Tissue-Specific Effects of Chronic Dietary Protein Restriction and Gastrostomy on the Insulin-Like Growth Factor-I Pathway in the Liver and Colon of Adult Rats

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Dietary protein restriction decreases plasma concentrations of insulin-like growth factor-I (IGF-I) and reduces IGF-I mRNA levels in the liver. In addition to the actions of systemic IGF-I, locally produced IGF-I is thought to mediate autocrine and paracrine growth effects in the colon. The objectives of the present study were to investigate the IGF-I pathway in the colon and liver of adult rats under conditions of dietary protein restriction, surgical stress, and dietary protein repletion. Two groups of rats were placed on either a 20% or 2% casein diet for 19 days. Two additional groups of rats underwent gastrostomy after a 2% casein diet for 2 weeks, and then were either kept on the 2% casein diet or changed to a 20% casein diet until day 19. Dietary protein restriction reduced plasma concentrations of IGF-I and IGF-binding proteins (IGFBPs) and hepatic IGF-I mRNA content, while increasing colonic IGF-I receptor mRNA. Gastrostomy in protein-depleted animals had no effect on hepatic IGF-I mRNA, but led to a marked increase in colonic IGF-I mRNA levels. Dietary protein repletion resulted in a decrease in colonic IGF-I receptor mRNA. The distinct effects of dietary protein depletion and operative stress on the IGF pathway in the colon as compared with the liver may serve to maintain the level of IGF-I signaling in the colon by autocrine or paracrine mechanisms under these conditions.

MALNUTRITION as a result of dietary protein deficiency is a common problem in hospitalized patients.[1-3] A positive correlation between nutritional status and clinical outcome in surgical patients has been well established.[4,5] Metabolic stress due to surgery, infection, or inflammation often worsens the degree of protein malnutrition and its pathological consequences.[4,6] Therefore, provision of an adequate nutritional regimen is of critical importance for malnourished and postoperative patients.[6,7]

Insulin-like growth factor-I (IGF-I) is an anabolic hormone produced by many tissues, which exerts actions via endocrine, paracrine, and autocrine pathways.[8-11] IGF-I has been shown to be markedly affected by nutritional status and stress.[12] The level of circulating IGF-I is particularly sensitive to dietary manipulation, as evident from a dramatic reduction in plasma IGF-I concentrations in response to dietary protein restriction.[13] In comparison to other plasma proteins used in nutritional assessment (eg, albumin and transferrin), circulating IGF-I has a shorter half-life and responds rapidly to dietary alterations, making it a sensitive indicator of malnutrition.[14,15]

In addition to declining plasma IGF-I concentrations, expression of IGF-I mRNA is decreased in several tissues in response to malnutrition.[16] In particular, IGF-I mRNA levels in the liver, a major source of circulating IGF-I, are decreased by dietary protein malnutrition in rats. The lack of detectable IGF-I receptors on hepatic parenchymal cells indicates that the liver is not an important IGF-I–responsive tissue.[17] However, hepatic IGF-I production, through its effects on circulating IGF-I levels, is believed to be an important determinant of the endocrine actions of IGF-I on extrahepatic tissues. By contrast with the liver, the colon represents a tissue that expresses significant amounts of IGF-I receptor and IGF-I mRNA.[18,19] Systemic treatment with exogenous IGF-I after small-bowel resection has been shown to increase colonic mucosal mass in rats,[20] and in addition to systemic IGF-I, locally produced IGF-I is thought to mediate autocrine and paracrine growth effects on colonic cells.[9,21] This capacity for endocrine, paracrine, and autocrine responsiveness to IGF-I may help to maintain colon morphology during periods of dietary nutrient deficiency. Intestinal IGF-I mRNA levels are known to be altered by fasting,[22-24] but the specific effects of dietary protein restriction have not been established. Although stress is associated with decreased plasma IGF-I levels,[25-26] it is not known what effects minor surgical procedures such as gastrostomy placement have on IGF-I and IGF-I receptor expression in the liver and colon of protein-restricted rats. The objectives of the present study were to investigate the IGF-I pathway in the liver and colon of adult rats following (1) chronic (19-day) dietary protein restriction alone, (2) dietary protein restriction both before and after gastrostomy surgery, and (3) dietary protein repletion for 3 days after surgery.

MATERIALS AND METHODS

Animal Procedures and Experimental Design

Male Sprague-Dawley rats (n = 30; Taconic Farms, Germantown, NY) weighing 180 to 200 g were acclimated for 5 days in a controlled environment with respect to light (12-hour light/dark cycle) and temperature (24°C). Rats were housed individually in wire-bottomed cages and given free access to water and a standard laboratory rat diet (Prolab; Agway Country Foods, Syracuse, NY). On day 1 of the experiment, two groups of rats were placed on semipurified diets containing either 20% casein (n = 10, group I) or 2% casein (n = 8, group II) and kept on these diets without surgical intervention until

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termination of the study (day 19). The composition of the diets is described in Table 1. Body weight and food intake were measured every other day. A third group of rats ($n = 12$) were fed the 2% casein diet for 14 days, and then, under diethyl ether anesthesia, a silastic gastrostomy catheter (ID 0.027 in, OD 0.047 in) was surgically inserted into the antrum of the stomach and advanced approximately 1 cm beyond the pylorus. The catheter was tunneled subcutaneously, exteriorized at the midscapular region, and connected to a flow-through swivel device (Instech Laboratories, Philadelphia, PA) that allowed for uninterrupted infusion of 0.9% saline and free movement by the rats. Twenty-four hours after surgery (day 15), these rats were randomly assigned to two dietary groups and were either kept on the 2% casein diet (group III) or changed to a 20% casein diet (group IV). The animals were fed these diets for 3 days, during which time body weight and food intake were measured daily.

On day 19, the rats were killed by decapitation (10 to 11 AM). Blood was collected into chilled EDTA-containing tubes and centrifuged, and the plasma was stored at −20°C for subsequent analysis. The right lobe of the liver and the full length of the colon (contents flushed out with cold saline) were removed, weighed, quickly frozen in liquid nitrogen, and stored at −80°C for subsequent RNA analysis.

Plasma Measurements

Plasma glucose level was measured by the glucose oxidase method using a glucose analyzer (Glucose Analyzer II; Beckman Instruments, Brea, CA). Plasma leucine concentrations were determined by high-performance liquid chromatography as previously described.[27] Plasma albumin was determined by colorimetry with a commercially available kit (Sigma, St Louis, MO). Plasma insulin was determined by radioimmunoassay using a commercial kit (Binax, Portland, ME). Total plasma IGF-I was separated from binding proteins by acid-ethanol extraction[28] and determined with a radioimmunoassay kit (Nichols Institute, San Juan Capistrano, CA).

RNA Extraction and Analysis

RNA was extracted from the liver and colon by the acid guanidinium thiocyanate-phenol-chloroform method,[29] and Northern blotting was used to measure IGF-I and IGF-I receptor mRNA levels in the liver and colon. Twenty micrograms of total RNA per sample was denatured and electrophoresed in 1% formaldehyde agarose gels. RNA integrity was assessed by ethidium bromide staining and visualization of 28S and 18S ribosomal subunit bands. The RNA was then transferred to nylon membranes (Gene Screen Plus; Dupont NEN Products, Boston, MA) by capillary blotting and immobilized by UV cross-linking. The blots were hybridized overnight with a ^{32}P-labeled rat IGF-I cDNA probe (from Dr P. Rotwein, Washington University, St Louis, MO) or a rat IGF-I receptor cDNA probe isolated in our laboratory.[23] Blots were washed according to procedures described previously,[23] and the relative amounts of RNA transcripts were detected and quantified using a Phosphorimager system (Molecular Dynamics, Sunnyvale, CA). The blots were subsequently stripped, and a mouse 18S ribosomal cDNA probe (from Dr P. Bachierre, Centre de Recherche de Biochimie et Genetique Cellulaire, Toulouse Cedex, France) was used to verify equal total RNA loading.

Table 1. Dietary Composition (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. Diets were purchased from Dyets (Bethlehem, PA). 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, plus vitamin A 4,000 IU, vitamin E 175 IU, and vitamin D_3 1,000 IU.

Plasma IGF-Binding Proteins

Plasma IGF-binding proteins (IGFBPs) were determined by ligand blotting according to published methods.[30] Briefly, plasma samples were electrophoresed on 12.5% discontinuous sodium dodecyl sulfate–polyacrylamide gels under nonreducing conditions using the Laemmli buffer system.[31] The proteins were transferred to nitrocellulose membranes by electroblotting under constant current (0.4 A) at 15°C for 4 hours. After transfer, blots were incubated overnight in Tris-buffered saline, pH 7.2, at 4°C with 400,000 cpm/mL ^{125}I-IGF-I and washed several times in Tris-buffered saline. Labeled bands corresponding to IGFBPs were identified and quantified by phosphorimaging.

Statistical Analysis

Data are presented as the mean ± SEM. Group means were compared by one-way ANOVA using the SYSTAT statistical software package (SYSTAT, Evanston, IL). Significance is defined as P less than .05. Comparisons of multiple groups were performed according to the Tukey test when ANOVA was found to be significant at the 95% confidence level.

RESULTS

Body Weight and Energy Intake

Mean body weights were similar for all dietary groups at the onset of the study. Animals fed the 2% casein diet lost 16% of their starting body weight (248.8 ± 2.2 *v* 209.0 ± 3.5 g, $P < .05$) during the initial 2-week feeding period. Over this same period, the mean body weight for rats on the 20% casein diet increased 36% from 250.4 ± 3.8 g to 340.6 ± 8.8 g ($P < .05$). After surgery, the mean body weight of protein-restricted rats refed the 20% casein diet between days 16 and 19 increased by 23%, whereas there was only a small (<2%) increase in body weight in animals continued on the 2% casein diet (Table 2). During this period, protein-restricted rats that underwent gastrostomy and were refed with the 20% casein diet gained more weight than rats without surgery receiving the 20% casein diet. No significant difference in body weight gain was observed between the operated and nonoperated groups receiving the 2% casein diet, indicating that gastrostomy did not result in a further decrease in body weight in protein-restricted animals. Chronic dietary protein restriction (19 days) decreased liver and colon weight by 48% and 36%, respectively. Dietary protein repletion with 20% casein for 3 days (group IV) almost entirely restored liver weight to control levels, but colon weight was not affected.

During the 3-day postsurgical refeeding period, rats fed the 20% casein diet consumed more food than rats fed the 2% casein diet with or without gastrostomy. However, since body

Table 2. Body Weight Gain and Energy Intake During Days 16 to 19

Group	No.	Body Weight Change (g)	Liver Weight (g)	Colon Weight (g/15 cm)	Energy Intake (kcal/kg · d)
I: 20% casein	10	27.9 ± 1.4	15.9 ± 0.3‡	0.96 ± 0.1‖	251.6 ± 8.7
II: 2% casein	8	−0.2 ± 0.9*	8.3 ± 0.3	0.61 ± 0.1	279.7 ± 14.1
III: 2% casein preop 2% casein postop	6	3.3 ± 1.3*	9.5 ± 0.5	0.55 ± 0.1	303.0 ± 35.9
IV: 2% casein preop 20% casein postop	6	47.9 ± 1.7†	14.2 ± 0.5§	0.6 ± 0.1	319.8 ± 31.0

NOTE. Results are the mean ± SE; significance was established by 1-way ANOVA with Tukey test correction.

*$P < .001$ *v* groups I and IV.

†$P < .01$ *v* group I.

‡$P < .05$ *v* all other groups.

§$P < .001$ *v* groups II and III.

‖$P < .01$ *v* all other groups.

weight was lower in animals fed the 2% casein diet, dietary energy intake was not different among all experimental groups after correction for body weight (kilocalories per kilogram per day) (Table 2).

Plasma Glucose, Insulin, Leucine, and Albumin

Consistent with the dietary protein-restricted state, rats fed the 2% casein diet (with or without gastrostomy) had lower plasma levels of insulin, leucine, and albumin than animals receiving the 20% casein diet (Table 3). In contrast, plasma glucose concentrations were not altered by the 2% casein diet, demonstrating maintenance of glucose homeostasis during chronic dietary protein restriction. In the protein-restricted state, gastrostomy resulted in a 16% decrease in plasma glucose levels without a further decrease in insulin, leucine, or albumin levels. Dietary protein repletion for 3 days after surgery increased plasma glucose, insulin, leucine, and albumin to levels similar to those in control rats fed continuously with the 20% casein diet (Table 3).

Table 3. Plasma Levels of Glucose, Insulin, Leucine, and Albumin

Group	No.	Glucose (mg/dL)	Insulin (μU/mL)	Leucine (μmol/mL)	Albumin (g/dL)
I: 20% casein	10	140 ± 2	78.0 ± 4.6	0.23 ± 0.02‡	3.5 ± 0.1§
II: 2% casein	8	141 ± 4	25.6 ± 0.5†	0.15 ± 0.01	2.8 ± 0.1
III: 2% casein preop 2% casein postop	6	119 ± 5*	32.3 ± 3.8†	0.17 ± 0.01	2.6 ± 0.1
IV: 2% casein preop 20% casein postop	6	135 ± 5	74.1 ± 7.7	0.28 ± 0.02§	3.6 ± 0.2§

NOTE. Results are the mean ± SE; significance was established by 1-way ANOVA with Tukey test correction.

*$P < .001$ *v* all other groups.

†$P < .001$ *v* groups I and IV.

‡$P < .05$ *v* group II.

§$P < .01$ *v* groups II and III.

Plasma IGF-I and Hepatic and Colonic mRNA

Plasma IGF-I concentrations decreased markedly (65%) when rats were fed the 2% casein diet compared with the 20% casein diet (Fig 1). Since the liver is a major source of circulating IGF-I, this decrease may be explained, in part, by the observed 50% decrease in hepatic IGF-I mRNA levels in rats fed the 2% casein diet (Fig 2). In contrast to these findings in the liver, there was a smaller decrease in colonic IGF-I mRNA levels with chronic dietary protein restriction that was not statistically significant (Fig 3). Colonic IGF-I receptor expression was significantly increased in rats fed the 2% casein diet ($P < .01$; Fig 4).

In the protein-restricted state, gastrostomy led to a further 40% decrease in plasma IGF-I concentration (Fig 1). This response was prevented, and instead, there was a 40% increase in plasma IGF-I if protein-restricted rats were refed a 20% casein diet for 3 days following gastrostomy. The decrease in plasma IGF-I levels after gastrostomy in protein-restricted animals cannot be explained by changes in hepatic IGF-I mRNA abundance (Fig 2), although the increase in plasma IGF-I in rats fed the 20% casein diet postoperatively correlated with a complete normalization of hepatic IGF-I mRNA (Fig 3). In the colon, there was a greater than twofold increase in the level of IGF-I mRNA after gastrostomy, which was not altered by refeeding animals the 20% casein diet (Fig 3). The surgical procedure did not affect colonic IGF-I receptor mRNA expression in protein-restricted rats, and IGF-I receptor mRNA was decreased after 3 days of postoperative dietary protein repletion to the same level as in nonoperated rats maintained continuously on the 20% casein diet (Fig 4).

Plasma IGFIBPs

Western ligand blotting was used to determine the effects of dietary protein restriction and surgical stress on plasma IGFBP

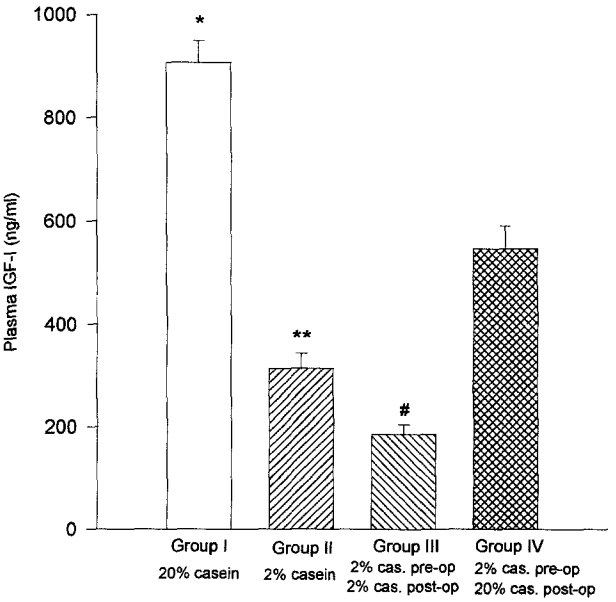

Fig 1. Effects of dietary protein and gastrostomy on plasma IGF-I levels. Plasma was subjected to acid-ethanol extraction, and IGF-I was determined by radioimmunoassay. *$P < .001$ *v* group IV; **$P < .001$ *v* groups I and IV; #$P < .001$ *v* all other groups.

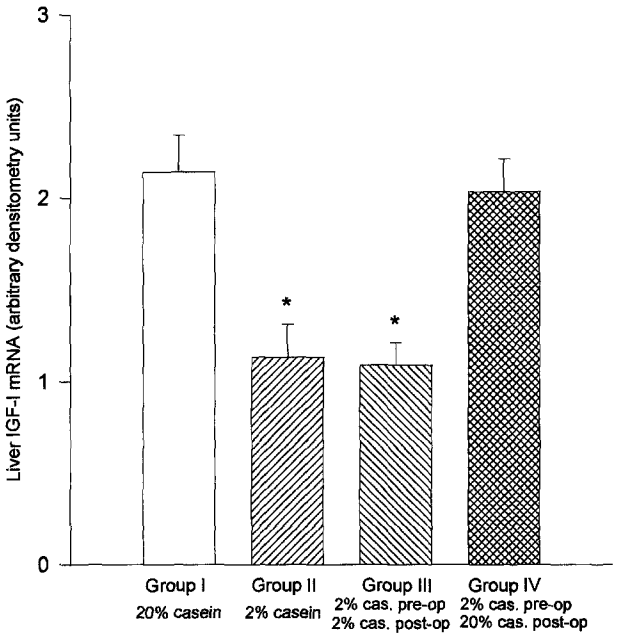

Fig 2. Liver IGF-I mRNA content. The 7.8-kb IGF-I mRNA transcript identified on Northern blots was quantified by phosphorimaging. The 18S ribosomal control RNA was shown to be similar in all groups by stripping and reprobing the same blots. *$P < .01$ v groups I and IV.

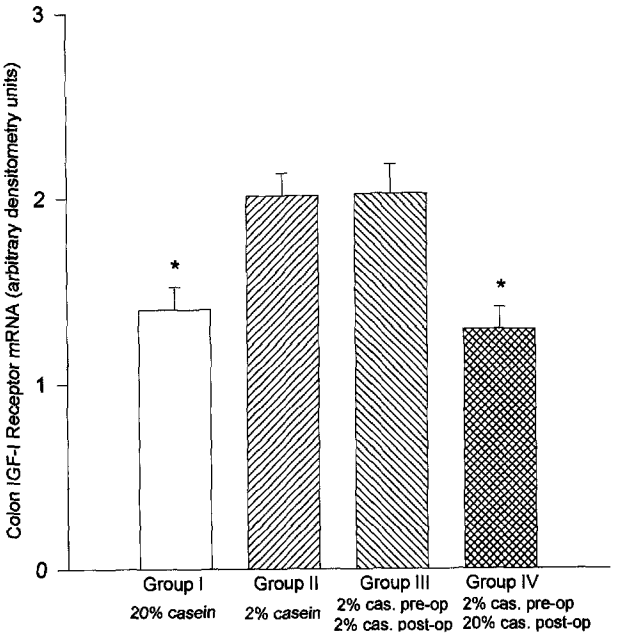

Fig 4. Colon IGF-I receptor mRNA content. The 11.0-kb IGF-I receptor transcript mRNA identified on Northern blots was quantified by phosphorimaging. The 18S ribosomal control RNA was shown to be similar in all groups by stripping and reprobing the same blots. *$P < .01$ v groups II and III.

levels (Fig 5). IGFBPs with approximate molecular weights of 30,000 (IGFBP-1 and -2) and 45,000 (IGFBP-3) were significantly decreased in rats fed the 2% casein diet. Gastrostomy did not alter the levels of these IGFBPs in the protein-restricted state. Postsurgical dietary repletion for 3 days with the 20% casein diet fully normalized levels of the 30,000– and 45,000–molecular weight IGFBPs. The concentration of plasma IGFBP-4, detected as a band migrating at 24,000 molecular weight, was not significantly affected by dietary protein restriction or gastrostomy.

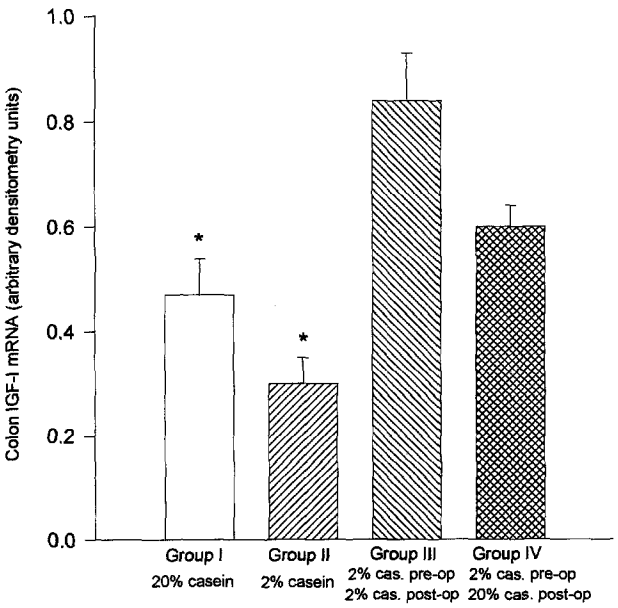

Fig 3. Colon IGF-I mRNA content. The 7.8-kb IGF-I mRNA transcript identified on Northern blots was quantified by phosphorimaging. The 18S ribosomal control RNA was shown to be similar in all groups by stripping and reprobing the same blots. *$P < .005$ v groups III and IV.

DISCUSSION

Administration of a 2% protein diet to adult laboratory rats resulted in significant malnutrition at the whole-body level manifested by weight loss and decreased plasma concentrations of leucine, albumin, and IGF-I, consistent with published reports.[32] Plasma levels of IGF-I were reduced by 65% after 2 weeks of dietary protein restriction, confirming observations that circulating IGF-I is a sensitive nutrient-dependent factor.[13,32] This change in plasma IGF-I concentration was associated with a decrease in IGF-I mRNA in the liver, a major source of circulating IGF-I, as previously reported in protein-depleted rats.[33] The mechanisms responsible for the decrease of hepatic IGF-I mRNA are unclear. Although growth hormone (GH) and its binding protein are major factors in regulating the IGF-I axis,[13,33] recent evidence indicates that GH resistance plays a more important role than decreased GH secretion or GH binding in protein-restricted rats.[33,34] In contrast to IGF-I mRNA in the liver, IGF-I mRNA content in the colon was not significantly altered by feeding a protein-depleted diet. This finding is suggestive of persistent local paracrine or autocrine effects of IGF-I, which may help to explain our previous observation that dietary protein restriction does not alter colonic crypt-cell proliferation.[35] The failure of a low-protein diet to affect IGF-I mRNA abundance in the colon compared with the liver may be a consequence of tissue-specific nutrient utilization. Since short-chain fatty acids produced by bacterial fermentation represent a substantial energy source for the colon, and endogenous protein secretion and desquamated intestinal mu-

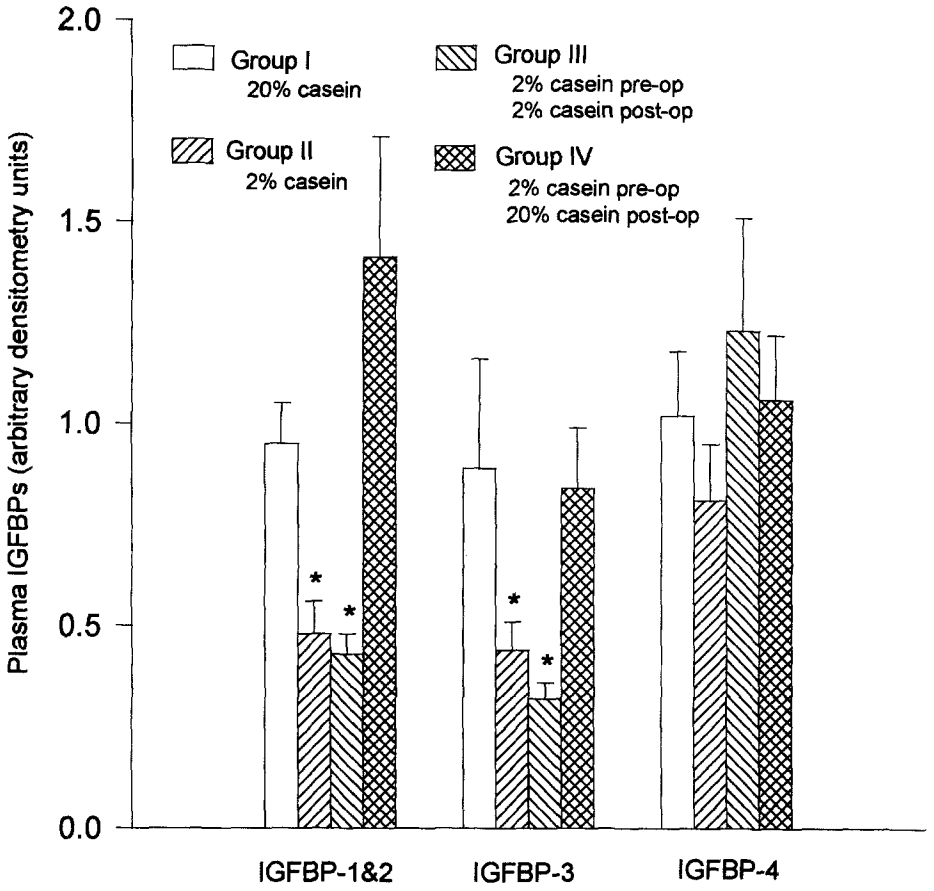

Fig 5. Effects of dietary protein and gastrostomy on plasma levels of IGFBPs. Bands corresponding to the indicated IGFBPs on radioligand blots were quantified by phosphorimaging. Data are expressed as arbitrary densitometry units. *$P < .05$ *v* groups I and IV.

cosa provides a source of protein,[36-38] the colon may be relatively protected during periods of severe nutrient deprivation in comparison to other nutrient-sensitive organs such as the liver.

The colonic response to dietary protein restriction includes not only persistent expression of IGF-I mRNA, but also an increase in colonic IGF-I receptor mRNA. A similar increase in IGF-I receptor mRNA abundance has previously been observed in other extrahepatic tissues in fasted rats.[39] During times of nutrient restriction, when the circulating (endocrine) source of IGF-I is diminished, increased IGF-I receptor expression and maintained local IGF-I production in the colon may provide a compensatory response that serves to preserve the colonic mucosa.

Gastrostomy surgery resulted in a further decrease in plasma IGF-I concentrations in protein-restricted rats without affecting plasma albumin levels. The change in circulating IGF-I levels in response to the acute catabolic stress of surgery is consistent with some[25-26,40] but not all[41] published reports. Hepatic expression of IGF-I mRNA and plasma IGFBPs was not altered after gastrostomy, suggesting that these components of the IGF-I system did not contribute to the stress-induced decrease in plasma IGF-I levels. Further studies will be required to determine whether stress-induced changes in hepatic IGF-I mRNA translation, IGF-I gene expression in extrahepatic tissues, or IGF-I clearance rates contribute to the decrease in plasma IGF-I concentrations following surgical stress in protein-restricted rats. In contrast to the liver, colonic IGF-I mRNA was markedly increased after gastrostomy despite continued dietary protein restriction. This is similar to previously reported findings demonstrating increased colonic IGF-I mRNA levels in rats following partial small-bowel resection.[20] The underlying mechanisms responsible for this induction of IGF-I are unknown and require further investigation. The increase in colonic IGF-I mRNA as a consequence of surgical procedures involving noncolonic gastrointestinal tissues suggests that the response is systemic or at least regional, which is consistent with the present knowledge about systemic effects of surgical stress.[42]

The mechanism of the selective increase in liver versus colon weight in response to dietary protein repletion is unknown. This could result from tissue-specific differences in the kinetics of the growth response to nutrients in the liver and colon. The rapid increase in liver weight also could reflect, in part, acute-phase protein synthesis in the liver induced by the surgical stress. Dietary protein repletion after gastrostomy increased plasma IGF-I and hepatic IGF-I mRNA levels, although plasma IGF-I concentrations were still depressed in comparison to levels in operated rats fed the 20% casein diet throughout the study. This may reflect persistent translational or posttranslational defects in IGF-I synthesis during the relatively brief refeeding period.[43] Alternatively, partial restoration of IGF-I levels may represent the balance between the effects of nutrient depletion and stress associated with the surgical procedure.

In the present study, dietary protein restriction decreased plasma levels of IGFBP-3 (45 kd) and the sum of IGFBP-1 and

IGFBP-2 (30 kd). The reduction in the circulating IGFBP-3 level is consistent with reports demonstrating a decrease in serum IGFBP-3 and hepatic IGFBP-3 mRNA levels in rats fed a low-protein diet.[44,45] Although effects of protein restriction on serum concentrations of IGFBP-1 and -2 in rats have not been extensively investigated, our data are consistent with the findings in one study describing a decrease in 30-kd plasma IGFBPs using ligand blotting analysis, which correlated with a reduction in serum IGFBP-2 levels determined by immunoblotting with a specific IGFBP-2 antibody.[44] This contrasts with reports of increased circulating IGFBP-1 and -2 in chronically malnourished humans,[46,47] possibly reflecting differences in nutrient deprivation or species differences between rats and humans. In protein-restricted rats, IGF-I is preferentially bound to IGFBPs as a small 30-kd complex.[13] These small binding protein complexes in the systemic circulation may facilitate the transport of IGF-I across the capillary endothelium and increase the bioavailability of IGF-I to the colon.[48] In support of this possibility, accelerated clearance of IGF-I in dietary protein-restricted rats has been shown to result from more rapid distribution of IGF-I into tissues and not from a change in IGF-I degradation.[49]

In summary, chronic protein malnutrition leads to decreased plasma IGF-I and IGFBP concentrations. Hepatic IGF-I mRNA content is decreased in accordance with the role of the liver as an important source of circulating IGF-I. Although the mean colonic IGF-I mRNA level was lower in protein-restricted rats, this difference was not significant. There was an upregulation of colonic IGF-I receptor mRNA levels, which may serve to maintain the level of anabolic IGF-I signaling in the colon by autocrine or paracrine mechanisms. Tissue IGF-I concentrations appear to be important for some organs in states of protein deprivation, and growth under these conditions may be dependent on local production of IGF-I rather than systemic IGF-I.[50,51] Although the underlying mechanism is unknown, the present study demonstrates that minor gastrointestinal surgery has specific effects on IGF-I gene expression in the colon that highlight the importance of both endocrine and paracrine/autocrine regulation by this important tissue growth factor.

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