Effects of contraceptives on mother's serum and milk zinc

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The changes in concentration of zinc in serum and milk were studied in 81 nursing mothers following different contraceptive practices. Blood and milk samples from users of combined pills (levonorgestrel 0.15 mg + ethinylestradiol 0.03 mg; levorgestrel 0.25 mg + ethinylestradiol 0.05 mg; n = *15), minipill (norethindrone 0.35 mg;* n = *29), injectable progesterone (depot medroxiprogesterone acetate 150 mg;* n = *13), intrauterine devices (IUD) (plastic or copper;* n = *5), or no contraceptives (barrier and natural methods or sterilization;* n = *22) were collected before and 20 (range, 7–103) days after initiation of study. Mean length of lactation prior to taking contraceptives were 88, 73, 36, 57, and 108 days, respectively, with follow-up observation for 26, 20, 20, 18, and 22 days, respectively. Mean concentrations of serum zinc before contraceptives ranged from 0.34 to 1.14 µg/mL and no significant differences (paired* t *test) existed within groups after use of contraceptives, except for IUD (*$P = 0.01$*). The normal decrease in milk zinc observed during lactation was not affected by contraceptive practices. Covariate analysis showed the concentration of zinc in samples of milk before contraceptives is a significant (*$P = 0.0001$*) and stronger determinant of milk zinc concentration in subsequent samples than contraceptive practices. There was no significant correlation between concentrations of zinc in serum and milk (*$r = -0.134$*,* $P = 0.10$*).*

Keywords: zinc; human milk; serum; contraceptives

Introduction

Oral contraceptive agents are believed to affect mineral metabolism.[1] During pregnancy there is a decrease in serum/plasma zinc attributed to the circulating high levels of estrogens. Earlier literature reviewed by Smith and Brown[1] indicated that hormonal contraceptives have in most, but not all cases, caused a significant decrease in plasma/serum zinc.

Since the inception of oral contraceptive use there have been considerable changes in hormonal formulations and practices.[2] Combined pills have had marked declines in both progestogen and estrogen, with substantially more changes in the progestogen formulation. The estrogen most employed in the combined pill has been either mestranol or ethinylestradiol. The dose has varied from 0.01 to 0.3 mg, with the most frequent dose at 0.05 mg. Minipill formulations have also shown a marked change in the progestogen concentration, which declined from 30 to 0.03 mg of d-Norgestrel. Nearly all studies that have tested injectable progestogen have used depot medroxy-progesterone acetate (DMPA) in different regimens, with the 150 mg formula every three cycles as the most common prescription.[2] The use of hormonal contraceptives is worldwide, and combined pills are reported to be prescribed to nursing mothers more in developing regions than in industrialized countries.[3] Complaints of decreased milk production by women using combined pills were also higher in developing regions.[3]

The effects of hormonal contraceptives on lactation are controversial or at best inconclusive. Hull[4] summarized the literature showing increase, decrease, maintenance, or no change in lactation due to any kind of hormonal therapy: combined pill, minipill, or injectable progesterone. The report concluded that "the balance of evidences does appear to indicate, as reviewers have claimed, that preparations containing estrogen are more often implicated in cases of suppression of lactation than are progestogen-only preparations."

Termination of lactation per se seems to influence the composition of human milk.[5] In the particular case

This work was partially supported by the National Research Council of Brazil (grants 40.6383/84, 0269-90.2, 404866/90-5).
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Received February 19, 1992; accepted July 30, 1992.

of zinc, during gradual weaning Casey et al.[6] found no significant changes in milk zinc while others[7-9] reported a significant drop in milk zinc.

Zinc-rich colostrum is replaced by mature milk with a substantial decline in zinc concentration as lactation proceeds, irrespective of regions, race, time of delivery (term or preterm), socioeconomic, and nutritional status.[6,9-28] During early lactation zinc declines to a level that is 50% of its initial levels.[18] However, it seems that in the first 3 months of lactation total zinc intake by the infant is not affected because of a compensatory increase in milk intake.[29] In spite of that, to meet the requirement in the first 6 months of life, it has been suggested that there must exist an increase of 30% in absorption of milk zinc.[20] This delicate balance between supply and demand for optimal well-being of exclusively breast-fed infants has been recognized. There are indications of beneficial effects of supplemental zinc to normal babies when given directly[30] or through mothers,[19] while clinical zinc deficiency in infants has been associated with low concentration of zinc in mother's milk.[31]

Although there are many studies dealing with hormonal contraceptives and proximate composition of milk,[32] it seems that none has dealt with the effects of such contraceptives on zinc during lactation.[4] Therefore we studied the effects of contraceptive practices (such as hormones and IUDs) believed to affect zinc metabolism and/or lactation. We found no evidence that hormonal contraceptives cause serum zinc and/or milk zinc changes in nursing mothers.

Materials and methods

Eight-one mothers were recruited from private and public clinics after explaining the objectives of the study and obtaining their consent. One sample of blood and serum was taken before and during treatment. Although we had scheduled the second sample (posttreatment) to take place 2 weeks after initiation of contraceptive treatment, this was not always possible. Because we could control neither the day of lactation when the mothers chose to start with the contraceptive nor the length of observation, we kept records of these variables. All mothers were in full lactation; their characteristics and contraceptive methods are described in *Table 1*. The choice of contraception method was decided by the mothers and their physicians without our interference. The mothers referred from public clinics were slum dwellers living in the squatter village of Paranoa on the outskirts of Brasilia and are classified here as of low socioeconomic status. The mothers of high socioeconomic status were middle-class residents of the city of Brasilia and were referred by private and University Hospital clinics.

Samples of blood and milk were collected on the same day, between 7:00 a.m. and 11:30 a.m., before and at the end of the study. All glassware was rendered clean by washing in acid and rinsing with EDTA solution followed by distilled and deionized water, as routinely done in our laboratory.

The determination of zinc in both serum and milk was done directly by atomic absorption spectrophotometry (AAS) in a spectrophotometer (Perkin Elmer, model 603 Norwalk, CT USA) equipped with a background corrector, according to the manufacturer's instructions. The AAS operational settings were wavelength, 214.4 nm; slit, 4; mode, absorbance, 1.0 for reading; lamp current, 15 mA. All tests were completed in duplicate and precision and accuracy was checked with Bovine Liver Standard Reference Material (National Institute of Standards and Technology, Washington, DC USA).

Blood was taken after an overnight fast by venipuncture in sterile plastic syringes (Unaplic, Produtos Medicos do Parana SA, Brazil), cooled, and taken to the laboratory. There it was allowed to clot, centrifuged, and serum harvested with care to avoid hemolysis. The procedure to determine zinc in serum was that of Arnaud et al.[33] A 0.2 mL aliquot of serum was diluted in 1 mL of 6% butanol:water (Merck do Brasil SA, Brazil) solution (1:6 vol/vol) and read. Working standards were prepared from a stock solution (Standard per Absorbimento Atomico, Carlo Erba, Italy)

Table 1 Background information on the groups studied

	Groups*				
	NC	CP	MP	IP	IUD
Number of mothers	22	15	29	12	5
(Primipara/multipara)	(8/14)	(7/8)	(17/12)	(4/8)	(1/4)
Status (L/H)†	(8/14)	(13/2)	(4/25)	(9/3)	(3/2)
Previous lactation (N/Y)‡	(8/14)	(9/6)	(18/11)	(5/7)	(1/4)
Age in years, mean ± SD	31 ± 5	20 ± 3	24 ± 6	22 ± 4	25 ± 3
Duration of study, days mean and range	22 (14–35)	26 (14–103)	20 (7–43)	20 (15–40)	18 (15–22)
Days of lactation, mean and range					
–before contraceptive	108 (31–254)	88 (17–380)	73 (23–190)	36 (15–140)	57 (18–200)
–after contraceptive	130 (61–270)	134 (31–394)	80 (38–215)	52 (31–155)	80 (32–221)

*NC, No contraceptive (barrier and natural methods or sterilization); CP, combined pill (levonorgestrel 0.15 mg + ethinylestradiol 0.03 mg; levorgestrel 0.25 mg + ethinylestradiol 0.05 mg); MP, minipill (norethindrone 0.35 mg); IP, Injectable progesterone (depot medroxy-progesterone acetate 150 mg); IUD, intrauterine device (plastic or copper).
†(N/Y), no/yes.
‡(L/H), low socioeconomic status/high socioeconomic status.

and diluted with 6% (vol/vol) butanol:water solution. The working solutions of zinc standard had final concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 μg/mL.

Milk samples (2–10 mL) were collected by manual expression after cleaning the nipples with distilled and deionized water. Nursing interval (time between last nursing and the sample collection) and day of lactation were recorded. After collection, samples were divided into two or three aliquots and stored at −20° C until analysis. After thawing, a 0.1 mL aliquot was diluted in 0.7 mL (1:8 vol/vol) in the same solution of butanol and processed as for serum samples.

The data were summarized as means ± SEM and ranges. Paired *t* test, Pearson correlations between variables, linear regression analysis, and data summarization were performed with an SAS computer program for PC (SAS Institute, Cary, NC USA). A *P* value of less than 0.05 was considered significant. Differences between before and after serum samples were examined by paired *t* tests. The effects of contraceptives on changes in concentrations of milk zinc were studied by covariate analysis taking into account milk zinc concentrations before contraceptives, day of lactation, length of observation, type of oral contraceptives, and the nested effect of length of observation within type of contraceptives. To measure the interactive effects of these five variables, covariate analyses were run sequentially after deleting the one with least significant effect according to equation 1.

$$Y_{ij}(k) = \mu + \alpha_i + e_{ij} + \sum_{l=0}^{k} B_l X_{lj}$$

Here $X_{oj} = 1$ for all j, and k = 1–5 represents the number of variables.

We also ran a Pearson correlation between the concentrations of zinc in serum and in milk.

Results

Table 1 summarizes characteristics of mothers, including day of lactation at start of the study and duration of observation. The mean length of study was comparable among the groups. However, the day of lactation at start of contraceptive and the duration of study varied a great deal.

Figure 1 shows zinc concentration in both milk and

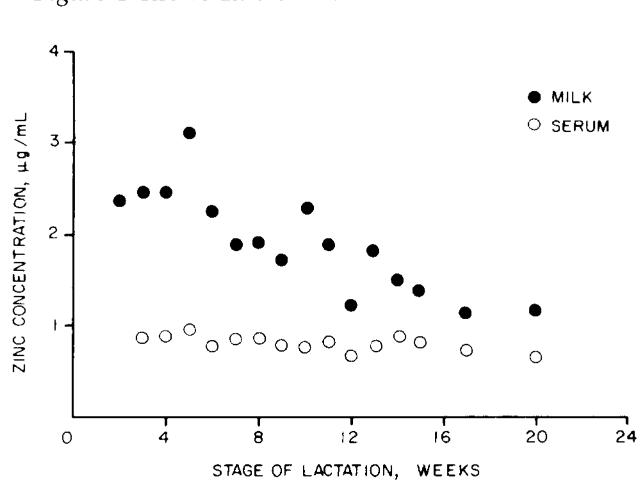

Figure 1 Concentrations of zinc in milk and serum according to stage of lactation of mothers before taking any type of contraceptive. Each point represents the average of at least three mothers.

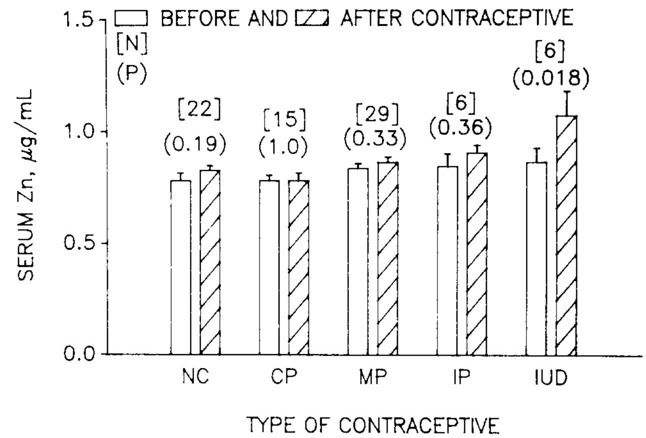

Figure 2 Mean and SEM of serum zinc according to type of contraceptives. NC, No contraceptives; CP, combined pill; MP, minipill; IP, injectable progesterone; IUD, intrauterine device (plastic/copper).

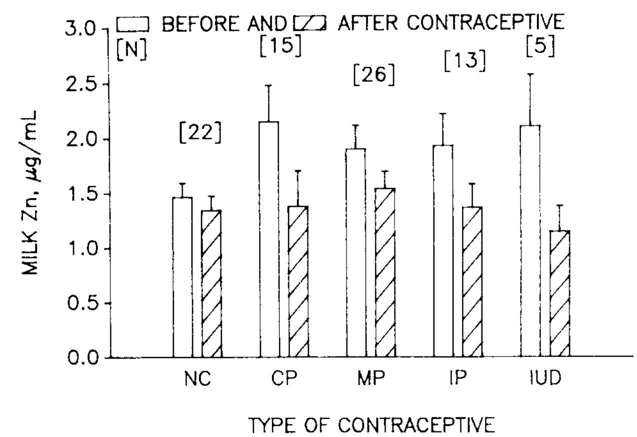

Figure 3 Mean and SEM of milk zinc according to type of contraceptive. NC, No contraceptives; CP, combined pill; MP, minipill; IP, injectable progesterone; IUD, intrauterine device (plastic/copper).

serum of mothers at different stages of lactation before any type of contraceptives. Due to the different pattern of occurrence of zinc in milk and serum, the effects of contraceptives were evaluated by different statistical tests. *Figure 2* illustrates zinc concentration in serum before and after the observation period. Comparisons by paired *t* test showed that only the IUD users had significantly higher concentrations of serum zinc.

In *Figure 3* we show the zinc concentration in milk. Due to the normal decline in concentration of milk zinc *(Figure 1)*, rather than comparisons of means between treatments within each group, we ran regression analysis adjusting for variables known to affect milk zinc and/or lactation. Milk zinc concentration before any contraceptive, length of observation nested within contraceptive treatments, type of contraceptive, and length of observation were fit into several covariate analyses summarized in *Table 2*. Zinc concentration after treatment with contraceptives showed the strongest association with initial levels of zinc (P = 0.0001). In this interactive model, when considered separately

Table 2 Summary of covariate analysis (F values and *P*) of concentration of milk Zn before contraceptives, length of observation nested in type of contraceptives, stage of lactation, length of observation, and type of contraceptive, on milk Zn after treatment according to different models

Number of variables (k):	1	2	3	4	5
Variables					
Milk Zn before contraceptives	77.3 0.0001	80.0 0.0001	73.40 0.0001	73.40 0.0001	71.31 0.0001
Length of observation nested in contraceptive		1.52 0.20	1.50 0.21	1.94 0.13	0.90 0.45
Stage of lactation			0.54 0.46	0.54 0.46	0.78 0.38
Length of observation				0.01 0.91	0.30 0.59
Type of contraceptive					0.62 0.60

Variables with the least significant effect were dropped from each model.

neither contraceptive treatment nor its length of use showed significant effects. However, the combined effect of both variables gave the lowest *P* values (0.13–0.20) in the interactive covariate analysis, although not significant.

Figure 4 shows the correlation between zinc concentration in serum and milk (before and after treatment). The correlation coefficient was low and not statistically significant ($r = -0.13$, $P = 0.10$).

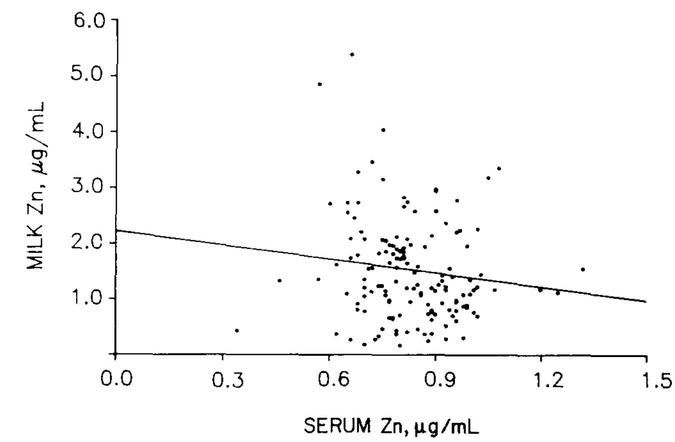

Figure 4 Plot of concentrations of zinc in milk and in serum ($r = -0.134$, $P = 0.10$).

Discussion

The decline in plasma zinc concentration that occurs during pregnancy has been accepted to be due to estrogens. However, hormonal contraceptives containing estrogens have not always caused a decrease in serum/plasma zinc.[1] Publications that appeared after the review of Smith and Brown[1] have also shown both types of results, i.e., decrease in serum/plasma zinc[34–36] or no effect.[37–40] It seems that these studies were done on nulliparous or non-lactating women whose hormonal profiles are quite different from those of nursing mothers. Therefore our study, although not showing any effect of hormonal contraceptives on serum zinc, should not be directly compared with those reports.[37–40] The low serum zinc observed during pregnancy seems to persist up to day 5 postpartum,[41] when it rises to prepregnancy levels. Indeed, at 2 weeks postpartum we found that serum zinc had already achieved concentrations that persisted throughout the study. The behavior of serum zinc illustrated in *Figure 1* is quite distinct from milk zinc. While the latter tended to decrease, the former showed a consistent oscillation without any particular trend from 2–20 weeks postpartum. Similar observations were reported by others.[16,17,19] The concentration of serum zinc we found before use of any type of contraceptive is comparable to values reported for nursing mothers in Brazil[19,42–44] and elsewhere.[11,17,45]

We do not know how to explain the significant increase in serum zinc concentration in the IUD group. The effects of contraceptive practices on lactation reviewed earlier[46] indicated that "IUD appears to stimulate lactation possibly through its mechanical action provoking a neuroendocrine reflex that increases the excretion of endogenous oxytocin." A more recent review by McCann et al[47] however, points out that the effects of IUD on lactation are as conflicting or inconsistent as the reports for hormonal contraceptives.

As reported by others,[10,11,17] we also did not find a significant correlation *(Figure 3)* between serum and milk zinc. Individual samples showed a consistent decrease in milk zinc concentration as lactation proceeded that had no parallel with serum zinc *(Figure 1)*. This physiological decrease in milk zinc universally observed during lactation occurs irrespective of concentrations in plasma zinc and seems to be controlled by the mammary gland.[48] Attempts to attenuate the fall in milk zinc by raising serum zinc through supplementation of mothers have failed.[11,19,48] Therefore it seems that in nursing mothers the mechanisms that control secretion of zinc into milk are different from the ones that control serum zinc. Even in the presence of significant differences in serum zinc no significant effect was seen in the concentration of milk zinc due to IUD use. This is consistent with other findings showing that significantly higher levels of serum zinc (due to supplemental zinc) in nursing mothers had no parallel with milk zinc.[17,19,48]

The concentrations of milk zinc *(Figure 3)* are in agreement with previous work in Brazil[18,23,24,43,44] at the corresponding stage of lactation. It can be inferred from the literature that milk zinc can decrease at different rates depending on the length of lactation.[6] It is not known, however, if or how abrupt or gradual termination of lactation can affect the rates of decrease in milk zinc. During late lactation or in controlled and non-controlled weaning studies, what may appear as conflicting results regarding milk zinc behavior[6-9] could be due to improper statistical evaluation.[7-9] Estimated rates of fall in milk zinc are quite different at stages of lactation between 1 and 3 months[18] or 9 and 12 months.[9] Due to the nonlinear rate of decrease of milk zinc as lactation proceeds, comparisons between means of zinc concentrations at two points in time are not justifiable.[7-9] Even comparisons of mean percentages of changes[9] are fraught with difficulty in assessing the proper rate of fall in milk zinc concentration when the observations are not done at the same period of lactation. Therefore, studies that made such comparisons[7-9] reported a significant decrease in milk zinc with weaning. However using regression statistics, Casey et al.[6] demonstrated no significant effect of weaning or termination of lactation on milk zinc. Indeed with covariate analysis we showed that initial milk zinc concentration was a stronger determinant of subsequent milk zinc concentration than use of contraceptives.

The nutritional consequences to the nursing infant of contraceptive practices during lactation are still unsettled.[4] Given the peculiarities of zinc metabolism in the nursing mother and its secretion into milk, direct (mean comparisons) or inferential statistical testing (adjusted covariate analysis) showed no evidence that hormonal contraceptives can affect the concentration of zinc in human serum or milk. In cases of altered zincemia (IUD group) no significant effects were demonstrable in milk zinc concentration.

Acknowledgments

We thank the mothers that contributed to the study and the interest and cooperation of Drs. Lucilla D.C. Mota, Iraci D. Oliveria, and H. Borato and nurses Sonia M.B.A. Silveira and Celina M. Santana from Hospital Docente Assistencial-UnB and Posto de Saude 15-FHDF. We also thank Dr. John Grove (School of Public Health, University of Hawaii) and Dr. Edina Myazaki (Department of Statistics, University of Brasilia) for the statistical help and Prof. Ira Lichton (Dept. Food Science & Human Nutrition, University of Hawaii) for discussion and help in the preparation of the manuscript.

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