ORIGINAL ARTICLE

CALCIUM, MAGNESIUM, IRON, ZINC AND COPPER, COMPOSITIONS OF HUMAN MILK FROM POPULATIONS WITH CEREAL AND 'ENSET' BASED DIETS

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ABSTRACT

BACKGROUND: As breast milk is normally the only source of food in the early stages of life, the dietary levels of the essential elements in the milk of lactating mothers are significantly important. Ethiopia is a country of many nations and nationalities with distinct dietary habits. This variation in food habit may result in the variation of the nutritional quality of milk of lactating mothers who live in different part of the country, which in turn may affect the intake of nutrients by breast-fed infants. Therefore, a cross-sectional study of the levels of Ca, Mg, Fe, Zn and Cu in milk of mothers from societies with cereal and 'enset' based dietary habits was carried out to assess the influence of maternal diet on the levels of the elements in human milk.

METHODS: Milk samples were collected from 27 voluntary mothers in Jimma and in 18 rural areas of Welkite. Breast milk samples were collected within four days postpartum and the concentrations of the elements were determined by using FAAS.

RESULTS: Average concentrations (mg/L) of the elements determined in the milk of mothers from Jimma and rural Welkite respectively were: Ca (758 ± 107, 579 ± 168); Mg (22.6 ± 7.87, 30.5 ± 13.4); Fe (0.50 ± 0.08, 0.41 ± 0.17); Zn (2.3 ± 1.2, 2.49 ± 0.88) and Cu (0.28 ± 0.14, 0.16 ± 0.08).

CONCLUSIONS: Milk samples from Jimma were found to have significantly higher levels of Ca and Cu than those of rural Welkite (P < 0.05). Breast milk Ca and Cu levels were thus found to be influenced by dietary intake.

KEYWORDS*:* Human milk, Ethiopia, Welkite, Jimma, Essential elements, 'Teff', 'Enset'.

INTRODUCTION

Optimal growth of infants can be guaranteed only when the intake of food and water provides the required doses of all the essential elements. Calcium and magnesium are among the macro-elements that are essential for the proper growth and development of a child. Calcium, a major nutrient in human milk, contributes to the development of bones, muscle contraction, the transmission of nerve impulses and clotting of blood (1). Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor (2, 3).

Iron, zinc and copper are among the micro-elements that are essential for the normal growth of infants. Iron functions as haemoglobin in the transport of oxygen and as essential component of enzymes involved in biological oxidation (4, 5). Copper is necessary for the growth and formation of bone and myelin sheaths in the nervous systems (4, 6). Zinc functions as a cofactor and is a constituent of many enzymes; the primary roles of zinc appear to be in cell replication and gene expression and in nucleic acid and amino acid metabolism (4, 7). Thus, deficiency or excess amount of these microelements can alter enzyme activities and

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influence important biological processes in the body. As breast milk is normally the only source of food in the early stages of life, the dietary levels of the essential elements in the milk of lactating mothers are critically important (1, 5). The concentrations of some essential elements determined in human milks of similar time postpartum are given in Table 1.

'Teff' (*Eragrostis abyssinica Schrad*) is the staple food for people in the north and central parts of Ethiopia. The traditional staple crop in many parts of the densely populated south and south-western Ethiopia, however, is 'enset' (*Ensete ventricosum*) also known as "false banana" (8, 9). A detailed study on the health and nutritional status of mothers and children carried out around rural Welkite, indicated that 'enset' is the staple food in the area; and people frequently eat 'enset' bread with kale (10). In Jimma, foods of 'enset' product are not common and people mainly eat 'injera' (prepared from 'teff') with 'wot'. This leads to the question of whether there exists a difference in the levels of some of the essential elements in the milk of mothers of the two areas.

Table 1: Concentrations of Ca, Mg, Fe, Zn and Cu (mg/L) reported in the literature

	Concentration (mg/L)					
Country	**Ca**	**Mg**	**Fe**	**Zn**	**Cu**	**Ref.**
Australia	NR	NR	0.46	4.1	0.86	[11]
Bangladesh	NR	NR	NR	0.28 – 1.8	0.12 – 0.25	[12]
Canada	NR	NR	NR	1.17 – 5.31	0.21 – 0.57	[13]
Egypt	261	28.8	NR	2.35	NR	[14]
Ethiopia						
Addis Abeba*	321 ± 76	22.85 ± 2.34	0.46 ± 0.25	6.66 ± 2.71	0.17 ± 0.04	[15]
Addis Abeba†	462 ± 133	25.62 ± 2.87	0.47 ± 0.19	6.59 ± 2.06	0.37 ± 0.20	[15]
Rural Arsi‡	NR	NR	NR	0.60 ± 0.03	0.15 ± 0.01	[8]
Rural Arsi§	NR	NR	NR	0.68 ± 0.02	0.14 ± 0.01	[8]
Germany	NR	NR	0.43	2.06	0.8	[16]
Honduras	NR	NR	0.21 ± 0.25	0.7 ± 0.18	0.16 ± 0.21	[17]
India	NR	NR	NR	1.77	0.195	[18]
Iran	NR	NR	0.43±0.04	2.95 ± 0.77	0.36 ± 0.11	[19]
Italy	NR	NR	0.881	3.49	0.40	[20]
Jeddah	NR	NR	0.33	1.37	0.62	[21]
Kuwait	NR	NR	0.33 – 0.70	3.2 ± 0.12	0.71 ± 0.02	[22]
Sudan	NR	NR	NR	1.3	0.12	[23]
Sweden	165 ± 51.3	26 ± 5.3	0.50 ± 0.12	5.14 ± 2.86	0.61 ± 0.27	[15]
Taiwan	230 ± 16	27 ± 2.1	0.25 ± 0.03	2.53 ± 0.34	0.34 ± 0.04	[24]
USA	258	29.05	0.29 ± 0.21	0.12 - 1.09	0.05 – 0.15	[14,25]

**milk from privileged mothers, †milk from non-privileged mothers, ‡milk from mothers of stunted infants, §milk from mothers of non-stunted infants*

To date, no study has been conducted in Ethiopia on the relative concentrations of the essential elements in the milk of populations exposed to different dietary habits. Therefore, this research was undertaken to assess the concentrations of Ca, Mg, Fe, Zn, and Cu in milk of mothers from Jimma, whose major diet is based on the cereal 'teff', and mothers from rural Welkite areas, whose major diet is based on 'enset' products.

MATERIALS AND METHODS

Study Subjects: Convenience samples of 18 women from around rural Welkite (Arge, Werbeche, Burat, Akuna, and Gume) who gave births in Atat hospital (18 km to the West of Welkite) and, 27 women from Jimma Town, who gave births in Jimma University Specialized Hospital between March 1st to May 1st 2010 and who fulfilled the inclusion criteria participated in the study. Mothers who were in good health; delivered healthy full term infants and reported having used no minerals or trace-element supplementation except pharmaceutical dose of iron sulfate/folic acid supplement which was given to all participant mothers during the 2nd trimester, were selected for the study. The study protocol was approved by Jimma University Ethical Review Committee. Before collection of milk samples was carried out, the purpose of the study was clearly explained to each of the participating mothers and written informed consent was obtained from all participants.

Milk Collection and Analysis: About 8 mL milk samples were collected from each of the mothers by manual expression into pre-cleaned polyethylene containers during the first week postpartum. Samples were transported to the laboratory in an ice bag and transferred into a freezer (-20 ^oC) until analysis. Then, 3.0 mL aliquot of whole milk from each sample was wet digested according to Rodroguez et al. (26). Metal concentrations were determined by FAAS using Nova AAS 300.

Data Analysis: Data analysis was performed using SPSS for Window version 16.0. The Mann-Whitney-Wilcoxon test was used to compare the difference in measured values. Spearman rank-order correlation was used to assess the relationship between metal concentrations and, age or weight of mothers. All data were expressed as mean ± SD and the level of significance was determined at $p < 0.05$.

Definitions of terms

'**Injera**' is leavened bread prepared by fermentation of teff, wheat, barley, maize or sorghum, or from a mixture of these.

'**Wot**' is a spiced sauce prepared from legumes, vegetables or meat depending on the income of the family.

RESULTS

The influence of maternal age and weight on human milk composition has been extensively studied but the conclusions of researchers do not agree. Ages and weights of the mothers who participated in the current study were recorded using questionnaire and the mean age and weight of the mothers from the two areas are given in Table 2 below.

Table 2: Mean age (Year) and weight (kg) of subjects from Jimma and rural Welkite

Group	n	Age (Years)		Weight (kg)	
		mean±SD	Range	mean±SD	Range
Jimma	27	24.0 ± 5.0	18 - 35	57.5 ± 8.7	43 - 86
Rural Welkite	18	25.2 ± 5.1	18 - 35	50.8 ± 5.4	44 - 59

The ages of the mothers in both groups ranged 18 to 35 years. The mean age of rural Welkite mothers (25.2 ± 5.1 years) was slightly higher than that of Jimma mothers (24.0 ± 5.0 years) but the difference was not significant, $p > 0.05$. The mean weight of Jimma mothers, however, was significantly greater than that of rural Welkite participants ($p < 0.05$). Results of the human milk analysis for the samples collected from Jimma and rural Welkite are given in Table 3.

Calcium and Magnesium: The concentration of Ca in Jimma milk was significantly higher than in that of rural Welkite ($p < 0.01$). The difference in the Mg levels in the milk of the two groups of mothers, however, was not significant ($p > 0.05$). The levels of both Ca and Mg were found to show wide variability among milk of rural Welkite mothers (342 – 979 mg/L and 15.5 – 64.4 mg/L, respectively) than in those of Jimma (508 – 998 mg/L and 10.76 – 41.3 mg/L, respectively). The distributions of Ca and Mg in the milk of each of the two groups in this study are shown in Figure 1 (a) and (b), respectively.

Table 3: The mean, median, range and the 95% confidence intervals of the concentrations of Ca, Mg, Fe, Zn and Cu in human milk samples of Jimma and rural Welkite

	Element	Mean, median, range and confidence intervals (mg/L)			
		Mean ± SD	Median	Range	95% CI
Jimma (n = 27)	Ca	758 ± 107	738	508 – 998	717 – 799
	Mg	22.6 ± 7.9	23.37	10.8 – 41.3	19.46 – 25.7
	Fe	0.499 ± 0.08	0.5	0.26 – 0.62	0.47 – 0.53
	Zn	2.27 ± 1.18	2.14	0.05 – 5.2	1.8 – 2.73
	Cu	0.28 ± 0.14	0.26	0.12 – 0.58	0.23 – 0.34
RW (n = 18)	Ca	579 ± 168	543	342 – 979	493 - 665
	Mg	30.3 ± 13.4	24.7	15.4 – 64.4	23.6 – 36.9
	Fe	0.41 ± 0.17	0.38	0.10 – 0.71	0.326 – 0.496
	Zn	2.49 ± 0.88	2.23	0.92 – 4.25	2.05 – 2.93
	Cu	0.16 ± 0.08	0.16	0.05 – 0.33	0.118 – 0.198

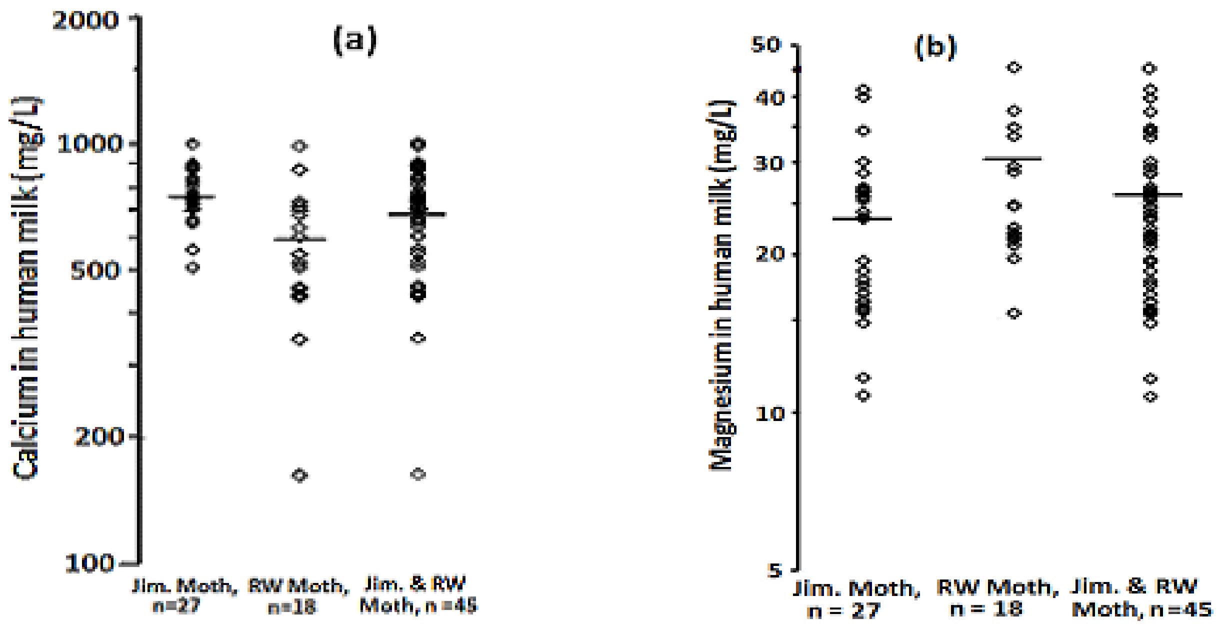

Fig 1: Logarithmic distributions of Ca (a) and Mg (b) in Jimma (Jim.) and rural Welkitie (RW) mothers. (N.B. Horizontal lines indicate mean values)

The Spearman correlation coefficients between the concentrations of Ca and each of, Mg, Fe, Zn, and, Cu were all statistically non-significant ($p > 0.05$). Similarly, Mg did not correlate with each of the other elements ($p > 0.05$) but with Zn ($rs = 0.37$, $p = 0.01$). Mother's age or weight did not also correlate with Ca and Mg levels in milk.

Iron, Zinc, and Copper: The concentration of Cu in the milk of Jimma mothers was found to be significantly higher than in that of rural Welkite ($p < 0.01$). Zinc and Fe concentrations, however, were not significantly different ($p > 0.05$). The distributions of Fe, Zn and Cu in the milk of Jimma and rural Welkitie mothers are given in Fig 2 (a, b and c).

The Spearman correlation coefficients were calculated to investigate whether maternal age or weight is related to the concentrations of each of Fe, Zn and Cu. But, none of the correlations were found to be statistically significant ($p < 0.05$).

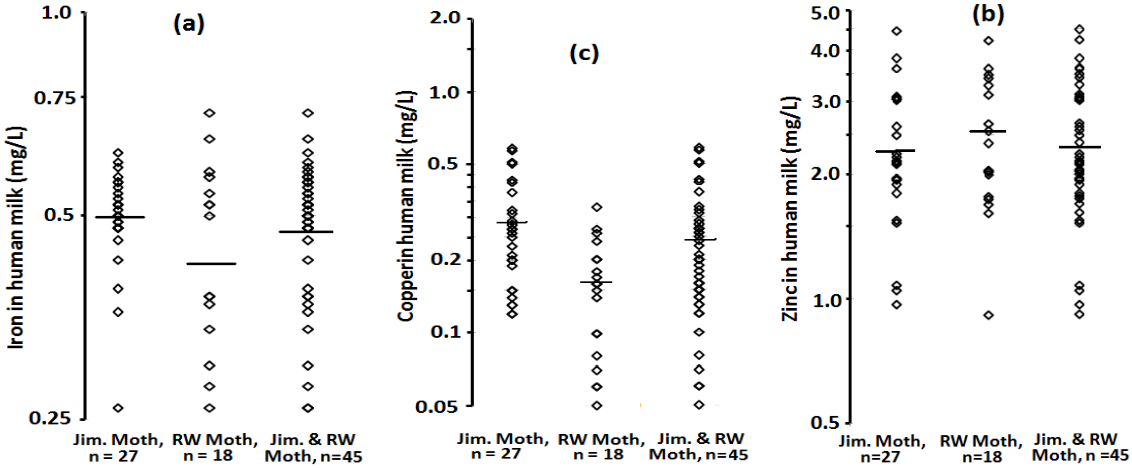

Fig.2: Logarithmic distributions of Fe (a), Zn (b), and Cu (c), in the milk of Jimma (Jim) and rural Welkitie (RW)mothers.

DISCUSSION

The Ca content of breast milk examined between 1950 and 1999 based on the works of 169 authors varied between 84 – 462 mg/L (on average 252 mg/L), and that of Mg varied over a wide range of 15 to 64 mg/L with a median of 31 mg/L (27, 28). Compared with the range given by Salamon and Csapo (28), and other literature values (Table 1), the mean concentrations of Ca determined in the milk of mother both from Jimma and rural Welkite were higher. The mean Ca level in the total samples in this study (687.67 ± 159) was 4 times higher than that of Sweden and, 3 times higher than that of Taiwan, USA, or Egypt. From the two groups in the current study, however, the Ca level in the milk of Jimma mothers was significantly greater than in that of rural Welkite mothers. In line with the findings of this study, Fransson et al. (15) observed higher concentrations of Ca in the milk of Ethiopian women than in that from Swedish women.

According to the researchers, the high altitude, clear air, and abundant sunshine environment of the Ethiopian mothers than that of the Swedish, might have contributed to the higher level of Ca in the Ethiopian mothers' milk. It would, however, be difficult to attribute the same reason for the observed difference in Ca between the milk of the two groups of mothers in this study since both the groups reside in geographical locations with slight difference in altitude (Rural Welkite area 1891–1935 meters, and, Jimma about 1750 meters above sea level) and enjoy similar atmospheric air and sunshine abundance. Nutritionally, 'enset' based dietary system was found to be extremely deficient in Ca than cereal based system. The Ca level in 'enset' was determined to be 320 mg/kg edible yield while in 'teff' it was 650 mg/kg of edible yield and is not hindered by the existence of phytate since the Ca:phytate molar ratio of fermented 'teff-injera' is above the critical molar ratio of 6:1 (8, 29, 30). Therefore, the difference in the concentrations of Ca between the milk of the two groups of mothers could be attributed to the difference in the Ca contents of the major diets of the groups. The lower level of Ca in the diet of rural Welkite mothers and the cultural restriction of consuming milk and its products during pregnancy might have contributed to the lower level of Ca in the milk of rural Welkite mothers.

The mean Mg levels in the Jimma and rural Welkite milk were not significantly different. Related to this finding, Fransson et al. (15) reported that they found no significant difference between the Mg levels in milk of non-privileged and privileged mothers who were living in Addis Ababa and were exposed to similar dietary habits. A number of other studies have also reported that milk Mg level is not correlated with the content in maternal diet (3, 28, 31, 32).

Relative to the studies reported in Table 1, the Mg level determined in the current study was very close to that of the two Ethiopian groups in Addis Ababa and those of Egypt, Sweden, Taiwan and USA.

The difference between the level of each of Fe and Zn in the milk of Jimma and rural Welkite mothers was not significant ($p > 0.05$). The findings of other researchers also indicate that there exists no correlation between maternal dietary intake of Zn and Fe and, their level in breast milk (31, 33, 34). The mean concentrations of Zn and Fe in the milk of the two groups of mothers in the current study fall within the range of values reported in the literature (Table 1). The mean Fe level of the total milk of the two groups, 0.46 mg/L, was close to the values reported from Australia, Germany, Ethiopia (Addis Ababa), Iran, Kuwait, Jeddah and Sweden. The value reported from Italy, however, was about 2 times higher, and those of Honduras, Jeddah, Taiwan and USA, were about 2 times lower than the level determined in the current study. On the other hand, the mean concentration of Zn in the privileged and non-privileged groups in Addis Ababa was about 3 times higher than in that of the current study. Relative to values reported from other countries, the mean Zn level in the total milk samples in the current study (2.35 mg/L) was very close to those of Canada, Kuwait, Egypt, Taiwan, USA and Iran, about 2 times higher than in those of Bangladesh, Jeddah and Sudan, and about 3 times than in that of Honduras. The Zn levels reported from Sweden, Australia, and Italy, however, were about 2 times higher than in that of the current study.

The level of Cu, determined in Jimma and rural Welkite milk was found to conform to most of the literature values in Table 1. Higher concentration of Cu was noted in the milk of Jimma mothers than in that of rural Welkitie mothers. When compared with previous studies carried out in Addis Ababa and Arsi (Table 1), the Cu level in the milk of rural Welkitie mothers was close to that of the privileged mothers in Addis Ababa, while the level in Jimma mothers' milk was close to that of the non-privileged mothers in Addis Ababa (15). On the other hand, the mean concentrations of each of Zn and Cu determined in the milk of the two groups in the current study were about 4 and 2 times higher than the respective concentrations in the milk of mothers of stunted and non-stunted infants in rural Arsi respectively (8). Relative to the reports from other countries, the mean concentration of Cu determined in the current study was about 2 times higher than those of Sudan and USA; close to those of Bangladesh, Honduras, India, and Taiwan; about 2 times lower than those of Italy, Iran, and Canada; about 3 times lower than those of Germany, Jeddah, Kuwait and Sweden; about 4 times lower than that of Australia.

Although studies indicate that most fractions of 'enset', except leaf lamina (which is not edible), are deficient in Cu (35), and 'teff' grain has very high Cu content (30), the amount of Cu in the human diet usually far exceeds the requirement (36). There have been no reported instances of copper deficiency in adults that have not resulted from repeated and prolonged diarrhea combined with poor general nutrition (15). Therefore, it seems unlikely that the differences in the copper content between the milk samples of Jimma and rural Welkitie mothers can be accounted for by variations in dietary intake. In line with the findings of the current study, Domellof et al. (17) reported that they found higher milk Cu in Honduran women than in Swedish women. Some other studies, however, reported that the Cu content in mothers' milk did not show significant differences when different nutritional habits are compared in the same cultural circle, and also there is no difference even between the vegetarians, the non-vegetarians and different nationalities (32, 37).

The correlation between mothers' age or weight and each of Mg, Ca, Fe, Zn and Cu level in milk was determined by Spearman rank correlation and, the findings indicated that mothers' age and weight were not related with the levels of all the five elements in milk ($p > 0.05$). Regarding the relationship between mothers' age and trace element content in human milk, however, the literature provides controversial reports. Some studies reported findings that are in line with the current study (20, 38, 39) while some others reported findings that indicated existence of correlation (40, 41).

In conclusion, the present study was conducted to determine the concentrations of Mg, Ca, Fe, Zn and Cu in breast milk samples collected from lactating mothers in Jimma and rural Welkitie areas in Ethiopia. The concentrations of Ca and Cu in Jimma milk samples were found to be significantly greater than those of rural Welkite ($p < 0.05$) and, the levels of these elements in breast milk seem to be influenced by dietary intake of the mothers. The concentrations of Mg, Fe and Zn in the milk of the two groups of mothers, however, were similar. The mean concentrations of Mg, Cu, Zn and Fe in the milk of the two groups of mothers in the current study showed a high level of agreement with literature values. The level of Ca in both milk samples, however, was higher than concentrations reported in the literature.

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