Determination Of Copper, Zinc, Cadmium And Lead In Breast Milk Of Yemeni Khat Users And Non-Users

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ABSTRACT

The concentration of copper, zinc, cadmium and lead in human milk of Yemeni women were determined by anodic stripping voltammetry and the daily intake of these elements were calculated. Zn concentrations in breast milk declined throughout lactation from 3706 ± 1233 µg/l at one month to 816 ± 226 µg/l at 12 month. Cu levels declined from 664 ± 139 µg/l at one month to 284 ± 84 µg/l at 12 month with little change thereafter. Based on these data and the volume of milk produced by Yemeni mothers it was estimated that fully breast-fed infants would receive approximately 1853 to 775 µg of zinc and 332 to 270 µg of copper daily during the first year of lactation. These values are higher than those observed in other poorly nourished countries but are considerably lower than dietary recommendations. The weekly intake of Cd and Pb by Yemeni infants from breast milk alone is below the FAO/WHO Provisional Tolerable Weekly Intake (PTWI).
The breast milk of regular khat users had significantly higher concentrations of cadmium and lead, but almost the same amounts of copper and zinc. The concentration of Cu and Zn in breast milk seems to be stabilized via the ability of mammals and vertebrate to maintain homeostasis by a combination of decreased absorption and enhanced excretion. The most dramatic consequence of khat chewing found in this study was a marked decreases in the breast-milk volume of khat-users which is expected to reduce the daily Zn and Cu intakes of breast-fed infants.

INTRODUCTION

A vast body of research from all over the world sustains the recommendation that only breast-milk be fed to infants for the first six months of life. Breast-milk provides a proper balance and quantity of nutrients ideal for the human infant. Zinc and copper are two trace minerals essential for normal growth and development. Zinc deficiency results in a variety of immunologic defects whereas copper deficiency is characterized by anemia, neutropenia and skeletal abnormalities (Lonnerdal & Uauy, 1998; Prentice, 1993; Linder & Azam 1996; Fung *et al.*, 1997). Yemeni women with poor or marginal status at the time of delivery may be less likely to maintain adequate copper and zinc nutriture during lactation when nutrient demands are increased. Such deficiencies may limit the rate of infant growth and development. The first objective of this study was to determine the concentration of copper and zinc in breast milk of Yemeni lactating women and to use these concentrations to evaluate the adequacy of these elements to the fully breast-fed infants.
A further concern related to the Cu and Zn balance of the fully breast-fed infants in Yemen, is that an increasing number and percentage of Yemeni lactating women chew khat (*Catha edulis Forsk*) regularly for its stimulating effect. In prior studies (Matloob, in press) we found that urinary cadmium, lead, copper and zinc of regular khat users were significantly higher than those of the controls. The second objective

of this study was to evaluate the possible role of khat chewing in disturbing the trace element levels of Cu and Zn in breast milk and to monitor the concentration of Cd and Pb as a reflection of the overall environmental and dietary influences during lactation. In milk, trace metals often occur at very low concentrations. Several techniques have been used in trace metal analysis with varying degree of success and convenience. Among the various techniques, anodic stripping voltammetry have demonstrated great sensitivity for Cu, Zn, Cd and Pb and can easily determine these metals quantitatively in the low parts per billion ranges (Kissinger & Heineman, 1996; Staden, & Matoetoe, 2000). This remarkable sensitivity of stripping analysis is attributed to the deposition step that takes place before the actual measurement (stripping) step. In this paper, an accurate and reproducible differential pulse anodic stripping voltammetric (DPASV) procedures for the determination of Cu, Zn, Cd and Pb in breast milk are described.

MATERIALS AND METHODS

Subjects

The study was carried out during 2001-2002 in Ibb province, 190 km south-west of Sana'a (Yemen Republic). The study group included 60 lactating women aged 19-30 y and weighing 45-65 kg. Thirty of them were khat non-users and the other thirty were regular khat users chewing between 100 and 500 grams of freshly khat daily. The subjects were of low-income background and largely illiterate. All subjects were in good health, encountered no unusual difficulties during delivery and delivered healthy full-term infants.

Milk Sample collection

Milk samples were provided daily by the volunteers from day 15 to 1 month and thereafter monthly up to 12 month postpartum. Milk was obtained at mid morning feed, from both breast. This protocol gives a sample representative of the entire breast contents and of the 24 h mean value for zinc and copper (Casey *et al*., 1989). The volunteers also measured their 24-h milk production monthly by a hand breast pump. The samples were frozen and stored at – 20° C until thawed for analysis. To avoid the possibility of contamination all collection equipments were acid-washed and rinsed thoroughly with deionized water. All chemicals used were Merck, Suprapure, Analar or Electronic grade.

Dietary intake

Lactation raises nutrient needs, mainly because of the loss of nutrients first through colostrums and then through breast-milk. For this reason ten lactating women were requested to collect monthly duplicate samples of all foods that they consumed during a 24-h period. The samples were weighed, homogenized, dried, powdered and stored until analysis.

Digestion procedure and trace metal analysis

An aliquot of approximately 3.5 ml of milk was accurately weighed into a high-form porcelain crucible. After being dried to a constant weight in an oven at 90 ° C, 1 ml of concentrated nitric acid was added to the dried solids and the sample was heated to dryness on an electric hot plate. The dried residue was ashed in a muffle furnace overnight at 480 ° C, thereafter dissolved in 10 ml of 0.25% nitric acid (pH = 2).

Cu, Zn, Cd and Pb were determined by differential pulse anodic stripping voltammetry at the hanging mercury drop electrode (Model 746 – 747 VA, Metrohm, Herisau - Switzerland). Parameters in all measuring procedure were as follows: deposition potential –1.20 V, deposition time 30-90 s, scan rate 6 mV s^{-1}, pulse amplitude 50 mV and final potential +0.3 V. Standard solutions (1 g l^{-1}), of Cd, Pb, Cu and Zn were prepared and necessary dilutions were made as and when required. Food samples were burned to ash at 550° C and dissolved in 0.5 ml of concentrated nitric acid. They were diluted appropriately and analyzed in duplicates for Cu and Zn as described above.

The limits of detection were 0.15 µg l^{-1} Cd, 0.30 µg l^{-1} Pb, 2.80 µg l^{-1} Cu and 1.20 µg l^{-1} Zn. The precision and accuracy of the proposed method were checked with nonfat milk powder (BCR Milk powder 151 Belgium) after digestion. Our mean results agree to 95% with the certified values. Recoveries were done by five replicate voltammetric determinations of the metals under consideration in five milk samples. The average recoveries were 99% (range 90 – 108%).

Statistical analysis

Quantification of metal concentrations in the samples was carried out by use of the standard addition method. This is the preferred method as the sensitivity of the stripping voltammetric analysis may vary between samples of different ionic strength. The best fitting line through the data pairs was calculated by linear least-squares regression analysis. The concentration of each element in the sample is equal to the quotient of the intercept and the regression coefficient.

Unless otherwise indicated, data are presented as mean ± SD. A value of $p < 0.05$ was considered statistically significant. The metal concentrations in milk of khat users are compared with the corresponding non-users values by Students *t*-test.

RESULTS AND DISCUSSION

The concentration of Cu, Zn, Cd and Pb, in breast milk off khat non -users

An anodic stripping voltammogram of a digested milk sample is shown in Figure 1. Well-defined peaks for cadmium, lead, copper and zinc were observed, indicating that the digestion of the sample was relatively complete.

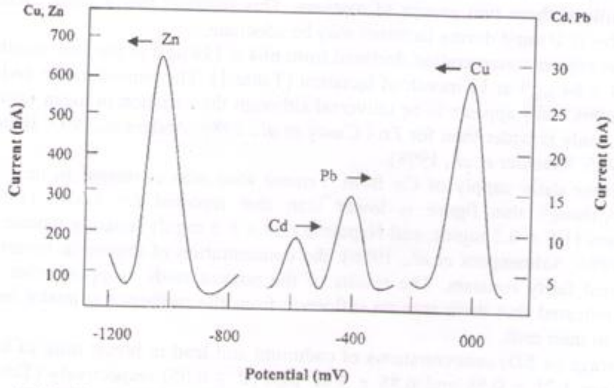

Fig. 1 Typical voltammogram of digested milk (3.5 ml) after dilution to 20 ml. The peaks represent 3 ppb Cd, 10 ppb Pb, 160 ppb Cu and 380 ppb Zn. Deposition potential –1200 mV; deposition time 90 s for Cd and Pb, and 30 s for Cu and Zn; scan rate 6 mV/s; pulse amplitude 50 mV.

Because of the rapid, often very marked changes known to occur in the concentrations of a number of milk constituents during the onset of lactation, and the rapid increases in volume in the first weeks, results are reported for the intervals 0.5 to 12 months (Table 1).

Table 1 Concentrations of Cu and Zn in breast milk during the first year of lactation*.

	Khat non-users		Khat users	
Stage of lactation	Cu	Zn	Cu	Zn
Month	μg/l		μg/l	
0.5 – 1	664 ± 139	3706 ± 1233	ND**	ND
3	417 ± 117	1368 ± 438	422 ± 101	1474 ± 389
6	356 ± 117	1140 ± 447	364 ± 129	1065 ± 454
9	304 ± 83	823 ± 255	291 ± 99	877 ± 297
12	284 ± 84	816 ± 226	273 ± 91	822 ± 208

*number of subjects = 30
** not determined

The mean zinc concentration declined from 3706 ± 1233 μg/l at 0.5-1 month to 816 ± 226 μg/l at 12 months ($P < 0.05$), with the sharpest decline occurring during the first 3 month (Table 1). The present data are in good agreement with those intensively reported by others (Gross *et al.*, 1998; Dorea, 2000; Casey *et al.*, 1989). The decline in Zn concentration reflects the rapidly changing physiological state of the mammary gland.
The mean dietary zinc intake for the low-income Yemeni women was estimated to be 9.8 ± 4.1 mg/d, which did not change significantly at any stage of lactation. The figure is lower than that reported by Krebs *et al.* (1995) for well-nourished populations (13 mg/d). Despite this, there was no significant differences in the Zn concentration of the breast milk of these two groups of mothers. This suggests that a mean dietary zinc intake of ~ 10.0 mg/d during lactation may be adequate.
The mean copper concentration declined from 664 ± 139 μg/l in the first month to less than 284 ± 84 μg/l at 12 month of lactation (Table 1). The general range and pattern found in this study appears to be universal although the variation in mean values from study to study is wider than for Zn (Casey *et al.*, 1989; Anderson, 1993; Benemariya *et al.*, 1995; Krachler *et al.*, 1998).
The average daily supply of Cu from Yemeni food was estimated to be 1.4 ± 0.3 mg/d. Although this figure is lower than that reported for Finish (1.7 mg/d), Americans (1.5 ± 0.2 mg/d), and Nepalese (2.8 ± 0.5 mg/d) lactating women (Moser *et al.*, 1988; Salmenpera *et al.*, 1986), the concentration of copper in breast milk is maintained fairly constant. The results of the present study support other findings which indicated that there was no influence from the mothers Cu intake on the Cu content in their milk.
The average (± SD) concentrations of cadmium and lead in breast milk of khat non-users were 1.21 ± 0.58 and 6.88 ± 3.82 μg/l ($P < 0.05$) respectively (Table 2). In contrast to Cu and Zn, the concentration of Cd and Pb did not vary significantly with duration of lactation. Compared to literature data these levels proved to be in the normal range for uncontaminated human milk (Dabeka *et al.*, 1986; Rodrigues *et al.*, 1999; Frkovic *et al.*, 1997).

Table 2 Comparison of cadmium and lead concentrations* in breast-milk of Yemeni khat users and non-users.

Group	Cd	Pb
	µg / l	
Khat non - users	1.21 ± 0.58 (0.25 – 2.23)	6.88 ± 3.82 (1.41 – 12.30)
Khat users	2.48 ± 1.72 (0.32 – 9.47)	13.11 ± 7.42 (1.57 – 48.9)

*Each value is the mean ± SD with the range shown in parenthesis

Infant Zn and Cu intake

Table 3 gives the average volumes of milk produced by Yemeni women during the first year of lactation. The values are comparable with that found in developed countries and higher than those observed in poorly nourished countries. These observations led to the conclusion that the nutritional status of Yemeni lactating women (in urban areas) seems to be satisfactory. Based on milk concentration and volume, the daily intake of the breast-fed Yemeni infants was found to range from 1853 to 775 µg zinc and 332 to 270 µg copper during the first year of lactation. These intakes are astonishingly similar to those reported by developed countries (Casey *et al.*, 1989; Simmer *et al.*, 1990; Moser *et al.*, 1988), which again emphasizes that Yemeni lactating women are adequately nourished.

Table 3 Calculated average infantile daily Cu and Zn intakes from Breast-milk of khat non-users: (milk concentration × milk volume).

Age	Volume	Cu	Zn
Month	ml	µg	µg
1	500	332	1853
3	800	333	1094
6	850	303	969
9	900	270	740
12	950	270	775

The daily Cu intake reported in this study is at the lower end of the estimated safe and adequate intake (ESAI) range (0.4-0.7 mg/d), whereas the daily Zn intake is remarkably lower than the recommended dietary allowance (RDA) (5 mg/d) (Recommended Dietary Allowance, 1989). Since no signs of copper and zinc deficiency were seen in Yemeni infants, we like others believe that the recommended levels for breast-fed infants may be inappropriately high.

Infant Cd and Pb intake

The average quantity of milk consumed by Yemeni infants is ranging from 500 to 1000 ml daily throughout lactation. According to Table 2 this quantity contributes 1.1

to 2.1µg kg^{-1} body mass of cadmium and 6.0 to 12.0 µg kg^{-1} body mass of lead weekly (assuming an average Yemeni infant weight of 4 kg). The FAO/WHO Joint Expert Committee on Food Additives recommended a provisional maximum tolerable weekly intake of Cd and Pb from all sources of 7.0 and 25.0 µg kg^{-1} body mass, respectively (Marquardt, 1999). According to these directives the weekly intake of Cd and Pb by Yemeni infants from khat non-users is much below the FAO/WHO recommendations.

Mean weights of Yemeni infant

Infants and children have greater requirements on a unit weight basis than adults, mainly because they are growing. A satisfactory gain in the infant s weight is the best way to judge the adequacy of the diet of the infant. The mean weights of 417 Yemeni infants from 0 to 12 months are demonstrated in Table 4. Referred to the growth standards of the United States National Center for Health Statistics (NCHS) (Latham, 1997), the weights of Yemeni infants (Figure 2) are very close to the median for children in developed countries and much better than the average weights in poor countries like Bangladesh (Simmer *et al.*, 1990). These results suggest that if lactating women have adequate breast-milk, breastfeeding alone with no added food or medicinal supplementation is all that is needed for the normal infant.

Table 4. Weight (kg) of infants in Yemen. (no. of infants in parentheses)

Age (months)	Median	SD
0	3.50	0.69 (36)
1	4.00	0.80 (84)
2-3	5.00	1.07 (88)
4-5	6.50	1.18 (51)
6-7	7.40	1.34 (36)
8-9	8.30	1.49 (86)
10-12	9.95	1.57 (36)

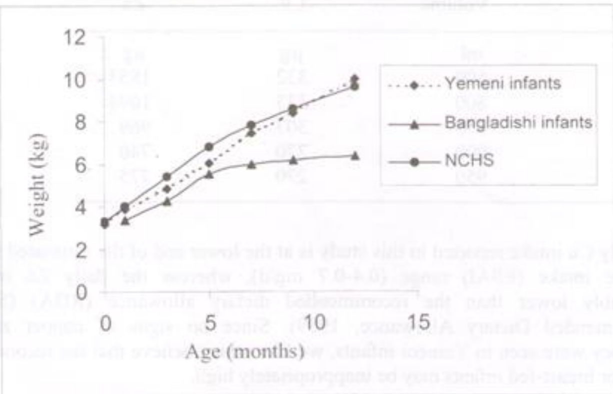

Fig. 2. Mean weights of Yemeni infants (both sexes), compared with Bangladeshi infants and the growth standards of the United States National Center for Health Statistics (NCHS) as published by WHO.

The effect of khat chewing on breast feeding

In a survey conducted in Ibb city in April 2002, 2400 mothers were interviewed about khat chewing practice, it was found that 34% of lactating women are chewing khat regularly. Only one quarter of khat chewers were heavy consumers. The average quantity of fresh khat chewed by heavy khat user is ranging from 100 to 500 gram daily. These amounts supplied the body with an extra daily intake of 0.530 – 2.654 mg copper and 0.662 – 3.311 mg zinc daily (Matloob, in press). Despite these extra intakes, there was no significant impact of khat chewing on milk copper and zinc concentrations (Table 1). These observations supported other findings that copper and zinc concentrations in breast milk are unrelated to intakes of Cu and Zn from the diet or supplements (Benemariya *et al.*, 1995; Krachler *et al.*, 1998). On the other hand, our previous observation indicated that urinary copper and zinc of regular khat users were significantly higher than those of khat non-users (Matloob, in press). The sum of these results suggest that the normal mammary gland controls and limits the amount of copper and zinc secreted into the milk and is obviously not an excretory route for excessive maternal copper and zinc intake. This mechanism may be hormonal in which prolactin may have a central role.

The mean cadmium and lead levels in breast milk of heavy khat users were 2.48 ± 1.72 and 13.11 ± 7.42 µg/l ($p<0.05$) respectively (Table 2). Based on these data and the volume of milk produced by Yemeni mothers it was estimated that fully breast-fed infants would receive approximately 2.2 to 4.3 µg kg^{-1} body mass of Cd and 11.5 to 23.0 µg kg^{-1} body mass of lead weekly during the first year of lactation. Both are higher than that of the normal controls but did not present any risk to infants health according to the Provisional Tolerable Weekly Intake (Marquardt, 1999). The present results, however, reinforce other findings that certain minerals such as cadmium, lead, selenium and iodide in human milk are recognized as reflecting maternal intake (Krebs et al., 1995), and may serve as a good biomarker for body burden exposure.

If khat is digested without washing (as most Yemeni mothers do), the daily intake may exceed the recommended levels. Continuous exposure to Cd and Pb results in their gradual accumulation in human vital organs, which may cause profound biochemical and neurological changes in the body. Lead poisoning is recently considered the most important environmental health problem for young children (Piomelli & Wolf, 1994).

The most dramatic effect of khat chewing was a marked decrease in the 24-milk volumes of khat users compared with non-users (Figure 3). The effect was attained 2-3 h subsequent to khat chewing and remains remarkable as long as khat chewing continues (and even after). The fall in breast milk volume was found to depend on the type and quantity of khat digested. In one of the 30 women from whom samples were obtained, the excretion of milk stopped entirely for few days after heavy khat chewing. These interesting results plus the evidence already presented by Halket *et al.*, (1995), that the peak plasma cathinone levels were attained after about 1.5-3.5 h following khat-chewing, support our assumption that cathinone and cathine are the constituent mainly responsible for the unusual reduction in human milk.

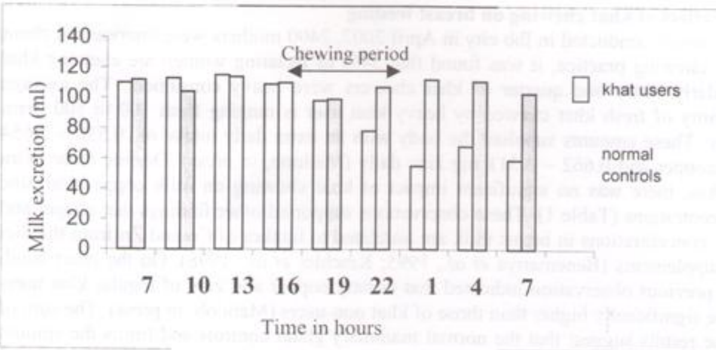

Fig. 3. Influence of khat chewing on the volume of breast milk excreted over 24 h.

Milk production is influencd by hormones, particularly prolactin and oxytocin, and by reflux. Cathinone and cathine seems effecting these hormones. The exact mechanism of these hormonic effects waits further investigations. Contraceptive pills, especially high-oestrogen pills, also reduce a woman's ability to produce breast milk.

The daily Zn and Cu intakes of breast-fed infants from khat chewers are expected to be low mainly due to the low volume of milk produced. Since inadequate elemental intake may contribute to abnormal growth and several disease, lactating mothers should be advised to cease khat chewing soon after childbirth.

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