The effects of a dietary zinc supplement during lactation on longitudinal changes in maternal zinc status and milk zinc concentrations1–3

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ABSTRACT Dietary zinc intakes, selected biochemical indices of zinc status, and milk zinc concentrations were determined at monthly intervals throughout lactation for 53 middle-income lactating women, 14 of whom received a daily supplement of 15 mg zinc. Overall mean dietary zinc intake for the non-supplemented group (NZS) was 10.7 ± 4.1 mg/day (x ± SD). The mean dietary zinc intake of the zinc supplemented group (ZS) was 12.2 ± 3.5 mg/day, with an additional 12.8 ± 1.5 mg/day from the supplement. For the NZS group, the highest mean plasma zinc concentration of 79 ± 10 μg/dl, which occurred at month 4, was significantly less than the mean for non-lactating control women (86 ± 10 μg/dl). ZS plasma zinc levels had a pattern similar to that of the NZS group for months 1–7. The rate of decline in milk zinc during lactation was significantly less for the ZS group compared to that of the NZS group (p = 0.02). It is concluded that milk zinc concentrations are influenced by maternal zinc intake within a physiological range and that the effects of low maternal intakes are most apparent with prolonged lactation. Am J Clin Nutr 1985;41:560–570.

KEY WORDS Milk zinc concentration, longitudinal study of lactation, zinc supplementation, plasma zinc concentrations, dietary zinc intake

Introduction

Published data on the zinc content of human milk are growing steadily (1–22) and there are some reports of calculated dietary zinc intakes of women during lactation (11, 12, 20, 23, 24, 25). However, relatively little is known about the effects of lactation on maternal zinc status (20) and about potential interactions among dietary intake, maternal status, and the concentration of zinc in human milk as lactation progresses. The broad objectives of this study were to calculate dietary zinc intakes, evaluate maternal zinc nutritional status, and determine zinc concentrations in milk longitudinally at monthly intervals throughout lactation in a group of apparently healthy well-nourished women. As this study progressed, it became apparent that the calculated dietary zinc intakes were uniformly low in relation to the Recommended Dietary Allowance (RDA) of 25 mg/day for lactating women (26). Accordingly, maternal zinc status and milk zinc concentrations were determined in another similar group of lactating women who received a daily zinc supplement throughout lactation. This supplement was designed to increase the total daily zinc intake to a level of approximately 25 mg/day in order to assess the effects of changes in daily zinc intake within a physiological range.

Methods

Experimental design

The study was designed as a longitudinal investigation of lactating women. Subjects who gave informed consent were enrolled during the first month of lactation. At one

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Apart of percent subjects from tablet subjects respectively, primarily who 30 years. The data have been analyzed using glass capillary pipettes that had been washed in HCl and deionized water and were stored at -20°C in Eppendorf tubes. For analysis 2 x 150 μl aliquots of plasma were transferred to small circular quartz dishes for overnight ashing in a low temperature asher (LFE Corp., Waltham, MA). The ashed samples were dissolved with 150 μl of 0.1 N HCl. A 100 μl aliquot of the dissolved ash was diluted with 500 μl of 6% n-butanol in 0.1 N HCl (28). The samples were analyzed by flame atomic absorption spectrophotometry with a modified Perkin-Elmer 503 fitted with deuterium arc background correction and AS-3 auto sampling system (Perkin Elmer Corp., Norwalk, CT). Standards were also made up in 6% butanol in 0.1 N HCl. The value obtained for National Bureau of Standards bovine liver using the same analytical techniques was 129 ± 3 μg zinc/g dry weight (certified value = 130 ± 13).

Urine zinc. Twenty-four hour urine samples were collected in EDTA washed containers. Samples were analyzed directly for zinc by flame atomic absorption spectrophotometry.

Hair zinc. A small hair sample (approximately 10 mg) was cut close to the scalp in the suboccipital region and treated as described previously (29). Zinc levels were measured by flame atomic absorption spectrophotometry.

Milk zinc. Milk samples of approximately 5 ml were collected by manual expression directly into zinc-free polypropylene containers, and immediately frozen at -20°C until thawed for analysis. Sample ashing and analytical procedures were identical to those employed for plasma, except that further dilutions of dissolved ash were necessary for samples with relatively high zinc concentrations that were collected during the first two months of lactation. Within run precision was 4.7%. Recovery of added zinc averaged 99.6 ± 3.1%. Most subjects provided multiple samples at each cycle. An initial objective of this study was to determine if there were diurnal or within feed changes in milk zinc concentration. Neither of these factors had a significant effect (30). Therefore, the concentrations for all samples from each individual at each month were averaged to obtain a single mean milk zinc concentration per subject per day. Analytical procedures were identical to those employed for plasma zinc determinations.

Ancillary laboratory assays. Alkaline phosphatase activity was determined by a modification of Bowers’ and McCombs’ kinetic method at 37°C (31). Serum albumin was measured by the bromocresol green procedure (32).

Dietary analyses
Of the 32 NZS subjects who provided monthly dietary data, 16 completed 1 day records and 16 completed 3 day records. All NZS subjects completed 3 day records at each cycle. Records were completed before monthly cycle visits and were reviewed for accuracy and completeness by the research nutritionist at the time of sample collection. Nutrient intakes were computer calculated using a data base derived from the USDA Handbook of Food Composition (34). As described previously (27) zinc values were added to the data base from various published references, manufacturer’s data,
laboratory analyses, and calculations from recipes. When no analytical data were available, zinc values were imputed from similar foods, thus avoiding systematic underestimation of zinc intakes due to missing data in the base.

Statistical analyses

Changes in biochemical indices over the course of lactation were evaluated by regression analysis (linear or quadratic) on each individual subject's longitudinal data. Though all monthly means are plotted in Figures 2-4, only the first 9 months' data were included in regression analyses, unless otherwise indicated. The validity of the regression analysis for a given parameter was evaluated by consideration of $r^2$ values on each individual's regression. For parameters for which regression analysis was applicable, the coefficients of the regression equations on individuals' data were then averaged and the means compared between groups and to zero by two-tailed "Student's" t tests. An important rationale for use of this technique of averaging individual regressions was the avoidance of undue weighting of the more limited data in the later months which would have resulted from direct linear regression on the monthly means. For the milk zinc data, log transformations (to the base 10) were performed on both milk zinc concentrations and months of lactation prior to linear regression analysis as described above. The validity of a linear model was assessed further by comparison of the observed monthly geometric means with the predicted geometric means derived from the mean regression equations for the 2 groups.

At selected months, differences between concentrations for lactating subjects and the non-lactating controls were compared by t tests. Pearson correlation coefficients were computed between selected variables at each month of lactation. All statistical analyses were performed with the Minitab II (34) computer program.

Results

Dietary data. For the NZS subjects there were no significant differences between monthly overall means for 3 day vs 1 day diet records. Hence, 1 day and 3 day data were combined to give total monthly means. For zinc, these ranged from 11.7 ± 5.4 mg/day at month 4 to 8.8 ± 4.3 mg/day at month 10. Mean zinc intakes did not change significantly at any stage of lactation and linear regression of dietary zinc intake on month of lactation was not significantly different from zero. The overall mean calculated dietary zinc intake for the NZS group was 10.7 ± 4.1 mg/day. Figure 1 depicts the frequency distribution of calculated daily dietary zinc intakes based on individual 3-day records only. Only 2% of these provided two-thirds or more of the RDA. Seventy-seven percent failed to reach one half the RDA,

![Zinc Intake Distribution](image)

**FIG 1.** Frequency distribution of calculated dietary zinc intakes.
including 29% that were less than one-third the RDA.

Monthly means for energy, protein and dietary iron did not vary significantly with duration of lactation. Overall mean daily intakes were 2010 ± 510 kcal, 80 ± 25 g protein and 12.5 ± 4.5 mg iron. Zinc and iron intakes were both significantly correlated (p < 0.01) with protein and energy intakes. The overall mean zinc intake was 5.3 ± 1.5 mg per 1000 kcal and 0.13 ± 0.03 mg per g protein. Individual zinc (mg):protein (g) ratios ranged from 0.06 to 0.25.

Ninety percent of NZS subjects took a multivitamin tablet containing iron on a daily basis for at least 5 months of lactation. Over the same time interval 37% of subjects took, in addition, a specific iron supplement. From the combination of the multivitamin preparation and the iron supplement, the subjects comprising this 37% took an average of 122 ± 65 mg supplemental iron per day. The remaining 54% who took only a multivitamin with iron preparation, had an average of 47 ± 22 mg supplemental iron per day.

The mean calculated monthly dietary zinc intakes for the ZS group also did not vary significantly with duration of lactation. The overall mean was 12.2 ± 3.5 mg zinc/day, ranging from 13.4 ± 3.4 mg/day at month 9 to 11.4 ± 2.3 mg/day at month 4. Compliance for the supplementation averaged 85% which provided an additional 12.8 ± 1.5 mg zinc/day. The mean dietary zinc intake was 5.9 ± 1.6 mg per 1000 kcals and 0.13 ± 0.03 mg per g protein. Although the overall mean energy intakes did not differ between the two groups, the zinc:energy ratio for the ZS group was significantly (p < 0.05) higher than that for the NZS group. Nine of the 14 ZS subjects took multivitamin preparations containing iron for at least 5 months of lactation, and these provided an average of 54 ± 11 mg iron per day. Only one of these subjects took an additional separate iron supplement.

**Plasma zinc.** The mean plasma zinc concentration of the NZS group at one month lactation (70 ± 11 µg/dl) was significantly (p < 0.001) higher than that at 36 weeks gestation (54 ± 9 µg/dl) (27). The maximum value of 79 ± 10 µg/dl occurred at 4 months

![Plasma zinc concentrations (mean ± SEM) at each month of lactation. Standard errors not indicated for months at which data were available for <3 subjects.](https://academic.oup.com/ajcn/article-abstract/41/3/560/4691532)
lactation (Fig 2); this value was significantly lower than that of the non-lactating control women (86 ± 10 μg/dl). Neither linear ($r^2 = 1\%$) nor quadratic ($r^2 = 3\%$) regression analysis fitted these data. Mean plasma zinc concentrations for the ZS group are also shown in Figure 2. These data were similar to corresponding data for the NZS group, at least through the first 7 months of lactation. Because these longitudinal data could not be satisfactorily analyzed by regression analysis, no additional statistical comparisons of the NZS and ZS concentrations were attempted.

**Urine zinc.** Twenty-four hour urine zinc excretion rates are depicted in Figure 3. With the exception of the fourth month, all monthly means were below the control mean of 424 ± 163 μg/24 hours. Mean urine zinc concentrations of the ZS group followed a similar longitudinal pattern to that of the NZS group, but at 7 of the 9 months for which data were available, means for the ZS group were above the control mean. Urine zinc:creatinine ratios closely paralleled the 24-hour urine zinc excretion rates for both groups.

**Hair zinc.** The mean slope of the regression line of hair zinc on month of lactation was not significantly different from zero for either group. Mean monthly hair zinc concentrations for the NZS group ranged from a maximum of 178 ± 61 at month 5 to a minimum of 148 ± 4 μg/g at month 11. None of the monthly means differed significantly from the control mean of 168 ± 37 μg/g. The monthly means for the ZS group overlapped those of the NZS group, and generally showed a similar pattern. The overall means for the NZS and ZS groups were 164 ± 47 μg/g and 168 ± 32 μg/g, respectively.

**Serum alkaline phosphatase.** The mean slope of the regression line of alkaline phosphatase activity on month of lactation was
not significantly different from zero for either the NZS or ZS group. Overall means for the NZS and ZS groups were 80 ± 23 and 86 ± 22 IU/L, respectively. The mean for the control subjects was 62 ± 16 IU/L.

Serum albumin. At 1 month lactation mean serum albumin levels for both the NZS and ZS groups, 4.03 ± 0.51, 4.16 ± 0.63 g/dl respectively, were significantly (p < 0.001) lower than the control mean of 4.52 ± 0.25 g/dl. For the remaining months, mean concentrations for both groups were similar to the control mean, with overall means of 4.56 ± 0.49 and 4.41 ± 0.50 g/dl for the NZS and ZS groups, respectively.

Milk zinc concentrations. Mean milk zinc concentrations at each month of lactation are shown in Figure 4 for both NZS and ZS groups. The mean monthly zinc concentration of the NZS group declined from 2.65 ± 0.81 μg/ml at 1 month to 0.67 ± 0.40 μg/ml at 9 months. Corresponding means for the ZS group were 2.83 ± 1.05 μg/ml and 0.82 ± 0.54 μg/ml, respectively. Log transformations were performed on both the milk zinc concentrations and the month of lactation.
tion. Linear regression analysis was then performed on each individual's longitudinal data. The mean $r^2$ values for these linear regression analyses were 85 ± 12% and 83 ± 17% for the NZS and ZS groups respectively, indicating that the log–log transformations had adequately straightened the curves of the longitudinal data for use of a linear model. The mean slopes for the NZS and ZS groups from 1 through 9 months (Fig 5) were $-0.6944 \pm 0.2750$ and $-0.5396 \pm 0.1363$, respectively. Comparison by a two-sample $t$ test indicated that the mean slope of the regression equations for the ZS group was significantly less than that of the NZS group ($p = 0.02$). Identical regression analyses were performed with the inclusion of all available data beyond 9 months, and this also showed a significant difference ($p = 0.03$) in mean slopes between the NZS and ZS groups. Finally, analysis of data limited to those subjects (8 NZS; 4 ZS) who remained in the study for at least 9 months also revealed a significant difference ($p < 0.05$) in mean slopes between the 2 groups.

Correlations. No consistently significant correlations were found across lactation between a wide selection of dietary and laboratory parameters.

Discussion

The calculated mean dietary zinc intakes of the subjects in this study were similar to an earlier report from this laboratory (35) and to results of other investigators in this country (11, 12, 20, 24, 25) and elsewhere (23). The adequacy of this level of intake remains uncertain. Undoubtedly this will depend in part on variations in bioavailability of zinc from the particular composite diets selected by individual lactating women. Though much remains to be learned about this topic, there are suggestions that there could be particular factors in the diets of lactating women that may affect zinc absorption. For example, increased calcium/milk intakes can have significant effects, depending on the other constituents of the meal (36, 37). Although there are indications from animal studies that zinc absorption is increased during lactation (38) independent of maternal zinc nutritional status, it is presently unknown whether this also applies to humans. These are examples of areas requiring extensive research before it is possible to precisely define dietary zinc requirements during lactation. Meanwhile it is unjustifiable to conclude either that the RDA is excessive or that current levels of intake are optimal merely because they are common.

The mean zinc to protein ratios of the subjects in this study were also similar to those reported elsewhere (25). However, the fourfold difference observed between individual records indicates that it is possible to have considerable variation in zinc intake at the same level of protein intake. Twenty-one records had average zinc (mg) to protein (g) ratios of 0.18 or more. At the average protein intake of the NZS subjects (80 g/day), the average zinc intake given a ratio of 0.18 would be 14.4 mg/day. This is 35% higher than the actual calculated mean intake and, though far short of the RDA, could be of practical significance.

Among additional dietary factors that merit consideration in the context of this report, the level of self-administered iron supplementation is noteworthy. Especially in view of recent observations during pregnancy (27), it is tempting to hypothesize that this level of iron intake may have had adverse effects on zinc absorption. However, no correlation was observed in this study between the level of iron supplementation and either plasma or milk zinc concentrations.

Plasma zinc remains the most widely accepted and useful laboratory index of zinc status even though it lacks adequate sensitivity and discrimination for the detection of marginal zinc deficiency states (39). The early post-partum increase in plasma zinc concentrations in this study can be attributed to physiological changes associated with delivery, including restoration of normal non-pregnant blood volume, hormonal changes, and an increase in serum albumin. Serum albumin normalized by the second month, which is in agreement with some (40, 41) but not all (42) previous reports. In contrast to pregnancy there are no recognized physiological factors operative during lactation that depress plasma zinc concentrations. Thus the consistently
low levels of the NZS group observed in this study throughout lactation in comparison with the mean for control subjects suggests some impairment of zinc nutritional status. Additional studies with modified experimental design including more data after 6 months lactation will be necessary to ascertain whether a zinc supplement such as that administered in this study is sufficient to normalize plasma zinc concentrations, especially with prolonged lactation. The length of time required for plasma zinc to normalize after either short-term or prolonged lactation is unknown. This question is of potential concern with respect to a subsequent pregnancy, particularly if the latter commences before lactation has been completed and while plasma zinc levels may be depressed. The pattern of mean urine zinc excretion rates for the NZS group across lactation resembled that for plasma zinc with a maximum value at 4 months and nadir at 9 months, suggesting that both reflected the same factor(s) affecting zinc status during lactation.

As has been reported elsewhere (43, 44, 45, 46), serum alkaline phosphatase activity of both groups was higher throughout lactation than for control women. The increased activity has been attributed to increased bone remodelling secondary to the substantial increase in calcium requirements during lactation. In these circumstances it is unclear how the level of activity would be influenced by a co-existing mild zinc deficiency state.

The mean one month milk zinc concentration of both the NZS and ZS groups in this study are both similar to other recently reported values at a comparable stage of lactation which have ranged from 2.5–2.9 μg/ml (10, 13, 18, 20, 24). This represents a notable decline from zinc concentrations in colostrum and transitional milk (1, 7). The decline in milk zinc concentrations continues as lactation progresses. The mean for the NZS group declined by 60% between 1 and 3 months. Comparable figures from other recent studies ranged from 50–56% (10, 20). By 6 months, the mean value was only 33% of the 1 month means, compared with 20 and 42% from 2 other recent studies (10, 20). The similarities between different studies at comparable stages of lactation suggest that these figures may to a large extent be accepted as part of a normal physiological pattern of decline in zinc concentrations of human milk as lactation progresses. However, this does not exclude subtle but potentially important differences within this broad framework due to variations in maternal dietary zinc intake and/or maternal zinc nutritional status, a possibility which is supported by the findings in this study.

Milk zinc concentrations have been reported not to correlate with maternal zinc status or dietary zinc intake (9, 11, 12, 20). However, effects of long-term administration of a separate daily zinc supplement have not been reported previously. In this study a modest zinc supplement sufficient only to raise the total daily zinc intake to a level comparable to the RDA of 25 mg, was associated with a rate of decline in milk zinc concentration that was significantly less than that for the NZS group. The mammary gland appears to have remarkable homeostatic mechanisms to control milk zinc. An increase in milk zinc concentrations does not occur relatively early in lactation when mothers are given short-term pharmacological quantities of zinc that are sufficient to elevate serum zinc concentrations (47). Hence, pending confirmation with a randomized controlled supplementation study, a reasonable interpretation of the milk zinc data in this study is that the long-term maternal zinc supplement was preventing an abnormally steep rate of decline in milk zinc caused by suboptimal maternal zinc intake. This explanation also fits with data from experimental animals, in whom maternal zinc deficiency is associated with abnormally low milk zinc concentrations (48). The latter do not occur if a zinc supplement is added to the zinc-deficient maternal diet. A substantial (23%) reduction in milk zinc concentration with only minimal changes in plasma zinc has been observed in dairy cows fed a “practical” moderately zinc deficient diet (49), an observation which appears to be similar to those pertaining to human lactation in this study.

During the first 6 months of lactation the rate of decline of milk zinc in the NZS group may not be a major concern, as differences between ZS and NZS averaged less than 10%.
On the other hand, differences averaged approximately 50% between 7–9 months lactation. The latter could well have implications of nutritional importance in countries where older infants typically continue to derive a large percentage of their nutrients from breast milk (50) and in this country among infants whose mothers elect to feed primarily from the breast after the first 6 months. These nutritional implications may be appreciated with the following simple calculations. Assuming that the zinc content of the fat free body is approximately 30 μg/g (51), the zinc required for growth of the normal infant aged 7–8 months would be 330 μg/day. Urine zinc excretion would be approximately 80 μg/day, and from extrapolation from adults (52) a similar loss may occur via sweat. Hence even if there are no obligatory losses of endogenous zinc via the gastrointestinal tract, calculated absolute zinc requirements at this age are approximately 500 μg/day. If the infant continues to be solely breast-fed at this age, and assuming the volume of breast milk intake is 750 ml/day (53), the average zinc intake for infants aged 7–8 months of mothers who did not receive the zinc supplement would amount to only 420 μg/day. This would be inadequate to meet requirements even with 100% absorption. However, applying parallel calculations for the zinc-supplemented subjects, requirements would be met at 75% absorption. Thus these superficially quite small differences in zinc intake could have a profound effect on the zinc status of the infant over a period of a few weeks or months.

Incidentally, the estimated absolute requirement of 500 μg/day appears to be remarkably low in comparison with earlier calculations (51) and in comparison with the RDA (26). However, earlier calculations erred on the “safe” (ie, high) side, especially with respect to urine and sweat losses in the young infant. Dietary requirements as opposed to absolute requirements will depend of course, on percentage absorption. Zinc absorption from infant formulas appears to be relatively low (54, 55); hence, current RDA’s appear to be reasonable. However, it is a misuse of the RDAs to describe breast milk as deficient in a specific nutrient on the basis of comparison with the RDA.

From a practical viewpoint, the type of maternal zinc supplement may be of considerable importance in determining the effect, if any, on milk zinc concentrations. Self-supplemented lactating women who took 15 mg zinc per day in a multi-vitamin/mineral preparation did not have any detectable increase in milk zinc concentration above that of unsupplemented controls (Krebs and Hambrdige, unpublished data), an observation consistent with the findings of other investigators (8, 9, 18). Possible explanations for this lack of effect include impaired bioavailability due to the iron present in these preparations and to additional specific iron supplements taken simultaneously by many of this self-supplemented group.

Finally, in the authors’ opinion these findings do not necessarily suggest that routine zinc supplements during lactation are either essential or desirable. Adequate dietary counselling may well prove a more desirable and effective alternative, especially in countries such as the United States where changing dietary habits is a viable practical possibility. Such changes would be geared not only toward increasing total daily zinc intake without substantially increasing protein intake, but would also take into account factors influencing bioavailability.

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References

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