Original Research

The influence of gestational zinc deficiency on the fetal insulin-like growth factor axis in the rat

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Abstract

The insulin-like growth factor (IGF) axis, a key regulator of embryonic growth and development, is exquisitely sensitive to the nutrient status of the animal. In addition to macronutrient deficiencies, zinc deficiency can impact the IGF axis. Gestational zinc deficiency is teratogenic, resulting in intrauterine growth retardation and structural abnormalities. The aim of this study was to investigate the effects of gestational zinc deficiency on the fetal IGF axis in a rat model. From gestation day (GD) 0.5, dams consumed zinc-deficient (ZD, 0.3 mg zinc/kg) or control (25 mg zinc/kg) diet *ad libitum*, while a third group of dams consumed the control diet in amounts equivalent to the food intake of the ZD dams (Paired group). On GD 19.5 fetal tissue, blood and amniotic fluid were collected. Fetal growth was significantly reduced by zinc deficiency compared with the Paired and Control groups. Fetuses from the Paired group were smaller compared with the Control, but only ZD fetuses had structural malformations. Amniotic fluid IGF-1 concentrations were significantly lower in the Paired group than in the ZD and Control groups. Plasma of ZD fetuses contained lower levels of IGF binding protein-1 when compared with fetuses in the Paired and Control groups. Fetal liver IGF-1 mRNA levels were lower in the ZD fetuses than in the Paired and Control fetuses. These observations suggest that differences in the fetal IGF axis between ZD and Paired groups contribute to the poor pregnancy outcome and enhanced fetal growth retardation observed with zinc deficiency.

Keywords: pregnancy, growth, birth defects, caloric restriction, teratology, placenta, IGF, IGFBP-1, IGFBP-2

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Introduction

Low maternal zinc status has been associated with numerous pregnancy complications, including gross structural malformations, intrauterine growth retardation (IUGR), premature birth and small for gestational age in multiple species including humans.[1,2] It has been estimated that among women of child-bearing age, approximately 50% have dietary zinc intakes that are less than what is currently recommended.[3] Importantly, even with an adequate consumption of zinc, an individual's plasma zinc concentrations can be low due to acute or chronic insults (e.g. exposure to pathogens or chemical toxicants) that trigger an acute phase response. Studies with experimental animals have demonstrated causal links between an acute phase response, lower maternal plasma zinc, lower delivery of zinc to the fetus and increased risk for developmental defects.[4,5] Zinc has both direct structural and enzymatic roles in many proteins, and as a consequence, a portion of the teratogenicity of zinc deficiency may be due to altered protein function in the embryo.[1] However, the condition of zinc deficiency can also result in numerous secondary effects, including, inanition, alterations in iron homeostasis[6] and impaired growth factor signaling pathways.[7] Growth factor signaling is exemplified by the insulin-like growth factor (IGF) axis, which serves to regulate the processes of cell proliferation, differentiation and survival within the embryo and fetus.

The IGF axis consists of two ligands (IGF-1 and IGF-2) and two receptors (IGF-1R and IGF-2R). Both ligands activate IGF-1R, a receptor tyrosine kinase, triggering a phosphorylation cascade that ultimately regulates the activity of numerous proteins involved in crucial metabolic functions and gene expression. The role of the IGF-2R is controversial. One function may be to serve as a sink and a possible degradation pathway for the IGF ligands, but recent studies suggest that it may also promote placental

function.[8] The delivery of ligand to the receptor is regulated by a family of binding proteins (IGFBP), which, depending on cellular context, can facilitate the delivery or the sequestration of IGF-1 or -2 to, or from, the IGF-1R.[9] The major source of circulating IGFBP is the liver, and once secreted, they are the primary regulators of IGF half-life in the blood. In healthy non-pregnant animals, the majority of IGF-1 in circulation is bound to IGFBP-3 and the acid labile subunit (ALS) forming a 150 kDa ternary complex. The ternary complex prolongs the half-life of IGF-1 in circulation by preventing either its movement into the endothelium or its proteolysis. When IGF-1 is associated with the low molecular weight (LMW) IGFBP (<40 kDa), its half-life in circulation is shortened, as these complexes can cross the endothelium.[9]

While the IGF axis in maternal circulation is endocrine in nature, it also functions in a paracrine/autocrine manner to support placental function and fetal growth.[10,11] During fetal development, IGF-1 expression is up-regulated in the liver and it acts to promote fetal growth and regulate maturation of many systems including the skeleton.[12] The fetal liver also expresses IGFBP-1 and -2, as well as the ALS, but no significant amounts of IGFBP-3; thus, circulating IGF ligands complex with the LMW IGFBP in the fetal circulation.[11] IGF-1 is also found in the amniotic fluid, primarily emanating from the placenta and bathing the fetus.[13]

The IGF axis of adult humans and experimental animals is sensitive to nutrition in general[14] as well as specific nutrients such as zinc.[15–18] In pregnant experimental animals, caloric or protein restriction markedly reduces the level of circulating maternal IGF-1, combined with an increase in IGFBP-1 and -2, and relative reductions in the levels of IGFBP-3 and the ALS.[19–22] Similarly, zinc deficiency in non-pregnant animals is characterized by significant reductions in circulating IGF-1 and alterations in circulating IGFBP profiles.[15,16,18] In humans, an observational study by Akman *et al.*[23] reported a positive relationship between infant IGF-1 and birth weight; however, they did not detect a correlation between maternal serum zinc levels and infant birth weight or IGF-1 levels. In contrast, another report in human pregnancies found that small for gestational age infants had significantly lower cord blood zinc levels than larger babies.[24]

While the severe impact on the IGF axis can be partially attributed to zinc deficiency-induced inanition,[18] others have found that circulating and hepatic mRNA levels of IGF-1 are impacted to a greater extent than can be accounted for by food restriction alone.[15,16,25] It is well characterized in non-pregnant rat models that consuming a zinc-deficient (ZD) diet results in decreased circulating IGF-1, decreased hepatic IGF-1 mRNA, decreased liver growth hormone receptors and alterations in the binding profiles of IGFBP.[15–18] While zinc deficiency can result in severe reductions in food intake, food restriction alone typically does not result in gross morphological defects, although it can result in fetal growth retardation. In the present study, we used a rat model to compare the effects of food restriction and zinc deficiency on the fetal IGF axis. This work suggests that zinc deficiency results in alterations in both the levels and distribution of fetal IGF-1 that differ from the effects of food restriction alone. We suggest that these differential effects contribute to the developmental defects associated with poor maternal zinc status.

Materials and methods

Animals and diets

Virgin Sprague–Dawley female rats were handled in accordance with protocols approved by the University of California at Davis Institutional Animal Care and Use Committee following the *NIH Guide for the Care and Use of Laboratory Animals*.[26] Females were mated overnight (dark: 18:00–06:00) with a male until copulatory plugs were observed (designated gestation day [GD] 0.5). Rats were housed in stainless-steel wire bottom cages to permit the measurement of food intake and to reduce the likelihood of coprophagy. The diets were formulated to the AIN93 recommendations using dried egg white protein supplemented with biotin.[27] The ZD diet had a concentration of 0.3 mg zinc/kg. The control diet was supplemented with zinc to a concentration of 25 mg zinc/kg. During a one-week acclimation period and during mating, all females were given the control diet. As animals became pregnant, they were assigned to the control (Control group) or the ZD (ZD group) diet beginning on GD 0.5. To control for the cycling reductions in food intake commonly associated with zinc deficiency, subsequently mated dams were matched (Paired group) by body weight at GD 0.5 to specific ZD dams. Each Paired dam received the control diet in the amount equivalent to that consumed by their ZD pair on each GD. Food intake was recorded each morning. Each diet group consisted of 7–8 dams.

Tissue collection

Food cups were removed the evening of GD 18.7, and the following morning (09:00, GD 19.5) dams were euthanized by CO_2 overexposure. Maternal tibias were collected for zinc analysis. The gravid uterus was removed and placed on a warming table at 37ºC where the uterine muscle was dissected away. Amniotic fluid from each litter was collected from the fetal sacs by needle and syringe and pooled before freezing at −80ºC. Fetal blood was collected from the umbilical vessels in heparinized capillary tubes, pooled in heparinized vials on ice, centrifuged for 15 min at 1800 *g* at 4ºC and fetal plasma was frozen at −80ºC. Fetuses and placentas were removed from their membranes and placed on ice. Gross morphologic evaluations were conducted on the fetuses as they and their placentas were weighed. Fetal liver was dissected on ice and pooled, and placentas were pooled before freezing at −80ºC.

Zinc determination

Maternal tibias were wet ashed in 16 mol/L nitric acid and diluted in Ultrex nitric acid (0.1 mol/L; JT Baker, VWR, Brisbane, CA, USA).[28] The diluted digests were analyzed for zinc by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Trace Scan, Thermo Electron Corp,

Waltham, MA, USA). The ICP-AES was calibrated with multielement standards (SPEX CERTIPREP, Metuchen, NJ, USA) diluted in 2 mol/L Ultrex nitric acid. The zinc concentration in bovine liver obtained from the National Bureau of Standards was used as an external control. Blanks were generated by the same method and their values were subtracted from the sample measurements.

Serum and amniotic fluid IGF-1 concentrations and IGFBP profiles

IGF-1 concentrations in amniotic fluid were determined by radioimmunoassay (RIA) after acidic molecular sieve chromatography to completely separate IGF-1 from IGFBP as described previously.[18] Acid denaturation is critical for rat serum due to the high affinity of IGF-1 for IGFBP. An internal standard was run with each analysis with an observed coefficient of variation of 6%. The amount of fetal plasma collected from the litters was too small for RIA; therefore, we chose to reserve the plasma for native IGFBP analysis.

IGF-1 binding profiles in fetal plasma or amniotic fluid (125 μL) were determined by native molecular sieve chromatography by fast protein liquid chromatography (GE Healthcare, London, UK) utilizing tandem Superose S-12 columns (1.5 $\times$ 48 cm, GE Healthcare).[18] This chromatography technique relies on equilibrium of tracer binding under native conditions and was chosen over methods of quantification that require denaturing conditions (i.e. ligand blots). The latter can lead to artificially decreased ligand binding and/or loss of IGFBP post-translational modifications as a consequence of the relatively harsh isolation procedures. Furthermore, the native chromatography technique, in conjunction with Western immunoblot analysis, can provide a picture of IGF-1 binding that reflects both the *in vivo* affinity of IGF-1 to IGFBP and the relative amounts of IGFBP present in samples during a variety of physiological and nutritional states. The data are expressed as % counts per minute (CPM) (i.e. the CPM for each fraction was normalized to the total counts recovered from the columns) versus relative elution volume (V_e/V_0). The molecular weight (MW) of the peak fractions containing the tracer ^{125}I were calculated from a calibration curve (Log MW versus K_{av}; $R^2 = 0.98$) generated by chromatography of known MW protein standards. Chromatography results are shown as the averaged chromatograms for each dietary group. To quantify the relative distribution of the ^{125}I-IGF-1 containing protein fractions, binding area under each Gaussian curve (AUC) was determined by ORIGINPRO (Version 7.5) curve-fitting software (OriginLab Inc, Northampton, MA, USA).

Western immunoblot analysis

Equal amounts of plasma (100 μg total protein per lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12% Bis–Tris denaturing gel (Criterion XT, BioRad, Hercules, CA, USA). Separated proteins and MW standards (Kalidoscope/Western C, BioRad) were transferred to Immun-Blot polyvinylidene fluoride membranes (BioRad) for one hour at 100 V at 4ºC. A solution of 5% (w/v) bovine serum albumin in Tris-Tween buffered saline (0.5 mol/L Tris-HCl, pH 7.5, 0.5 mol/L sodium chloride, 0.1% v/v Tween 20) was used for blocking and antibody dilutions (anti-rabbit-hIGFBP-1 at 1:1000, anti-rabbit bIGFBP-2 at 1:1000; Millipore, Billerica, MA, USA). The antibody–antigen complexes were visualized by chemiluminescence (anti-rabbit IgG-conjugated horseradish peroxidase at 1:5000; Cell Signaling Technology, Danvers, MA, USA) following the manufacturer's instructions (Western C Kit, BioRad). Blot images were captured by a charge-coupled device camera in a Chemi-Doc XRS (BioRad) gel documentation system with densitometry analysis software (Quantity One, BioRad).

Expression of fetal IGF and IGFBP genes

The relationship between plasma IGF-1 protein and hepatic transcript levels have correlated well in previously published work,[20] leading us to rely on fetal liver transcripts for IGF-1 as a quantitative measure, especially given the insufficient amount of serum available for RIA quantification. Fetal liver total RNA was isolated with RNAqueous kits (Applied Biosystems/Ambion, Foster City, CA, USA) using the manufacturer's protocol. Ultraviolet fluorescence of ethidium-stained RNA after formaldehyde-agarose electrophoresis was used to verify that all RNA isolates were intact. Two-step semi-quantitative reverse transcriptase-polymerase chain reaction (PCR) was conducted using random hexamer or decamer (Applied Biosystems) and Moloney mouse leukemia virus (Applied Biosystems) reverse transcription of cDNA from total RNA isolates. The same cDNA samples were used for realtime PCR reactions to detect targets. Realtime PCR for IGF ligands and IGFBP cDNA was conducted using Taqman™ probes with FAM fluorescence (Applied Biosystems) multiplexed with 18 s ribosomal RNA (rRNA) using a Taqman™ probe with VIC fluorescence (Applied Biosystems) as an endogenous control. Cycle number at the threshold of detection (C_t) for target and 18s rRNA was determined using iQ software and the iCycler thermocycler (BioRad). Conditions were optimized by determining the concentration for primers to the 18s cDNA that provided a constant C_t value with the lowest fluorescence so that the amplification of this more abundant cDNA did not over compete with the amplification of the targets. Before multiplexing, the linearity of the ΔC_t relationship was determined over a serial dilution of a cDNA sample in separate reactions for 18 s and each target.

Statistical analyses

One-way analysis of variance (ANOVA) was used to detect differences among treatment groups (Sigma Stat 3.5; Systat, San Jose, CA, USA) followed by Fisher least significant difference (LSD) *post hoc* analysis. Kruskall–Wallace ANOVA on ranks was used when data were not normally distributed. The dam or the litter was the statistical unit.[29] Values were designated as significantly different when $P \leq 0.05$. Data are reported as means with standard error of the mean (SEM), unless stated otherwise.

For realtime PCR data, the ΔC_t was normalized to the Control values by calculating $2^{\Delta\Delta Ct}$. One-way ANOVA was conducted on both the $2^{\Delta\Delta Ct}$ values, and the Ln transformed ΔC_t followed by Kruskall–Wallace *post hoc* on ranks or Fisher LSD, as appropriate. When comparing transcript levels among groups, we verified that the ANOVA analysis of both the $2^{\Delta\Delta Ct}$ and Ln transformed ΔC_t were in agreement. *P* values reported are from Ln transformed ΔC_t analysis and data are expressed as average fold difference and SEM from Control ($2^{\Delta\Delta Ct}$) for each group.

Results

Maternal food intake, body weight, zinc status and reproductive outcome parameters

During gestation, average maternal body weight in the Control group was significantly higher than in the ZD and Paired groups at GD 9.5 and from GD 11.5 to GD 19.5 (Figure 1a). Average maternal weights in the Paired group tended to be higher than in the ZD group ($P = 0.07$) until GD 17.5, when the Paired group became significantly heavier than the ZD group (Figure 1a). Maternal food intake was cyclic and lower in the ZD group than in the Control group (Figure 1b). Cumulative food intake for the Control dams was significantly different from the ZD and Paired groups from GD 9.5 to GD 19.5 (Figure 1c). Overall, there was a 29% lower cumulative food intake in the ZD and Paired groups compared with the Control group over the experimental period (Figure 1c). Tibia zinc, a marker of zinc status in the dam, was significantly lower in the ZD group than in the Paired and Control groups; the latter two groups were not significantly different from each other (Table 1). Similar to the pattern for maternal body weights, fetal body weights, crown rump lengths and placental weights were significantly less in the ZD and Paired groups than in the Control group (Table 1). In the Paired group, these fetal parameters were also greater than those found in the ZD group (Table 1). The ratio of fetal body weight to placenta weight was similar among the groups (Table 1). Gestational zinc deficiency resulted in fetal morphological malformations (data not shown), a significant increase in embryonic resorptions and a reduction in the number of live fetuses per litter (Table 1). Fetal gross abnormalities were not observed in the Paired and Control groups (data not shown), and resorption frequencies were low in these groups (Table 1).

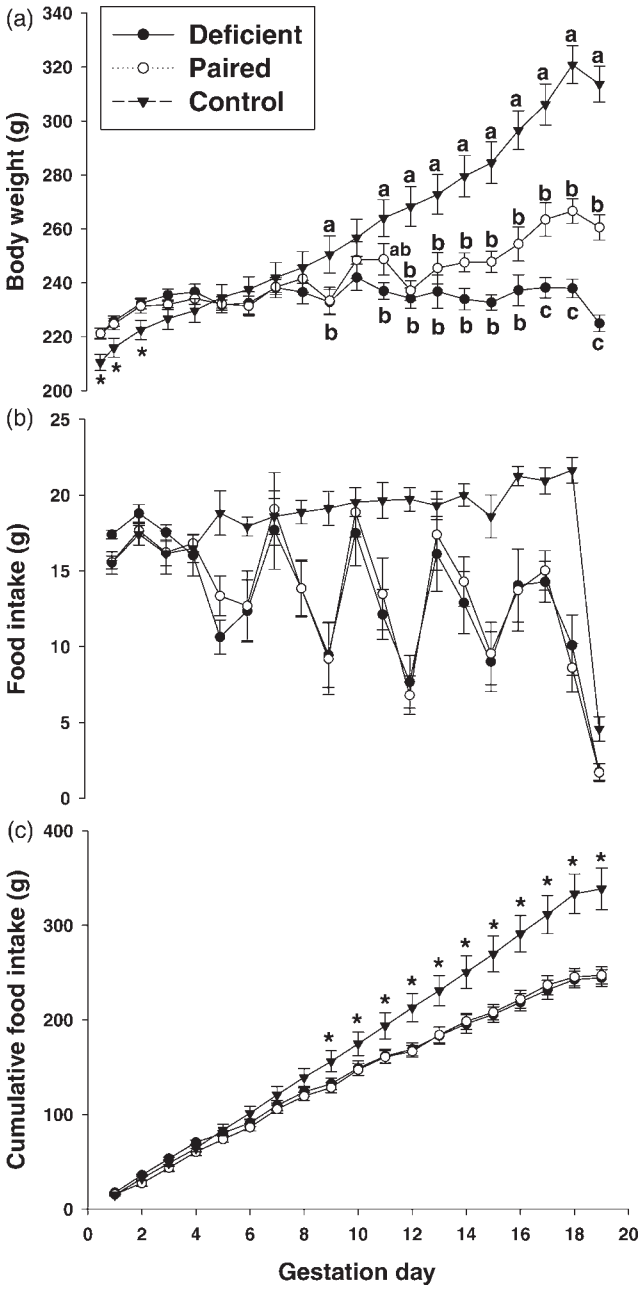

Figure 1 Average ± SEM for maternal body weight (a), daily food intake (b) and cumulative food intake (c) of dams from the ZD, Paired and Control groups during gestation. Body weights with different letter notation are significantly different among groups on the indicated GD ($P \leq 0.05$). *Control group is significantly different from either the Paired or ZD groups on this GD ($P \leq 0.05$). GD, gestation day; ZD, zinc deficient; SEM, standard error of the mean

Amniotic fluid IGF-1 and IGFBP profiles

Amniotic fluid IGF-1 concentrations were significantly lower in the Paired group than in the ZD or Control groups; the latter two groups were not significantly different from each other (Figure 2a). All dietary groups were characterized by low amounts of the tracer binding to a 54,000 MW peak consistent with the MW of glycosylated IGFBP-3 in complex with IGF-1 (Figure 2b). A subsequent broad distribution of the tracer was observed between the 54,000 MW peak and the peak of unbound tracer at 8000 MW. The large free tracer peak present in amniotic fluid suggests that less IGF-1 binding occurs in this fluid relative to fetal plasma (described below). Although small changes in the chromatogram were noted, no significant differences in IGFBP binding profiles in the amniotic fluid were demonstrated among the groups (Figures 2a and b).

Fetal plasma IGFBP profiles

Native column chromatography was chosen for characterizing IGFBP/IGF-1 interactions as this technique is less harsh, preserves post-translational modification of the IGFBP and

Table 1 Reproductive outcome, maternal zinc and IGF-1 status in dams from the ZD, paired and control groups*

Group (no. analyzed)	Live fetuses per litter†	Resorptions per litter†	Fetal body weight (g)	Crown–rump length (mm)	Placenta weight (g)	Ratio of placenta:fetal body weight	Maternal tibia Zn (nmol/g)
ZD (7)	6^a	9.5^a	1.328 ± 0.052^a	24.7 ± 0.6^a	0.225 ± 0.017^a	0.178 ± 0.012	1543 ± 107^a
Paired (8)	15^b	0^b	1.708 ± 0.050^b	28.4 ± 0.3^b	0.305 ± 0.009^b	0.176 ± 0.004	2544 ± 131^b
Control (7)	14^b	0.5^b	1.993 ± 0.038^c	30.1 ± 0.3^c	0.388 ± 0.023^c	0.194 ± 0.011	2572 ± 89^b

IGF, insulin-like growth factor; ZD, zinc deficient
*Data are mean ± SEM, and values with different superscripts within a measurement significantly differ ($P \leq 0.05$)
†Shown as median values

is more sensitive than denaturing techniques such as ligand blotting.[21] At GD 19.5, fetal plasma contained two peaks of ^{125}I-IGF-1 binding activity. The first was at 32,000 MW representing the combined binding activity of LMW IGFBP, and the second, a peak at 8000 MW representing free tracer (Figure 3a). The IGFBP-3 ternary complex was not detected in fetal plasma from any of the dietary groups, but was present in serum of the dams (data not shown) as expected. Fetuses in the ZD group demonstrated significantly lower ^{125}I-IGF-1 binding to LMW IGFBP compared with fetuses in the Paired and Control groups; the latter two groups did not significantly differ from each other (Figures 3a and b). Western immunoblot (Figure 3c) confirmed the decreased tracer binding to the 32,000 MW peak in the ZD group most likely could be attributed to lower IGFBP-1 levels relative to the Paired or Control groups, with the latter two groups not differing from each other (Figures 3c and d). The level of plasma IGFBP-2 among the three fetal groups was similar (Figures 3c and e).

Fetal hepatic IGF and IGFBP transcript levels

Fetal liver IGF-1 transcript levels in the ZD group were significantly lower than in the Paired and Control groups; the latter two groups did not differ from each other (Figure 4a). Fetal liver IGF-2 transcript levels were similar among the groups (Figure 4b). Both IGFBP-1 and -2 transcript levels in the livers of fetuses from the Paired dams were significantly higher (four- and two-fold, respectively) than were levels in fetuses from the ZD and Control dams; the latter two groups did not differ from each other (Figures 4c and d).

Placental IGF-2 transcript levels

Placental IGF-2 transcripts were significantly higher in the ZD group than in the Paired group (Figure 5), and tended ($P = 0.07$) to be higher than the Control group. IGF-2 expression did not differ between the Paired and Control groups.

Discussion

The critical role of IGF in fetal growth was clearly established by knockout studies showing that ablation of one or more copies of the *Igf-1*, *Igf-2*, *Igf-1r*, or combinations of these genes, resulted in severe IUGR or death.[30] Similarly, the teratogenicity of zinc deficiency is well recognized, leading to IUGR and severe malformations in multiple fetal organ systems.[1] Pregnancy complications, such as IUGR, have been associated with alterations in the IGF axis of the mother and fetus. The fetus responds during

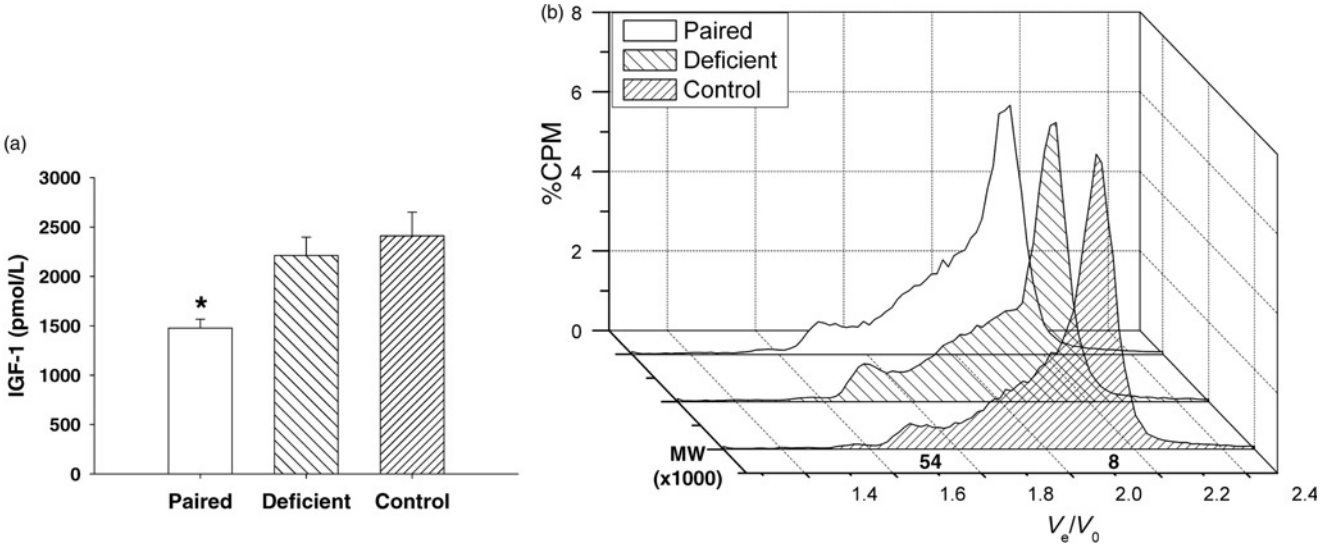

Figure 2 Average ± SEM for IGF-1 concentrations (a) and chromatogram profiles of ^{125}I-IGF-1-labeled IGFBP (b) in amniotic fluid from litters in the ZD, Paired and Control groups. *Significantly different among groups ($P \leq 0.05$). IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; ZD, zinc deficient; SEM, standard error of the mean

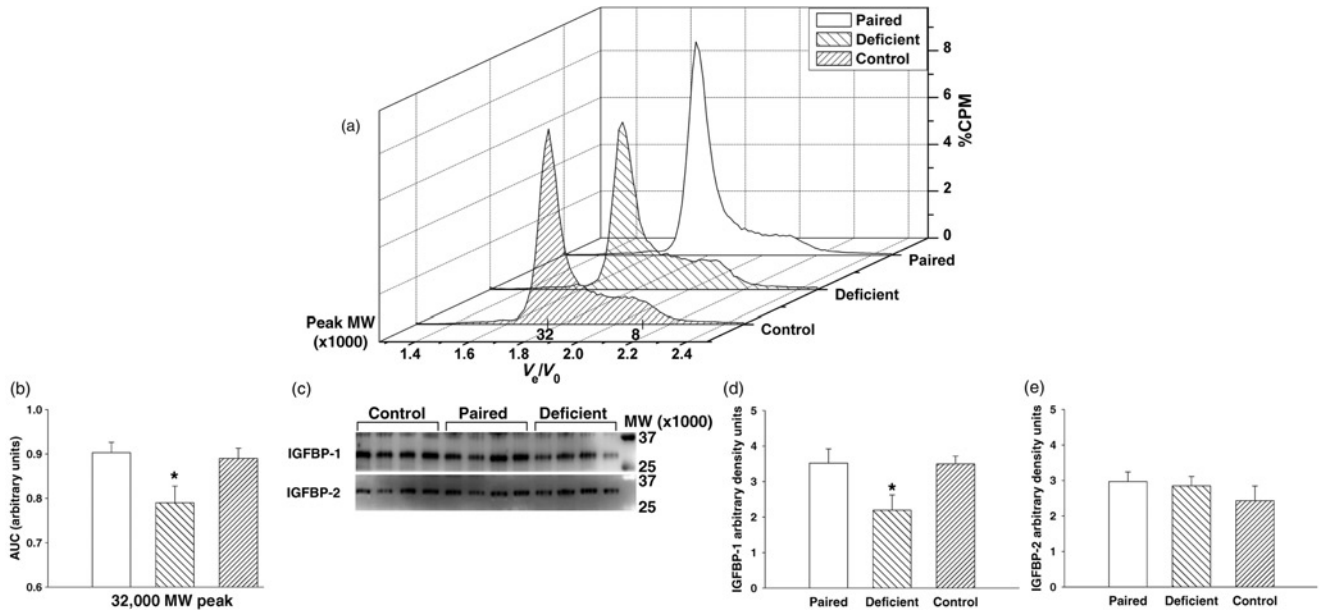

Figure 3 Average chromatogram profiles of ^{125}I-IGF-1-labeled IGFBP in fetal plasma from litters in the ZD, Paired and Control groups detected by native molecular sieve chromatography (a) and AUC ± SEM (b). Representative Western immunoblot of IGFBP-1 and -2 (c) with densitometry analysis of IGFBP-1 (d) and IGFBP-2 (e) of fetal plasma from litters in the ZD, Paired and Control groups. *Significantly different among groups ($P \leq 0.05$). IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; ZD, zinc deficient; AUC, area under the curve; SEM, standard error of the mean

maternal caloric or protein restriction with reductions in circulating fetal IGF-1 and increases in LMW IGFBP, the combination of which can result in IUGR.[11] Given the above, we hypothesized that zinc deficiency *per se* produces a more severe alteration to the fetal IGF axis than mere food restriction alone.

Consistent with this concept, the fetuses in the Paired group were moderately growth impaired relative to the Control group (14% decrease in fetal body weight). Pair feeding, rather than conventional food restriction, was implemented in this study due to the fact that dams consuming ZD diet are characterized by cyclic patterns of

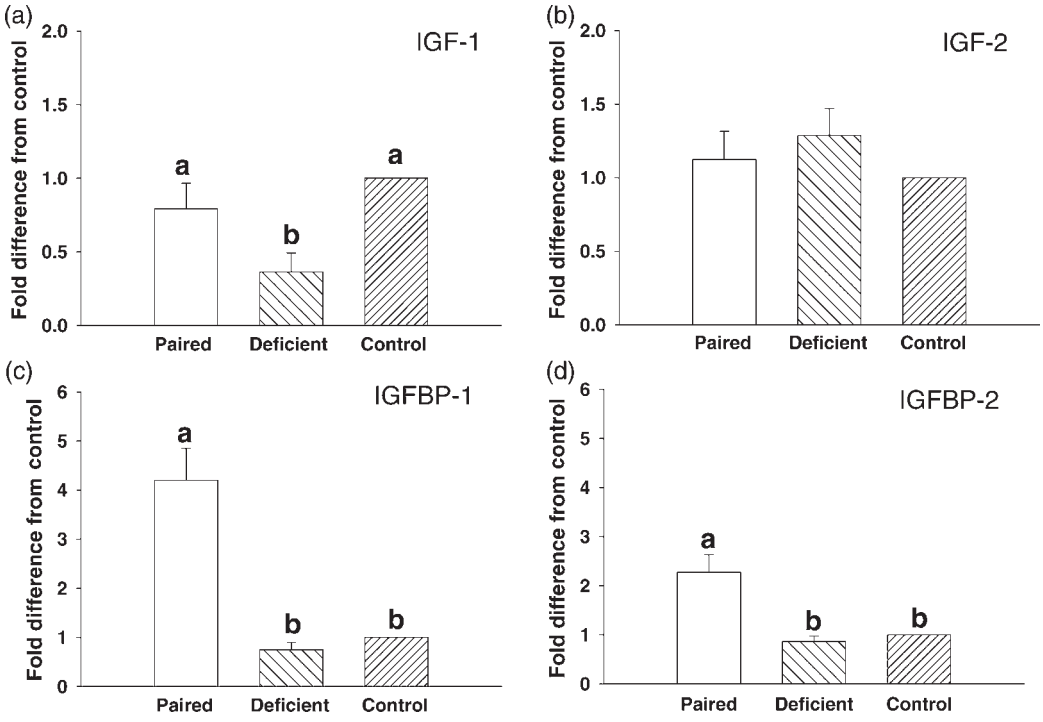

Figure 4 Relative mRNA levels in fetal liver for IGF-1 (a), IGF-2 (b), IGFBP-1 (c) and IGFBP-2 (d) measured by realtime RT-PCR. Data are expressed as the average fold difference from Control ± SEM. Bars with different letter notation are significantly different for each transcript ($P \leq 0.05$). IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; SEM, standard error of the mean; RT-PCR, reverse transcriptase-polymerase chain reaction

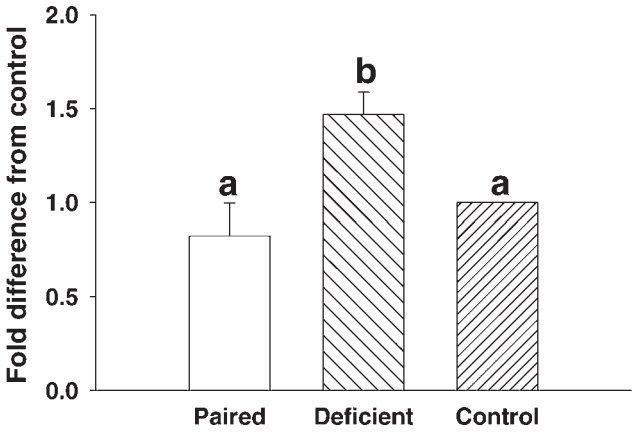

Figure 5 Relative placenta IGF-2 mRNA levels measured by realtime RT-PCR. Data are expressed as the average fold difference from Control ± SEM. Bars with different letter notation are significantly different ($P \leq 0.05$). IGF, insulin-like growth factor; SEM, standard error of the mean; RT-PCR, reverse transcriptase-polymerase chain reaction

food intake. Our model of IUGR differs from the classic model of Woodall *et al.*,[31] who severely reduced maternal food intake (i.e. 70% reduction of the *ad libitum* intake of controls compared with a 29% reduction in this report) and found that at GD 20 fetal weights declined by 21%. In contrast to the Paired group, the ZD group suffered a more severe reduction in these parameters. The severity of IUGR in the ZD group was associated with a significantly smaller placental weight when compared with the Paired and Control groups; however, the placental to fetal body weight ratio was similar among groups. This outcome differs from that observed for macronutrient deficiencies in the rat[32,33] or mouse.[34] Feeding a low protein diet (6–10% protein by weight compared with approximately 20% in control diets) through GD 20 did not result in any alteration in placenta or fetal weight, or the ratio between them. However, a 6–10% protein diet fed through birth resulted in reduced pup weights[35] and placental weights,[33] but the magnitude of this reduction was much smaller than that exhibited by the ZD fetuses in the current study.

There are many physiological factors that could contribute to IUGR and reduced placental size. Inadequate maternal nutrition has been suggested to result in altered maternal IGF-1 metabolism leading to the reapportioning of maternal nutrients away from fetal compartments, thereby depriving the placenta and fetus of essential macronutrients required for growth.[35] Maternal IGF-1 levels are significantly reduced during gestational zinc deficiency and food restriction.[19–22] Interestingly, augmentation of the malnourished dam with exogenous growth factors (growth hormone and/or IGF-1) does not restore placental and fetal growth in the rat, while maternal weight gain is only marginally increased.[15,16,31,36,37] Another factor potentially affecting placenta size, and covariant fetal size, is the expression of the placental *Igf-2* gene. Thus, mice with global knockout of the *Igf-2* gene are worth noting for their dramatically reduced placental and fetal size.[38] Later findings established that the placental-specific P0 transcript of *Igf-2* accounted for most of the reduction in placental size in this global knockout model.[39] We are not aware of any studies concerning the nutrient regulation of the placental specific P0 *Igf-2* transcript *per se*; however, we found perturbations in the expression of total placental IGF-2 transcripts among groups, although these observations did not correlate with placental weight. In humans, one report found small for gestational age infants had significantly lower IGF-2 expression in the placenta compared with larger babies.[24] In any event, it is likely that in addition to placental *Igf-2* expression and restricted maternal nutrient flow, other factors play a role in controlling placental size during caloric restriction and zinc deficiency. One such factor may be fetal attenuation of its own growth by controlling the bioactivity of locally produced IGF. Hence, direct supplementation of the malnourished fetus with exogenous IGF-1 attenuates IUGR.[40]

The current report suggests that the accompanying decreased bioactivity of the IGF ligands in the fetus contributes to IUGR. The decrease in bioactive ligand concentration can be a consequence of either decreased ligand transcription and expression, or their post-translational sequestration by IGFBP. Several studies of fetal IUGR have observed reduced IGF-1 and/or increased IGFBP-1 or -2 in cord blood,[41,42] amniotic fluid[43] and fetal plasma.[19,20,40,44–46] Studies of transgenic IGFBP-1-overexpressing mice have also reported retarded fetal growth as a consequence of reduced IGF-1 bioactivity.[47–49] As the severest reduction in fetal body weight occurred in the ZD group, we hypothesized that this group's hepatic IGF-1 levels would be the lowest, while its circulating LMW IGFBP levels would be the highest. Consistent with this hypothesis, the ZD fetuses displayed the lowest hepatic IGF-1 mRNA levels, yet surprisingly displayed the lowest levels of circulating IGFBP-1. Our data suggest that the slightly retarded growth in the Paired fetuses was a consequence of poor maternal nutrition, while the more severe growth retardation in the ZD group was a combination of the former event, and decreased fetal IGF-1 transcription. Thus, during fetal zinc deficiency, growth retardation was primarily associated with the downregulation of fetal IGF-1 rather than reduction in IGF bioactivity caused by IGFBP sequestration. Food restriction of the dam clearly leads to fetal malnutrition via decreased access of the fetal compartment to both macro- and micronutrients. However, zinc deficiency imposes a heavier burden on the fetus by not only reducing the same macro- and micro-nutrients as food restriction, but also, in addition, superimposing the removal of an essential trace element. Thus, it is not unexpected that this outcome results in more severe and unique alterations to the fetal IGF axis than food restriction alone. Often lost in the translation is the fact that food intake regulation is just one parameter altered by zinc deficiency, which adds to the myriad of physiological, biochemical and molecular defects underlying the pathological consequences of zinc deficiency.

Lastly, the growth hormone or IGF resistance to exogenous supplementation, observed during malnutrition and zinc deficiency,[15,16,31,36,37] suggests attenuation in signal transduction pathways controlling cell proliferation and cell survival that are downstream of ligand/receptor production and/or receptor binding. In this regard, we have reported that ZD cells *in vitro* are refractory to a multitude

of exogenous growth factors that commonly signal through receptor tyrosine kinases.[7] Thus, defects in postreceptor binding events may augment the effects of decreased ligand bioactivity and ensure that maternal and fetal growth is attenuated under conditions of nutrient deprivation. Supporting a role for postreceptor binding defects in growth factor signaling events is the study of Laviola *et al.*[50] that demonstrated disruption of the IGF-1 signaling pathway in placentas derived from IUGR births. Further studies are warranted to address this mechanism in other maternal and fetal tissues, in particular the placental transporters that regulate the delivery of maternal nutrients to the fetus.

In summary, the main findings in this study were that zinc deficiency leads to differential effects in measures of fetal IGF-1 and IGFBP profiles. Decreases in fetal IGF-1 transcripts contribute to the more severe IUGR seen in zinc deficiency relative to food restriction alone.

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