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Authors

Arsenault, Joanne E
Havel, Peter J
de Romaña, Daniel López
et al.

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Longitudinal measures of circulating leptin and ghrelin concentrations are associated with the growth of young Peruvian children but are not affected by zinc supplementation[1–3]

Joanne E Arsenault, Peter J Havel, Daniel López de Romaña, Mary E Penny, Marta D Van Loan, and Kenneth H Brown

ABSTRACT

Background: Leptin, ghrelin, and insulin are hormonal regulators of energy balance and, therefore, may be related to growth during infancy. Zinc is essential for growth, and its growth effects may be mediated through these hormones.

Objective: We examined the effects of supplemental zinc on plasma leptin, ghrelin, and insulin concentrations among young children at risk of zinc deficiency and examined the relations between these hormones and physical growth.

Design: Children ($n = 142$) aged 6–8 mo were randomly assigned to receive 3 mg Zn/d as a supplement, in a fortified food, or as a placebo for 6 mo. Relations between hormones and anthropometric *z* scores, body composition, and growth rates were examined at baseline and 3 and 6 mo after the start of the intervention.

Results: No treatment group–related differences were found in plasma leptin, ghrelin, or glucose concentrations or in anthropometric *z* scores at 3 or 6 mo after the start of the zinc intervention. Neither plasma leptin nor ghrelin concentrations at baseline or 3 mo were predictive of subsequent changes in growth. However, changes in weight-for-age *z* scores over the two 3-mo time intervals were positively associated with subsequent leptin concentrations and inversely associated with subsequent plasma ghrelin concentrations.

Conclusions: Supplemental zinc did not affect the children's growth, anthropometric indexes, or plasma hormone concentrations in this study population. Our results suggest that plasma leptin and ghrelin concentrations in later infancy are a consequence of previous weight changes rather than predictors of short-term growth. *Am J Clin Nutr* 2007;86:1111–9.

KEY WORDS Zinc, leptin, ghrelin, insulin, growth, infants

INTRODUCTION

Supplemental zinc increases linear growth and weight gain in zinc-deficient animals (1) and in young, stunted children (2). Zinc may stimulate dietary intake, possibly through hormonal or neuroendocrine transmitters that affect appetite and thereby promote increased growth (3). Hormonal factors may also affect growth directly.

Leptin is a hormone produced primarily in the adipose tissue and is positively associated with body fat mass (FM) and recent energy intake. Circulating leptin signals the brain that body fat stores and energy intake are adequate, thereby suppressing appetite (4). In contrast, ghrelin, which is secreted into the circulation primarily from the stomach, provides a hunger signal to the brain. Insulin stimulates leptin production and secretion and is likely to be involved in suppressing ghrelin secretion after meals (5). Zinc is necessary for insulin synthesis and secretion (6). One human trial of induced zinc deficiency conducted in men showed lower leptin concentrations during zinc deficiency and higher leptin concentrations after zinc repletion (7).

Leptin and ghrelin may influence growth during infancy. Leptin concentrations are high in cord blood (8), and a positive relation between plasma leptin concentrations and body weight or body mass index (BMI) has been reported in infants (8–11). In contrast, circulating ghrelin is negatively associated with body weight in infants (10, 12–14).

The primary objective of the present set of analyses was to determine whether supplemental zinc would affect plasma leptin, ghrelin, and insulin concentrations in children assumed to be at risk of zinc deficiency. Additionally, we examined longitudinally the relations between these hormones and anthropometric indicators of physical growth. We hypothesized that *1*) children receiving supplemental zinc would have higher plasma hormone concentrations at 3 mo, 6 mo, or both after starting supplementation than would children not receiving additional zinc; and *2*) the hormone concentrations would be predictive of subsequent growth.

SUBJECTS AND METHODS

Subjects

The children included in this study were a subset of a larger cohort enrolled in a randomized, double-blind, community-based trial designed to examine the effects of additional zinc intake on children's growth, morbidity, and energy intake. Children were selected, by location of residence, to participate in

[1] From the Program in International and Community Nutrition (JEA, MDVL, and KHB) and the Department of Nutrition (JEA, PJH, MDVL, and KHB), University of California, Davis, Davis, CA; Instituto de Investigación Nutricional, La Molina, Lima, Perú (DLdR and MEP); and the US Department of Agriculture, Agricultural Research Center, Western Human Nutrition Research Center, Davis, CA (MDVL).

[2] Supported by the Bill and Melinda Gates Foundation.

[3] Reprints not available. Address correspondence to KH Brown, Department of Nutrition, University of California, One Shields Avenue, Davis, CA 95616. E-mail: khbrown@ucdavis.edu.

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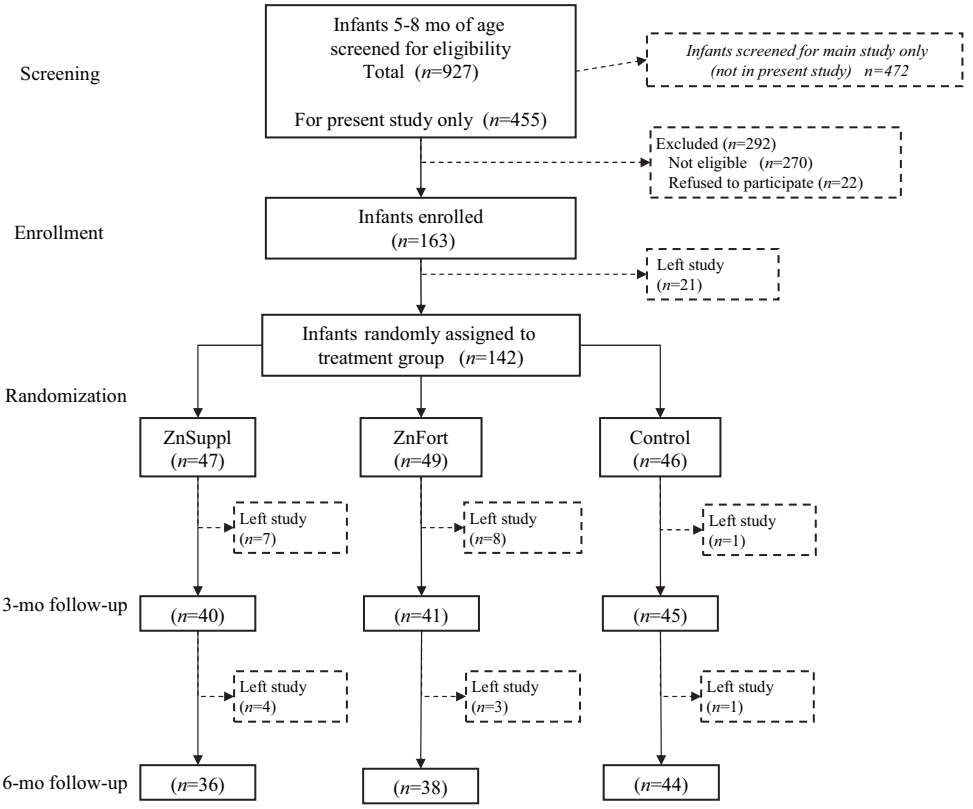

FIGURE 1. Flow chart of subjects in the study. ZnSuppl, zinc-supplementation group; ZnFort, zinc-fortification group.

substudies to measure hormones involved in energy balance and assessment of their body composition. The trial was conducted in a low-income, periurban community in Trujillo, a coastal city in northern Peru. Children aged 5–7 mo of age were identified initially by conducting a census, and they were then invited to attend a screening examination to measure their weight, length, and hemoglobin concentration. Children were selected if they met the following inclusion criteria: length-for-age *z* score (LAZ) < -0.5, weight-for-length *z* score (WLZ) > -3, hemoglobin >8 g/dL, no congenital or chronic conditions affecting growth, no use of infant formula (providing >1 mg Zn/d, ≥ 5 times/wk), no twin enrolled in the study, and an intention to live in the study community during the next 7 mo. Within 2 d of the screening exam, a worker visited the home of eligible children whose families were interested in participating in the intervention study to obtain written informed consent. A total of 163 children were enrolled in the present study. The protocol was approved by the Institutional Review Boards of the University of California, Davis, and the Instituto de Investigación Nutricional, Lima, Perú.

Zinc treatments

Randomization to treatment group occurred 1 mo after enrollment. During the month before randomization, the caregivers of 21 children decided not to continue in the study, and the remaining 142 children were assigned to 1 of 3 groups: *1*) wheat-based porridge without added zinc and a liquid multivitamin supplement with zinc (ZnSuppl), *2*) the same porridge fortified with added zinc and a liquid multivitamin supplement without zinc (ZnFort), or *3*) porridge without zinc and a liquid multivitamin supplement without zinc (control) (**Figure 1**). Details of the zinc treatments were described previously (15). In brief, the zinc-fortified porridge was designed to provide an additional 3 mg Zn/d (as zinc sulfate), assuming an average porridge consumption of 20 g/d dry weight, as was observed in a previous study in Peru (16). The liquid multivitamin supplement with added zinc (as zinc sulfate) provided 3 mg Zn/d along with vitamins A, B-6, C, D, and E; thiamine; riboflavin; pantothenic acid; biotin; and niacin.

Biochemical analyses

Venous blood was collected at 0, 3, and 6 mo after the initiation of the zinc intervention. Because of the impracticality of obtaining fasting blood samples from predominately breastfed infants in a community-based setting, the children were brought to the study office at ≈0730–0800 and allowed to breastfeed. All food was then withheld until blood was drawn 90 min after the end of the feeding. The amount of breast milk consumed during this feeding was measured by weighing the children before and after the feeding with an electronic infant balance with 5 g precision (Baby Scale model 231; Seca, Hamburg, Germany). Children who were not breastfeeding were fed the typical non-human milk substitute used in this community (reconstituted evaporated or powdered cow milk), and the amounts of milk and water were weighed with an electronic balance with 0.1 g precision (MX200; MyWeigh, Phoenix, AZ). Venous blood (≈2 mL) was collected into EDTA-coated tubes and centrifuged immediately. Plasma was stored initially at −20 °C in the field lab and transferred to Davis on dry ice for storage at −80 °C until analyzed.

Plasma leptin and insulin were measured by radioimmunoassay (LINCO Research, St Charles, MO) with the use of human antiserum and ^{125}I-labeled peptides. Samples were measured in duplicate, and all samples from the same subject were measured in the same analytic run. The mean CVs of the duplicate leptin and insulin measures were 8.1% and 9.3%, respectively. The detection limits of the leptin and insulin assays are 0.5 ng/mL and 2 μU/mL, respectively. Plasma glucose was measured by the glucose oxidase method with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). The mean CV of duplicate glucose measures was 0.6%.

Plasma ghrelin was measured by radioimmunoassay (Phoenix Pharmaceuticals Inc, Belmont, CA) with the use of a rabbit antiserum and [^{125}I]ghrelin as a tracer. The mean CV of the duplicate ghrelin measures was 4.1%. The detection limit of the assay is 64 pg/mL. Because of financial constraints, plasma ghrelin was measured in only 75% of the study population. The subjects omitted from ghrelin analyses were randomly selected from the zinc-fortification group because of the potential variability in consumption of the porridge and presumed lower zinc absorption from wheat-based foods.

Anthropometric measures

Anthropometric measures were obtained at baseline and at 3 and 6 mo. Weight was measured on an infant balance with ±15 g precision (model 345; Seca). Supine length was measured to 0.01 cm by using a digital infantometer (447 Infantronic Digital Infantometer; Quickmedical, Snoqualmie, WA). All measurements were performed by the same person. The mean CVs for duplicate measures of weight and length for each infant were between 0.02% and 0.04% throughout the study. Maternal weight and height were measured at the time that baseline measures of the infants were made. The birth weights of the infants were obtained from the mothers and was verified with written records if available. The infants' midupper arm circumference was measured to the nearest 0.1 cm with a flexible, nonstretch tape at baseline and 6 mo. Anthropometric *z* scores for weight and length were calculated by using EPIINFO software (version 3) with Centers for Disease Control and Prevention 2000 reference data (17). Anthropometric *z* scores for arm circumference were calculated by using World Health Organization 1997 reference data (18).

Body composition

Body composition was assessed at baseline and at 6 mo by using deuterium oxide dilution. A preweighed 0.8-g dose of deuterium oxide tracer (^2H_2O) was administered quantitatively to the children orally with a syringe. Urine samples were collected in plastic urine collection bags before dosing and ≥2 h after the dosing. The first urine sample collected 2 h after dosing was discarded to avoid collection of a nonequilibrated sample, and the next urine sample was saved for analysis. The average length of time from dosing to the third urine sample collected (used for analysis) was 3.6 ± 0.9 h (range: 2.3–9.1 h). The urine samples were transferred to screw-top vials and stored at −20 °C until processed. The children were allowed to breastfeed during the body-composition study, and the amount of breast milk consumed was measured as described above. Consumption of other fluids, such as water and juice, and occasionally other food was allowed, and these items were weighed with a portable balance with ±0.1 g precision (MX200; MyWeigh).

The urine samples were vacuum distilled to obtain pure water, and ^2H_2O concentrations were measured in duplicate by infrared spectrometry (Miran-IFF; Foxboro Co, Foxboro, MA). Total body water (TBW) was calculated by using the following formula:

$$TBW = [(^2H_2O \text{ dose}/^2H_2O \text{ enrichment})] \times 900 \times 0.96 \times 0.944] - \text{water intake (L)} \quad (1)$$

Corrections were made for the molecular weight of ^2H_2O relative to water (900), the nonaqueous hydrogen exchange (0.96) (19), the fractionation of the isotope (0.944) (20), and the amount of water consumed during the equilibration period. Water intake included the water used to rinse the ^2H_2O vial, breast milk, and other food and fluids. Fat-free mass (FFM) was calculated by dividing TBW by the proportion of water in FFM, obtained from age- and sex-specific reference data (21).

Sample size and statistical analyses

To detect treatment-related differences in plasma concentrations of leptin, ghrelin, and insulin, having an effect size of 0.7 SD, with the use of 3-factor group-wise comparisons and 2-sided statistical tests, a sample size of 41 subjects per treatment group was necessary ($P < 0.05$, power of 80%).

Anthropometric measures and plasma hormone concentrations at baseline were compared between zinc-treatment groups by ANOVA with the use of SAS for WINDOWS (release 8.02; SAS Institute, Cary, NC). Plasma hormone concentrations were log transformed because they were not normally distributed. A mixed model with repeated measures (PROC MIXED) was used to compare anthropometric indexes and hormone concentrations by treatment group at 2 times during the study. The models with anthropometric indexes as the dependent variables included the following covariates: time, group × time, initial *z* score, and initial *z* score × time. The models with leptin and ghrelin concentrations as dependent variables included the covariates time, group × time, initial hormone concentration, initial hormone concentration × time, weight, weight × time, and sex. The models with insulin and glucose as dependent variables also included energy intake 90 min before the blood sampling as a covariate. Spearman correlations were used to assess relations between hormones and between hormones and anthropometric indexes. Partial correlations were used to assess relations between hormones and presampling meal components, with control for body weight. Analysis of covariance (PROC GLM) was used to examine relations between hormone concentrations and growth. To determine whether leptin or ghrelin concentrations were more strongly predicted by current weight status or prior growth, the independent variables were standardized, and the β coefficients from the regression analyses using the standardized variables were compared by *t* test.

RESULTS

Plasma hormone and glucose concentrations

There were no differences in initial plasma ghrelin, insulin, or glucose concentrations by treatment group. However, plasma leptin concentrations at baseline were significantly lower in the ZnSuppl group than in the ZnFort group (**Table 1**). After baseline values were controlled for sex, and body weight, there were no group × time interactions in plasma concentrations of leptin,

TABLE 1
Plasma leptin, ghrelin, insulin, and glucose concentrations by zinc-treatment group at baseline (0 mo) and 3 and 6 mo[1]

	ZnSuppl	ZnFort	Control	*P* for model 1[2]	All groups combined	*P* for model 2[3]
Leptin (ng/mL)	—	—	—	0.99	—	<0.0001
0 mo	2.9 ± 0.9 [47]	3.4 ± 1.1 [49]	3.4 ± 1.2 [46]	—	3.2 ± 1.1[a] [142]	—
3 mo	2.6 ± 0.8 [40]	2.7 ± 0.7 [39]	2.9 ± 1.3 [44]	—	2.7 ± 1.0[b] [123]	—
6 mo	2.6 ± 0.8 [36]	2.8 ± 0.9 [37]	2.7 ± 0.8 [44]	—	2.7 ± 0.8[b] [117]	—
Ghrelin (pg/mL)						
0 mo	371 ± 94 [40]	396 ± 131 [20]	390 ± 113 [45]	0.82	384 ± 110[a] [105]	0.02
3 mo	403 ± 111 [40]	418 ± 138 [20]	415 ± 146 [44]	—	411 ± 131[b] [104]	—
6 mo	403 ± 136 [36]	404 ± 115 [19]	423 ± 111 [44]	—	412 ± 121[a,b] [99]	—
Insulin (μU/mL)						
0 mo	3.9 ± 1.5 [47]	4.6 ± 2.1 [49]	5.0 ± 3.7 [46]	0.007	4.5 ± 2.6[a] [142]	0.0005
3 mo	3.4 ± 1.6 [40]	4.2 ± 1.8 [38]	4.5 ± 4.0 [44]	—	4.1 ± 2.8[a,b] [122]	—
6 mo	3.4 ± 1.3 [36]	4.2 ± 1.6 [37]	3.4 ± 1.6 [44]	—	3.7 ± 1.5[b] [117]	—
Glucose (mg/dL)						
0 mo	77.4 ± 6.0 [39]	78.7 ± 6.6 [20]	78.1 ± 6.4 [44]	0.41	78.0 ± 6.2[a,b] [103]	0.04
3 mo	77.1 ± 5.9 [40]	80.3 ± 9.3 [20]	80.4 ± 9.0 [44]	—	79.1 ± 8.1[a] [104]	—
6 mo	76.6 ± 6.7 [36]	77.1 ± 5.7 [19]	77.2 ± 5.5 [44]	—	76.9 ± 5.9[b] [99]	—

[1] All values are $\bar{x}$ ± SD; *n* in brackets. ZnFort, zinc-fortified group; ZnSuppl, zinc supplement group.

[2] Model 1 is a mixed model with repeated measures (PROC MIXED) that examined the effect of treatment group over time. The *P* value is for the treatment group × time interaction. Leptin, ghrelin, and insulin were log transformed. The models with leptin and ghrelin as the dependent variables included the following covariates: time, group × time, initial hormone, initial hormone × time, weight, weight × time, and sex. The models with insulin and glucose as the dependent variables included the following covariates: time, group × time, initial insulin/glucose value, initial insulin/glucose × time, weight, weight × time, sex, and presampling energy intake. ANOVA was used to examine initial (0 mo) differences among treatment groups. There was a significant difference in 0-mo leptin values between the ZnSuppl and ZnFort groups ($P = 0.03$).

[3] Model 2 is a mixed model with repeated measures (PROC MIXED) that examined the effect of time. The *P* value is for the variable time. Means in a column with different superscript letters are significantly different, $P < 0.05$.

ghrelin, or glucose. In other words, treatment group did not affect these outcomes at either time point. There was a significant group × time effect on insulin concentrations ($P = 0.007$). In particular, regression analyses of the change in log insulin showed a significantly greater decrease in insulin concentrations from baseline to 6 mo in the control group than in the zinc-fortification group ($P = 0.01$), but neither of these groups differed significantly from the ZnSuppl group.

Considering all treatment groups combined, plasma leptin concentrations decreased and ghrelin concentrations increased from baseline to 3 mo with no further change from 3 to 6 mo (Table 1). Insulin concentrations decreased from baseline to 6 mo. Glucose concentrations decreased from 3 to 6 mo. Girls had higher concentrations of plasma leptin and ghrelin, and the interaction between sex and time was significant for both leptin and ghrelin ($P = 0.01$) after BMI was controlled for. Plasma leptin concentrations were significantly greater in girls at all time points, and plasma ghrelin concentrations were greater in girls at baseline only (**Figure 2**). Girls had significantly higher concentrations of plasma leptin per kg of body fat than did boys at 6 mo (1.1 compared with 0.8 $ng \cdot mL^{-1} \cdot kg^{-1}$; $P < 0.0001$). Leptin concentrations were positively correlated with insulin concentrations ($r = 0.20–0.43$, $P < 0.01$) at all time points and with glucose at baseline ($r = 0.20$, $P < 0.05$) and 3 mo ($r = 0.24$, $P < 0.05$). Ghrelin concentrations were not correlated with the other hormones or glucose at any time point. Insulin and glucose concentrations were positively correlated at all time points ($r = 0.29–0.46$, $P < 0.01$).

Anthropometric measures and body composition

No group-wise differences were found in anthropometric *z* scores or body composition at baseline or 3 or 6 mo after the start of the zinc intervention. Therefore, the anthropometric and body-composition indexes of all children were combined for all treatment groups, and the results are presented for the 3 time points in **Table 2**. The mean weight-for-age *z* scores (WAZ) and LAZ decreased at each follow-up time ($P < 0.0001$). WLZ decreased at 3 mo ($P < 0.0001$), but did not change further from 3 to 6 mo. The children gained an average of 1.3 kg over the 6 mo. The majority of the weight gain consisted of FFM (94 ± 94%; median = 74%), and percentage body fat (%FM) decreased over the 6 mo ($P < 0.001$). Body weight was positively associated with %FM at baseline and 6 mo ($P < 0.001$). After body weight was controlled for, girls had a higher %FM than did boys at both time points ($P < 0.05$).

Dietary energy intake before blood sampling

The average energy intakes from the milk feeding 90 min before the blood sampling were 50 ± 31, 36 ± 211, and 43 ± 22 kcal at baseline, 3 mo, and 6 mo respectively. At baseline only, energy intakes were positively correlated with plasma concentrations of insulin ($r = 0.35$, $P < 0.001$) and glucose ($r = 0.25$, $P < 0.05$) after body weight was controlled for. Plasma leptin and ghrelin concentrations were not related to the energy content of the milk feeding before the blood sampling.

Relations between plasma hormone concentrations and anthropometric measures and body composition

Cross-sectional correlations between both leptin and ghrelin concentrations and anthropometric indexes at all 3 time points are presented in **Table 3**. In general, leptin concentrations were positively correlated with anthropometric indexes. There was a positive correlation between leptin and FM at both baseline and

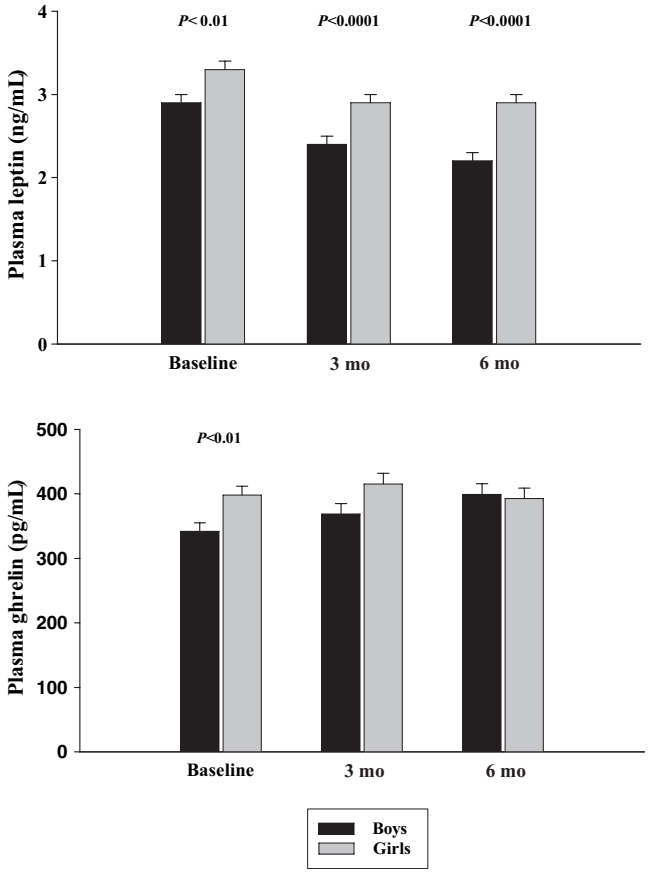

FIGURE 2. Mean (±SE) plasma leptin and ghrelin concentrations by sex and month of study after control for BMI in boys and girls aged 6–8 mo. A mixed model was used (PROC MIXED; SAS Institute Inc, Cary, NC) to examine the relation between sex and time. There was a significant interaction between sex and month of study for both leptin and ghrelin ($P = 0.01$).

6 mo. On the other hand, ghrelin was inversely correlated with weight, WAZ, BMI, and arm circumference. Ghrelin was also inversely correlated with FFM at 6 mo, but not with FM.

Multiple regression analyses were performed to examine the relations of leptin and ghrelin concentrations with prior and subsequent growth. Analyses were conducted with the 2 growth intervals: baseline to 3 mo and 3 to 6 mo. The results were similar during both growth intervals; therefore, for simplicity, we are presenting just the models relating to the 6-mo growth interval (baseline to 6 mo) for leptin (**Table 4**) and ghrelin (**Table 5**). Leptin at baseline was positively related to WAZ at baseline (model 1), but was not predictive of the subsequent change in WAZ (model 2). However, change in WAZ (ΔWAZ) from baseline to 6 mo was positively predictive of leptin at 6 mo (model 3). Current WAZ at 6 mo was also positively related to leptin at 6 mo (model 4). When both ΔWAZ and current WAZ were included in the same model, neither was significant (model 5). To determine whether one of these variables was more strongly associated with leptin at 6 mo, WAZ at 6 mo and ΔWAZ were standardized, and regression analyses were repeated with the standardized variables. There was no difference in the standardized β coefficients for WAZ at 6 mo and ΔWAZ (model 5: $t = 0.17$, $P = 0.43$), which indicated that neither of these variables was more predictive of leptin than the other. Ghrelin at baseline was not related to WAZ at baseline (model 1) or with subsequent ΔWAZ (model 2). Both ΔWAZ (model 3) and current WAZ (model 4) at 6 mo were negatively predictive of ghrelin at 6 mo in separate models. However, when both were included in the same model, only ΔWAZ was significant (model 5).

The relations of leptin and ghrelin concentrations with prior and subsequent changes in WLZ were similar to the relations previously described for changes in WAZ. That is, ΔWLZ was positively predictive of subsequent leptin concentrations and negatively predictive of subsequent ghrelin concentrations. There were no differences in the strength of the relations with hormones between WAZ and WLZ when the standardized β coefficients were compared. There was no relation between changes in LAZ and leptin or ghrelin concentrations.

Despite the positive relation between leptin concentrations and FM at baseline and 6 mo (Table 3), there were no associations between leptin and prior or subsequent changes in FM or %FM (data not shown). In contrast, ΔFFM was a positive predictor of

TABLE 2
Anthropometric indexes and body-composition variables measured at 3-mo intervals[1]

	Baseline ($n = 142$)	3 mo ($n = 126$)	6 mo ($n = 118$)	P^2
Anthropometric indexes				
Weight (kg)	7.6 ± 0.8[a]	8.3 ± 0.9[b]	8.9 ± 0.9[c]	<0.0001
Length (cm)	65.2 ± 1.8[a]	69.1 ± 1.9[b]	71.9 ± 1.9[c]	<0.0001
WAZ	−0.71 ± 0.84[a]	−1.19 ± 0.88[b]	−1.33 ± 0.84[c]	<0.0001
LAZ	−1.19 ± 0.51[a]	−1.28 ± 0.56[b]	−1.44 ± 0.52[c]	<0.0001
WLZ	0.51 ± 0.91[a]	0.12 ± 1.02[b]	0.11 ± 0.92[b]	<0.0001
BMI (kg/m^2)	17.8 ± 1.5[a]	17.3 ± 1.5[b]	17.3 ± 1.4[b]	<0.0001
MUAC (cm)	14.5 ± 1.1[a]	—	14.6 ± 1.1[a]	0.19
MUAC z score	−0.14 ± 0.92[a]	—	−0.70 ± 0.80[b]	<0.0001
Body composition[3]				
Fat-free mass (kg)	4.96 ± 0.66[a]	—	6.00 ± 0.69[b]	<0.0001
Fat mass (kg)	2.66 ± 0.66[a]	—	2.94 ± 0.77[b]	<0.0001
Fat mass (% of body weight)	34.8 ± 7.0[a]	—	32.6 ± 6.9[b]	0.0002

[1] All values are $\bar{x}$ ± SD. WAZ, weight-for-age z score; LAZ, length-for-age z score; WLZ, weight-for-length z score; MUAC, midupper arm circumference.
[2] Variables were compared by time with the use of a mixed model with repeated measures (PROC MIXED; SAS Institute, Cary, NC); values in a row with different superscript letters are significantly different, $P < 0.001$.
[3] $n = 127$ at baseline and $n = 116$ at 6 mo.

TABLE 3
Spearman correlation coefficients between hormones and anthropometric measures at the same time point[1]

	Leptin						Ghrelin					
	Baseline		3 mo		6 mo		Baseline		3 mo		6 mo	
	r	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*
Maternal BMI[2]	0.17[3]	(139)	0.20[3]	(121)	0.01	(115)	−0.10	(103)	−0.07	(102)	−0.02	(97)
Birth weight[2]	−0.12	(134)	−0.08	(119)	0.02	(113)	0.11	(101)	−0.02	(100)	−0.09	(95)
Weight	0.28[4]	(142)	0.27[5]	(123)	0.12	(117)	−0.28[5]	(105)	−0.32[5]	(104)	−0.31[5]	(99)
WAZ	0.46[6]	(142)	0.39[6]	(123)	0.30[5]	(117)	−0.16	(105)	−0.23[3]	(104)	−0.25[5]	(99)
Length	−0.18[3]	(142)	−0.13	(123)	−0.21[3]	(117)	−0.12	(105)	−0.25[3]	(104)	−0.17	(99)
LAZ	0.01	(142)	−0.05	(123)	0.03	(117)	0.08	(105)	−0.09	(104)	−0.05	(99)
WLZ	0.51[6]	(142)	0.45[6]	(123)	0.35[4]	(117)	−0.22[3]	(105)	−0.20[3]	(104)	−0.27[5]	(99)
BMI	0.48[6]	(142)	0.43[6]	(123)	0.30[4]	(117)	−0.25[3]	(105)	−0.22[3]	(104)	−0.26[5]	(99)
MUAC	0.50[6]	(142)	—		0.37[6]	(117)	−0.23[3]	(105)	—		−0.25[3]	(99)
MUAC *z* score	0.56[6]	(142)	—		0.46[6]	(117)	−0.11	(105)	—		−0.22[3]	(99)
FFM	−0.09	(127)	—		−0.07	(115)	−0.17	(97)	—		−0.22[3]	(97)
FM	0.42[6]	(127)	—		0.24[5]	(115)	−0.08	(97)	—		−0.12	(97)
Percentage FM	0.39[6]	(127)	—		0.21[3]	(115)	−0.01	(97)	—		−0.04	(97)

[1] WAZ, weight for age *z* score; LAZ, length-for-age *z* score; WLZ, weight-for-length *z* score; MUAC, midupper arm circumference; FFM, fat-free mass; FM, fat mass.
[2] The $\bar{x}$ (± SD) maternal BMI (in kg/m^2) was 25.4 ± 3.7, and the reported birth weight was 3.1 ± 0.4 kg.
[3] $P < 0.05$.
[4] $P < 0.001$.
[5] $P < 0.01$.
[6] $P < 0.0001$.

leptin at 6 mo ($P < 0.05$); however, this relation did not remain significant when FFM at 6 mo (which was not significant) was included in the model. In separate models with ΔWAZ as the dependent variable, both ΔFFM and ΔFM were positively associated with ΔWAZ ($P < 0.001$). However, the standardized β coefficient for ΔFM ($\beta = 0.32$) was significantly greater than that for ΔFFM ($\beta = 0.20$; $t = 2.3$, $P = 0.01$). In addition, the percentage of the 6-mo weight gain that was FM was positively associated with ΔWAZ. In contrast, the percentage of weight gain that was FFM was negatively associated with ΔWAZ. In other words, infants with the greatest weight gain or ΔWAZ had a greater percentage of that weight gain as FM. However, the percentage of weight gain that was FM was not predictive of leptin at 6 mo, nor was the change in %FM or %FFM. The only relation found between ghrelin concentration and body-composition measures was a negative association between ghrelin at 6 mo and FFM at 6 mo ($P < 0.05$). In a regression model of the change in ghrelin over 6 mo, there was a negative relation between the changes in ghrelin and FFM ($P = 0.01$). There was a significant interaction between change in FFM and change in WAZ ($P < 0.05$), ie, only among children with weight gain less than the median value of ΔWAZ was there a negative relation between change in ghrelin and change in FFM.

TABLE 4
Multiple regression models that examined the relation between plasma leptin concentrations and weight-for-age *z* score (WAZ) and change in WAZ (ΔWAZ) during a 6-mo period (from baseline to 6 mo)

Model[1]	Dependent variable	Independent variable	β Coefficent (*P*)	Standardized β Coefficient ± SE[2]
1[3]	log Leptin, baseline	WAZ, baseline	0.17 (<0.0001)	—
2[4]	ΔWAZ, from baseline to 6 mo	log Leptin, baseline	0.08 (0.67)	—
3[5]	log Leptin, 6 mo	ΔWAZ, from baseline to 6 mo	0.14 (0.003)	0.075 ± 0.025
4[6]	log Leptin, 6 mo	WAZ, 6 mo	0.10 (0.003)	0.082 ± 0.027
5[7]	log Leptin, 6 mo	ΔWAZ, from baseline to 6 mo	0.09 (0.07)	0.051 ± 0.028
		WAZ, 6 mo	0.07 (0.07)	0.056 ± 0.030

[1] Data were analyzed by using ANCOVA (PROC GLM; SAS Institute Inc, Cary, NC).
[2] The primary independent variables for models 3–5 were standardized, and β coefficients for standardized variables were determined by using ANCOVA (PROC GLM); *t* tests conducted to compare standardized β coefficients from models 3 and 4, and with standardized β coefficients from model 5, determined that there was no difference in the strength of the relation between WAZ at 6 mo and ΔWAZ from baseline to 6 mo and leptin at 6 mo ($t = 0.17$, $P = 0.43$).
[3] Model 1 ($n = 142$) controlled for sex (NS).
[4] Model 2 ($n = 118$) controlled for WAZ at baseline ($\beta = -0.21$, $P = 0.004$) and sex (NS).
[5] Model 3 ($n = 117$) controlled for log leptin at baseline ($\beta = 0.37$, $P < 0.0001$) and sex (F > M, $P = 0.0001$).
[6] Model 4 ($n = 117$) controlled for log leptin at baseline ($\beta = 0.24$, $P = 0.005$) and sex (F > M, $P < 0.0001$).
[7] Model 5 ($n = 117$) controlled for log leptin at baseline ($\beta = 0.29$, $P = 0.001$) and sex (F > M, $P < 0.0001$).

TABLE 5
Multiple regression models that examined the relation between plasma ghrelin concentrations and weight-for-age *z* score (WAZ) and change in WAZ (ΔWAZ) during a 6-mo period (from baseline to 6 mo)

Model[1]	Dependent variable	Independent variable	β Coefficient (*P*)	Standardized β coefficient ± SE[2]
1[3]	log Ghrelin, baseline	WAZ, baseline	−0.04 (0.18)	—
2[4]	ΔWAZ, from baseline to 6 mo	log Ghrelin, baseline	−0.09 (0.70)	—
3[5]	log Ghrelin, 6 mo	ΔWAZ, from baseline to 6 mo	−0.14 (0.002)	−0.081 ± 0.025
4[6]	log Ghrelin, 6 mo	WAZ, 6 mo	−0.07 (0.02)	−0.060 ± 0.026
5[7]	log Ghrelin, 6 mo	ΔWAZ, from baseline to 6 mo	−0.12 (0.02)	−0.068 ± 0.027
		WAZ, 6 mo	−0.04 (0.24)	−0.033 ± 0.028

[1] Data were analyzed by using ANCOVA (PROC GLM; SAS Institute Inc, Cary, NC).
[2] The primary independent variables for models 3–5 were standardized, and β coefficients for standardized variables were determined by using linear regression; *t* tests conducted to compare standardized β coefficients from models 3 and 4, and with standardized β coefficients from model 5, determined that there was no difference in the strength of the relation between WAZ at 6 mo and ΔWAZ from baseline to 6 mo and ghrelin at 6 mo ($t = 1.25$, $P = 0.11$).
[3] Model 1 ($n = 105$) controlled for sex (F >M, $P = 0.0006$).
[4] Model 2 ($n = 99$) controlled for at baseline WAZ ($\beta = -0.22$, $P = 0.003$) and sex (NS).
[5] Model 3 ($n = 99$) controlled for log ghrelin at baseline ($\beta = 0.50$, $P < 0.0001$) and sex (NS).
[6] Model 4 ($n = 99$) controlled for log ghrelin at baseline ($\beta = 0.47$, $P < 0.0001$) and sex (NS).
[7] Model 5 ($n = 99$) controlled for log ghrelin at baseline ($\beta = 0.48$, $P < 0.0001$) and sex (NS).

DISCUSSION

Contrary to our hypotheses, we did not find differences in plasma leptin, ghrelin, or glucose concentrations between infants who received zinc supplements or zinc-fortified food for 6 mo and infants who did not receive additional zinc. Although children in the control group had a significantly greater decrease in plasma insulin concentrations during the 6-mo study than did children who received the zinc-fortified porridge, the change in insulin concentrations among children receiving zinc in a liquid supplement did not differ from that of the control group. Thus, it is possible that the insulin results observed in the ZnFort group were spurious because we would have expected to see the same effect with the liquid zinc supplement if these results were due to zinc. In a previous analysis we found that children in the ZnSuppl group had a significant increase in plasma zinc concentrations, but children in the ZnFort group did not (15). Thus, it seems unlikely that the insulin results could have been due to differences in zinc absorption.

Supplemental zinc also had no effect on the infants' growth. Possible explanations for the lack of zinc-induced differences in child growth and appetite-related hormone concentrations could be poor compliance with the intervention, inadequate dose of zinc, or absence of zinc deficiency in the study population. In fact, compliance with taking the liquid zinc supplement was very good; children in all groups consumed the supplement on 98% of the days, and the average porridge consumption was ≈23 g/d. Thus, poor compliance is an unlikely explanation for the observed results. The zinc dose of 3 mg/d used in this study was less than the amounts administered in many previous supplementation trials (22–26). Nevertheless, this dose is consistent with the recently revised Recommended Dietary Allowance of 3 mg/d and less than the Upper Tolerable Limit of 5 mg for young children between the ages of 7–12 mo (27). Thus, the dose of zinc provided should have been adequate to satisfy the theoretical requirement.

Although there is no valid indicator to identify mild-to-moderate zinc deficiency in individuals, the risk of zinc deficiency in a population can be assessed on the basis of the prevalence of low plasma zinc concentrations or inadequate dietary zinc and the percentage of the population with a low height-for-age *z* score (28). The children in the present study were selected to be at high risk of zinc deficiency based on their initial LAZ < −0.5. However, the final mean LAZ was −1.3, so the children did not become as stunted as did those in earlier studies, and they may not have been sufficiently stunted to respond to zinc. Current guidelines suggest that a population is considered to have a high risk of zinc deficiency when >20% of the plasma zinc concentrations are less than reference cutoffs. However, only 17% of infants in our study had low plasma zinc concentrations (<65 μg/dL) at baseline, which suggests that this population may not have been zinc deficient.

Despite a lack of effect of zinc on the hormone concentrations or growth of the children, our study design allowed us to examine both cross-sectional and longitudinal relations between the hormones and anthropometric indexes and body composition. Our study was unique in that we measured plasma leptin and ghrelin concentrations multiple times and examined periods of growth both before and after the hormones were measured while controlling for other factors related to these hormones, such as previous hormone concentrations and child sex. We found that plasma concentrations of leptin and ghrelin were not predictive of subsequent growth, but were related to prior growth as well as current weight status. The strength of the associations between current weight status and prior weight change were similar, so we were unable to determine whether prior growth or current status is a more important determinant of these hormone concentrations. Nevertheless, these findings suggest a role for leptin and ghrelin that may be compensatory for, rather than causative of, weight changes during later infancy.

A compensatory role for leptin and ghrelin has been noted in other studies. Leptin circulates in proportion to FM and decreases with weight loss (29). On the other hand, plasma ghrelin concentration are increased during weight loss and in patients with anorexia nervosa and are higher in obese than in lean individuals (30). In Gambian infants, leptin concentrations were positively related to prior changes in BMI *z* scores, but not with subsequent growth (8). Another study in Italian infants reported a negative association between weight gain since birth and subsequent

ghrelin concentrations during the first year of life (31). Our findings of a positive relation between current body weight or BMI and leptin and of an inverse relation between weight and ghrelin are also consistent with other reports on leptin (8–11) and ghrelin (10, 12–14) concentrations during infancy.

In contrast with our findings and those of others of a compensatory role for leptin and ghrelin during infant growth, some studies have found an association between these hormones measures at birth and subsequent growth during infancy. Ong et al (9) reported that cord blood leptin was a negative predictor of weight gain from birth to 4 mo of age ($P < 0.005$). James et al (32) found that low ghrelin concentrations in cord blood at birth were associated with slow weight gain over the first 3 mo of life, although they found no relation between leptin and subsequent growth. We found no associations between leptin or ghrelin and subsequent weight change during later infancy; however, it is possible that hormone concentrations in the newborn have a role in growth early in infancy, but not later.

Our finding that leptin concentrations were significantly higher in girls than in boys after BMI was controlled for agrees with the findings of other studies (8, 33). The female children in our study had a higher %FM; however, leptin concentrations were higher at 6 mo even after %FM was controlled for. The reason for higher leptin concentrations in females is unclear; however, it has been reported that leptin expression in vitro and in vivo is suppressed by androgens and promoted by estrogens (34). In contrast with leptin, most studies have found no sex differences in ghrelin concentrations during infancy (10, 12, 14, 31, 35). We found higher ghrelin concentrations in girls at baseline, but not at the later time points.

The disparity between the relations between hormones and changes in WAZ compared with FFM or FM is puzzling. Changes in WAZ was more strongly related to changes in FM than with changes in FFM, although both were related to changes in WAZ. The changes in WAZ and changes in FFM predicted final plasma leptin concentrations at 6 mo, whereas changes in FM did not. Because 94% of the weight gained by these children over the 6 mo of the study was due to increased FFM, it appears likely that changes in WAZ would be more closely related to changes in FFM. However, children who gained more weight had a greater percentage of that weight gain as FM. There was great variability in the percentage of weight gain that was FM; therefore, the lack of measurement precision may explain the lack of an association with subsequent leptin concentrations.

In conclusion, supplemental zinc did not influence plasma leptin or ghrelin concentrations in this population of young children. Plasma leptin and ghrelin concentrations were associated with both current weight status and prior growth but not with subsequent growth during a 3- or 6-mo period. However, some evidence suggests that early metabolic programming may affect the future risk of obesity (36). Despite our findings of no association with short-term growth, it is possible that these hormones may have more long-term effects on weight and body composition in later childhood. Further research is needed to determine whether leptin and ghrelin during periods of undernutrition in infancy may affect long-term body weight and body composition.

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The authors' responsibilities were as follows—JEA: study design, implementation of the project in the field, data analysis, interpretation of results, and preparation of the manuscript; KHB: study design, overall supervision of the research team, interpretation of results, and preparation of the manuscript; PJH and MDV: study design, interpretation of results, and preparation of the manuscript; DLdR: study design, implementation of the project in the field, interpretation of results, and preparation of the manuscript; and MEP: study design, training of field staff, interpretation of results, and preparation of the manuscript. None of the authors had any conflict of interest.

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