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Copper, Iron, and Zinc Contents in Human Milk During the First Three Months of Lactation

A Longitudinal Study

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ABSTRACT

The aim of the study has been to analyze the evolution of copper, iron, and zinc contents in human milk, from colostrum to the third postpartum month, following a longitudinal design, under specific conditions of sample collection and to apply an analytical procedure previously optimized to reduce any variation outside physiological lactation.

The copper, iron, and zinc concentrations in 144 milk samples from 39 healthy puerpera women, were analyzed in five stages by flame atomic absorption spectrometry, following a standardized protocol.

Copper presented a gradual decrease from 0.38 mg/L to 0.19 mg/L by the 90th day; the particular analysis from colostrum to transitional milk manifested the following two tendencies. Whereas an increase from 0.19 to 0.42 mg/L was observed in some women, a decrease from 0.53 to 0.45 mg/L was detected in others; therefore, copper presented two significant behaviors in the evolution from colostrum to transitional milk. In both cases, the evaluated changes were significant. The

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iron content varied from 0.56 to 0.40 mg/L by the 30th day, remaining constant until the first trimester concluded. The average zinc concentration decreased sharply from 7.99 to 3.3 mg/L on d 15; the rate of decrease slowed down gradually until 1.05 mg/L.

Index Entries: Copper; iron; zinc; human milk; trace elements; lactation.

INTRODUCTION

Human milk composition is complex and presents continuous changes *(1)*. Presently, its nutrient contents comprise the reference values used to establish the Dietary Reference Intakes for healthy infants from birth to 6 mo of age *(2)*.

Numerous studies have attempted to establish trace element contents in human milk, especially those of copper, iron, and zinc *(3–6)*, because these elements play essential roles in the proper functioning of metabolic systems as components of metalloproteins and metalloenzymes *(3)*. The various studies that have been done on the contents of these elements consistently show a variability, attributed to factors such as interindividual differences, lactation period, analytical procedure, or sample collection conditions *(7–11)*. Precisely because of these variabilities, it has been impossible to determine a series of specific reference values for trace elements in human milk.

In this work, a longitudinal study has been designed under monitored conditions in order to evaluate copper, iron, and zinc concentrations during the first 3 mo of lactation.

MATERIALS AND METHODS

Sample Collection

Group I

To determine copper, iron, and zinc contents in human milk during lactation, 110 human foremilk samples provided by 22 women and collected at 5 lactation stages were analyzed as follows: colostrum, sampled on the d 2 and 4 postpartum (stage 1); transitional milk, sampled during the second postpartum week (stage 2); mature milk on the 30th day (stage 3); mature milk on the 60th day (stage 4); mature milk on the 90th day postpartum (stage 5).

Group II

To evaluate the particular changes of copper content from colostrum to transitional milk, 34 additional samples were taken from another 17 women and were added to the 44 from stages 1 and 2 of the previous collection.

Samples were voluntarily provided by well-nourished healthy women with an adequate gestational follow-up and full-term delivery. All of them resided in the metropolitan area of Valencia (Spain). All women gave their written informed consent to participate in the study.

To avoid sampling errors, the following conditions were applied: (1) Similar volumes of milk from both breasts were collected in the same recipient before feeding; (2) sampling collection was carried out from 11 AM to 4 PM; (3) the nipples and hands were thoroughly cleaned with abundant water before each sampling.

An automatic breast milk pump (Mamilat SM 122) was used together with polypropylene containers, in which about 10 mL of the samples were directly collected. In order to avoid contamination as much as possible, the glassware utilized and the breast-milk-pump accessories (suckling funnels and polypropylene containers) were washed with concentrated nitric acid and later with abundant deionized water (Millipore; Milli Q System). Then, the samples were frozen to −18°C, reducing handling before entering the analytical process.

Analytical Determinations

The three metal elements' analytical determination was carried out through flame atomic absorption spectrometry (atomic absorption spectrophotometer, Perkin Elmer 2380), preceded by the milk samples' wet digestion procedure (Microwave Digestor, Milestone MS 1200) as described by Silvestre et al. *(12)*. The analytical parameters of the method were as follows: interday precision (% relative standard deviation [RSD]): 6.8 for iron, 8.9 for zinc, and 7.3 for copper; accuracy (recovery %): 100.4 ± 6.8 for iron, 95.9 ± 6.7 for zinc, and 98.8 ± 11.0 for copper.

Statistical Analysis

The existence of significant differences among the five stages considered was tested by the application of a one-way variance analysis (ANOVA).

Data was also analyzed by Tukey's test of highly significant differences, using the statistical systems for Personal Computers Package (Statgraphics v.5.1). When $p < 0.05$, differences were considered significant.

The copper content variation between the colostrum and transitional stages was verified applying the two-factor variance analysis and the posterior calculation of the minimum significant differences.

Prior to the ANOVA application, the population's normality was verified via the χ^2 test or, if considered appropriate, the Kolmogorov–Smirnov test; the variance homogeneity was verified by Cochran's test. When dealing with nonhomogeneous variances, the results obtained by the variance analysis were compared to those arising from the nonparametric Kruskal–Wallis test.

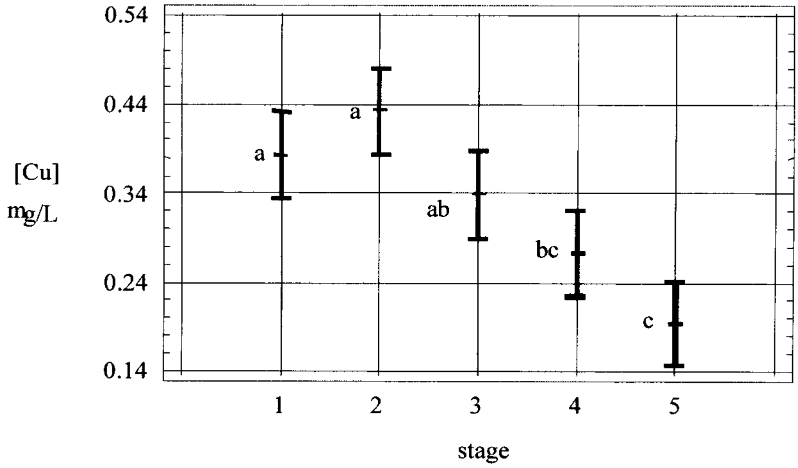

Fig. 1. Copper. Intervals for the means of the contents obtained in the five stages of the study (n = 22). Tukey test = 95%. Mean value and standard deviation (mg/L) for each step are shown. Noncoincidence in the letters indicates statiatically significant differences. Stages: 1: colostrum (0.38 ± 0.20); 2: transitional milk (0.43 ± 0.07); 3: mature milk by d 30 (0.34 ± 0.07); 4: mature milk by d 60 (0.27 ± 0.07); 5: mature milk by d 90 (0.19 ± 0.10).

RESULTS

As a whole, the three studied elements' concentrations obtained in each step turned out to be greatly variable among women. For each population, the normality of the distribution was verified, obtaining homogeneous variances for the iron content and nonhomogeneous for the copper and zinc contents.

Copper

Copper concentrations obtained in the five stages varied between 0.82 mg/L and nondetectable values. Figure 1 shows the characteristic mean values for each period. A greater dispersion in the data was observed in the colostrum stage as opposed to the other stages. The significant levels obtained via ANOVA (p = 0.000) and the Kruskal–Wallis analysis ($p = 3.2 \times 10^{-9}$) confirmed the existence of statistically significant differences among the studied stages. Application of the Tukey test showed the significant differences. The copper contents was unchanging from colostrum to mature milk at d 30 postpartum. Its content decreased from this stage to a mean content of 0.19 mg/L after d 90 postpartum.

In most of the cases, this statistical result contrasted with the individual behavior determining the following study:

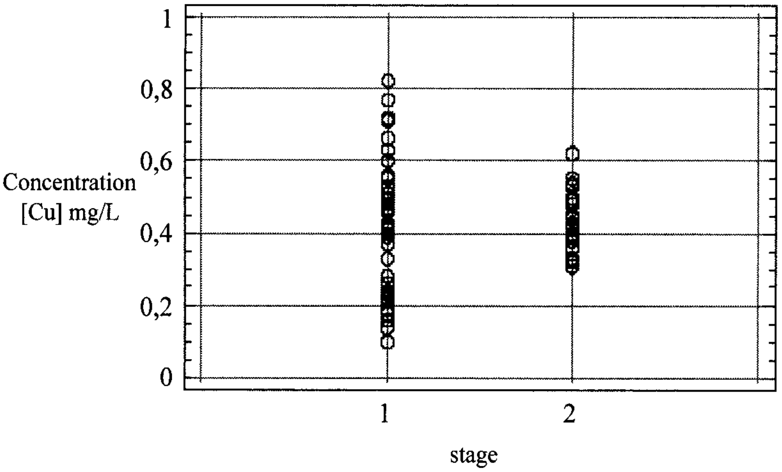

Fig. 2. Copper concentration (mg/L) in colostrum (stage 1) and transitional milk (stage 2) (39 women).

Evolution of Copper Concentration from Colostrum to Transitional Milk (Samples of Group II)

The majority of women manifested a considerable variation of copper concentration in the evolution from colostrum to transitional milk. In some cases, this variation meant an increase in concentration; in others, the concentration decreased. Figure 2 shows that in the colostrum stage, the concentrations varied within a large interval (x_{mean} = 0.43 mg/L; S.D. = 0.193; x_{minimum} = 0.10 mg/L; x_{maximum} = 0.82 mg/L), whereas in the transitional milk stage, the values were more homogeneously distributed (x_{mean} = 0.44 mg/L; S.D. = 0.072; x_{minimum} = 0.31 mg/L; x_{maximum} = 0.62 mg/L). The mean values for the two stages were similar, and taking into account these data altogether, the observed variations were masked.

Most copper concentrations in the colostrum stage were distributed in two well-differentiated groups, distributed around an intermediate data area of 0.35 mg/L. This value was chosen as the cutoff point between the two groups, and the following hypothesis was established. Copper content has two possible behaviors in the evolution from colostrum to transitional milk: (1) higher copper contents in colostrum that would decrease in the following stage and (2) lower copper contents in colostrum that would increase in the transitional milk stage.

This hypothesis gained strength with the statistical analysis of the data (ANOVA, $p \ll 0.01$). Table 1 shows that two statistically different populations in terms of copper content (less than or greater than 0.35 mg/L) appeared at stage 1. The difference between the two populations disappeared at stage 2. The group with copper contents less than 0.35 mg/L in colostrum experienced an increase in this element's concentration at

Table 1
Copper from Colostrum to Transitional Milk, Group II (n = 78);
Minimum Significant Differences (MSD) Estimation

	Stage 1	**Stage 2**	**MSD**	**d**
[Cu] < 0.35 μg/mL	(n = 11) $\bar{x}$ = 0.19	(n = 11) $\bar{x}$ = 0.42	0.0833	0.2318*
[Cu] > 0.35 μg/mL	(n = 28) $\bar{x}$ = 0.53	(n = 28) $\bar{x}$ = 0.45	0.0522	0.0785*
MSD	0.0690	0.0690		
d	0.3383*	0.0279		

*Significance at 95%.

Note: d = difference between means, absolute value; $\bar{x}$ = mean value; stage 1: colostrum; stage 2: transitional milk.

the following stage, from a mean value of 0.19 to 0.42 mg/L; the population with copper contents greater than 0.35 mg/L in colostrum experienced a decrease in this element's concentration in transitional milk, from a mean value of 0.53 to 0.45 mg/L. Both variations were significant, and the two groups reached similar values by d 15 postpartum.

Iron

The iron contents ranged from 0.98 mg/L to nondetectable values. Figure 3 shows the wide dispersion of results in all stages. The ANOVA analysis applied indicated significant differences (p = 0.0175); the Tukey test showed the significant differences between the colostrum period and d 30 and 90 postpartum. The iron concentration decreased during the lactation period from an initial mean value of 0.56 mg/L in colostrum to 0.39 mg/L at d 30 postpartum, remaining stable until the end of the study.

Zinc

The zinc contents ranged from 17.22 to 0.13 mg/L; the main difference among the five stages was clearly outlined by the ANOVA and Kruskal–Wallis tests application ($p \ll 0.01$ in both). Figure 4 shows the mean values for each stage; the Tukey test showed the significant differences. Zinc contents manifested a sharp decrease in the evolution from colostrum to transitional milk (from a mean value of 7.99 to 3.3 mg/L). Then, the decrease in zinc contents was steady until d 60 postpartum (reaching a mean value of 1.40 mg/L) and then remained constant until day 90.

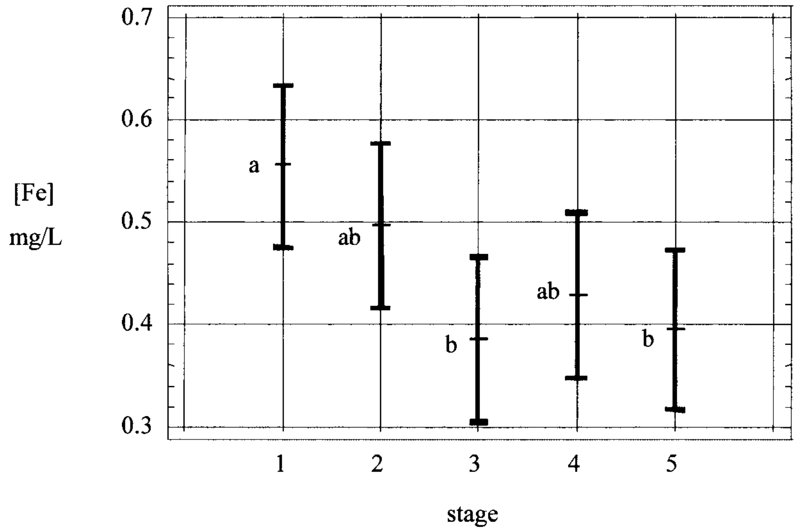

Fig. 3. Iron. Intervals for the means of the contents obtained in the five stages of the study ($n = 22$). Tukey test = 95%. Mean value and standard deviation (mg/L) for each step are shown. Noncoincidence in the letters indicates statiatically significant differences. Stages: 1: colostrum (0.56 ± 0.22); 2: transitional milk: (0.50 ± 0.19); 3: mature milk by d 30 (0.39 ± 0.19); 4: mature milk by d 60 (0.43 ± 0.15); 5: mature milk by d 90 (0.40 ± 0.17).

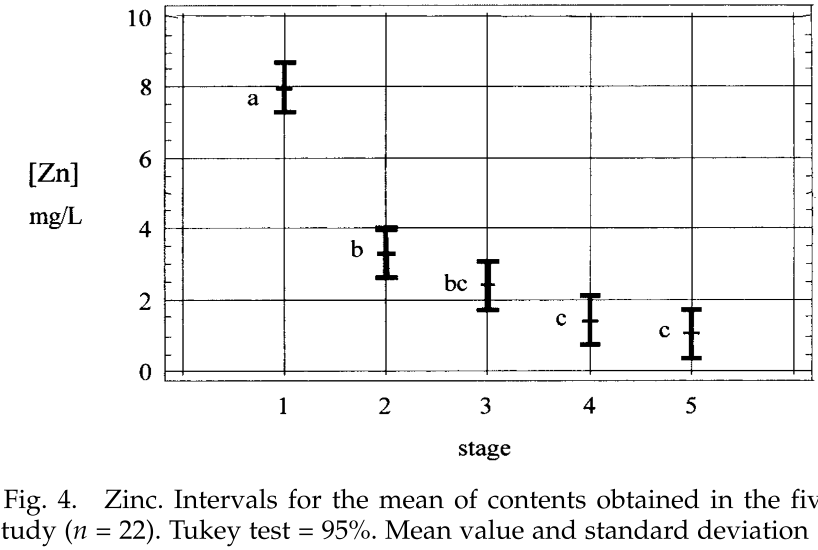

Fig. 4. Zinc. Intervals for the mean of contents obtained in the five steps the study ($n = 22$). Tukey test = 95%. Mean value and standard deviation (mg/L) for each step are shown. Noncoincidence in the letters indicates statiatically significant differences. Stages: 1: colostrum (7.99 ± 3.23); 2: transitional milk (3.31 ± 1.06); 3: mature milk by d 30 (2.41 ± 0.90); 4: mature milk by d 60 (1.40 ± 0.65); 5: mature milk by d 90 (1.05 ± 0.71).

DISCUSSION

There are many well-documented studies regarding the content of trace elements in human milk. However, as a consequence of the wide range of concentrations obtained for each element and their common variation throughout lactation, inferred conclusions are frequently unclear. It is difficult to draw general conclusions because the studies' designs, analytical and statistical procedures, and maternal factors are different. Moreover, cross-section studies, the most often published analysis, introduce high interindividual variability capable of masking the natural variations during the individual lactation stages.

In order to approach the actual biological situation, this study attempted to minimize the influence of factors unrelated to the lactation period. This was done by using a longitudinal design with samples obtained at each stage in unrestricted conditions and by evaluating the quality of the applied analytical method, maintaining standardized conditions with respect to which breast was sampled and the time of the day and moment of intake at which all of the samples were collected.

The obtained values for copper, iron, and zinc were within the widespread ranges documented by other researchers *(3,13–16)*. As a whole, the obtained results indicated higher concentrations during the first lactation stages with a subsequent decrease. Each element behaved differently.

The decrease in copper concentration throughout lactation is a well-know fact, although there is some discrepancy regarding the onset of this decrease *(5,16–22)*. The results exhibited a smooth continuous decrease in concentration from d 30 postpartum until the end of the study.

The longitudinal design of the study permitted the discernment of two different behaviors in the evolution of copper concentration during the first 15 d postpartum. In the first one, the copper concentration was particularly low and increased in transitional milk; in the second, the concentration was initially high and decreased in the next stage (Table 1). The differences between both groups were highly significant, but they were masked when both groups were considered together.

Studies carried out by other researchers disagree with respect to the evolution of copper during the first week of lactation. In some cases, an initial increase is reported *(23,24)*, whereas in others, the copper content remains constant until the further decrease *(25)*. Although no references to the double evolution observed in this study have been found, some authors have pointed out a high variability of copper content in colostrum. These researchers report that this variability is greater than that detected in subsequent stages and that the particular behavior of each woman does not always coincide with the general trend observed in the rest of the women studied *(13)*. These conclusions agree with those drawn from our study.

The origin of this double evolution is unknown; alternative studies are required in order to ascertain whether copper values oscillate depending on the day of sampling or on other maternal factors.

Variations in iron content throughout lactation were less pronounced than those of copper and zinc. No variation was detected in the transition from colostrum to transitional milk; the concentration decrease was significant only at d 30 postpartum, and iron concentrations remained constant throughout the rest of the studied time period (Fig. 3). This decrease has been documented by some researchers *(19,26)*, whereas others *(4,27,28)* maintain that iron concentration in milk stays constant; therefore, a large number of samples is necessary in order to rule out variations in concentration as a result of extraneous factors. The real concentration curve could be masked by these factors if the number of samples taken was too small, and no variations would be detected *(5,14,19,29)*.

In agreement with the values reported by other authors, zinc contents decreased sharply during the first weeks of lactation *(3,13,18,30)*, followed by a gradual decrease (Fig. 4). The significant differences between the following stages occurred in intervals greater than 30 d. This smooth decrease of zinc concentration in mature human milk is similar to that documented in other studies *(31–33)*.

CONCLUSIONS

The interindividual discrepancies in the content of these elements in human milk require the design of a longitudinal study, under very controlled conditions, if the evolution of these elements throughout lactation is to be appreciated. This study has thereby evidenced that copper presents two significant behaviors in the evolution from colostrum to transitional milk, the result of which no previous references have been found.

A global decrease is observed in the longitudinal study as the lactation period develops, being particularly significant in the case of zinc. Thus, variability requires the specification of the considered lactation stage if reference values for these elements are to be established.

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