Zinc Deficiency–Induced Anorexia Influences the Distribution of Serum Insulin-Like Growth Factor–Binding Proteins in the Rat

Michael S. Clegg, Carl L. Keen, and Sharon M. Donovan

Zinc (Zn) deficiency can result in severe growth retardation in mammals, and in a number of animal model systems it leads to low circulating insulin-like growth factor-I (IGF-I) concentrations. Using a weanling male rat model and a number of feeding schemes, we show that in addition to lower circulating IGF-I concentrations, Zn deficiency leads to alterations in the distribution of serum IGF-binding proteins (IGFBPs). Serum from Zn-deficient animals labeled in vitro with [^{125}I]IGF-I displayed three peaks of tracer activity: 150 kd (IGFBP-3), 37 kd (IGFBP-2 and -1), and 8 kd (free [^{125}I]IGF-I). Relative to controls, Zn-deficient animals demonstrated more tracer binding in the 37-kd region, whereas less was found in the 150- and 8-kd peaks. Serum from chronically calorie-restricted fed animals displayed [^{125}I]IGF-I binding profiles similar to Zn-deficient serum, implicating Zn deficiency–induced anorexia as the principle factor underlying both the lower circulating IGF-I and the alterations in IGFBP profiles. Concentrations of IGFBP-4 were unaffected by diet manipulation based on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)/Western ligand blot (WLB) analysis.

THE ESSENTIALITY of zinc (Zn) in mammalian systems was established by Todd et al[1] in 1934. Subsequent to this publication, a concerted effort has been directed toward understanding the mechanism(s) underlying the myriad of structural, physiological, and biochemical defects common to Zn deficiency.[2,3] One of the most striking of these defects is the extensive growth retardation that occurs in animals subjected to a Zn-deficient diet in utero and/or during early postnatal development. Anorexia is a common trait noted in Zn-deficient animals,[4] which contributes significantly to their poor growth and tissue maintenance. Indeed, it has been hypothesized that abnormal Zn metabolism may be an etiologic factor in the development of anorexia nervosa.[5]

Although the mechanisms underlying the wasting and growth retardation in Zn-deficient animals are poorly understood, it is reasonable to speculate that alterations in growth factor metabolism contribute to these phenomena. Normal tissue growth and maintenance is greatly influenced by circulating growth hormone acting alone and/or through its influence on the synthesis and action of insulin-like growth factor-I (IGF-I). Biological activity of IGF-I is modulated to a large extent by a number of high-affinity binding proteins collectively termed the IGF-binding proteins (IGFBPs).[6]

It is known that both IGF-I and IGFBPs are developmentally and nutritionally regulated.[7-10] Animals consuming Zn-deficient diets have low circulating levels of IGF-I, consistent with their poor nutritional status. For example, Oner and Bor[11] reported a 50% decrease in circulating IGF-I levels in Zn-deficient rats. Subsequent studies found that Zn-deficient rats had significantly lower serum IGF-I levels as compared with pair-fed groups and Zn-adequate control groups.[12-14] In contrast, Bolze et al[15] did not detect differences in serum IGF-I levels between Zn-deficient animals and conventional pair-fed controls. The effect of Zn deficiency on the distribution of circulating IGFBPs was not examined in the above studies.

In the present study, weanling male rats were used to examine the effects of Zn deficiency on the distribution of serum IGFBPs using native molecular-sieve chromatography and denaturing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)/Western ligand blot (WLB) techniques. Furthermore, we compared the state of Zn deficiency with food restriction by using a number of conventional and depletion-repletion feeding schemes in an attempt to distinguish the effects specifically caused by the lack of Zn from those indirectly caused by inanition.

MATERIALS AND METHODS

Animals and Diet

Male weanling rats (day 17) were purchased from a commercial source (Bantin and Kingman, Richmond, CA). Upon arrival, the animals were randomly assigned to one of eight groups: control (CON), 25 μg Zn/g diet fed ad libitum for 17 days; restricted-fed (RES), 25 μg Zn/g diet fed at 5.5 g/d for 17 days; restricted-fed, zinc-deficient (RZD), 0.5 μg Zn/g diet fed at 5.5 g/d for 17 days; ad libitum–fed, zinc-deficient (ADLIBZD), 0.5 μg Zn/g diet fed ad libitum for 17 days; RZDtoCON, RZD diet and feeding protocol for 13 days followed by CON diet and feeding protocol for 4 days; REStoCON, RES diet and feeding protocol for 13 days and CON diet and feeding protocol for 4 days; CONtoRZD, CON diet and feeding protocol for 13 days and RZD diet and feeding protocol for 4 days; and CONtoRES, CON diet and feeding protocol for 13 days and RES diet and feeding protocol for 4 days.

Detailed components of the diet have been described previously.[16] Animals were individually housed in suspended stainless steel cages and received distilled water ad libitum. The experimental protocol was approved by the University of California, Davis Animal Use and Care Administrative Committee, and animals were cared for in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals.* Food intakes and body weights were monitored daily. Animals were killed by CO_2 exposure followed by decapitation. Trunk blood was collected and incubated on ice for 30 minutes. Serum was collected by centrifuga-

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tion at 800 × *g* at 4°C for 30 minutes and stored in siliconized tubes at −70°C until analysis.

Trace Element Analysis

Serum samples (10 μL) were diluted with 4.0 mL metal-free 0.5-mol/L ammonium acetate, pH 6.8. Zinc concentrations were determined by flameless atomic absorption using a Thermal Jarrell Ash Video 12E atomic absorption spectrophotometer and associated Model 188 furnace atomizer with Fastac deposition (Thermal Jarrell Ash, Franklin, MA).

Native Column Chromatography

Serum samples (125 μL) were thawed and incubated with 150,000 cpm [^{125}I]IGF-I (specific activity, 2,000 Ci/mmol/L; Amersham, Arlington Heights, IL) and 125 μL elution buffer (25 mmol/L HEPES/150 mmol/L NaCL) for 4 hours at 4°C. Samples were then centrifuged at 10,000 × *g* for 10 minutes at 4°C, and 200 μL of the supernates were applied to a molecular-sieve Superose 12 column preequilibrated with elution buffer and attached to a fast protein liquid chromatography (FPLC) chromatography system (Pharmacia, Piscataway, NJ). Flow rate was 0.25 mL/min, and 0.25-mL fractions were collected for subsequent isotope quantitation by gamma counting (Packard Instruments, Meriden, CT). The Superose 12 column was calibrated with various molecular-weight standards including β-amylase (200 kd), alcohol dehydrogenase (150 kd), alkaline phosphatase (92 kd), serum albumin (66 kd), egg albumin (45 kd), carbonic anhydrase (29 kd), cytochrome *c* (12.5 kd), and aprotinin (6.5 kd).

Radioimmunoassay of IGF-I

To dissociate IGFs from the associated binding proteins, serum samples (500 μL) were chromatographed in 0.2 mol/L formic acid on a 0.9 × 100-cm column containing Sephadex G-50 (fine; Pharmacia). Serum was applied to the column, and fractions containing the IGF peptide (46 to 71 mL) were collected in 50-mL tubes containing 0.25 mL radioimmunoassay buffer (0.03 mol/L sodium phosphate, 0.25% bovine serum albumin, and 0.02% sodium azide, pH 7.5); samples were then frozen and lyophilized. IGF peptide fractions were resolubilized in radioimmunoassay buffer without added bovine serum albumin. IGF-I content was measured using [^{125}I]IGF-I as radioligand and a polyclonal anti–somatomedin C/IGF-I antibody generated by Drs Underwood and Van Wyk, University of North Carolina at Chapel Hill, and distributed through the National Hormone and Pituitary Program. Interassay and intraassay coefficients of variation for the assay were 7% and 9%, respectively.

WLB

Molecular forms of serum IGFBPs were characterized by WLB using the method reported by Hossenlopp et al.[17] Serum samples (3 μL) were mixed with sample buffer (β-mercaptoethanol–free) and heated at 100°C for 5 minutes. Samples were loaded onto 12% SDS-PAGE gels that were electrophoresed overnight at 70 V and 4°C. Proteins were electrotransferred to nitrocellulose (0.45 μm; Micron Separations, Westborough, MA) using a Labconco semidry transfer apparatus (Labconco, Kansas City, MO). Nitrocellulose membranes were sequentially incubated with Tris-buffered saline (TBS) (0.15 mol/L sodium chloride and 0.01 mol/L Tris hydrochloride) containing 3% Nonidet P-40, TBS containing 1% bovine serum albumin, and TBS containing 0.1% Tween 20 at 4°C. Membranes were then incubated overnight at 4°C with 0.5 μCi [^{125}I]IGF-I, washed with TBS, and air-dried. IGFBPs were visualized by exposure to Kodak X-Omat AR film (Rochester, NY) for 7 days at −70°C. Relative intensities of IGFBP bands on the autoradiographs were determined using the FotoAnalyst II Imager System and College Software (Fotodyne, New Berlin, WI).

Statistical Analysis

Data were analyzed using one-way ANOVA (SPSS for Windows; SPSS, Chicago, IL). When F values proved significant, post hoc group comparisons were made using Duncan's multiple-range test.

RESULTS

Body Weights

CON and ADLIBZD groups consuming experimental diet for 4 days had similar mean body weights, which were higher ($P < .05$) than mean body weights of animals in RZD and RES groups (Fig 1A). By day 5, this relationship was no longer evident, since the ADLIBZD group voluntarily reduced their food intake, which eventually developed into a cyclical food intake pattern (data not shown). The decrease in food intake resulted in lower body weights relative to the CON group ($P < .05$) after day 5, which were similar to the mean body weights of RES and RZD groups (Fig 1A). The relationship among body weights (ie, CON ≫ RES = RZD = ADLIBZD) remained through-

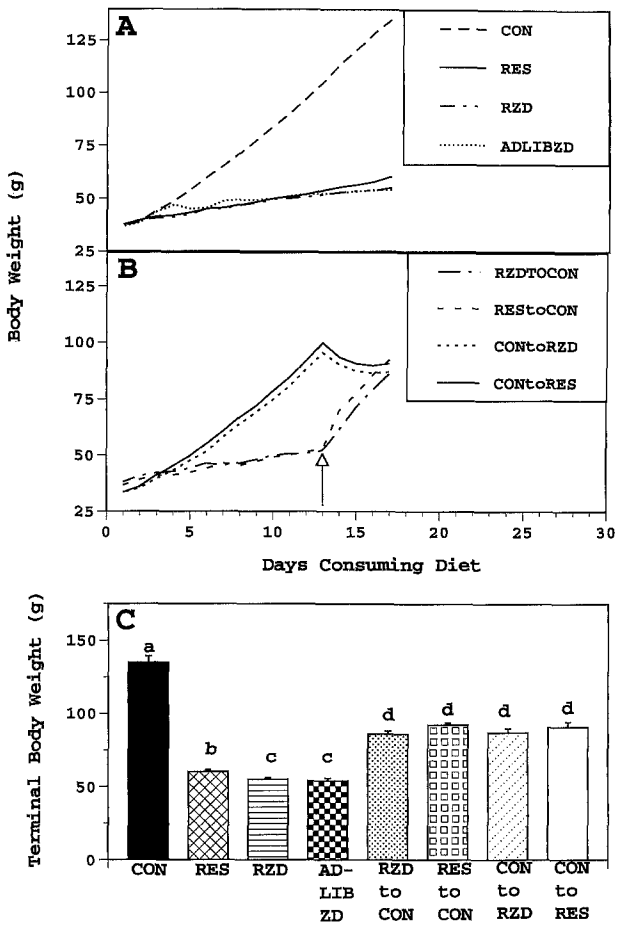

Fig 1. (A) and (B) Body weights as a function of days consuming experimental diet (mean). (C) Terminal body weights at conclusion of the 17-day study (mean ± SEM). Each group contained 4 to 10 animals. [b,c,d]Significantly different from the CON group at $P < .05$.

out the duration of the study. At termination of the study, mean body weight of the RES group was 50% that of the CON group, but was higher ($P < .05$) than that of the two Zn-deficient groups (ie, RZD or ADLIBZD; Fig 1A and C).

Mean body weights of the remaining dietary groups with depletion-repletion or repletion-depletion schemes are shown in Fig 1B. As expected, RZDtoCON and REStoCON groups had body weights similar to RZD and RES groups during the initial 12 days of the study. On day 13, both groups were switched to the 25-μg Zn/g diet and fed ad libitum (see arrow in Fig 1B indicating time line of the diet change); this change in dietary protocol resulted in increased body weights (Fig 1B) in these two groups. Growth rates of the two groups during the repletion period were compared (eg, linear regression analysis of the respective body weight *v* time plots and subsequent comparison of the slopes with an unpaired two-tailed *t* test). The REStoCON group (9.4 ± 0.4 g/d, mean ± SD) had a faster growth rate ($P < .05$) than the RZDtoCON group (8.5 ± 0.6 g/d).

The two repletion-depletion groups, CONtoRES and CONtoRZD animals, had body weights similar to those of the CON group during the first 12 days of the study (Fig 1B). On day 13, these animals were switched from the ad libitum–fed CON protocol (25 μg Zn/g diet) to the RZD diet (0.5 μg Zn/g diet at 5.5 g/d) or RES diet (25 μg Zn/g diet at 5.5 g/d), which quickly resulted in decreased body weights for both groups. The decrease in growth rates experienced by both groups was curvilinear and similar in magnitude (Fig 1B).

In summary, at termination of the study, all four repletion-depletion or depletion-repletion groups had (1) similar mean body weights, (2) lower mean body weights relative to the CON group ($P < .05$), and (3) higher mean body weights relative to RES, RZD, and ADLIBZD groups ($P < .05$; Fig 1C).

Circulating IGF-I and Zn Concentrations

Serum IGF-I concentrations were significantly higher in the CON group (Table 1) than in all other groups. The RES group and both Zn-deficient groups had low circulating IGF-I concentrations as compared with all other groups; there were no significant differences among RES and Zn-deficient groups (Table 1). Depletion-repletion and repletion-depletion groups (ie, RZDtoCON, REStoCON, CONtoRES, and CONtoRZD) had serum IGF-I concentrations that were higher ($P < .05$) than those of the RES, RZD, and ADLIBZD groups (Fig 2). The depletion-repletion groups (RZDtoCON and REStoCON) had higher ($P < .05$) serum IGF-I concentrations than the repletion-depletion groups (CONtoRZD and CONtoRES). There were no significant differences in IGF-I concentrations between the two depletion-repletion groups (ie, RZDtoCON *v* REStoCON) or the two repletion-depletion groups (CONtoRZD *v* CONtoRES). A linear regression analysis of the relationship between mean IGF-I concentrations and final body weights for all groups yielded a significant correlation coefficient of .80 ($P < .05$; data not shown).

Serum Zn concentrations in the two Zn-deficient groups (ie, RZD and ADLIBZD) and the Zn repletion-depletion group (CONtoRZD) were similar; these three groups had lower serum Zn ($P < .05$) than all other dietary groups (Table 1). The two depletion-repletion groups (RZDtoCON and REStoCON) did not differ from the CON group; these three groups had higher serum Zn concentrations than RZD, ADLIBZD, and CONtoZD groups ($P < .05$; Table 1). The short-term restriction group (CONtoRES) had lower ($P < .05$) serum Zn concentrations than the CON group, the long-term restriction (RES) group, and the two depletion-repletion groups, RZDtoCON and REStoCON. The CONtoRES group had higher serum Zn concentrations than RZD, ADLIBZD, and CONtoRZD groups ($P < .05$; Table 1).

Table 1. Serum IGF-I and Zinc in Rats Consuming Various Experimental Diets for 17 Days

Group	Serum IGF-I (ng/mL)	Serum Zn (μmol/L)
CON	375 ± 17[a]	27.5 ± 1.2[a]
RES	68 ± 11[b]	25.8 ± 2.6[a]
RZD	60 ± 6[b]	3.8 ± 0.9[b]
ADLIBZD	63 ± 4[b]	3.5 ± 0.3[b]
RZDtoCON	184 ± 15[c]	29.9 ± 1.3[a]
REStoCON	199 ± 21[c]	27.9 ± 2.3[a]
CONtoRZD	97 ± 16[d]	6.8 ± 2.2[b]
CONtoRES	109 ± 14[d]	17.7 ± 1.5[c]

NOTE. Data are expressed as the mean ± SEM. Different superscripts indicate values that are significantly different ($P < .05$). The number of animals varied from 4 to 11 per group.

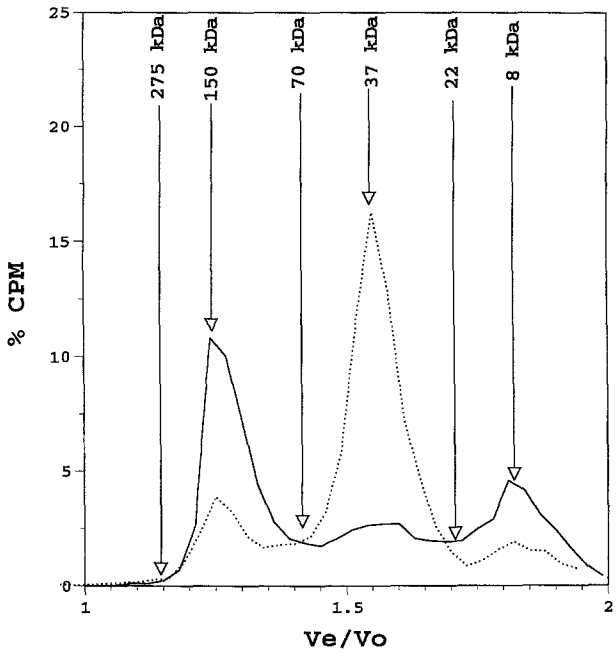

Fig 2. Native gel-permeation chromatography (Superose 12) of serum. Serum (125 μL) was incubated with sample buffer (125 μL) and 1 × 10^5 cpm [^{125}I]IGF-I for 4 hours at 4°C. Representative chromatograms for animals experiencing a high plane of nutrition (CON, RZDtoCON, and REStoCON groups, ——) and a low plane of nutrition (RES, RZD, ADLIBZD, CONtoRZD, and CONtoRES groups, ...) are shown. Vo, volume.

Serum Tracer Binding/Native Molecular-Sieve Chromatography Studies

Consistent with other studies,[18-20] equilibration of [^{125}I]IGF-I with serum and subsequent size fractionation under native conditions yielded three major peaks of [^{125}I]IGF-I activity (Fig 2). Peak 1 encompassed the molecular weight range of 250 to 70 kd and had an elution volume (Ve) of 150 kd, corresponding to the complex of IGFBP-3, the acid-labile subunit, and IGF-I. Peak 2 encompassed the molecular weight range of 70 to 22 kd, with a Ve of 37 kd; this peak contains several IGFBPs including IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-3 minus the acid-labile subunit, and the 29-kd glycosylated amino-terminal fragment of IGFBP-3 minus the acid-labile subunit.[18-20] Peak 3 encompassed a molecular weight range of 22 to 4.5 kd, and had a Ve of 8 kd, which represents the contribution of free [^{125}I]IGF-I tracer and any proteolyzed IGFBP products that still may be able to bind IGF-I. The small (ie, $<1\%$ of total counts) high–molecular-weight peak that was frequently seen at 275 kd most likely represents [^{125}I]IGF-I bound to circulating IGF-II receptor.[21] The consistent shoulder seen at 120 kd most likely represents the 29-kd amino-terminal IGFBP-3 fragment associated with the 85-kd acid-labile subunit and the 7.5-kd IGF-I and/or the underglycosylated IGF BP-3 complex. Attempts to consistently resolve this peak from the 150-kd peak using commercially available deconvolution/gaussian-fit software failed, and thus the area associated with this binding activity is included in the area for the 150-kd complex.

The three major [^{125}I]IGF-I peaks were found in all dietary groups examined; however, the magnitude of each peak was dependent on the animal's nutritional status. Profiles for all dietary groups could be represented as the two extreme patterns displayed in Fig 2 or amalgamations thereof. Figure 3A to C illustrates the integrated areas contained under each of three major [^{125}I]IGF-I–containing peaks. Specifically, the CON group, which experienced the highest plane of nutrition throughout the study, had the majority of the [^{125}I]IGF-I binding activity associated with the 150-kd peak (Figs 2 and 3A). A similar finding was made for the depletion-repletion groups (REStoCON and RZDtoCON; Figs 2 and 3A). In addition, these three groups had significantly less [^{125}I]IGF-I associated with the peak centered in the 37-kd region and significantly more free tracer than the remaining dietary groups (Figs 2 and 3B).

In contrast, animals experiencing an extremely poor plane of nutrition (ie, RZD, RES, and ADLIBZD groups) or a relatively poor plane of nutrition (CONtoRZD and CONtoRES groups) showed the highest percentage of [^{125}I]IGF-I tracer binding in the 37-kd region (Figs 2 and 3B). In contrast to the groups with a high plane of nutrition, these five groups had significantly less tracer binding associated with either the 150-kd or the 8-kd/free tracer region (Fig 3A and C).

WLB

Representative WLBs of IGFBPs in rat serum from the various dietary groups are illustrated in Fig 4A and B. CON and ADLIBZD groups were included in both WLB. All

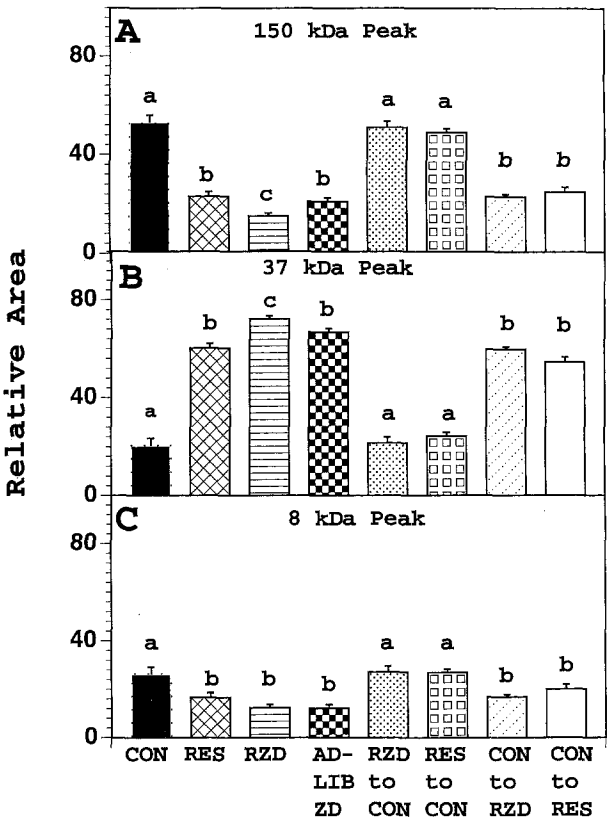

Fig 3. Relative peak areas of [^{125}I]IGF-I binding obtained under native conditions. Serum was labeled in vitro with [^{125}I]IGF-I as described in Fig 2. Chromatograms were integrated using commercially available software; integrated areas represent [^{125}I]IGF-I binding to proteins found centered at (A) 150 kd, (B) 37 kd, or (C) 8 kd (free tracer). Each individual peak area was normalized to the total summed area of the three peaks. Values are the mean ± SEM from 4 to 11 animals in each group. [b,c]Significantly different from the CON group at $P < .05$.

dietary groups demonstrated three major areas of [^{125}I]IGF-I ligand binding: four bands at 36 to 42 kd, a triplet at 28 to 30 kd, and a single band at 24 kd. The quartet at 36 to 42 kd represents various glycosylated species of IGFBP-3, and the triplet at 28 to 30 kd most likely represents the combined contribution of IGFBP-1, IGFBP-2, and the proteolytic *N*-terminal fragment of IGFBP-3.[22] The activity associated at 24 kd most likely represents the contribution of IGFBP-4.

Figure 5A to C illustrates the integrated areas obtained from densitometric scans of the WLBs displayed in Fig 4A and B; data have been normalized to the respective areas found for the CON group. With respect to the quartet of bands found in the 36- to 42-kd region, CON and REStoCON groups showed significantly more [^{125}I]IGF-I tracer binding ($P < .05$) than any of the other dietary groups. The ADLIBZD group had less tracer binding ($P < .05$) in this molecular-weight region than all other dietary groups. In the 28- to 30-kd region, RES, RZD, and ADLIBZD groups displayed significantly less ($P < .05$) [^{125}I]IGF-I tracer binding than CON, RZDtoCON, CONtoRZD, REStoCON, and CONtoRES groups. The latter four groups were not significantly different from each other. Finally, in reference to the 24-kd band, only the

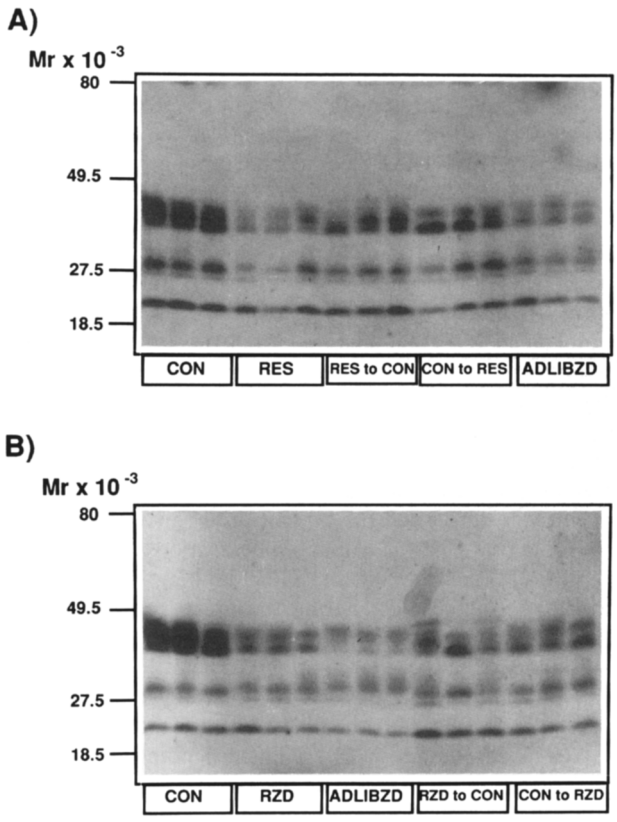

Fig 4. WLB of serum. Serum (3 μL) was mixed with sample buffer and fractionated under denaturing conditions (without β-mercaptoethanol) on a 12% acrylamide gel. Proteins were electrotransferred to nitrocellulose, probed with 0.5 μCi [^{125}I]IGF-I, and subjected to autoradiography. Three representative sera are shown for (A) CON, RES, REStoCON, and CONtoRES and (B) CON, RZD, ADLIBZD, RZDtoCON, and CONtoRZD groups. Molecular weights represent various purified standards chromatographed prior to serum.

ADLIBZD group differed ($P < .05$) from the CON group, displaying less [^{125}I]IGF-I tracer binding than all other dietary groups.

DISCUSSION

The expression of IGF-I and the IGFBPs are exquisitely sensitive to the general well-being of the animal. Dietary treatments or disease states known to reduce growth and/or induce tissue catabolism such as marasmus,[23] consumption of diets low in protein,[24-26] vitamin C,[27] magnesium,[14] or Zn,[11-15,28,29] and surgical trauma[30] all have been associated with decreased circulating concentrations of IGF-I. Cossack,[12] Oner et al,[13] and Dorup et al[14] reported that Zn-deficient animals had significantly reduced circulating IGF-I concentrations. In this report, we confirmed that Zn deficiency results in significant decreases in serum IGF-I. However, we did not observe a specific effect of Zn on circulating IGF-I levels; that is, neither of the two Zn-deficient groups differed significantly from the chronically food-restricted group. In this regard, our results are similar to those of Bolze et al,[15] who reported that circulating IGF-I concentrations in absolute pair-fed controls were not significantly different from those in their Zn-deficient counterparts. Differences among the studies may be due to the types of pair-fed controls used. Due to the inherent difficulty in finding an adequate control group for Zn-deficient groups, we used in our experimental design, a number of nutrient repletion or depletion groups in an attempt to further examine if there were specific Zn effects on circulating IGF-I concentrations.

In a depletion-repletion design, two age- and sex-matched groups of animals received either 5.5 g/d of the CON diet (REStoCON) or 5.5 g/d of the Zn-deficient diet (RZDtoCON) for 13 days, and then both groups received ad libitum quantities of CON diet for 4 days. At the end of the repletion period, neither serum Zn or IGF-I differed between the groups. In a follow-up repletion-depletion experiment, two age- and sex-matched groups received CON diet ad libitum for 13 days, and then one group received 5.5 g/d Zn-deficient diet (CONtoRZD) while the other group received the CON diet at 5.5 g/d (CONtoRES). Results from these dietary crossover designs strongly imply that (1) Zn status of the animal, as monitored by serum Zn concentrations, does not directly correlate with circulating IGF-I levels, (2) macronutrients such as protein and energy have a much larger role in controlling circulating IGF-I

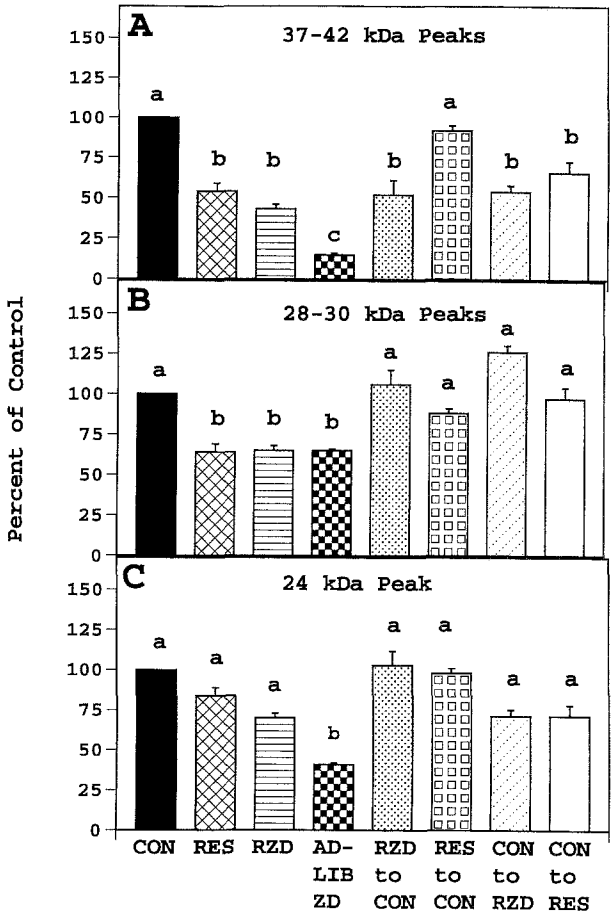

Fig 5. Densitometric analyses of autoradiographs obtained by WLB of serum samples displayed in Fig 4 were scanned, and areas of [^{125}I]IGF-I binding were quantified. [^{125}I]IGF-I binding was localized to 37 to 42 kd (A), 28 to 30 kd (B), and 24 kd (C). Data for individual groups were normalized to areas obtained from the CON group for each of the three indicated molecular weights. Values are the mean ± SEM. ^{b,c}Statistically different from the CON group at $P < .05$.

concentration than does Zn, and (3) changes in serum IGF-I level precede changes in body weight. For example, the two repleted-depleted groups and the two depleted-repleted groups had similar terminal body weights; however, serum IGF-I levels were significantly different, with the direction of change of the growth factor concentration indicating the future direction of body weight.

A second purpose of this study was to determine the effect of dietary Zn manipulation on the distribution of serum IGFBPs. Using native size-exclusion chromatography, three major serum IGFBP peaks were observed at 150, 37, and 8 kd. The 150-kd peak contains IGF-I, IGFBP-3, and the growth hormone–dependent acid-labile subunit.[31-33] Tracer binding to the 150-kd peak was reduced in malnourished animals (RES and CONtoRES groups) and Zn-deficient animals (RZD, ADLIBZD, and CONtoRZD groups). The apparent decrease in the 150-kd complex may be due to a decrease in serum IGFBP-3 expression, decreased production of the acid-labile subunit, proteolysis of serum IGFBP-3, and/or a reduced stability of the IGFBP-3 complex. Although circulating IGFBP-3 proteases have been observed in other catabolic states such as pregnancy[34] or after surgery, sera from CON, RES, and ADLIBZD animals did not show proteolytic activity when incubated with recombinant [^{125}I]IGFBP-3 (Michelle Oster, personal communication, November 1994).

The decrease observed in native 150-kd tracer binding was verified by denaturing WLB, which showed a decrease in the 38- to 41-kd glycosylated varients of IGFBP-3 from RES, RZD, and RES groups relative to the CON group. Total integrated areas of the IGFBP-3 bands were lower in the RZD than in the RES group; however, this does not appear to be a direct effect of Zn deficiency, since serum Zn concentrations were similar in RZD and ADLIBZD groups (ie, ADLIBZD group demonstrated tracer binding similar to that of the RES group).

The second major peak of native [^{125}I]IGF-I binding at 37 kd is heterogenous, consisting primarily of IGFBP-1 and IGFBP-2. Tracer binding in this molecular-weight region was higher in calorically deprived animals (RES and REStoCON) and Zn-deficient animals (ADLIBZD, RZD, and CONtoRZD) relative to CON animals. This observation is consistent with previous reports demonstrating an upregulation of IGFBP-1 and -2 in catabolic states such as chronic undernutrition, protein deficiency, surgical trauma, and type I diabetes.[35-39] These catabolic states are characterized by elevated circulating corticosterone and/or decreased circulating insulin; similar hormonal alterations have been reported in Zn-deficient animals.

When serum IGFBPs were examined by WLB, the density of 28- to 30-kd bands (corresponding to the 37-kd peak by column chromatography) was not higher in RES or Zn-deficient animals relative to CON animals. The 28- to 30-kd bands observed in the WLB consist of IGFBP-1, IGFBP-2, and a proteolytic fragment of BP-3. The WLB data appear to be at odds with data obtained by native chromatography. The former technique suggests that serum from RES, RZD, and ADLIBZD groups has less tracer binding in the 28- to 30-kd region, whereas the native chromatography technique conversely shows more tracer binding in this molecular-weight region relative to the CON group. This dichotomy may be explained by differences in endogenous IGF-I binding under native versus denaturing conditions. Under native conditions, the tracer is competing for binding with endogenous cold ligand, whereas under the denaturing conditions of SDS-PAGE and electroblotting, substantial endogenous IGF-I is stripped from the binding proteins, thus enhancing their ability to interact with tracer during the subsequent probing step. Additionally, potential posttranslational modifications of IGFBP-1/IGFBP-2[39] that may affect IGF-I binding under native conditions may be lost during the process of denaturing electrophoresis and electroblotting.

Hidden in the shoulder of the 37-kd peak detected under native conditions is the [^{125}I]IGF-I binding contributed by the 24-kd IGFBP-4. Although the native technique lacks sufficient resolution to discriminate binding among the dietary groups, WLB clearly resolves this binding activity and indicates that the expression of this binding protein was essentially independent of dietary treatment with the following exception: the ADLIBZD group showed less IGFBP-4 ($P < .05$) than all other dietary groups.

Finally, the third chromatographic peak (ie, 8 kd) represents mostly free tracer. Dietary conditions that downregulate IGF-I synthesis and concomitantly upregulate IGFBP-1 and IGFBP-2 levels consistently result in lower amounts of free tracer in serum when analyzed by native chromatography techniques. In our study, the malnourished groups (ie, RZD, ADLIBZD, RES, CONtoRZD, and CONtoRES) had significantly less free tracer relative to the nutrient-adequate groups (ie, CON, RZDtoCON, and REStoCON). Again, the effect appeared to be mostly a consequence of the anorexia, since both Zn-deficient groups were similar to the RES group.

In summary, Zn deficiency–induced effects on IGF-I and IGFBP metabolism appear similar to those generated by other catabolic states, including caloric deprivation.

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