

# Trace-Element Status in Milk and Plasma of Kuwaiti and Non-Kuwaiti Lactating Mothers

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There is a wide variation in the reported data on the concentrations of trace elements in human milk from different countries, but such data are not available for Kuwait. The objective of this study was to analyze the concentration of zinc, copper, manganese, and iron in milk and plasma of Kuwaiti and non-Kuwaiti mothers during prolonged lactation. Milk samples (from 34 donors) were collected early in the morning before feeding the infant. Trace elements were analyzed using atomic absorption spectrophotometry. Protein content and activity of superoxide dismutase were assayed spectrophotometrically. Concentration of zinc, copper, iron, and total protein and activity of superoxide dismutase in milk and of only zinc in plasma of Kuwaiti mothers were significantly higher than those of non-Kuwaitis. Concentration of zinc, copper, manganese, and total protein in milk of both groups decreased as lactation continued but that of milk iron and plasma trace elements remained unchanged. The data of Kuwaiti mothers are consistent with those of previous reports on hyperurecemia, and the prevalence of obesity was found to be higher in the Kuwaiti population than in other countries. High protein content in association with high concentration of trace elements in milk of Kuwaiti versus non-Kuwaiti mothers may indicate that protein content in milk is an important determining factor for the concentration and bioavailability of these elements. *Nutrition* 2000;16:1069–1073. ©Elsevier Science Inc. 2000

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## INTRODUCTION

Minerals and trace elements are essential micronutrients for growth, development, and maintenance of healthy tissues. The important factors that can influence the intake and bioavailability of minerals and trace elements include types of food consumed, environmental pollution, respiratory and gastrointestinal infections, extreme climatic conditions, and traditions and illiteracy.<sup>1</sup> Micronutrients important for the survival of all living organisms are available to the body only through diet. Human milk is the main source of nutrients to infants during the early stages of their lives; hence, the dietary levels of trace elements in the milk of the lactating mothers are significant. Analysis of the concentration of trace elements in blood plasma and serum of mothers is a poor indicator of the bioavailability of these elements to the infants. The concentration of trace elements in human milk differ from region to region (Table I),<sup>2–8</sup> but similar data for lactating mothers in Kuwait have not been reported.

Deficiency of trace elements such as zinc, copper, manganese, and iron can occur in infants for different reasons. It has been reported that zinc intake by infants from breast milk is inadequate during the weaning period, especially if weaning foods are introduced at an early stage.<sup>9</sup> Similarly, copper deficiency can occur because of infants' inability to use absorbed copper rather than a dietary insufficiency of this element.<sup>10</sup> The amount of manganese in breast milk and its recommended intake differ by a factor of 100; thus, it is often difficult to assess the availability of manganese from breast milk.<sup>11</sup> With regard to iron, based on theoretical estimations of the requirement for infants, the amount of iron

provided by breast milk alone would be insufficient.<sup>12</sup> Several studies have shown that infants are at a risk of developing iron deficiency if weaning foods are introduced before the age of 12 mo.<sup>13,14</sup> The availability of essential elements to infants depend solely on the trace-element content of the breast milk, length of breast feeding, and physiologic factors such as nutrient absorption and nutrient supplementation of the mother. In a previous study, we reported that the content of proteins in milk and that of selenium in milk and plasma of Kuwaiti lactating mothers were significantly higher than those of non-Kuwaiti mothers.<sup>15</sup> The present study was designed to investigate whether other trace elements (zinc, copper, and manganese) also follow a similar pattern during prolonged lactation (18 mo). Analysis of the concentration of iron in milk and plasma was included as an indicator of the health status of the donors.

## SUBJECTS AND METHODS

### Subjects

Thirty-four subjects aged 25 to 40 y and belonging to the middle class or upper middle class volunteered to participate in the study. Both Kuwaitis ( $n = 17$ ) and non-Kuwaitis ( $n = 17$ ) were subjects. Nationalities of the non-Kuwaiti group consisted of Americans, Egyptians, Indians, Czech, and Taiwanese who represent the major groups of expatriates living in Kuwait. All volunteers had been living in Kuwait for at least 2 y before the study. The lactation period extended from 0 to 18 mo after parturition, and the minimum lactation period included was 2 wk. All volunteer mothers and their infants were healthy, did not have any history of medical or breast-feeding problems, and reported not using any kind of mineral or trace-element supplementation. All infants were delivered normally after full-term pregnancy. The infants' age matched the duration of the breast feeding. The study

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TABLE I.

CONCENTRATION OF ZINC, COPPER, IRON AND MANGANESE IN HUMAN MILK FROM DIFFERENT COUNTRIES				
Country	Zinc (mg/L)	Copper (mg/L)	Iron (mg/L)	Manganese ( $\mu\text{g/L}$ )
Austria <sup>2</sup>	4.1	0.86	0.46	3.10
Canada <sup>3</sup>	1.17–5.31	0.21–0.57	—	4.0–17.0
Germany <sup>4</sup>	2.06	0.8	0.43	6.2
Italy <sup>5</sup>	2.2	0.25	—	4.1
Korea <sup>6</sup>	2.83–3.75	0.36–0.47	0.0021–0.0028	2.74–4.03
Taiwan <sup>7</sup>	0.55–4.6	0.09–0.27	0.09–0.22	—
USA <sup>8</sup>	0.12–1.09	0.05–0.15	—	0.66–1.23

protocol was in accordance with the guidelines of the Ethical Committee, Faculty of Medicine, Kuwait University.

### Sample Collection and Preparation

Milk samples (15 mL) from both groups were collected in metal-free tubes by manual expression early in the morning (between 6 and 8 AM) before the infants' first feeding. Blood samples (8 mL) were collected shortly after in heparinized metal-free tubes (Vacutainer, Becton and Dickinson, Franklin Lakes, NJ, USA). Plasma was separated after centrifugation at 2500 rpm for 10 min. Milk and plasma samples were stored at  $-80^{\circ}\text{C}$  until analyzed.

### Analytical Procedures

**DETERMINATION OF TRACE ELEMENTS IN MILK AND PLASMA.** Concentrations of zinc, copper, manganese, and iron in milk and plasma samples were analyzed using atomic absorption spectrophotometry. Whole-milk samples were first digested using a mixture of nitric and perchloric acid (4:1). All samples were run in duplicate. The accuracy and precision of the trace-element analytical procedures have been reported previously.<sup>15</sup> The concentrations of iron and manganese were measured using a graphite furnace, and those of zinc and copper were measured using flame atomic absorption spectrophotometry (Varian, Australia). The digested samples were diluted 1:20 with 3% nitric acid. Seronorm 103 serum standards (Nycomed, Oslo, Norway) were used for standardization of trace-element analysis in plasma. The mean ( $n = 7$ ) concentration of zinc, copper, iron, and manganese deviated  $-4\%$ ,  $-5\%$ ,  $-10\%$ , and  $-2\%$ , respectively, from the certified values. As reference material, bovine liver standard 1577a (National Institute of Standards and Technology, Gaithersburg, MD, USA) was used. The analytical values ( $n = 7$ ) for zinc, copper, iron, and manganese deviated  $-0.8\%$ ,  $-1.3\%$ ,  $-1.5\%$ , and  $-3\%$ , respectively, from the certified values.

**DETERMINATION OF TOTAL PROTEIN IN MILK.** Fat content was removed by centrifugation at 4000g for 30 min with a refrigerated Beckman centrifuge. The supernatants (skim milk) were then analyzed for the concentration of total protein, as previously described.<sup>15</sup> The supernatant (skim milk) samples were subjected to ultracentrifugation at 150 000g for 60 min at  $4^{\circ}\text{C}$  to obtain casein pellets and whey fraction. Protein concentrations of sample fractions were estimated at 280 nm by using standard solutions of pure casein and whey protein suspended in Tris-HCL buffer (pH 7.4). The sum of the casein and whey protein was considered as the total protein content. The accuracy of this procedure was tested on the basis of the recovery of compounds added

to some milk samples. Recovery of casein and whey protein differed from 91% to 97% and from 89% to 96%, respectively. Analytical precision, expressed as a coefficient of variation, was 6% (data from six analyses of the same milk samples).

**ASSAY OF ACTIVITY OF SUPEROXIDE DISMUTASE IN HUMAN MILK.** The zinc- and copper-dependent superoxide-dismutase activities in human-milk samples were assayed with a commercially available kit from Randox Laboratories Ltd. (Ardmore, Diamond Road, Crumlin Co., Antrim, UK). Briefly, milk samples were centrifuged at 4000g and the supernatant (skim milk) was used for the assay. The absorbance was measured with a Beckman spectrophotometer (DU 7500) at 505 nm at  $37^{\circ}\text{C}$ . The accuracy and precision of this method were checked with lyophilized whole blood, and the coefficient of variation was less than 10%.

### Statistical Analysis

Results are presented as mean  $\pm$  SD values. One-way analysis of variance followed by Student's *t* test was used to compare the mean values of different groups. When the data were not normally distributed, the Mann-Whitney method was used. A value of  $P < 0.05$  was accepted as statistically significant.

## RESULTS

### Concentration of Trace Elements in Milk

The concentration of zinc, copper, and iron in the milk of Kuwaiti mothers ( $n = 17$ ) was significantly higher (by 31%, 28%, and 37%, respectively) than that of the non-Kuwaiti mothers ( $n = 17$ ), but that of manganese was similar in both groups (Table II). The concentration of zinc, copper, and manganese in the milk of both Kuwaiti and non-Kuwaiti mothers decreased as lactation continued (approximately 21%, 13%, and 36%, respectively), but that of iron remained fairly unchanged (Table II).

### Content of Total Protein in Milk

The amount of total protein in the milk of Kuwaiti mothers was significantly higher (18%) than that of the non-Kuwaiti mothers during the initial lactation period of 0 to 6 mo ( $n = 14$ ,  $P < 0.03$ ) and 6 to 12 mo ( $n = 13$ ,  $P < 0.02$ ). However, this difference was not significant thereafter (12 to 18 mo,  $n = 7$ ). The total protein content of milk decreased in both groups as lactation progressed (Table II).

TABLE II.

Concentration	CONCENTRATION OF ZINC, COPPER, MANGANESE, AND IRON, ACTIVITY OF SOD, AND CONTENT OF TOTAL PROTEIN IN THE MILK OF KUWAITI AND NON-KUWAITI LACTATING MOTHERS*					
	0†–6 mo		6–12 mo		12–18 mo	
	Kuwaitis (n = 6)	Non-Kuwaitis (n = 5)	Kuwaitis (n = 6)	Non-Kuwaitis (n = 7)	Kuwaitis (n = 5)	Non-Kuwaitis (n = 5)
Zinc (mg/L)	3.2 ± 0.12‡	2.4 ± 0.06	2.4 ± 0.14‡§	1.9 ± 0.05§	2.0 ± 0.15‡§	1.7 ± 0.09§
Copper (mg/L)	0.71 ± 0.02‡	0.59 ± 0.04	0.57 ± 0.03‡§	0.4 ± 0.03§	0.53 ± 0.03‡§	0.41 ± 0.04§
Manganese (μg/L)	6.0 ± 0.04	5.7 ± 0.02	4.2 ± 0.2‡§	3.7 ± 0.3§	3.8 ± 0.2‡§	3.1 ± 0.1§
Iron (mg/L)	0.43 ± 0.04‡	0.33 ± 0.03	0.38 ± 0.05‡	0.27 ± 0.02	0.40 ± 0.02‡	0.29 ± 0.04
SOD (U/mL)	0.41 ± 0.04‡	0.32 ± 0.02	0.31 ± 0.01‡§	0.23 ± 0.02§	0.26 ± 0.04§	0.21 ± 0.03§
Total protein (mg/mL)	12.3 ± 0.8‡	9.7 ± 0.5	8.6 ± 0.4‡§§	5.9 ± 0.6§	5.4 ± 0.2‡§	5.0 ± 0.1§

\* Data are presented as mean ± SD.

† 0 indicates a minimum lactation period of 2 wk.

‡ Significantly higher than non-Kuwaitis ( $P < 0.05$ ).

§ Significantly lower than 0 to 6 mo ( $P < 0.05$ ).

SOD, superoxide dismutase.

### Activity of Superoxide Dismutase in Milk

The present study demonstrated activity of superoxide dismutase in the soluble fraction of human milk. The activity of this enzyme in the milk of Kuwaiti mothers was significantly higher than that of non-Kuwaiti mothers during 0 to 12 mo of lactation, and it remained low for the rest of the lactation period in both groups (Table II).

### Concentration of Trace Elements in Plasma

The concentration of zinc in plasma of Kuwaiti lactating mothers was significantly higher (11%) than that of the non-Kuwaiti mothers, but that of copper, iron, and manganese was similar in both groups (Table III). There was no significant change in the plasma concentrations of these elements as lactation progressed. None of the subjects had plasma levels of any of these trace elements below the lower limit of the reference values.<sup>16</sup> The differences in the concentrations of trace elements in milk and plasma of Kuwaiti and non-Kuwaiti mothers observed during the early stages of lactation (0 to 12 mo) did not persist at its final stages (12 to 18 mo).

### DISCUSSION

The concentration of zinc, copper, iron, and manganese in milk and plasma found in the present study were within the range reported in the literature (Table I). The concentration of zinc, copper, and manganese in the milk of the Kuwaiti and non-Kuwaiti mothers fell in the upper range reported from several other countries. The data for trace-element content of human milk differ widely from region to region. These variations may be due in part to differences in sampling and analytical techniques rather than to geographic variation. For example, interlaboratory variation of copper levels in reference materials was 23-fold in 25 laboratories.<sup>17</sup> Also, it has been reported that the concentration of manganese in human milk reported have been declining continuously during the past 18 y<sup>18</sup> and this change could be due to the introduction of better methodology including prevention of contamination. However, these technical factors did not contribute to the variation in the present data between the Kuwaiti and non-Kuwaiti groups because we have achieved satisfactory accuracy and precision in our analytical methods.

The data showed that concentration of zinc, copper, and iron in the milk and that of zinc in plasma of Kuwaiti mothers were

TABLE III.

Concentration	CONCENTRATION OF ZINC, COPPER, MANGANESE, AND IRON IN PLASMA OF KUWAITI AND NON-KUWAITI LACTATING MOTHERS*					
	0†–6 mo		6–12 mo		12–18 mo	
	Kuwaitis (n = 6)	Non-Kuwaitis (n = 5)	Kuwaitis (n = 6)	Non-Kuwaitis (n = 7)	Kuwaitis (n = 5)	Non-Kuwaitis (n = 5)
Zinc (μmol/L)	16.3 ± 2.4‡	11.6 ± 2.0	12.9 ± 3.6	10.6 ± 2.6	14.1 ± 2.3	11.0 ± 3.4
Copper (μmol/L)	19.3 ± 3.8	18.0 ± 2.6	17.5 ± 4.2	19.4 ± 3.3	18.5 ± 2.9	19.0 ± 4.9
Manganese (μmol/L)	21.4 ± 1.7	17.6 ± 2.1	19.6 ± 2.1	18.5 ± 2.9	19.5 ± 1.9	17.4 ± 1.6
Iron (mg/L)	0.48 ± 0.12	0.44 ± 0.16	0.46 ± 0.14	0.39 ± 0.16	0.41 ± 0.15	0.41 ± 0.19

\* Data are presented as mean ± SD.

† 0 indicates a minimum lactation period of 2 wk.

‡ Significantly higher than non-Kuwaitis ( $P < 0.05$ ).

significantly higher than those of the non-Kuwaiti mothers during the first 6 mo of lactation. The plasma level of trace elements remained unchanged throughout the lactation period, indicating that the plasma pool of these elements was unaffected by changes in the milk. The higher element levels in milk and plasma of the Kuwaiti mothers may be attributed to dietary factors. Unfortunately, data on dietary intake of minerals and trace elements by Kuwaitis are not available; hence, it is difficult to exclude factors other than the dietary effect. Previous studies on certain groups in Kuwait have reported that dietary habits differ widely between Kuwaitis and non-Kuwaitis, and this difference was reflected by a significant increase in body weight and hyperuricemia (the latter is a consequence of a high-protein diet).<sup>19–25</sup> These unusual data were attributed to increased food consumption due to affluence and the concomitant changes to a sedentary lifestyle during the past three decades. This may in part explain the occurrence of a higher concentration of protein and trace elements in the milk of Kuwaiti mothers versus their non-Kuwaiti counterparts.

Unlike zinc, copper, and iron, there was no significant difference in the concentration of manganese in the milk of Kuwaiti and non-Kuwaiti mothers. Some previous studies have reported a higher manganese concentration in milk after increased intake of this element.<sup>26</sup> Conversely, others have claimed that maternal intake of manganese does not appear to affect its concentration in milk.<sup>25</sup> However, the concentration of manganese in milk has been reported to be dependent on milk volume,<sup>27</sup> but none of the subjects in the present study noted a drastic decline in the daily total-milk volume as lactation progressed. Nevertheless, it could be argued that both Kuwaiti and non-Kuwaiti mothers were provided a similar supply of maternal manganese, if the idea that the concentration of this element in human milk is a reflection of maternal diet is true.

The concentration of zinc, copper, and manganese decreased 6 to 12 mo into the lactation period, regardless of nationality of the donors. We previously reported a similar longitudinal decrease of selenium, milk casein, and whey protein concentrations for the same subjects.<sup>15</sup> The present observation of a decline in zinc, copper, and manganese is consistent with the results of several other studies.<sup>5,7,21,27,28</sup> However, the present decrease in the concentration of copper (17% to 28% between 0 and 18 mo) was not as pronounced as found in other studies (25% to 35% between 1 and 12 mo).<sup>27</sup> It has been suggested that the decline in milk copper concentration is related to the rapid decrease in the total protein at the onset of lactation, although there was no correlation between copper and total protein content,<sup>27</sup> which may also be the case in the present study. Although there was a decline in the level of zinc in milk as lactation continued, the degree of decline was less pronounced in the Kuwaiti mothers and this is probably due to dietary factors. This suggestion is supported by another study where supplementation of lactating women with 230  $\mu\text{mol/d}$  of zinc, in addition to their dietary intake of 180  $\mu\text{mol/d}$ , minimized the decline in milk content of zinc with time.<sup>3</sup> Unlike the trace elements, concentration of iron in milk of both groups remained virtually unchanged throughout the lactation period, which is in accordance with several other studies.<sup>28–30</sup> It has been reported that the concentration of iron in human milk does not appear to be related to either maternal iron status<sup>29</sup> or iron supplementation.<sup>30,31</sup> The concentration of iron in milk was found to change considerably according to time of milk sample collection. For example, the iron content of foremilk and hindmilk was 50% higher than that found in samples collected at the morning feeding,<sup>32</sup> but in the present study the samples were collected in the morning before the first feeding of the infant. It seems that secretion of iron into the milk is homeostatically controlled rather than by dietary factors.

It is interesting to note that the concentration of total protein in the milk also decreased during the 6 to 12 mo of the lactation period. This decrease in total protein concentration paralleled that of trace elements, indicating a strong relation between concentration of trace elements and the protein content of milk.<sup>15</sup> It could be

assumed that protein content plays an important role in determining the concentration of these trace elements in milk and hence their bioavailability to infants. The parallel elevation in the concentration of zinc, copper, and protein in the milk of Kuwaiti and non-Kuwaiti mothers further support this suggestion. A statistically significant correlation between these parameters could be apparent if it is drawn between concentration of trace elements and their specific binding proteins, which is presently under investigation in our laboratory.

### Superoxide Dismutase Activity in Human Milk

The present study demonstrated a higher activity of zinc- and copper-dependent superoxide dismutase in the milk soluble fraction of Kuwaiti mothers than of non-Kuwaiti mothers. The activity of this enzyme decreased significantly after the first 6 mo of lactation (Table II). This observation indicated that this change was closely associated with the changes in the milk concentrations of zinc and copper, in addition to being associated with the variation of protein and trace elements between the Kuwaiti and non-Kuwaiti mothers. A previous study has reported that infants fed on human milk and evaporated-milk formula do not exhibit any difference in their serum copper/zinc superoxide dismutase activity.<sup>6</sup> The proportion of zinc and copper accounted for by this enzyme in human milk is still not known. It could be assumed that this enzyme serves as a transport mechanism by carrying zinc and copper into the milk, and this finding supports the suggestion that trace elements are secreted into the mother's milk as binding proteins rather than ionic forms.

## SUMMARY

The present observation of higher concentrations of protein and trace elements in the milk of Kuwaiti mothers versus non-Kuwaiti mothers is consistent with those of previous reports of obesity and hyperuricemia on different groups of the Kuwaiti population. This may be attributed to changes in lifestyle, dietary habits with high energy intake, and reduced physical activity associated with certain sociocultural norms. The differences in the protein levels and trace-element contents in the milk of the Kuwaiti and non-Kuwaiti mothers suggest that the concentration and thus the bioavailability of zinc, copper, and iron in milk is determined by the protein content but that the concentration of manganese is not.

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