,192.0 ± 106.7	0.05 ± 0.00	0.26 ± 0.02
20% Casein	Saline	6	247.2 ± 6.0	1,151.9 ± 30.1	0.49 ± 0.01	2.29 ± 0.06
	TNF	7	254.9 ± 10.2	1,170.0 ± 53.3	0.51 ± 0.02	2.32 ± 0.11

NOTE. Values are the mean ± SEM.

*$P < .001$, 20% casein *v* 2% casein by 2-way ANOVA.

Table 3. Body Weight Change, Liver Weight, and Liver Protein Content in Different Treated Groups

Group	Treatment	No.	Body Weight Change (g)*	Liver Weight (g)*	Liver Protein Content (%)*
2% Casein	Saline	5	3.20 ± 1.20	8.01 ± 0.37	45 ± 2
	TNF	5	2.26 ± 1.45	8.36 ± 0.16	52 ± 4
20% Casein	Saline	6	36.01 ± 2.67	10.69 ± 0.57	58 ± 5
	TNF	7	37.20 ± 2.27	11.31 ± 0.20	60 ± 2

NOTE. Values are the mean ± SEM.
*P < .001, 20% casein *v* 2% casein by 2-way ANOVA.

2). TNF did not further affect plasma IGF-I concentration in animals refed with either 20% or 2% casein. In contrast, 4 days of TNF injection significantly decreased plasma albumin in all rats independently of dietary protein content (Fig 2).

Hepatic IGF-I, Albumin, and IGFBP-2 mRNAs

Four days of 20% casein diet repletion significantly increased hepatic IGF-I and albumin mRNA concentrations as compared with the 2% casein diet (Figs 3 and 4). Hepatic IGF-I mRNA concentration was significantly increased by TNF injection in animals with 20% casein dietary repletion, but not in rats maintained on the low-protein diet. There was a slight decrease in liver albumin mRNA after TNF treatment, but this difference did not achieve significance (P = .08). In contrast to liver IGF-I and albumin mRNAs, hepatic IGFBP-2 mRNA concentration was significantly decreased by 20% casein dietary repletion compared with the 2% casein diet (Fig 5). TNF injection did not affect liver IGFBP-2 mRNA concentration with either refeeding diet.

There were significant correlations between plasma IGF-I and hepatic IGF-I mRNA and plasma albumin and hepatic albumin mRNA (r = .57 and .80, respectively, P < .05 for both).

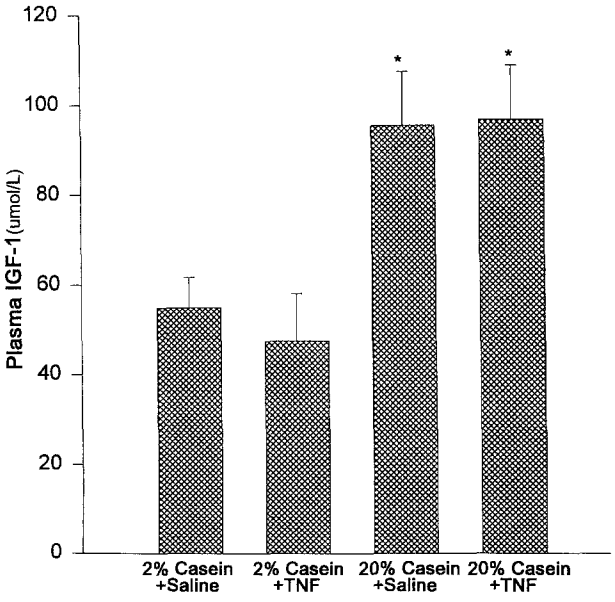

Fig 1. Plasma IGF-I concentration in different groups. Results are expressed as the mean ± SEM. *P < .001 *v* 2% casein + saline and 2% casein + TNF by 2-way ANOVA.

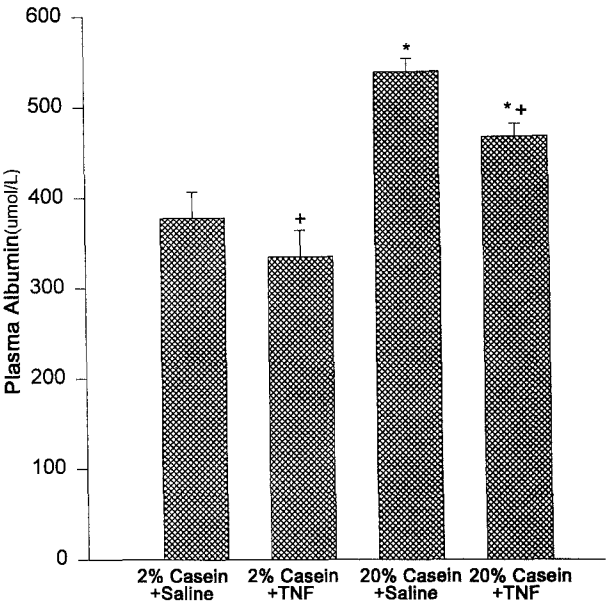

Fig 2. Effects of dietary protein content and TNF on plasma albumin concentration. Results are expressed as the mean ± SEM. *P < .001 *v* 2% casein + saline and 2% casein + TNF and †P < .01 *v* 2% casein + saline and 20% casein + saline by 2-way ANOVA.

DISCUSSION

Two weeks of dietary protein restriction resulted in significant malnutrition at the whole-body level, which is consistent with previously published reports.[19,30] Four days of adequate protein repletion significantly improved the nutritional status by increasing body weight, liver weight, liver protein content, and plasma IGF-I and albumin concentrations, as compared with isocaloric but low-protein refeeding. These data are consistent with other reports.[22,31-33]

It has been well demonstrated that chronic dietary protein restriction results in a significant decrease in plasma IGF-I and liver IGF-I mRNA.[19,21,34] It has been reported that refeeding a previously fasted human an essential–amino acid diet causes a large increase in serum IGF-I,[35] but there is less known about the changes in IGF-I induced by dietary protein repletion after chronic dietary protein restriction. In the present study, we observed a significant increase in plasma IGF-I following refeeding of adequate dietary protein compared with an equal-energy but low-protein diet. The increase in plasma IGF-I was associated with a significant increase in IGF-I mRNA synthesis in the liver. This indicates that the regulation of IGF-I in protein malnutrition is, in part, at the mRNA level, which is supported by findings from other studies.[21,34]

IGFBPs are considered to be involved in the change in plasma IGF-I concentration by prolongation of the plasma half-life of IGF-I, by alteration of its transport rate across the vascular endothelium, and by effects on the interaction between IGF-I and IGF-I receptor.[36-38] Fasting increases

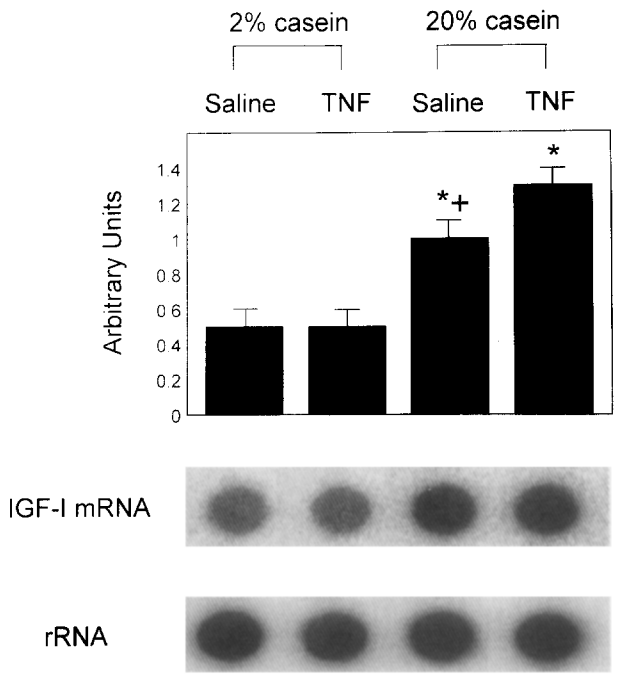

Fig 3. Effects of dietary protein content and TNF on liver IGF-I mRNA concentration. IGF-I mRNA concentration is expressed as arbitrary units relative to the 20% casein + saline group after being normalized to ribosome mRNA concentration. Data are expressed as the mean ± SEM. *$P < .001$ *v* 2% casein + saline and 2% casein + TNF and †$P < .05$ *v* 20% casein + TNF.

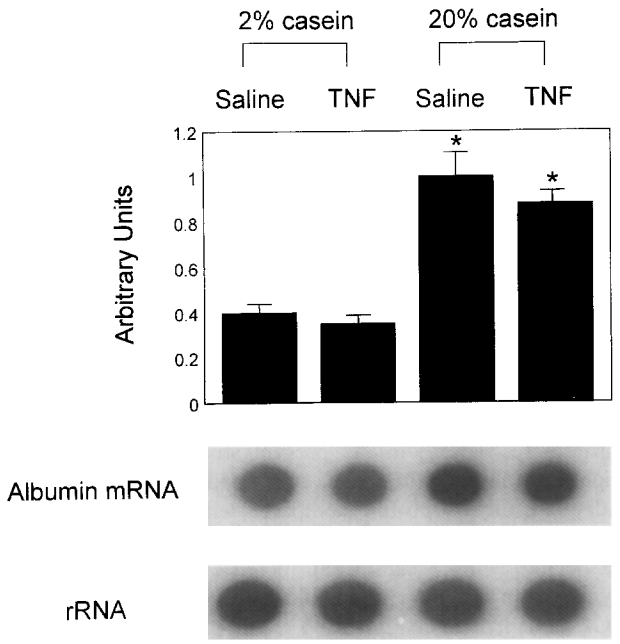

Fig 4. Liver albumin mRNA analysis in differ groups. Hepatic albumin mRNA concentration is expressed as arbitrary units relative to the 20% casein + saline group after being normalized to ribosome RNA concentration. Data are expressed as the mean ± SEM. *$P < .001$ *v* 2% casein + saline and 2% casein + TNF.

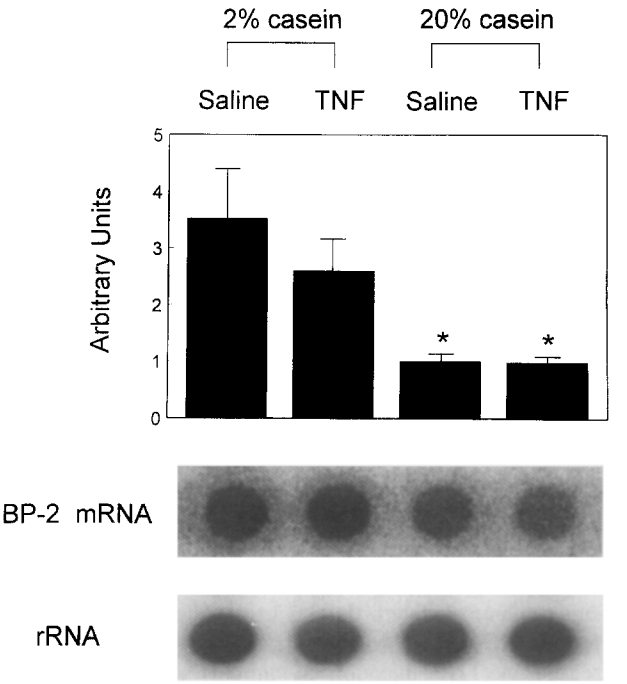

Fig 5. Effect of dietary protein content on IGFBP-2 mRNA. Hepatic IGFBP-2 mRNA concentration is expressed as arbitrary units relative to the 20% casein + saline group after being normalized to ribosome RNA concentration. Data are expressed as the mean ± SEM. *$P < .001$ *v* 2% casein + saline and 2% casein + TNF.

serum IGFBP-2 and produces a parallel increase in hepatic IGFBP-2 mRNA.[18,39] Straus and Takemoto[19] observed that 10 days of dietary protein (8% and 4%) restriction significantly increased IGFBP-2 mRNA in the liver. In the present study, the abundance of IGFBP-2 mRNA was greatly decreased by 20% casein diet refeeding. Since animals on the 20% casein diet consumed the same amount of energy each day as animals on the 2% casein diet, this change in IGFBP-2 mRNA in the liver presumably resulted from the differences in dietary protein. Since circulating IGFBP-2 inhibits the action of IGF-I[39,40] and the changes of serum IGFBP-2 are in parallel with the changes of hepatic IGFBP-2 mRNA,[18,38] the reduced IGFBP-2 mRNA in the liver may indicate that the inhibition of IGF-I activity has been relieved by dietary protein repletion.

The present data demonstrate that 4 days of dietary protein repletion significantly increased plasma albumin by increasing liver albumin mRNA, which is consistent with the observation by Sakuma et al[22] that a low-protein diet reduces albumin mRNA transcription. However, later study has shown that the decrease in albumin mRNA consequent to dietary protein restriction is caused mainly by a posttranscriptional mechanism rather than by a decrease in liver albumin gene transcription rates.[19]

TNF injection in normal rats produces different metabolic changes depending on the organ involved, such as increasing liver protein content while decreasing muscle protein content.[24,26] The present study demonstrated that 4 days of TNF treatment did not significantly increase liver

protein content in either the adequate- or low-protein refeeding group after chronic dietary protein restriction. Although the mechanism for this different observation is unknown, one hypothesis is that after malnutrition, net liver anabolism is already optimal when feeding adequate protein and is not responsive to TNF.

The present results also showed that TNF did not influence plasma IGF-I in any group, whereas IGF-I mRNA did not increase following TNF treatment in protein-repleted animals. This discrepancy between plasma IGF-I and liver IGF-I mRNA indicates that TNF may increase IGF-I gene transcription or mRNA stability in protein-repleted rats. However, there is also evidence of translational inefficiency of IGF-I mRNA after TNF administration, since plasma IGF-I concentration did not increase in response to this increase.[41]

There is strong and consistent evidence that TNF reduces plasma albumin and hepatic albumin mRNA concentrations in well-nourished animals.[25,42] The present study further demonstrated that TNF decreased plasma albumin concentration in all treated groups in addition to the separate effect of dietary protein content. However, TNF did not significantly alter hepatic albumin mRNA concentration, although there was a trend toward reduction and the correlation between mRNA and albumin concentration was excellent at .8. The differential effects of TNF on plasma albumin and hepatic albumin mRNA may be related to the following three factors. First, half-life values for rat plasma albumin (7 days) and albumin mRNA are decidedly different.[22] This is consistent with the observation by Sakuma et al[22] that the greatest increase in albumin mRNA is seen 1 day after protein refeeding, with a decrease thereafter even though the adequate-protein diet is continued. Second, there is evidence that TNF induces endothelial cell injury resulting in enhanced endothelial permeability to albumin, which consequently decreases plasma albumin concentration by an additional mechanism, extravascular extravasation.[25] Third, the impact of protein malnutrition on hepatic albumin mRNA concentration appears to be greater than that of TNF, whereas the effects of protein malnutrition and TNF on plasma albumin are similar in degree.

Plasma or serum albumin concentration is used clinically as an important measure of nutritional intake and as an indicator of the injury response. Even though plasma IGF-I and albumin may be regulated by dietary protein through different mechanisms at the molecular level with chronic protein restriction, both are increased by adequate dietary protein intake in association with increased hepatic mRNA concentration. Two important principles are demonstrated by this experiment that should be further confirmed. First, the response of plasma IGF-I and albumin to TNF is impaired by protein malnutrition, particularly for IGF-I. Second, with adequate nutrition, the response of hepatic IGF-I and albumin mRNA to TNF is discordant, being anabolic for IGF-I and catabolic for albumin. Thus, plasma albumin concentration shows the independent effects of both malnutrition and injury, whereas IGF-I concentration reflects most faithfully only the nutritional intake. This may explain why, clinically, plasma or serum albumin has traditionally been the best overall nutritional marker, since altered nutritional status is usually a consequence of both malnutrition and injury.

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