©Copyright 1991 by The Humana Press, Inc.
All rights of any nature, whatsoever, reserved.
0163-4984/91/2901-0051 $05.00

Minor and Trace Elements in Human Milk from Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire

Results from a WHO/IAEA Joint Project

ROBERT M. PARR,*[,1] EDOUARD M. DEMAEYER,[+,2] VENKATESH G. IYENGAR,[3] ANTHONY R. BYRNE,[4] GORDON F. KIRKBRIGHT,[+,5] GERHARD SCHÖCH,[6] L. NIINISTÖ,[7] OSCAR PINEDA,[8] H. L. VIS,[9] YNGVE HOFVANDER,[10] AND A. OMOLOLU[11]

[1]International Atomic Energy Agency, A-1400 Vienna, Austria; [2]World Health Organization, CH-1211 Geneva 27, Switzerland; [3]Institute of Medicine, Nuclear Research Center Juelich, Jeulich, FRG; and National Institute of Standards and Technology, Center for Analytical Chemistry, Gaithersburg, MD 20899; [4]Nuclear Chemistry Department, Jozef Stefan Institute, YU-61111 Ljubljana, Yugoslavia; [5]Department of Instrumentation and Analytical Science, UMIST, Manchester, M60 1QD, UK; [6]Forschungsinstitut fuer Kinderernaehrung, Heinsstueck 11, D-4600 Dortmund, FRG; [7]Laboratory of Inorganic and Analytical Chemistry, Helsinki University of Technology, SF-2150 Espoo, Finland; [8]Institute of Nutrition for Central America and Panama (INCAP), Guatemala City, Guatemala; [9]Department of Pediatrics, Free University, Brussels, Belgium; [10]Department of Pediatrics, University Hospital, Uppsala, Sweden; and [11]College of Medicine, University of Ibadan, Ibadan, Nigeria

Received January 29, 1990; Accepted March 19, 1990

*Author to whom all correspondence and reprint requests should be addressed.

+Now deceased.

ABSTRACT

Concentrations of As, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sn, V, and Zn were determined in human whole milk samples from Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire; in most of these countries, three groups of subjects representing different socioeconomic conditions were studied. Analytical quality control was a primary consideration throughout. The analytical techniques used were atomic absorption spectrophotometry, atomic emission spectrometry with an inductively coupled plasma, colorimetry, electrochemistry, using an ion-selective electrode and neutron activation analysis.

The differences between median concentrations of Ca, Cl, Mg, K, Na, and P (minor elements) were lower than 20% among the six countries. Among trace elements, concentrations observed in Filipino milk for As, Cd, Co, Cr, Cu, F, Fe, Mn, Mo, Ni, Pb, Sb, Se, and V were higher than for milk samples from other countries. The remaining five countries showed a mixed picture of high and low values. In the case of at least some elements, such as, F, I, Hg, Mn, Pb, and Se, the environment appears to play a major role in determining their concentrations in human milk. The nutritional status of the mother, as reflected by her socioeconomic status, does not appear to influence significantly the breast milk concentrations of minor and trace elements.

Significant differences exist between the actual daily intakes observed in this study and current dietary recommendations made by, for example, WHO and the US National Academy of Sciences. These differences are particularly large (an order of magnitude or more!) for Cr, F, Fe, Mn, and Mo; for other elements, such as, Ca, Cu, Mg, P, and Zn, they amount to at least a factor 2. In the opinion of the present authors, these findings point to the need for a possible reassessment of the dietary requirements of young infants, with respect to minor and trace elements, particularly for the elements Ca, Cr, Cu, F, Fe, Mg, Mn, Mo, P, and Zn.

Index Entries: Human milk; elemental composition; trace elements; geographic comparison; geochemical influence; lactation; infant nutrition; pediatric mineral nutrition; developing countries; urban; rural.

INTRODUCTION

A multinational study on breast feeding was initiated by the World Health Organization (WHO) in 1973. Nine countries from different geographical locations, namely, Chile, Ethiopia, Guatemala, Hungary, India, Nigeria, Philippines, Sweden, and Zaire, participated in the investigation that was carried out in three phases: contemporary patterns of breast feeding, measurement of volume and major components, and determination of minor and trace elements. The first two phases have

been summarized in 1981 (*1*) and 1985 (*2*), respectively. The third phase, that deals with the determination of minor and trace elements, and assessment of their daily intake by infants, was coordinated by the International Atomic Energy Agency (IAEA) in cooperation with the WHO and six of the countries listed above (all except Chile, Ethiopia, and India). A detailed account has just been completed (*3*). The purpose of the present report is to summarize the essential features of phase three. The elements selected included all the known essential trace elements (with the exception of Si), and some important toxic trace elements. Altogether, 24 elements were included: As, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sn, V, and Zn. Ca, Cl, Mg, P, K, and Na were also included since they, like many of the trace elements, are also biologically essential, and their analysis could be carried out by similar means.

Despite many important advances during the last 20 yr in the methodologies available for the determination of trace elements in biological materials, such analyses are still very commonly subject to significant errors (*4*). Consequently, literature data are inconsistent for many trace elements, and it is not possible to decide whether the differences are real (representing biological and/or geographical variability) or artifacts arising from unrecognized analytical errors (*5*). Elimination of analytical errors requires the development and use of suitable approaches and procedures for analytical quality assurance. This was a primary consideration in planning the studies. A second objective was to find out whether the nutritional status of the mother affected the breast milk concentrations of minor and trace elements.

MATERIALS AND METHODS

Sample Collection

Three groups of mothers were studied in each of the countries participating in this project. Details of the study areas, study groups, and number of samples collected are shown in Table 1. The communities from which the mothers were selected and the characteristics of the study samples have been described in great detail (*2*). It was decided to study the composition of human milk at one particular time after the birth of the baby, namely, at about 3 mo. At this stage of lactation, the milk is mature and its composition has reached stable levels for many of its constituents. Moreover, 3 mo is a stage at which many mothers start to wean their babies. After this age, therefore, the baby's intake of nutrients no longer depends exclusively on breast milk. Details of sample collection procedures are described in ref. *3*. Briefly, the collection steps involved complete emptying of one breast (using breast-pump) around noontime, approx 4 h after the previous feed. The collection centers were requested to provide 10 to 20 mL of whole milk for elemental analysis.

Table 1
Study Areas, Study Groups, and Number of Human Milk Samples Collected

Study area	Description[a]	No. of samples
Guatemala	A	31
	B	29
	C	40
Hungary	A	24
	B	33
	C	25
Nigeria	A	6
	B	2
	C	10
Philippines	A	46
	B	74
	C	63
Sweden	Only one group[b] (urban + rural)	64
Zaire[c]	A	37
	C	32

[a]Well-to-do mothers = A, Urban poor mothers = B, and mothers living in a rural environment = C.
[b]The distinction between urban and rural, as well as rich and poor, was not feasible.
[c]The distinction between really poor and not-so-poor urban mothers was thought to be impracticable.

The samples were frozen, and shipped to the IAEA's laboratory in Vienna in low temperature transport containers (Trans Temp, USA) capable of retaining the samples at −11°C during shipment.

Sample Preparation and Analysis

The workload was shared among a group of experienced analytical laboratories employing a variety of analytical methods. Table 2 identifies the laboratories involved and the methods employed (6–13).

The IAEA's laboratory in Vienna served as a central coordinating laboratory. Specimens received from the collection centers were stored at low temperature (−20°C) until the analysis could be started. After thawing, each sample was homogenized, and if sufficient material was available, six separate aliquots of 5 mL each were placed in precleaned plastic vials, carrying out the operations on a clean air workbench (laminar flow hood). Aliquots designated for the determination of Ca, Cr, Mg, K, and Na, were frozen pending analysis; the remaining aliquots were freeze-dried and shipped to the reference laboratories.

Table 2
Reference Analytical Laboratories, Elements Determined, and Analytical Methods Used

Analytical laboratory	Elements determined	Analytical method	Key* ref.
IAEA, Vienna Austria	Ca, Cr, K, Mg, Na	AAS	6
	Cl	INAA	6
	Cd, Mo	RNAA	7
Kernforschungsanlage (KFA), Juelich, FRG	Co, Fe, Hg, Sb, Se, Zn	INAA	8
	Cu, Mn	RNAA	7
Inst. Science and Tech., Manchester, UK	Ni	ICP-ES	6
	Pb	AAS	9
Jozef Stefan Inst. Ljubljana, Yugoslavia	V, Sn	RNAA	10
	As, I	RNAA	11
Helsinki Univ. Tech. Helsinki, Finland	F	ISE	12
Forschungsinstitut fuer Kinderernaehrung, Dortmund, FRG	P	COL	13

AAS = Atomic absorption spectrophotometry.
INAA = Instrumental neutron activation analysis.
RNAA = Radiochemical neutron activation analysis.
ICP-ES = Emission spectrometry.
ISE = Ion selective electrode (Electrochemistry).
COL = Colorimetry (Light absorption spectrometry).
*For additional references see Ref. 3.

Quality Assurance

An important feature of the analytical design used in this project is that each element was determined by only one reference laboratory, to eliminate interlaboratory differences. The quality of the data reported by that laboratory was checked by means of two milk-based analytical quality control (QC) materials, and by blind analysis of reference materials (RM). First of these was a cow milk powder, a certified RM (IAEA A-11) (*14*). At the time that this project was started, this was the only milk-based standard available. As a result of the experience gained during the course of this project work, it was recognized that A-11 was not the ideal standard for this project in the sense that cow's milk and human milk differ significantly in the concentrations of some analytes. Partly for this reason, but also because it was considered desirable to have a second standard for internal QC (to monitor the constancy of performance of the analytical methods during the full program of analysis), an additional RM was developed, based on human milk (HM-1). It may be mentioned that since the completion of this project, several other similar reference materials have become available (*15*).

Table 3
Summary of Analytical Results for the Milk Based RMs used in this Study for QC (concentration expressed in μg/g, dry)[a]

Element	A-11		HM-1
	Certified[b,c]	This work	This work
As	0.00485 ± 4	0.0048 ± 4	0.0013 ± 23
Ca	12,900 ± 3	13,100 ± 10	2,290 ± 4
Cd	0.0017 ± 11	<0.01	<0.006
Cl	9,080 ± 9.5	9,920 ± 3	2,748 ± 4
Co	0.0045 ± 18	0.0051 ± 21	0.0015 ± 47
Cr	0.0177 ± 21	0.018 ± 30	0.009 ± 60
Cu	0.378 ± 8	0.39 ± 6	1.60 ± 8.5
F	—	0.176 ± 13	0.054 ± 13
Fe	3.65 ± 10.5	2.24 ± 21	1.95 ± 29
Hg	0.0032 ± 20	—	0.008 ± 43
I	0.087 ± 7	0.09 ± 6	0.32 ± 8
K	17,200 ± 3	17,600 ± 10	3,840 ± 4
Mg	1,100 ± 3.5	1,130 ± 5	257 ± 3
Mn	0.257 ± 2.5	0.260 ± 9	0.072 ± 10
Mo	0.092 ± 10	0.085 ± 11	0.007 ± 50
Na	4,420 ± 4	4,100 ± 10	822 ± 4
Ni	—	—	0.11 ± 28
P	9,100 ± 5.5	11,219 ± 1	1,251 ± 2
Pb	0.054 ± 18	—	0.09 ± 32
Sb	—	—	0.011 ± 42
Se	0.034 ± 10.5	0.031 ± 13	0.074 ± 9
V	—	0.0009 ± 11	0.0006 ± 25
Zn	38.9 ± 3	34.7 ± 8	12.1 ± 7

[a]± = Uncertainty of the results expressed in percentage at one sigma level.
Tab[b]Results for As, Cd, Co, Cr, Cu, Hg, I, Mn, Mo, and Pb are for recent intercomparison data (Ref 11).
[c]Recommended concentrations by the IAEA.

Throughout the program, roughly 15% of the total workload was spent on analyzing a number of aliquots of A-11 and HM-1. Some of the HM-1 aliquots were included in the sample series designated as real samples, and their identity was released to the analysts only after the completion of the laboratory work. In addition to these two RMs, other certified RMs, such as, NBS Bovine Liver and Bowen's Kale, were used by some analysts to evaluate their methodologies. A summary of results for the two milk-based RMs is presented in Table 3. More details may be found in ref. *3*. Part of the investigations related to this project or related to the reference materials used in this project is reported elsewhere (*11,16*).

Table 4
Concentrations of Minor Elements in Human Milk from Different Study Areas: Median Values ± 1 SD*

Study area	Calcium (mg/L)	n	Chlorine (mg/L)	n	Magnesium (mg/L)	n
Guatemala	303 ± 7	81	326 ± 14	31	34.1 ± 0.9	81
Hungary	285 ± 6	71	411 ± 24	34	32.6 ± 0.7	71
Nigeria	226 ± 14	15	318 ± 44	16	29.0 ± 2.6	15
Philippines	270 ± 6	65	329 ± 22	31	29.7 ± 0.7	65
Sweden	235 ± 13	29	401 ± 35	20	34.2 ± 2.3	29
Zaire	274 ± 8	69	350 ± 20	24	37.8 ± 0.9	69

Study area	Phosphorus (mg/L)	n	Potassium (mg/L)	n	Sodium (mg/L)	n
Guatemala	154 ± 4	26	487 ± 10	81	106 ± 4	81
Hungary	136 ± 3	26	554 ± 9	71	105 ± 6	71
Nigeria	146 ± 26	3	410 ± 42	15	87 ± 13	15
Philippines	147 ± 8	15	469 ± 11	65	128 ± 7	65
Sweden	142 ± 7	4	548 ± 19	29	88 ± 17	29
Zaire	155 ± 7	33	511 ± 10	69	120 ± 5	69

*S.D. = Standard deviation of median as given by the quantity: $0.926R/\sqrt{N}$ where R is its interquartile range (the difference between the 75th and 25th percentiles) and N is the number of observations (17).

RESULTS

Data Evaluation and Presentation

Since, for many of the sets of data under consideration, the individual data points do not all conform to simple Gaussian statistics, it was decided to use a method of data evaluation (*17*) that is relatively insensitive to the exact form of the statistical distribution, and to the presence of outliners, namely, one based on the use of the median and its SD, s, as given by the relation $s = 0.926R\sqrt{N}$, where R is the interquartile range (the difference between the 75th and 25th percentiles), and N is the number of observations.

Dry Matter

Total dry matter was individually determined in all samples from various study groups and areas. Several significant differences between study areas were observed, though they were not of very large magnitude in strictly numerical terms. A statistical summary of the results for dry matter can be seen in the full report (*3*).

Table 5
Concentrations of Trace Elements in Human Milk from Different Study Areas: Median Values ± 1 SD*

Elem	Unit	Study area: Guatemala	Hungary	Nigeria	Philippines	Sweden	Zaire
As	µg/L	0.29 ± 0.08	0.24 ± 0.02	1.78 ± 1.44	18.9 ± 4.0	0.55 ± 0.15	0.26 ± 0.08
	n	26	11	6	14	8	20
Cd	µg/L	ND	ND	3.67 ± 2.03	2.67 ± 0.50	ND	ND
	n	13	11	9	15	13	15
Co	µg/L	0.24 ± 0.03	0.15 ± 0.01	0.64 ± 0.11	1.40 ± 0.12	0.27 ± 0.04	0.36 ± 0.04
	n	84	71	18	65	32	69
Cr	µg/L	1.17 ± 0.14	0.78 ± 0.21	4.35 ± 1.78	3.46 ± 0.60	1.48 ± 0.57	1.07 ± 0.55
	n	9	11	4	10	6	11
Cu	µg/L	263 ± 11	203 ± 10	278 ± 25	310 ± 18	186 ± 29	201 ± 16
	n	84	71	18	61	31	68
F	µg/L	9.4 ± 0.5	13.8 ± 0.8	24.7 ± 9.7	118 ± 13	17 ± 1.9	6.8 ± 0.6
	n	29	63	10	32	10	61
Fe	µg/L	346 ± 25	365 ± 23	523 ± 56	720 ± 64	446 ± 35	556 ± 74
	n	84	71	18	65	32	69
Hg	µg/L	1.56 ± 0.16	1.43 ± 0.16	2.15 ± 0.56	1.71 ± 0.30	3.34 ± 0.33	2.66 ± 0.66
	n	84	71	18	65	32	69
I	µg/L	60 ± 13	64 ± 9	62 ± 21	57 ± 14	56 ± 11	15 ± 5
	n	25	31	7	11	16	19
Mn	µg/L	3.79 ± 0.29	4.00 ± 0.45	15.84 ± 4.10	39.55 ± 3.54	3.23 ± 0.27	11.21 ± 2.45
	n	84	71	18	63	31	68
Mo	µg/L	2.12 ± 0.84	ND	2.65 ± 0.68	16.36 ± 3.88	0.40 ± 0.32	1.39 ± 0.78
	n	14	13	9	15	10	15
Ni	µg/L	12.9 ± 1.3	14.4 ± 0.7	12.2 ± 3.6	16.1 ± 1.2	11.0 ± 1.1	4.9 ± 1.7
	n	74	70	14	62	31	59
Pb	µg/L	2.9 ± 0.6	14.9 ± 0.9	4.9 ± 1.2	16.6 ± 1.2	16.8 ± 1.0	5.0 ± 0.8
	n	74	68	14	63	32	69
Sb	µg/L	1.0 ± 0.1	1.6 ± 0.2	4.1 ± 2.4	11.0 ± 1.5	3.0 ± 0.8	3.6 ± 0.4
	n	84	71	18	65	32	69
Se	µg/L	19.2 ± 1.2	13.9 ± 0.4	24.2 ± 2.4	33.2 ± 1.4	13.1 ± 0.9	19.3 ± 1.1
	n	84	71	18	65	32	69
V	µg/L	0.21 ± 0.05	0.11 ± 0.01	0.46 ± 0.11	0.69 ± 0.07	0.13 ± 0.02	0.27 ± 0.17
	n	26	19	6	14	8	20
Sn	µg/L	2.34 ± 2.82	—	—	—	—	1.26 ± 0.24
	n	4					8
Zn	µg/L	2610 ± 320	1200 ± 110	1680 ± 220	1980 ± 120	700 ± 100	1920 ± 120
	n	84	71	18	65	32	69

*see footnote Table 4

Elemental Concentrations

Concentrations of minor and trace elements in milks from various study areas are presented in Tables 4 and 5, respectively. The errors stated in these tables are total estimated uncertainties from a given technique (Table 2). The results shown are average concentrations derived from all the study groups. Analytical data on individual study group are recorded in the report (3). In the present report, differences between study groups, if any, are indicated by specific comments. Typical concentrations of minor and trace elements under "normal" conditions (i.e., after exclusing study areas where exceptionally low or high values were observed) are identified and listed in Table 6.

Table 6
Range of Median Minor and Trace Element Concentrations Observed Under Apparently "Normal" Conditions

Element	Unit	Range
As	μg/L	0.2–0.6
Ca	mg/L	220–300
Cd	μg/L	<1
Cl	mg/L	320–410
Co	μg/L	0.15–0.35
Cr	μg/L	0.8–1.5
Cu	μg/L	180–310
F	μg/L	7–17
Fe	μg/L	350–720
Hg	μg/L	1.4–1.7
I	μg/L	55–65
K	mg/L	410–550
Mg	mg/L	29–38
Mn	μg/L	3–4
Mo	μg/L	0.3–3
Na	mg/L	90–130
Ni	μg/L	11–16
P	mg/L	135–155
Pb	μg/L	2–5
Sb	μg/L	1–4
Se	μg/L	13–24
Sn	μg/L	~1
V	μg/L	0.1–0.3
Zn	mg/L	0.7–2.0

Daily Intake of Elements from Breast Milk

Daily intakes of various elements have been calculated by multiplying the concentration by the median daily intakes of breast milk in each of the study areas (Table 7). The following median values were used (g/d): Guatemala 636, Hungary 670, Nigeria 642, Sweden 800, Philippines 639, and Zaire 467. With the exceptions of Zaire, milk vol of this study compare well with recent mean vol reported for Swedish mothers (740 ± 150 g/d, ref. *18*) and American mothers (753 ± 89 g/d, ref. *19*).

DISCUSSION

Differences between Study Areas, Comparison with Literature Data, and Dietary Recommendations

A comparison of the measured daily intakes with recommended daily intakes proposed by WHO (*20,21*) and US National Academy of Sciences (NAS) (*22*), or estimated safe and adequate daily intakes (ES-

Table 7
Measured Daily Intakes of Minor and Trace Elements through Milk for 3-mo Old Babies and Comparison with WHO and NAS/NRC Recommendations

Elem	Unit	Daily intake, median value (range in brackets)*						Recommended daily allowances[1]		Maximum tolerable intake per day[2]
		Guatemala	Hungary	Nigeria	Phillipines	Sweden	Zaire	WHO[a]	NAS[b]	(FAO/WHO)
As	µg/d	0.18 (0.07–0.41)	0.16 (0.13–0.27)	1.1 (0.4–5.9)	12 (5–20)	0.44 (0.30–2.8)	0.12 (0.07–0.28)	—	—	12[d]
Ca	mg/d	193 (160–228)	191 (159–204)	145 (131–182)	173 (140–204)	188 (162–234)	128 (107–148)	—	360	—
Cd	µg/d	<0.6 (<0.6–1.2)	<0.7 (<0.7–1.2)	2.4 (<0.6–6.8)	1.7 (0.5–2.7)	<0.8 (<0.8–2.0)	<0.5 (<0.5–0.5)	—	—	6.4[e]
Cl	mg/d	207 (172–264)	275 (213–338)	204 (145–308)	210 (163–290)	321 (212–432)	163 (137–214)	—	275–700	—
Co	µg/d	0.15 (0.09–0.46)	0.10 (0.05–0.17)	0.41 (0.22–0.65)	0.89 (0.48–1.67)	0.22 (0.10–0.38)	0.17 (0.07–0.32)	—	—	—
Cr	µg/d	0.74 (0.34–1.2)	0.52 (0.42–1.4)	2.8 (2.2–5.3)	2.2 (1.1–3.1)	1.2 (0.6–3.2)	0.50 (0.26–1.3)	—	10–40	—
Cu	µg/d	167 (124–225)	136 (100–193)	178 (139–231)	198 (139–278)	149 (82–242)	94 (59–154)	480	500–700	3000[f]
F	µg/d	6.0 (4.5–9.5)	9.2 (6.5–13.2)	16 (6–32)	75 (33–104)	14 (8–17)	3.2 (7–35)	—	100–500	—
Fe	µg/d	220 (118–325)	245 (178–375)	336 (207–528)	460 (258–737)	357 (242–524)	260 (146–601)		10000	4800[g]
Hg	µg/d	1.0 (0.5–2.0)	1.0 (0.5–1.9)	1.4 (0.8–3.2)	1.1 (0.6–2.9)	2.7 (1.5–4.0)	1.2 (1.9–9.1)	—	—	4[h]
I	µg/d	38 (17–74)	43 (17–66)	40 (10–73)	36 (16–57)	45 (22–110)	7.0 (2.8–54)	—	40	—
K	mg/d	310 (256–354)	371 (338–423)	263 (209–360)	300 (255–349)	438 (379–495)	239 (206–276)	—	350–925	—
Mg	mg/d	22 (17–26)	22 (17–26)	19 (12–22)	19 (16–22)	27 (23–37)	18 (15–21)	40	50	—

Mn	µg/d	2.4 (1.6–4.3)	2.7 (1.4–5.5)	10 (6–21)	25 (13–42)	2.6 (1.9–3.8)	5.2 (1.6–14)	—	500–700	—
Mo	µg/d	1.3 (0.2–3.2)	<0.3 (<0.3–2.3)	1.7 (0.7-4.5)	10 (5–18)	0.3 (<0.3–1.9)	0.6 (<0.2–1.8)	12	30–60	—
Na	mg/d	67 (49–90)	70 (59–107)	56 (34–86)	82 (60–119)	70 (53–144)	56 (43–85)	—	115–350	—
Ni	µg/d	8.2 (3.0–14.5)	9.6 (5.9–14.9)	7.8 (3.0–13.7)	10.3 (6.6–16.9)	8.8 (5.8–14.0)	2.3 (<0.9–12.4)	—	—	—
P	mg/d	98 (89–112)	91 (80–105)	94 (82–114)	94 (86–212)	114 (105–120)	72 (57–86)	—	240	—
Pb	µg/d	1.8 (<0.6–4.3)	10 (7–17)	3.1 (1.5–6.0)	11 (6–15)	13 (11–19)	2.3 (<0.5–5.2)	—	240	—
Sb	µg/d	0.6 (<0.1–1.4)	1.1 (0.5–1.9)	2.6 (0.4–11)	7.0 (2.7–14)	2.4 (1.1–6.8)	1.7 (0.8–3.8)	—	—	21[i]
Se	µg/d	12 (7–18)	9.3 (7.8–11.4)	16 (11–23)	21 (15–28)	10.5 (7.7–15)	9.0 (6.0–13)	—	10–40	—
Sn	µg/d	1.5 (1.4–6.5)	—	—	—	—	0.6 (0.3–0.8)	—	—	12000[j]
V	µg/d	0.13 (0.09–0.31)	0.07 (0.05–0.15)	0.30 (0.19–0.44)	0.44 (0.26–0.61)	0.10 (0.05–0.12)	0.13 (0.05–0.70)	—	—	—
Zn	mg/d	1.7 (0.7–3.9)	0.8 (0.4–1.3)	1.1 (0.7–1.8)	1.3 (0.8–1.8)	0.6 (0.3–0.8)	0.9 (0.5–1.3)	3.1[c]	3	6[k]

[a]Daily intake (median concentration times median volume of milk consumed). Range (in round brackets) gives 16–84 percentile range (see text).
[b]These recommended daily allowances (RDA) are based primarily on the amount provided by human milk, but in some cases, they exceed the levels to provide for infants receiving formula and other infant feeds.
[c]Calculated on the basis of a 6 kg body wt.
[d]Recommended intake for 6 kg body wt (*20*).
[e]Recommended dietary allowance or estimated safe and adequate daily intakes for infants of 0–6 mos of age (*22*).
[f]Assuming a bioavailability of 40%.
[g]Maximum tolerable daily intake: 0.002 mg/kg body wt (*27*).
[h]Provisional tolerable weekly intake: 0.0067–0.0083 mg/kg body wt (*23*).
[i]Maximum tolerable daily intake: 0.5 mg/kg body wt (*26*).
[j]Maximum tolerable daily intake: 0.8 mg/kg body wt (*27*).
[k]Provisional tolreable weekly intake: 0.005 mg/kg body wt (*24,25*); refers to total Hg.
[l]Provisional tolerable weekly intake for infants and young children: 25 µg/kg body wt (*28*).
[m]Maximum tolerable daily intake: 2 mg/kg body wt (*26*).
[n]Provisional tolerable daily intake: 1 mg/kg body wt (*26*).

ADI) (NAS) is also shown in Table 7. The figures quoted are to be interpreted as recommended dietary allowances (RDA) or intakes, if a single figure is given, or as ESADI if a range is given. In the same Table, intakes are also compared with maximum tolerable intakes as they have been set by various FAO/WHO Expert Committees on Food Additives (23–28).

Minor Elements

Most median concentration values obtained were in the range of 200–300 mg/L for Ca, 300–400 mg/L for Cl, 120–180 mg/L for P, and 60 to 170 mg/L for Na. For Ca, Guatemala had significantly higher values than most other study areas. In Guatemala, also the three study groups differed significantly from each other: urban elite > rural > urban poor. For Mg and K, several significant differences were observed (Tables 4 and 7). For example, the Zairian data are relatively high, and the Nigerian and Philippine data relatively low. In the case of K, the Hungarian and Swedish values are relatively high. The Nigerian data cover a very wide range. In Guatemala, the rural group had significantly lower values than either of the urban groups.

A comparison of the concentrations of minor elements with data reported in the earlier scientific literature (*5*), as well as with some recent findings (*29,30*), reveals no serious discrepancies. Results for Na from the present study show slightly lower values than those found in the literature. However, the present findings are backed by analysis of reference materials, and therefore, are believed to be more reliable.

The daily intakes of minor elements through mother's milk fall in the relatively narrow range of 128–193 mg/d for Ca, 18–27 mg/d for Mg, 72–114 mg/d for P, and 56–82 mg/d for Na. For Cl and K, the ranges are 163–321 and 239–438 mg/d, respectively. All these ranges are generally below (in some cases < 50%) the RDA or the ESDDI prescribed by the NAS Report (*22*), indicating that these recommendations may have been set too high or may include an overgenerous margin of safety. However, the ratios of Ca:P fall within the desirable range of 1.5 to about 2.0, as discussed in the NAS report.

Trace Elements

Arsenic (As): Concentrations of As in specimens from the Philippines were rather high (18.9 ± 4.0 μg/L) in relation to those from all other study areas (0.24 to 1.78 μg/L). The Nigerian data had a relatively high median value and a very wide total range (Table 5).

Data reported for As in the literature vary from 3.2 to 36 μg/L (*5*). Because of difficulties in determining this element reliably in biological materials (*4*), these results should be interpreted with extreme caution. Recently, concentrations of 0.41 ± 0.12, 0.44 ± 0.23, and 3.04 ± 0.96 μg/

L (mean ± 1 SD) in mature milk for Indian (*31*), Yugoslavian (*32*), and Chilean subjects, respectively, have been reported. The high values cited for Chile are linked to high natural As in drinking water in a particular region. In contrast, in the Santiago region of Chile, the values were less than 0.1 μg/L (*33*).

The relatively high daily intake (Table 7) figure of 12 μg (range 5–20) by Filipino babies in relation to about 1 μg or less in the remaining five countries may perhaps be a cause for concern. Arsenic levels in tissues and body fluids have been linked to differences in dietary intake of this element. For example, high levels in blood of Japanese and Taiwanese, and in liver and hair of Japanese and Indians, have been reported (*34*). This probably reflects the geochemically high levels of As in those parts of Asia.

Cadmium (Cd): Many of the milk samples contained Cd at levels close to, or below, the limit of detection. For this reason, it was not always possible to obtain meaningful values for the median and other statistical parameters. Milk specimens from the Philippines had significantly higher levels than those from most other collection centers. All the values for specimens from Zaire were below the detection limit.

There is very little reliable information on Cd in the scientific literature, and the published data vary widely from 0.1 to 19 μg/L (*5*). Recent results obtained for Yugoslavian samples (*32*) are consistent with the values reported in Table 5, namely, < 0.3 μg/L.

With the exception of Nigeria, the median intake values reported in Table 7 are all considerably below the provisional tolerable intakes proposed by FAO/WHO (*23*). A close examination of the results from each country suggests that approx 20% of the samples from Nigeria and 10% of the samples from Guatemala would have been associated with intake values in excess of the provisional tolerable intakes. Since Fe deficiency, which is common in many developing countries, is believed to enhance cadmium absorption (*35*), this might be considered a possible explanation. Further investigations in these countries is advisable.

Cobalt (Co): Significant differences for this element between different study areas were observed. Highest values were measured in samples from the Philippines, whereas the Hungarian values were the lowest. In Guatemala, the urban poor had significantly higher values than the urban elite. In Zaire, the rural group has significantly higher values than the urban group.

Results reported in the scientific literature vary widely between 1 and 8.6 μg/L (*5*). Recently, mean concentrations of 0.5 and 0.37 μg/L in milk from Indian (*36*) and Chilean subjects (*33*), respectively, were reported.

The intake values for Co found in this study vary significantly between study areas. However, the assessment of total Co levels in human milk has only limited nutritional significance since this element is required only as vitamin B_{12}. The current RDA for infants (0.1 μg vitamin

B_{12}/d) corresponds to about 0.004 μg Co/d, a value that is easily exceeded in all study areas.

Chromium (Cr): Despite the fact that this is a difficult element to determine reliably in milk and other biological materials (*37*), and that at the time these measurements were carried out no appropriate certified reference materials for it were available, there are reasonable grounds for confidence in the accuracy of the analytical method used in this study. However, most of the measured values were close to the detection limit of the method, thus, leading to relatively large measurement errors (Table 5).

The specimens from the Philippines and Nigeria (3.50 and 4.35 μg/L, respectively), were exceptions with relatively high Cr concentrations, as compared with the other study areas (0.8–1.5 μg/L).

The results obtained for Cr in this study are lower than most literature data (*5*). However, our results differ from the findings of < 0.5 μg/L reported for Finnish (*38*) and American subjects (*39*). This raises some doubts about whether our findings are distorted by a systematic analytical error, or even by contamination. The analytical quality assurance data (annex 2, ref. *3*), argue against the existence of such errors. However, the analytical precision was poor, and therefore, the data obtained from this study must be viewed with caution. Nevertheless, it is probably valid to conclude that chromium concentrations in human milk are equal or less than the values obtained in this study.

The median intake values of Cr vary from 0.5 μg/d in Zaire to 2.8 μg/d in Nigeria, and all are considerably below the ESADI proposed by NAS. In view of the analytical difficulties associated with this element (*37*), a reassessment of the recommended intakes, on the basis of reliable analytical data, appears to be necessary. As discussed previously, if the results of this study are considered as upper limits, then, there is an even greater discrepancy with the present NAS recommendation.

Copper (Cu): Several significant differences were observed, though in numerical terms they were not of large magnitude. In general, the Philippines, Nigeria, and Guatemala had relatively high values (260 to 310 μg/L), and the remaining study areas, relatively low values (190–200 μg/L).

In general, the findings of the present study are in agreement with the results reported in the literature (*5*). Also, recent findings by various investigators (*29,31–33*) fall in the range of 250 to 340 μg/L). Exception to this on the high side and close to 400 μg/L (range 210 to 630) has been reported from the northern region of Chile with mining activities (*33*). On the other hand, very recently, a concentration of 200 ± 22 μg/L has been reported for Venezuelan (Merida region) mothers at one month postpartum (*40*).

Median intakes of Cu in this study (Table 7) varied between 94 μg/d (Zaire) and 198 μg/d (Philippines), all of which are lower than the 480 μg/

d recommended by WHO (*20*), or the 500–700 μg/d ESADI range recommended by NAS (*22*).

Fluorine (F): Fluorine is another element that is difficult to determine reliably at the levels found in human milk. It also suffers from a lack of appropriate reference materials. Nevertheless, there are reasonable grounds for confidence in the analytical methods used in this study. The results indicate that there are great variations between the Philippines (118 μg/L) and the remaining five countries (7 to 25 μg/L).

The results from Guatemala and Zaire (Table 5) compare well with the findings, namely 5 and 9 μg/L, reported for Finnish subjects living respectively, in low and high F areas (*41*), and in the Netherlands (*42*). The geochemical environment strongly influences the fluoride content of foods grown in any one area, which presumably explains the wide difference in fluoride concentrations observed in human milk in the present study.

Median intakes of F in this study varied between 3.2 μg/d (Zaire) and 75 μg/d (Philippines) (Table 7). Even the relatively high values from the Philippines is below the ESADI range of 100–500 μg/d recommended by NAS. However, even for breast-fed infants, drinking water may be assumed to be an additional source of F, and this may raise total intake to the recommended levels in many areas of the world.

Iron (Fe): Notable observations were the high levels in the Philippines and the relatively low levels in Guatemala and Hungary (Table 5). In Guatemala, the rural group had significantly lower values than either of the urban groups, and vice versa in Zaire.

The range of median concentrations (350 to 720 μg/L) observed for the present study areas indicates a certain degree of difference between the areas involved. However, it is similar to the overall tendency reported for Fe in the literature (*5*), showing a range of 200 to 1300 μg of iron/L. Some differences between rural and urban groups were identified, although no definite trends could be detected. For example, in Zaire, the rural group, that presented signs of malnutrition in mothers and children, had a higher concentration of Fe (850 μg/L) than the urban group (400 μg/L). In this study, Fe concentration in breast milk appears to be unrelated to the Fe status of the mother, which is consistent with other reports in the literature (*43,44*).

Median intakes for Fe in this study varied between 220 μg/d (Guatemala) and 460 μg/d (Philippines), all of which are much lower than the 10,000 μg/d recommended by NAS. Even lower values of 131 ± 6 μg/d and < 100 μg/d has been reported in recent studies from the USA (*29*) and Venezuela (*40*), respectively. However, the NAS (*22*) recommendation assumes a 10% bioavailability for Fe, that may be close to reality for breast-milk substitutes but not for breast milk. It should also be noted that the FAO/WHO maximum tolerable daily intake, when calculated on the basis of a 6 kg body wt, is less than the RDA value recommended by

NAS. There appears to be some inconsistency between these recommendations.

Mercury (Hg): The median concentration values all fall within a fairly narrow range, differing only by a factor of just over two between the lowest and highest (Table 5). The Swedish values stand out by being significantly higher than those for most other study areas (3.34 ± 0.33 μg/L), followed by Zaire (2.66 ± 0.66 μg/L).

The findings of the present study are in general agreement with the literature data (5), including recent results published for Yugoslavian subjects (32).

All the median intake values found in this study (Table 7) are less than the provisional tolerance limits of 4 μg/d proposed by WHO/FAO (23). The country with the highest median value of intake is Sweden with 2.7 μg/d. This is probably linked to the relatively high fish consumption of Swedes. A close examination of the results from each country suggests that approx 10–15% of the samples in Nigeria and Sweden, and 20–25% of the samples in Zaire would have been associated with intake values in excess of the provisional tolerable intakes. Further investigations in these countries are advisable.

Iodine (I): Iodine belongs to the group of elements that are difficult to determine at low concentrations in biological materials, besides lacking appropriate reference materials. However, there are reasonable grounds for confidence in the analytical method used in this study. No significant differences in I concentration between study areas were observed (56 to 88 μg/L), except for the Zairian data (15 μg/L) that stand out because of their low median values and large variability (Table 5). In Guatemala, the urban poor group had significantly lower values than the urban elite group.

Concentrations of I in human milk have been scarcely investigated. The commonly quoted sources (*45,46*) quote 50 μg/L, which is similar to the levels found in this study with the exception of Zaire, a region known for endemic goiter. In a recent study on Yugoslav subjects, a mean value of 88 ± 83 μg/L of I was found in mature milk (32). Much higher values have been reported in human milk specimens from Japanese subjects (*47*) who consume dietary algae, i.e., 150 μg/L (mode), with a range of 80–7000 μg/L.

Median intakes of I in this study (Table 7) were all, with one exception (Zaire), within the narrow range of 36–45 μg/d, in remarkable agreement with the RDA of 40 μg/d recommended by NAS (22). In Zaire, the study was conducted in the Kivu province that is notorious for its endemic goiter. It thus appears that the low I intake is reflected in the low I values in breast milk.

Manganese (Mn): Manganese is an element that is very susceptible to external contamination. The quality assurance data for this study indicate that the sampling steps were reliable and the analytical method was working with satisfactory accuracy and precision. Several significant

differences in Mn concentrations were observed between study areas. Once again, the Philippine data were by far the highest (39.6 μg/L). In contrast, Guatemala, Hungary, and Sweden all had relatively low values (3–16 μg/L). The urban poor group in Guatemala, and the rural group in Zaire had higher values than the remaining groups.

In the literature, concentrations of 4 μg/L in mid-lactation (2–6 mo) and 6–8 μg/L in late lactation have been reported (*5,31,32*). These values compare well with those found in this study in Guatemala, Hungary, and Sweden. In a study involving Indian subjects, a concentration of 23.0 ± 8.3 μg/L (mean value ± 1 SD) has been reported (*31*).

Median intakes of Mn in this study (Table 7) varied considerably between 2.4 μg/d (Guatemala) and 25 μg/d (Philippines), all of which are more than an order of magnitude lower than the ESADI range of 500–700 μg/d proposed by NAS (*22*). Obviously, some reassessment of the latter estimate is called for.

Molybdenum (Mo): In a number of samples, Mo concentrations were close to, or below the limit of detection. Therefore, it was not always possible to obtain meaningful values for the medians and other statistical parameters (Table 5). A notable exception was the specimens from the Philippines (16.4 ± 3.9 μg/L), characterized by their much higher concentrations than those from other study areas (0.4 to 2.7 μg/L).

There is very little information in the literature with which to compare these findings. In a study involving Indian subjects, a concentration of 6.4 ± 3.8 μg/L (mean value ± 1 SD) has been reported (*31*). Two more studies that appeared very recently reported 1–2 μg/L for American mothers (*48*) and 1.2 ± 0.5 μg/L in French subjects (*49*) in mature milks of 2 mo and 1 mo pp, respectively. Concentrations of Mo in food are known to vary considerably, depending on the environment in which the food was grown, and this is probably the origin of the large differences in Mo concentration in human milk observed in this study.

Median intakes of Mo in this study (Table 7) varied widely between less than 0.3 μg/d (Hungary) and 10 μg/d (Philippines). With the exception of the Philippines, these values are all one or more orders of magnitude lower than the ESADI (30–60 μg/d) proposed by NAS (*22*), and the daily intake recommended by WHO (ref. *20*, 12 μg/d), indicating that these recommendations may be in need of revision.

Nickel (Ni): Several significant differences were observed. The highest and the lowest median concentrations of 16.1 and 4.9 μg/L were measured in samples from the Philippines and Zaire, respectively, whereas in the remaining 4 countries, the results varied between 11 and 14.4 μg/L (Table 5). Between the study groups, subjects from the urban elite in Guatemala and the urban poor in Nigeria showed high values.

There is so little information on nickel in the literature that a meaningful comparison is not possible. In a very recent study, a concentration of 1.2 ± 0.4 μg/L was reported at 2 mo pp for American subjects (*48*).

Median intakes of nickel in this study (Table 7) were mostly in the

rather narrow range of 8–10 μg/d with the exception of Zaire where the median intake was only 2.3 μg/d. There are no recommended intakes from NAS (*22*) or WHO (*20*) with which to compare these figures. The American study (*48*) reported an average daily intake of 0.5 μg.

Lead (Pb): Although there are no *a priori* reasons for doubting the reliability of the analytical data, some quality assurance problems were encountered and considerable efforts were undertaken to understand them. Because of the very nature of the difficulty in determining Pb in milk, it was concluded that still more stringent quality assurance steps are desirable.

The concentration values obtained in the present study, that are in reasonably good agreement with data reported in the scientific literature (*5*), appear to fall into two groups: relatively high values in Hungary, the Philippines, and Sweden, and low values in others (Table 5).

Median intakes of Pb in this study (Table 7) varied widely between less than 1.8 μg/d (Guatemala) and 13 μg/d (Sweden). However, even the higher values observed in Sweden are much less than the provisional tolerable weekly intake proposed by the 30th Joint FAO/WHO Expert Committee on Food Additives (*28*).

Antimony (Sb): Several highly significant differences are apparent in the data (Table 5). The Philippines values are very high; the data for Guatemala and Hungary, on the other hand, are very low, whereas concentrations observed for Nigerian samples are highly variable.

There is almost no reliable information on this element in the literature (*5*). In a recent study on Yugoslavian subjects, a mean value of 0.16 ± 0.17 μg/L of Sb was found in mature milk (*32*). Clearly, further work is still required to establish well validated values for Sb in human milk.

Median intakes of Sb in this study (Table 7) varied widely between 0.6 μg/d (Guatemala) and 7 μg/d (Philippines). There are no recommended intakes from NAS or WHO with which to compare these figures.

Selenium (Se): The median concentration values obtained for samples from the different study areas varied between 13 and 33 μg/L (Table 5). Some of the differences are highly significant, the Philippine values being very high, and the Hungarian and Swedish values, relatively low.

Selenium is one of the few trace elements that can be determined in biological materials with reasonably good reliability (*6,50*). It is therefore not surprising that the results of this study agree well with the literature data (*5,34*), and that they fall within the range of values quoted in a recent review by Levander (*51*). This range extends from a low of 2.6 μg/L in a Keshan disease area to a high value of 283 μg/L in an endemic selenosis area. In other recent studies related to this element, the following concentrations have been reported: 20 μg/L in mature milk from Indian mothers (*36*), 20 ± 4 μg/L in mothers residing in the Santiago area of Chile (*33*), and somewhat lower values of 8.5 ± 2 μg/L for Yugoslavian subjects (*32*). In a very recent study, selenium concentrations of 16.4 to

20.2 μg/L were found in mature milk (up to 19 mo postpartum) in rural Gambian women (*52*). No differences in selenium concentrations were observed between milk samples from 1–6 mo postpartum and 13–19 mo postpartum in the Gambian study. The levels of Se in foodstuffs are known to vary widely according to the geochemical environments in which the foodstuffs are grown, and this presumably explains the variability observed in the present study. Such variations have been reported in clinical specimens, such as, whole blood and its components, liver, hair, and urine (*50*).

Median intakes of Se in this study (Table 7) varied between 9 μg/d (Zaire) and 21 μg/d (Philippines). These are at the lower end of the ESADI suggested by NAS.

Tin (Sn): Tin is a difficult element to determine in milk. Because of several analytical constraints (*6*), only a few samples from two study areas were analyzed. Samples from Guatemala and Zaire were found to contain about 1–2 μg/L of this element. It is not possible to make any statistical comment on the results. In the only other known study where tin analysis was undertaken, a lower concentration, namely 0.3 μg/L was reported for Yugoslavian subjects by the same group as did the analysis in this study (*32*).

Median intakes available from only two of the countries included in this study were 0.6 μg/d (Zaire) and 1.5 μg/d (Guatemala). These levels are several orders of magnitude lower than the maximum tolerable daily intake (Table 7). Since Sn is almost totally excreted via feces, mothers exposed to higher levels of Sn through their diets are unlikely to secrete a significant amount into their milk.

Vanadium (V): Vanadium is another difficult element to determine in milk. However, because of the suitability of radiochemical neutron activation analysis for the determination of V even at very low concentration levels, there are reasonable grounds for confidence in the results obtained. Samples from the Philippines and Nigeria had relatively high concentrations of V, as compared with Sweden and Hungary, that had relatively low ones (Table 5).

There is very little information on V in the literature with which to compare these findings. In a study on Yugoslavian subjects where V analysis was undertaken, a concentration of 0.15 μg/L, very similar to that found in Hungary and Sweden of 0.11 and 0.13 μg/L, respectively, has been reported for mature milk by the same group as did the analysis in this study (*32*).

Median intakes of V in this study (Table 7) varied widely between 0.07 μg/d (Hungary) and 0.44 μg/d (Philippines). There are no recommended intakes from NAS or WHO with which to compare these figures.

Zinc (Zn): Several significant differences in Zn concentrations are apparent in the data (Table 5). The values for Guatemala are relatively high whereas those for Hungary and, in particular, Sweden, are low. In

Guatemala, the urban elite group showed the lowest values, with the urban poor and rural groups having values higher by factors of about 2 and 4, respectively.

Zinc is one of the few trace elements that can be determined in biological materials with reasonably good reliability (*6*). It is therefore not surprising that the results of this study agree well with the literature data (*5*). In other recent studies related to this element, the following concentrations (mean ± 1 SD) have been reported: 2.4 ± 1.2 mg/L in mature milk from Indian Mothers (*31*), significantly higher values of 3.6 ± 1.0 mg/L in mothers residing in the Santiago area of Chile (*33*), and somewhat lower values of 1.44 ± 0.11 mg/L for Yugoslavian subjects (*32*).

Median intakes of Zn observed in this study (Table 7) varied by a factor of 3 between 0.6 mg/d (Sweden) and 1.7 mg/d (Guatemala). All these values are considerably lower than the dietary allowance recommended by NAS (*22*) or WHO (*20*), and the provisional tolerable intakes proposed by WHO/FAO (Table 7).

Comparisons between Actual Intakes Observed in this Study and Current Dietary Recommendations

Significant differences exist (Table 7) between the actual daily intakes observed in this study and current dietary recommendations made by, for example, WHO and the US National Academy of Sciences. These differences are particularly large (an order of magnitude or more!) for Cr, F, Fe, Mn, and Mo; for other elements, such as, Ca, Cu, Mg, P, and Zn, they amount to at least, a factor 2.

One of the sources of these discrepancies can probably be found in the fact that, for some elements, existing dietary recommendations are based, in part, on old analytical data of doubtful reliability. It was for this reason that analytical quality assurance received such close attention in the present study.

The authors recognize that in assessing dietary trace element requirements of infants, the situation with formula-fed infants is different from that of breast-fed babies. For example, the superior bioavailability of Zn from human milk, the elevated Zn concentrations in human milk during early stages of lactation, as well as reabsorption by breast-fed babies of endogenous Zn secreted into the gut, can make significant difference in requirements. Thus, existing dietary recommendations are justified in including a factor to take account of low bioavailability where applicable, from formula milk products. However, it is difficult to imagine in some cases, e.g., Fe, that this could possibly justify setting the recommended allowance as high as an order of magnitude greater than normal intakes by healthy infants from their mother's milk. A reassessment of some of the existing dietary recommendations for infant feeding may therefore be desirable.

Sources of Variation in the Elemental Composition of Human Milk

The composition of human milk is by no means constant, even from a single subject, and several factors have been identified that are responsible, either directly or indirectly, for the variations observed. These are both physiological and nonphysiological in origin, and many of them have been mentioned in previous publications (*2,5,53–55*).

Longitudinal variations (i.e., as a function of time) during the postpartum period, are known to occur for some elements, particularly for copper and zinc, both of which decrease in concentration as lactation continues (*56*). However, as explained in the "Materials and Methods" section, this source of variation was outside the scope of the present study, since all the samples were collected at about 3 mo postpartum.

Another potential source of variability is the socioeconomic status of the mother. Some information on the influence of this factor was available from the present study since, in most of the study areas, samples had been collected from three different socioeconomic groups (Table 1). As already stated in the section describing results for individual elements, some significant differences were occasionally observed between urban well-to-do, urban poor, and rural groups; however, no consistent trends could be identified.

Geographical influences are thought to be the main source of variability observed in this study. For example, the soil status of Cu, Fe, Mn, Mo, and Zn has been reported to be low in Hungary and high in the Philippines (*57*). It is interesting to note that a similar picture is reflected in the present study for these elements in milk samples from these two countries. Similarly, the Nigerian soil status has been classified as normal-to-low (with the exception of Mn which is high), and the milk composition reflects these findings. Unfortunately, not all elements and countries included in this investigation are covered in Silanpaa's report and therefore, an extended comparison is not possible. The observed variations are probably a reflection of the varying levels present in foods, that are generally dependent on the geochemical environment in which they are grown. For example, the consumption of foods rich in I (e.g., certain dietary algae), can rapidly affect the concentration of this element in breast milk (*47*). Similarly, high levels of fish consumption has been linked to high concentrations of Hg in the milk of Eskimo mothers (*58*).

Environmental contamination can also be a source of trace elements in human milk. For example, in an investigation involving subjects from a town with prominent Pb pollution of the atmosphere, highly significant increases in the Pb content of milk have been reported (*59*). The same factor is probably responsible for the high values found in this study in Hungary, Sweden, and possibly, the Philippines.

Some contamination of the samples prior to analysis is a possibility that cannot absolutely be ruled out, particularly for those elements that

occur only at levels of the order of μg/L, e.g., Pb. Although quality assurance was emphasized throughout this program, it was possible to apply active quality control only in respect of the analyses, but not of the sampling. Nevertheless, the experimental protocol prescribed rigorous procedures to ensure the validity of the samples, and there is no *a priori* reason to believe that any of the collection centers would have deviated from those procedures.

CONCLUSION

It can be concluded from this study that when minor and trace elements are determined under similar conditions in the breast milk of groups of mothers living in different parts of the world, environmental conditions appear to play a major role in determining the concentrations of most of them. However, for some of the elements, there appears to be little difference between groups and countries, and this is the case of Ca, Cl, K, Mg, Na, and P. The differences between median concentrations for these elements are lower than 20% between countries (difference from a median value) and may even be as low as 7% as in the case of phosphorus. Breast milk concentrations in these elements are probably controlled by homeostatic mechanisms that account for the small variations.

There is a second category of elements where differences between study areas may be very large although similar values may be found in a majority of countries. This is the case, for instance, of I where in five countries participating in the study concentrations in breast milk varied very narrowly, and it is in one study area only i.e. Zaire, that a low figure was found. These variations in breast milk concentrations, when they occur, are most likely reflecting variations in environmental conditions or food habits. The nutritional status of the mother as reflected in her socioeconomic status does not appear to influence significantly the breast milk concentrations of minor and trace elements. The range of concentrations found under usual conditions, i.e., after excluding study areas where exceptionally low or high values were observed is given in Table 6. These ranges may be useful in determining the desirable concentration of trace elements in breast milk substitutes; this, however, does not apply to toxic elements, such as, Cd, Pb, and Hg. It may be opportune in this context to formulate the concern expressed by a WHO expert committee in 1973 (*20*) which recommended then, with respect to formula products for babies, that such formulations should contain all the minor and essential trace elements at least in those levels that are present in human milk. Then the emphasis was on the need to meet minimum nutritional requirements. Nowadays, one might also wish to express concern over the possibility that such formulations based on existing dietary recommendations, may contain excessive levels of some trace elements, to an

extent far exceeding the normal nutritional requirements of babies during the first few months of life.

In the opinion of the present authors, the findings of this study point to the need for a possible reassessment of the dietary requirements of young infants, with respect to minor and trace elements, particularly for the elements Ca, Cr, Cu, F, Fe, Mg, Mn, Mo, P, and Zn.

ACKNOWLEDGMENTS

Many people at each of the collection centers and analytical laboratories participated in this project and have contributed to its success. The authors would particularly like to acknowledge the participation of the following: Federal Republic of Germany, L. E. Feinendegen, K. Kasperek and V. Galgan; Guatemala, J. J. Urrutia and B. Garcia; Hungary, I. Ory, P. Cholnoky, O. Gaal, M. Csaszar, J. Acs, M. Csiza, V. Markoczy, E. Dworschak, A. Gergely, and K. Linder-Szotyori; Nigeria, O. A. Ketiku and I. O. Akinyele; Philippines, V. B. Guzman, M. P. Macapinlac, L. V. del Castillo, and T. R. Lariosa; Sweden, U, Hagman, G. Fransson, L. Hambraeus, L. Whalberg, H. Jonsson, B. Larsson, C. E. Linder, R. Ras, and S. A. Slarach; UK, N. W. Barnett, L. S. Chen, and M. J. Cope; Yugoslavia, M. Dermelj and A. Vakselj; Zaire, P. Hennart, Ruchababisha-Migabo, and Nyampeta Uwaytu; IAEA, S. Clements, R. Ogris, F. Reichel, and E. Zeiller; WHO, A. Petros-Barvazian and A. Pradilla. Additional thanks are, of course, also owing to the more than 3000 mothers who, together with their families, took part in this and the earlier related WHO studies.

REFERENCES

1. Contemporary Patterns of Breast Feeding: World Health Organization Report, WHO, Geneva, 1981.
2. The Quantity and Quality of Breast Milk: World Health Organization Report on Collaborative Study on Breast-feeding, WHO, Geneva, 1985.
3. Minor and Trace Elements in Breast Milk: World Health Organization Report of a Joint WHO/IAEA Collaborative Study, WHO, Geneva, 1989.
4. R. M. Parr, *Nutr. Res.* Special Suppl. **I**, 5 (1985).
5. G. V. Iyengar, *Elemental Composition of Human and Animal milk*, IAEA-TECDOC-269, IAEA, Vienna, 1982.
6. Elemental Analysis of Biological Materials: International Atomic Energy Agency Technical Report Series, No. 197, Vienna, 1980.
7. G. V. Iyengar, *J. Radioanal. Nucl. Chem.* **110**, 503 (1987).
8. G. V. Iyengar, K. Kasperek, L. E. Feinendegen, X. Y. Wong, and H. Weese, *Sci. Total Environ.* **24**, 267 (1982).
9. R. F. Kirkbright, *Nutr. Res. Suppl.* **I**, 19 (1985).
10. A. R. Byrne and L. Kosta, *J. Radioanal. Chem.* **44**, 247 (1978). See also, *J. Radioanal. Chem.* **58**, 359 (1980), *J. Radioanal. Chem.* **20**, 627 (1974), and *Croat. Chim. Acta* **46**, 225 (1974).

11. A. R. Byrne, C. Camara-Rica, R. Cornelis, J. J. M. de Goeij, G. V. Iyengar, G. Krikbright, G. Knapp, R. M. Parr, and M. Stoeppler, *Fres. Z. Anal. Chem.* **326,** 723 (1987).
12. S. Esala, E. Vuori, and L. Ninisto, *Microchim. Acta* **1,** 155 (1983).
13. B. Estrin and F. E. Boland, *J. Assoc. Off. Agric. Chem.* **53,** 575 (1970).
14. Information Sheet for Reference Material A-11, Milk Powder: International Atomic Energy Agency, Vienna, July 1980.
15. Y. Muramatsu and R. M. Parr, Trace Elements in Biological and Environmental Reference Materials. Report IAEA/RL/128, Vienna, 1985.
16. G. V. Iyengar and R. M. Parr, *Proc. Symp. on Production, Regulation and Analysis of Infant Formula*, Association of the Official Analytical Chemists, Arlington, VA, 1985, pp. 233–245.
17. R. McGill, J. W. Tukey, and W. A. Larsen, *The American Statistician* **32,** 12 (1978).
18. M. C. Neville, B. A. Keller, and J. Seacat, *Am. J. Clin. Nutr.* **48,** 1375 (1988).
19. A. Sadurskis, N. Kabir, and J. Wager, *Am. J. Clin. Nutr.* **48,** 44 (1988).
20. Trace Elements in Human Nutrition: Technical Report Series No. 532, Geneva, 1973.
21. Report of an FAO/WHO Expert Group: Technical Report Series No. 230, Geneva, 1962.
22. Committee on Dietary Allowances and Food and Nutrition Board: Recommended Dietary Allowances, 9th Ed., Washington DC, National Academy of Sciences, 1980.
23. Joint FAO/WHO Expert Committee on Food Additives: Technical Report Series No. 505, Geneva, 1972.
24. Joint FAO/WHO Expert Committee on Food Additives: Technical Report Series No. 631, Geneva, 1978.
25. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission: CAC/VOL XVII, Contaminants. Rome, FAD of the UN and WHO, 1984.
26. Joint FAO/WHO Expert Committee on Food Additives: Technical Report Series No. 683, 1982.
27. Joint FAO/WHO Expert Committee on Food Additives: Technical Report Series No. 696, Geneva, 1983.
28. Joint FAO/WHO Expert Committee on Food Additives: Technical Report Series No. 751, Geneva, 1987.
29. N. F. Butte, C. Garza, and E. Smith, *Am. J. Clin. Nutr.* **45,** 42 (1987).
30. M. V. Karra, A. Kirksey, and O. Galal, *Am. J. Clin. Nutr.* **7,** 47 (1988).
31. H. S. Dang, D. D. Jaiswal, and C. N. Wadhwani, *Sci. Total Environ.* **44,** 177 (1985).
32. L. Kosta, A. R. Byrne, and M. Dermelj, *Sci. Total Environ.* **29,** 261 (1983).
33. E. Cortes-Toro E, (personal communication).
34. G. V. Iyengar, *Elemental Analysis of Biological Systems*, vol. I. Biomedical, Environmental, Compositional, and Methodological Aspects of Trace Elements. CRC Press, Boca Raton, FL, 1989.
35. P. R. Flanagan, J. S. McLellan, J. Haist, M. G. Cherian, M. J. Chamberlain, and L. S. Valberg, Gastroenterology 74, 841 (1978).
36. H. S. Dang, H. B. Desai, and S. R. Kayasth, *J. Radioanal. Nucl. Chem.* **84,** 177 (1984).
37. R. M. Parr, *J. Radioanal. Chem.* **39,** 421 (1977).
38. J. Kumpulainen, M. Uusitupa, and E. Vuori, *Sci. Total Environ.* **31,** 71 (1983).
39. C. E. Casey and K. M. Hambidge, *Br. J. Nutr.* **52,** 73 (1984).

40. M. Burguera, J. L. Burguera, and A. M. Garaboto, *Trace Ele. Med.* **5,** 60 (1988).
41. S. Esala, E. Vuori, and A. Helle, *Br. J. Nutr.* **48,** 201 (1982).
42. D. O. Backer, *Caries Res.* **8,** 181 (1974).
43. M. G. Karmarkar and C. V. Ramakrishnan, *Acta Paediatr.* **49,** 599 (1960).
44. M. J. Murray, A. B. Murray, N. J. Murray, and M. B. Murray *Br. J. Nutr.* **39,** 627 (1978).
45. Biology Data Book: P. L. Altman and D. S. Dittmer, eds., *Fed. Amer. Soc. Expt. Biol.* Bethesda MD, 1974.
46. Composition of Mature Human Milk: Report on Health and Social Security, 12, Dept. of Health and Social Security, London, Stationary Office, 1977.
47. M. Muramatsu, M. Sumiya, and Y. Ohmomo, *Hoken Butsuri,* **18,** 113 (1983).
48. C. E. Casey and M. C. Neville, *Am. J. Clin. Nutr.* **45,** 921 (1987).
49. D. Bougle, F. Bureau, and P. Foucault, *Am. J. Clin. Nutr.* **48,** 652 (1988).
50. G. V. Iyengar and J. R. W. Woittiez, *Clin. Chem.* **34,** 474 (1988).
51. O. Levander, *J. Nutr.* **119,** 1869 (1989).
52. M. A. Funk, L. Hamlin, M. F. Picciano, A. Prentice, and J. A. Milner, *Am. J. Clin. Nutr.* **51,** 220 (1990).
53. E. Vuori, PhD Thesis: Finland, University of Helsinki, 1979.
54. L. A. Vaughan, C. W. Weber, and S. R. Kemberlin, *Am. J. Clin. Nutr.* **32,** 2301 (1979).
55. M. F. Picciano, *Nutr. Rep. Int.* **18,** 5 (1978).
56. E. Roekens, H. Deelstra, and H. Robberecht, *Sci. Total Environ.* **24,** 267 (1982).
57. M. Sillanpaa, Micronutrients and the Nutrient Status of Soils: A Global Study. Rome, *FAO Soils Bull.* **48,** 1982.
58. W. A. Galster, *Environ. Health. Perspect.* **15,** 135 (1976).
59. N. W. Ghelberg, I. Ruckert, and H. Strans, *Igena, Hygiena, Bucharest* **21,** 17 (1972).

Acta Alimentaria, Vol. 27 (4), pp. 389–395 (1998)

EQUIVALENCY LAW IN THE METAL REQUIREMENT OF THE LIVING ORGANISMS?

Z. SÁNDOR

Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, H–1025 Budapest, Pusztaszeri út 59–67. Hungary

(Received: 9 February 1998; accepted: 3 June 1998)

From information referring to metal requirements of the human organism as well as metal contents of human and cow's milk and cereal grains it was concluded that an Equivalency Law exists in the metal balance of the living organisms. According to this law the alkali metal requirement (mainly potassium and sodium) is chemically equivalent to that of polyvalent metals (namely calcium, magnesium, zinc, iron, etc.). Theoretical considerations are given for proving the existence of the Equivalency Law.

Keywords: equivalency law, living organisms, metal requirements

With the development of science, more and more knowledge has been accumulated on nutrient requirements ensuring optimal function of human, animal (and plant) organisms. In addition to protein, carbohydrate, vitamin, etc., the requirements apply to mineral substances, macro-, meso- and microelements, as well. The values recommended on the basis of the most recent information concerning nutrient requirements of the human organism, are collected in the Recommended Dietary Allowances (RDA, 1989) by the Food and Nutrition Board of the National Research Council, the National Academy of Sciences in the US, and is republished from time to time. Surveying RDA data, the following regularity can be observed: the daily alkali metal requirement (ΣM^{+}, mainly potassium and sodium) of the human organism is chemically equivalent to the sum of the polyvalent metal requirement (ΣM^{Z+}, namely calcium, magnesium, zinc, iron, etc.). Taking into account metal contents of samples of biological origin (milk and cereal grains) as well as simple theoretical considerations, it can be concluded that this regularity exists in the metal balance of all the living organisms on the Earth.

0139-3006/98/$ 5.00 © 1998 *Akadémiai Kiadó, Budapest*

1. Discussion

The regularity mentioned may be termed as Equivalency Law, which can be described by the following formula: $\Sigma M^{+}=\Sigma M^{z+}$. Respective RDA data referring to adult men aged 19–24 years may represent the relevancy of the Equivalency Law (see Table 1).

The data in Table 1 show there is hardly any difference between alkali metal requirement, amounting to 92.085 milliequivalent/day (meq day^{-1}), and polyvalent metal requirement, which is 89.853 meq day^{-1}. According to RDA data, the difference is greater, for example, in the case of children aged 7–9 years (Ca: 800 mg, Mg: 170 mg, Fe: 10 mg, Zn: 10 mg, Mn: 2–3 mg, Cu: 1–2 mg, Na: 400 mg, K: 1600 mg); on the basis of which alkali metal requirement is 58.322 meq day^{-1} and the sum of polyvalent metal requirement is 54.849 meq day^{-1}. This difference, however, can not question the existence of the Equivalency Law, since RDA tables give data estimated as optimal values.

It is advisable to express the deviation from the Equivalency Law by calculating relative deviations, in percent, for example, by considering half the equivalents of the total amount of metals as standard. Thus, the relative deviation is 3.07% (A+) for children. ("A+" denotes alkali metal excess.) [E.g. (58.322+54.849)/2=56.586, the deviation from this is 58.322–56.586=1.736, which amounts to 1.736·100/56.586=3.07%]. Relative deviation is 1.23% (A+) for adult men.

Table 1

Metal requirement of adult men aged 19–24 years

Metal	mg day^{-1}	milliequivalent day^{-1}
Ca	1200	59.883
Mg	350	28.801
*Fe	10	0.448
Zn	15	0.459
*Mn	3.5 (2–5)	0.191
Cu	2.25 (1.5–3)	0.071
Na	500	21.749
K	2750 (2000–3500)	70.336
Alkali metals	ΣM^{+}	92.085
Polyvalent metals	ΣM^{z+}	89.853

* The equivalents are calculated for iron with 2.5+ charge, and for manganese with 3+ charge

The Equivalency Law can also be expressed by the formula: $\Sigma M^{+}/\Sigma M^{z+}=1$. In this way the deviation from the law ("quotient of equivalency") can be calculated by: $EQ=\Sigma M^{+}/\Sigma M^{z+}$. The value of EQ is greater than one for alkali metal surplus and is lower for the opposite case.

There are data referring to the Equivalency Law in the vegetable kingdom, as well. Results of my measurements concerning metal content of natural cereal flakes and cereal flake flours produced in 1994 in Hungary are given in Table 2. (After digestion with nitric acid and hydrogen peroxide, the metal contents were measured by a plasma emission spectrometer, Thermo Jarrell Ash Corp., AtomScan 25 type ICP.)

Table 2

Metal content of cereal flakes and flake flours [mg kg^{-1}]

Metal	Barley		Wheat		Rye		Oat	
	flake	f. flour	flake	f. flour	flake	f. flour	flake	f. flour
Ca	256.7	244.8	297.2	325.3	329.4	420.0	663.1	444.9
Cu	4.179	3.848	3.243	3.697	2.472	3.462	4.236	3.431
Fe	30.03	28.93	35.52	40.42	18.22	28.09	36.71	29.90
K	2890	3257	3429	3525	3942	5603	3632	2524
Mg	860.6	875.1	1088	1040	1027	1478	1357	921.0
Mn	10.13	10.06	34.20	35.93	18.62	27.96	46.26	39.42
Na	52.85	72.35	13.23	20.40	13.69	12.55	20.01	15.94
Zn	22.69	21.47	19.14	22.49	17.35	26.66	24.19	22.36

Table 3

Cereal flakes and flake flours and the Equivalency Law

	ΣM^{+} [meq kg^{-1}]	ΣM^{z+} [meq kg^{-1}]	$(\Sigma+\Sigma)/2$ [meq kg^{-1}]	Δ [%]	EQ
Barley flakes	76.216	86.349	81.283	6.23 (A–)	0.8827
Barley flake flour	86.451	86.848	86.650	0.229 (A–)	0.9954
Wheat flakes	88.278	108.50	98.389	10.28 (A–)	0.8136
Wheat flake flour	91.045	105.95	98.499	7.57 (A–)	0.8593
Rye flakes	101.42	103.39	102.41	0.96 (A–)	0.9810
Rye flake flour	143.85	146.29	145.07	0.840 (A–)	0.9833
Oat flakes	93.763	149.80	121.78	23.01 (A–)	0.6259
Oat flake flour	65.249	102.27	83.760	22.10 (A–)	0.6380

On the basis of data in Table 2, Table 3 shows alkali metal contents (ΣM^{+}), polyvalent metal contents (ΣM^{z+}), half of the total metal contents ($(\Sigma+\Sigma)/2$) and relative deviations from the Equivalency Law (Δ[%] and EQ respectively). Iron and manganese contents are calculated as above, "(A–)" denotes the lack of alkali metal.

Table 4

Metal content of human milk during the first 4 months of lactation [meq kg^{-1}]

Metal	Lactation (months)				Overall
	1	2	3	4	
Ca	14.8211	15.0207	14.5716	14.2223	14.6714
Mg	2.2218	2.4686	2.6332	2.7981	2.4686
Fe	0.0108	0.0091	0.0082	0.0072	0.0088
Zn	0.0704	0.0459	0.0336	0.0306	0.0459
Cu	0.0114	0.0100	0.0088	0.0084	0.0097
Na	5.8721	4.6107	4.6542	4.3497	4.8717
K	11.9188	11.5351	11.1770	10.6399	11.3305
ΣM^{+}	17.7909	16.1458	15.8312	14.9897	16.2022
ΣM^{z+}	17.1355	17.5543	17.2554	17.0666	17.2044
$(\Sigma+\Sigma)/2$	17.4632	16.8501	16.5433	16.0281	16.7033
Δ [%]	1.877 (A+)	4.180 (A–)	4.304 (A–)	6.479 (A–)	3.00 (A–)
EQ	1.0383	0.9198	0.9175	0.8783	0.9418

Table 5

Metal content of human and cow's milk [meq l^{-1}]

Metal	Human milk	Cow's milk
Ca	17.4659	59.8832
Mg	2.4686	9.8745
Fe	0.0313	0.0224
Zn	0.0092	0.0107
Cu	0.0126	0.0063
Na	6.5246	21.7486
K	15.3461	38.3651
ΣM^{+}	21.8706	60.1137
ΣM^{z+}	19.9877	69.7971
$(\Sigma+\Sigma)/2$	20.9292	64.9554
Δ [%]	4.498 (A+)	7.454 (A–)
EQ	1.0942	0.8613

The data for barley, wheat and rye seem to "conform" to the Equivalency Law surprisingly well, the highest deviation (for oat) is "only" 23%. At the same time, e.g. the ratios of sodium-potassium and calcium-magnesium in each sample considerably deviate from those considered to be optimal, according to RDA. Quantitative ratios of the various metals do not necessarily correspond to human requirements however, since the nutrients are stored in the seeds of cereals in order to satisfy the needs of their "descendants", the seedlings in their first phase of life.

The composition of milk also points to a genetically programmed supply of food requirements for descendants i.e. new-born children. BUTTE and co-workers (1987) gave data on the composition of human milk (and intake of human milk on 45 healthy mother-infant pairs) and to its changes during the first four months of lactation. Table 4 shows these data recalculated in accordance with the above.

PORTER (1978) published data of the composition of human and cow's milk. Table 5 gives values obtained by recalculating these data.

The data mentioned before unanimously verify the existence of the Equivalency Law, but are not sufficient to answer all questions. It is not evident from the data of BUTTE and co-workers (1987), whether there is any significant change in the metal composition of human milk from the range of alkali excess to the steadily increasing deficiency. This question could be answered either by re-elaboration of the original, individual data, taking into account the Equivalency Law or by new measurements. An answer to this question may be of particular significance for producing baby food of optimal composition. In 1989 a dairy product for babies (namely Drikkeklar Allomin, Beauvais Industri A/S, Tåstrup, Sweden) contained the following metals [mg l^{-1}]: Na: 160, K: 585, Ca: 470, Mg: 50, Fe: 7, Zn: 4, Cu: 0.4. Relative deviation from the Equivalency Law is calculated 12.2% (A–). Investigations related to the Equivalency Law may lead to significant practical results e.g. in food science, stock-raising, plant cultivation and in biotechnology as well. It would be feasible to carry out research related to the Equivalency Law for determining concentration of further metals (e.g. Li, Co, Cr, etc., and Al, Cd, Pb, etc.), micro or toxic metals, present in low concentration. The metals mentioned could influence, however, the sum of equivalents only at a small extent. The equivalents of metals with varying valences, e.g. Fe, Mn, etc., should be calculated by taking into consideration a uniform degree of oxidation; e.g. the average of the most common degrees of oxidation in biological systems. Thus, iron and cobalt could be calculated with 2.5+ and manganese with 3+ charge.

2. Theoretical conclusions

A long list of literature data could be enumerated here to verify the existence of the Equivalency Law, but it can be proved theoretically as well. As it is well known, in the aqueous electrolytes of living organisms metals change ligands as positive ions. As strict chemical stoichiometrical rule of these cation exchange processes is that they proceed with the exchange of an equivalent amount of positively charged counter-ions. The various polyvalent metals can hardly act as counter-ions of each other, since they are present as free ions in the electrolytes of organisms only in low concentration and form far more stable compounds (complexes) with various proteins and simple acids. Apart from a few exceptions, the different polyvalent metals cannot act as counter-ions of each other because they cannot substitute each other at the specific bonding sites of the individual protein molecules (enzymes, transport proteins, etc.). Ammonium ions cannot act as counter-ions of polyvalent metals either, since they are present in the electrolytes of living organisms only in rather low concentration. Hydrogen ions (or more precisely, H_3O^+ ions) can also be counter-ions of polyvalent metals only in a very low ratio, as the pH value in living organisms is nearly neutral, thus, the concentration of hydrogen ions is very low; it is in the range of 10^{-7} mol l^{-1}. Contrarily, the total concentration of alkali metals (mainly potassium and sodium ions) is of 0.1 mol l^{-1} order of magnitude. Thus, in ligand exchange processes of polyvalent metal ions, primarily alkali metal ions can act as counter-ions. Life on Earth is uniformly based on proteins. Chemical and physical properties of proteins and other macromolecules allow vital processes in the electrolytes of organisms only at a very low concentration range. Economical utilization of energy and food is a common characteristic of living creatures. It does not allow any surplus of metal ions. Finally, it is commonplace that every living organisms are related genetically. Consequently, it may be supposed that the Equivalency Law, referring to the alkali and polyvalent metals, must exist in the metal metabolism processes of various living creatures on Earth.

It should be mentioned, that the metal content of oceanic and sea-water is significantly shifted from the Equivalency Law towards alkali metal excess ($\Delta \approx 57\%$). During the evolution, after the appearance of living creatures with calcareous skeleton, limestone layers constituting today the enormous calcareous mountain ranges of continents have accumulated in the depths of oceans and seas. Thus, it may be assumed that many million years ago the water of primordial oceans and seas contained more calcium and more magnesium, iron, zinc, etc. than recently. This greatly depended on the carbon dioxide content of the primordial atmosphere. In the course of time, the amount of calcium, magnesium, etc. decreased in water. At the same time, due to the higher solubility of alkali metal compounds, the amount of these did not change substantially. Consequently, millions of years ago the metal content of the water of

primordial oceans and seas must have been closer to the EQ=1 value, as compared to present-day metal content, which may have played an important role in the course of biological evolution.

References

BUTTE, N. F., GARZA, C., O'BRIAN SMITH, E., WILLS, C. & NICHOLS, B. L. (1987): Macro- and trace-mineral intakes of exclusively breast-fed infants. *Am. J. Clin. Nutr.*, *45*, 42–48.

PORTER, J. W. G. (1978): Milk as a source of lactose, vitamins and minerals. *Proc. Nutr. Soc.*, *37*, 225–230.

RDA (1989): Recommended Dietary Allowances 10th ed., National Academy Press, NW, Washington DC.

Contact: sandor.zoltan@ttk.mta.hu

The institute's new name and address:
Institute of Materials and Environmental Chemistry
Research Centre for Natural Sciences
Hungarian Academy of Sciences
H-1117 Budapest, Magyar tudosok korutja 2. Hungary

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Trace Elements in Medicine and Biology 19 (2005) 171–181
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

EPIDEMIOLOGY

Macronutrient, mineral and trace element composition of breast milk from Japanese women

Namiko Yamawaki, Mio Yamada, Takahiro Kan-no, Tadashi Kojima*, Tetsuo Kaneko, Akie Yonekubo

Nutrition Research Institute, Meiji Dairies Corporation, 540 Naruda, Odawara, Kanagawa 250-0862, Japan

Received 3 December 2004; accepted 4 May 2005

Abstract

The aim of the study was to determine the concentrations of macronutrients and the mineral and trace element composition in maternal milk of Japanese women. We collected human milk samples from mothers living throughout Japan from December 1998 to September 1999, and defined as group A the 1197 samples among them that met the following conditions: breast milk of mothers who were under 40 years old, not in the habit of smoking and/or using vitamin supplements, and whose babies showed no symptoms of atopy and whose birth weights were 2.5 kg or more. We then analyzed their contents individually. We also analyzed the amino acid and free amino acid composition of the breast milk of pooled samples from various lactation stages.

Large differences were found to exist among the contents of individual human milk samples. The mean contents of each component were as follows: energy, 66.3 ± 13.3 kcal/100 mL; solid matter, 12.46 ± 1.56 g/100 mL; ash, 0.19 ± 0.06 g/100 mL; total nitrogen, 0.19 ± 0.04 g/100 mL; lipids, 3.46 ± 1.49 g/100 mL; carbohydrates, 7.58 ± 0.77 g/100 mL; lactose, 6.44 ± 0.49 g/100 mL; pH, 6.5 ± 0.3; osmotic pressure, 299 ± 14 mOsm/kg $\cdot H_2O$; chloride, 35.9 ± 16.2 mg/100 mL; sodium, 13.5 ± 8.7 mg/100 mL; magnesium, 2.7 ± 0.9 mg/100 mL; phosphorus, 15.0 ± 3.8 mg/100 mL; potassium, 47.0 ± 12.1 mg/100 mL; calcium, 25.0 ± 7.1 mg/100 mL; chromium, 5.9 ± 4.7 μg/100 mL; manganese, 1.1 ± 2.3 μg/100 mL; iron, 119 ± 251 μg/100 mL; copper, 35 ± 21 μg/100 mL; zinc, 145 ± 135 μg/100 mL; and selenium, 1.7 ± 0.6 μg/100 mL. The content of each component varied greatly as the duration of lactation increased. In conclusion, it appears to be necessary to evaluate individual differences of human milk in order to perform valid research regarding infant formula.
© 2005 Elsevier GmbH. All rights reserved.

Keywords: Human milk; Macronutrients; Trace elements; Mineral composition; ICP-AES

Introduction

Human milk is considered to be the best source of nutrition for the human infant [1,2], but nursing using milk-based formulas and/or milk substitutes is generally recommended in Japan when it is difficult to bring up an infant on mother's milk. Nutrient levels in formulas for infants are generally modeled on the composition of human milk and one goal of the improvement of infant formulas is to make them even more similar to human milk [3]. However, this goal is far from being attained. One of the reasons for this is that not much data is available about "mother's milk" which can be used for reference.

*Corresponding author. Tel.: +81 465 37 3681; fax: +81 465 36 2776.
E-mail address: TADASHI_KOJIMA@MEIJI-MILK.COM (T. Kojima).

0946-672X/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2005.05.001

Many excellent studies have made measurements of the components of human milk [4–17], but data that could be used for comparisons of samples are limited, since researchers often used pooled milk samples and did not have a well-defined study population. In addition, they did not use a uniform sampling procedure or the same methods of biochemical analysis and/or instrumental analysis with the same degree of precision and accuracy, and in some cases did not store samples properly. Therefore, data that allow reliable comparisons from one study to another are not available.

There have been a few comprehensive studies regarding the composition of breast milk of Japanese women, but all of them were performed before 1990 [18–20]. However, the recent drastic changes in eating habits of Japanese people, such as the increased consumption of processed foods, seem likely to have had significant influence on the composition of breast milk of mothers.

Furthermore, trace elements such as iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) are known to be essential for normal growth and development in infants, but the literature on this subject is very limited [21–24]. Ferris et al. [8] pointed out that data useful for comparison of samples are limited, because researchers have often used pooled samples and did not have a well-defined population. Butte et al. [9] examined the concentration of macronutrients in the milk from five mothers, and reported that variation among women was greater than variation for a given woman over time for all nutrients. They also pointed out that the reliability of group estimates could be improved primarily by increasing the number of subjects.

The present study aimed to clarify the lactational influences on the concentrations of macronutrients – nitrogen, carbohydrates, lactose, lipids, total solid matter, ash, and energy content – and minerals and trace elements – calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorous (P), chloride (Cl), chromium (Cr), manganese (Mn), Fe, Cu, Zn, and Se – in the breast milk on days 1–365 postpartum of approximately 1190 mothers living in various regions in Japan. The composition of total and free amino acids in breast milk was also determined.

Materials and methods

Milk collection

The overall methods have been described previously [25]. Briefly, human milk samples were randomly collected twice, i.e., in summer (from July to September 1998) and winter (from December 1998 to March 1999), from approximately 4000 mothers at various stages of lactation (1–365 days postpartum), living in various regions throughout Japan. Informed consent was obtained orally from the subjects prior to enrollment in this study. Approximately 50 mL of human milk was obtained at an intermediate time during suckling, placed in a nylon bag (Kaneson, Osaka, Japan) and stored in a freezer. At the same time, a personal information sheet (date and time of milk collection, right and/or left breast, mother's age, smoking habits and/or use of vitamin supplements, birth weight of infant, atopic symptoms of her baby, and so on) was filled in by the mother herself. Frozen milk samples and information sheets were collected periodically by employees of Meiji Dairies Corporation, and were transported by delivery service at −20 °C to the Nutrition Research Institute, and stored at −40 °C in a Sanyo Medical Freezer Model U-442 (Osaka, Japan) until preliminary sample preparation.

Preliminary milk preparation

Frozen human milk was thawed using tap water for 30 min and sonicated in ice water with a Branson ultrasonic cleaner model 5510J-MTH (Branson Ultrasonics Co., Dranbury, CT, USA) twice for 15 min. The milk was divided into aliquots in 5 mL polypropylene tubes (Assist Co., Tokyo, Japan), and then stored at −40 °C until analysis.

Classification of human milk

Human milk samples were classified into four groups (A, B, C, and D) according to information on the conditions of the mother (age, smoking habits and use of vitamin supplements) and/or of the infant (birth weight, symptoms of atopy).

Group A: Breast milk of mothers who were under 40 years old, not in the habit of smoking and/or using vitamin supplements, and whose babies showed no symptoms of atopy and had birth weights of 2.5 kg or more.

Group B: The conditions of the mothers' age and the birth weight of the babies were almost the same as those of group A, but the mothers were habitual smokers, had a habit of taking vitamin supplements, and/or had a possibility of having taken some kind of medicine around the time of the sample collection, according to the personal information sheet.

Group C: The conditions of the mothers and babies were almost the same as those of group A, but the age of the mothers was over 40 years.

Group D: The conditions of the mothers were the same as those of group A, but the birth weights of the infants were under 2.5 kg.

Of 4056 samples that were collected in this study, approximately 3170, 630, 30, and 200 samples were

assigned to groups A, B, C, and D, respectively. Group B was considered unsuitable for this study because of the possibility that the mothers had taken some kind of medicine. Furthermore, not a sufficient number of samples could be collected from group C for analysis. For group D, unfortunately no information could be obtained on the gestational periods of the mothers at delivery.

The number of specimens that could precisely be analyzed for macronutrients, mineral and trace element concentration was approximately 1350, and out of these 1197 belonged to group A.

Human milk samples of group A were further classified into subgroups according to several characteristics such as the season (summer or winter) and lactation stage (1–5, 6–10, 11–20, 21–89, 90–179, 180–365 days), and were also divided into 17 subgroups according to the regions where the mothers were living in Japan. All of the mothers in this group were healthy and the average age was 29.12 ± 3.99 years. They gave birth to infants whose birth weight was 3135 ± 346 g.

Analytical determinations

Nitrogen and lipid analysis of human milk

Human milk samples were analyzed for their total nitrogen using the Tecator Kjeltec system (2040 Digester and 1026 Distilling units, Foss Japan, Tokyo, Japan) based on the Kjeldahl method. Protein contents were calculated from total nitrogen $\times$ 6.38 [26]. Lipid contents were analyzed by the Röse-Gottlieb method.

Total solid matter and ash

Total solids in whole milk were measured after drying 1 mL aliquots of thoroughly mixed samples at 100 ± 2 °C for 3 h in porcelain crucibles until they reached constant weight. Ash was further incinerated at 450 °C for 12 h for determination of ash content. Results were expressed in mg/100 mL.

Carbohydrate and lactose content

Carbohydrates were calculated as: Carbohydrates = total solids−proteins−lipids−ash. Lactose content was analyzed using a Bio-Flow®-4 (Oji Scientific Instruments, Hyogo, Japan) based on amperometric-enzymatic methods.

Energy content

Gross total energy content was calculated as: Energy = proteins $\times$ 4 + fat $\times$ 9 + carbohydrates $\times$ 4 according to the Atwater general factor system.

Minerals and trace elements

Inductively coupled plasma atomic emission spectrometry (ICP-AES) measurements were done using a sequential plasma spectrometer ICPS-7500 system (Shimadzu Corporation, Kyoto, Japan) for simultaneous determination of multielements in breast milk, namely Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, and Se. For preparation for the analysis, except for Se, 10 mL aliquots of the whole milk samples were dried at 450 °C for 2 h in ceramic evaporating dishes, and then dissolved in 10 mL of distilled deionized water supplemented with 2 mL of 6 mol/L HCl (solution A). Samples for Se analysis were mineralized by heating in a microwave oven using nitric acid and peroxide. In order to determine the Fe, Cu, Zn, and Mn contents of the sample, 1 mL of solution A was transferred into a 10 mL volumetric flask and the flask was filled with Milli-Q water (solution B). To determine the Na, K, Ca, Mg, and P contents of the sample, 10 mL of solution B were transferred into a 100 mL volumetric flask and the flask was filled with Milli-Q water.

The plasma source used for spectrophotometry was 99.998% argon, and the wavelengths used for analysis of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, and Se were 589.592, 279.553, 178.287, 766.491, 422.673, 267.716, 257.610, 259.940, 324.754, 206.191, and 196.026 nm, respectively. The calibration was performed using a blank and two standard concentrations for each element measured. The optimal operation conditions for ICP-AES analysis of minerals/trace elements other than Se were as follows: power, 1.2 kW; carrier gas flow rate, 0.7 L/min; plasma gas flow rate, 1.2 L/min; cooling gas flow rate, 14.0 L/min. On the other hand, the optimal operation conditions for ICP-AES analysis of Se were as follows: power, 1.0 kW; carrier gas flow rate, 0.6 L/min; plasma gas flow rate, 0.6 L/min; cooling gas flow rate, 8.0 L/min.

Cl was measured with a coulorimetric titration method using a chloride counter (CL-6M, Hiranuma Sangyo Co., Ltd., Japan).

Total amino acids and free amino acids

For analysis of total and free amino acids, pooled samples of human milk were used. One milliliter aliquots of five or 10 human milk samples from a given lactation stage, region, or season were mixed together well. For the lactation stages of the 21st–89th and 90th–179th day, one pooled sample was prepared for every region except the Tokai area, where five pooled samples were prepared. For each lactation stage of days 0–5, 6–10, 11–20, and 180–365, separate pooled samples were prepared for the Kanto area (the area surrounding Tokyo), the Kanagawa prefecture area, the Tokyo metropolitan area, and the 23 wards of Tokyo because adequate sample numbers were available. The total number of pooled samples prepared for the amino acid analysis was 119.

For the analysis of total amino acids, 1.0 mL of pooled milk and 1.0 mL of HCl were mixed well and

allowed to stand for 22 h at 110 °C under nitrogen. After evaporation to remove HCl, 1 mL of 0.02 mol/L HCl was added to these samples, which were then filtered through a 0.45-μm pore size filter. A 0.5 mL aliquot was subjected to automated amino acid analysis using a JLC-500V amino acid analyzer (JEOL Ltd., Tokyo, Japan). Tryptophan, however, was assayed separately by the modified fluorometric method of Nielsen and Hurrell [27]. For the analysis of free amino acids, after deproteinization with 2% sulfosalicylic acid and centrifugation, the samples were analyzed using an amino acid analyzer (L-8500A amino acid analyzer, Hitachi, Ltd. Tokyo, Japan).

SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a Mini-PROTEAN® electrophoresis cell and a gradient gel (Ready Gel J 10–20%) (Bio-Rad Laboratories, USA). The pooled samples were prepared for the analysis of total amino acid composition according to the method of Laemmli. Molecular weight calibration was achieved with an LMW Marker kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). The protein concentration of each sample applied to the gel was 1 mg/mL. The gel was stained with Coomassie blue R-250, and images of bands on the gel were captured and analyzed using the KODAK EDAS 120 system (Eastman Kodak, NY, USA).

Chemicals and reagents

All reagents were of HPLC grade and purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan).

Statistical analysis

Values are expressed as means ± SD. Effects of the different stages of lactation, season, and living district on the concentrations were tested by the Kruskal–Wallis test followed by the Mann–Whitney two-sample test.

Differences between means were considered significant at $p<0.05$. All statistical analyses were conducted using a statistical computer program for Windows (Stat View, Abacus Concepts, Inc., Berkeley, CA, USA).

Ethical considerations

This study was approved by the Research and Ethics Committee of the Nutrition Research Institute of Meiji Dairies Corporation.

Results

The average macronutrient, mineral and trace element contents of human milk samples are presented in Table 1.

Although a large number of samples were obtained for Group A (1197 samples), and the content of each sample was analyzed individually, the number of samples analyzed for each component was somewhat smaller due to some problems in the analytical procedures, e.g., lack of sample volume.

Because the milk samples were collected in two different periods, namely in summer and winter, we compared the data between them. Compared with solid matter, ash, total nitrogen, and carbohydrates, the lipids and mineral/trace element contents had large standard deviations. Neither significant seasonal nor regional variation was detected in the average level of any component; thus, we next focused on possible lactation period-dependent differences.

The concentration of each nutritional ingredient according to the lactation stage is shown in Table 2. Although there were large variations in the sample numbers obtained at different lactation stages, the size of the standard deviation of the levels of the ingredients was almost the same at all stages.

The lipid content showed a time-dependent increase, being higher in the period of days 11–89 than in colostrum or mature milk. On the other hand, the protein nitrogen concentration decreased significantly during the year of lactation at different rates depending on the stage of lactation.

The concentrations of total and free amino acids in human breast milk according to the lactation stage are shown in Tables 3 and 4, respectively. Although almost all of the individual amino acid constituents of total amino acids were decreased as the lactation stage advanced, the constituent amino acids of free amino acids could be roughly classified into three groups, namely, (1) amino acids whose level increased as the lactation stage increased: glutamic acid, glycine, and alanine, (2) amino acids whose level decreased as the lactation stage increased: phosphoserine, taurine, proline, isoleucine, leucine, tyrosine, ornithine, lysine, and arginine, (3) amino acids whose level remained almost constant as the lactation stage increased: aspartic acid, threonine, methionine, and histidine.

Fig. 1 shows representative results of the SDS-PAGE analysis of the pooled samples. Comparison with the protein bands of molecular weight markers revealed that five proteins were detected in each pooled sample, i.e., lactoferrin, albumin, β-casein, κ-casein, and α-lactalbumin. The level of lactoferrin decreased gradually as the stage of lactation increased, as indicated by the relative intensity of each protein spot on the gel (Fig. 2).

Table 1. Composition of milk samples[a]

	Average ± SD (*n*)	Time of collection	
		Summer (*n*)	Winter (*n*)
Energy (kcal/100 mL)	66.3 ± 13.3 (1180)	65.6 ± 13.2 (587)	66.9 ± 13.3 (593)
Solid matter (g/100 mL)	12.46 ± 1.56 (1180)	12.41 ± 1.59 (587)	12.50 ± 1.53 (593)
Ash (g/100 mL)	0.19 ± 0.06 (1180)	0.18 ± 0.05 (587)	0.19 ± 0.06 (593)
Total N (g/100 mL)	0.19 ± 0.04 (1180)	0.19 ± 0.04 (587)	0.19 ± 0.05 (593)
Lipids (g/100 mL)	3.46 ± 1.49 (1180)	3.39 ± 1.46 (587)	3.53 ± 1.51 (593)
Carbohydrates (g/100 mL)	7.58 ± 0.77 (1180)	7.62 ± 0.80 (587)	7.55 ± 0.75 (593)
Lactose (g/100 mL)	6.44 ± 0.49 (1172)	6.45 ± 0.49 (579)	6.43 ± 0.48 (593)
pH	6.5 ± 0.3 (1180)	6.5 ± 0.2 (587)	6.5 ± 0.3 (593)
Osmotic pressure (mOsm/kg · H_2O)	299 ± 14 (1179)	301 ± 12 (587)	298 ± 16 (592)
Cl (mg/100 mL)	35.9 ± 16.2 (1180)	38.7 ± 18.1 (587)	33.1 ± 13.5 (593)
Na (mg/100 mL)	13.5 ± 8.7 (1160)	13.8 ± 9.6 (567)	13.2 ± 7.7 (593)
Mg (mg/100 mL)	2.7 ± 0.9 (1170)	2.6 ± 0.9 (577)	2.7 ± 0.9 (593)
P (mg/100 mL)	15.0 ± 3.8 (1170)	14.6 ± 3.4 (577)	15.3 ± 4.1 (593)
K (mg/100 mL)	47.0 ± 12.1 (1167)	45.5 ± 11.9 (574)	48.5 ± 12.2 (593)
Ca (mg/100 mL)	25.0 ± 7.1 (1170)	23.7 ± 6.6 (577)	26.2 ± 7.4 (593)
Cr (mg/100 mL)	5.9 ± 4.7 (1166)	6.7 ± 3.9 (579)	5.1 ± 5.2 (587)
Mn (μg/100 mL)	1.1 ± 2.3 (1167)	0.9 ± 1.6 (580)	1.2 ± 2.9 (587)
Fe (μg/100 mL)	119 ± 251 (1155)	108 ± 252 (579)	129 ± 249 (576)
Cu (μg/100 mL)	35 ± 21 (1169)	36 ± 10 (582)	34 ± 29 (587)
Zn (μg/100 mL)	145 ± 135 (1165)	132 ± 127 (582)	159 ± 142 (583)
Se (μg/100 mL)	1.7 ± 0.6 (303)	1.8 ± 0.6 (169)	1.7 ± 0.7 (134)

[a]Average ± SD (*n*).

On the other hand, the concentration of carbohydrates was almost the same throughout lactation except in the sample from days 1–5, but the lactose content increased during the course of lactation.

Concerning minerals and trace elements, the Na and K concentration decreased significantly over time. The Mg, P, Ca, and Cl concentrations showed significant lactation-stage-specific differences, but the standard deviation was large. The Cr concentration was higher during days 21–89 and days 90–180, but the standard deviation was also very large. On the other hand, the concentrations of Mn and Fe remained almost constant during all lactation stages. The Cu concentration was also almost the same throughout lactation except in the samples from days 181–365, whereas the Zn concentration decreased with increasing time of lactation.

We also determined the Se content of human milk obtained at different stages of lactation from the Japanese mothers by ICP-AES analysis. The Se content of human breast milk from Japanese mothers was almost constant at a level of about 2.5 μg/100 mL during days 1–20 and then decreased with increasing time of lactation. Although the Se concentrations in human milk were not affected by the region in Japan in which the mothers lived ($p = 0.506$), the Se concentration of mothers who lived in eastern Japan was significantly higher than that of mothers in western Japan ($p = 0.0213$, data not shown).

Discussion

The purpose of the present analysis was to quantify parameters of human milk energy, nitrogen, lipids, carbohydrates, lactose, mineral and trace element concentration among presumably well-nourished Japanese women. Our results were generally in agreement with the results of investigations of Japanese mother's milk reported in the past [18–20]. However, it became clear that there is a large standard variation in the composition of Japanese mother's milk. Yamamoto et al. [18] reported differences in crude protein, ash, total solids, fat, lactose and phosphorus contents due to regional differences, but we could not find such region-dependent differences because of a wide range of standard variation.

We also found that the free amino acid composition of human milk varied according to the stage of lactation, and the pattern of this variation differed depending on the amino acid. We did not determine the physiological meaning of these variations of amino acid levels, but the SDS-PAGE analysis of human breast milk showed that protein components such as lactoferrin varied according to the lactation stage; thus, we suppose that this variation of protein composition must be related to the free amino acid composition.

We used the ICP-AES technique for assessing various trace elements simultaneously. In agreement with many

Table 2. Composition of milk samples*

	Day					
	1–5	6–10	11–20	21–89	90–180	181–365
Energy (kcal/100 mL)	60.0±9.5 (21) a	63.0±10.3 (39) a	68.5±15.0 (40) b	69.1±12.5 (557) b	63.6±13.8 (483) a	62.6±11.3 (39) a
Solid matter (g/100 mL)	11.92±1.13 (21) a	12.53±1.30 (39) ab	13.25±1.56 (40) c	12.78±1.48 (557) bc	12.09±1.60 (483) a	11.86±1.41 (39) a
Ash (g/100 mL)	0.27±0.05 (21) a	0.25±0.05 (39) a	0.21±0.04 (40) b	0.20±0.05 (557) c	0.17±0.05 (483) d	0.17±0.03 (39) d
Total N (g/100 mL)	0.29±0.06 (21) a	0.30±0.07 (39) a	0.26±0.03 (40) b	0.20±0.03 (557) c	0.17±0.03 (483) d	0.16±0.02 (39) e
N × 6.38 (g/100 mL)	1.84±0.38 (21) a	1.90±0.48 (39) a	1.66±0.20 (40) b	1.25±0.20 (557) c	1.10±0.18 (483) d	0.99±0.12 (39) e
Lipids (g/100 mL)	2.68±1.00 (21) a	2.77±1.14 (39) a	3.90±1.53 (40) b	3.75±1.42 (557) b	3.20±1.53 (483) a	3.17±1.36 (39) a
Carbohydrates (g/100 mL)	7.13±0.45 (21) a	7.60±0.91 (39) b	7.48±0.61 (40) b	7.58±0.83 (557) b	7.61±0.67 (483) b	7.53±1.15 (39) b
Lactose (g/100 mL)	5.59±0.56 (21) a	5.88±0.40 (39) b	5.92±0.52 (40) b	6.40±0.39 (557) c	6.62±0.46 (475) d	6.46±0.61 (39) d
pH	6.9±0.4 (21) a	6.6±0.2 (39) b	6.5±0.5 (40) bc	6.5±0.2 (556) c	6.5±0.2 (484) b	6.4±0.3 (39) c
Osmotic pressure (mOsm/kg · H_2O)	293±8 (21) a	301±20 (39) b	306±49 (40) b	300±8 (555) b	298±13 (484) b	299±14 (39) b
Cl (mg/100 mL)	34.1±12.5 (21) a	33.8±14.8 (39) a	38.3±27.0 (40) a	33.4±16.3 (556) a	39.3±14.6 (484) b	28.6±14.0 (39) c
Na (mg/100 mL)	32.7±17.0 (21) a	24.1±11.1 (38) b	24.2±10.1 (39) b	13.9±7.2 (541) c	10.7±6.9 (481) d	11.6±6.1 (39) d
Mg (mg/100 mL)	3.2±0.5 (21) a	3.0±0.9 (38) ab	2.9±0.6 (40) b	2.5±0.7 (550) c	2.7±1.1 (481) b	3.3±0.7 (39) a
P (mg/100 mL)	15.9±4.0 (21) a	19.0±6.1 (38) ab	17.6±3.0 (40) ab	15.6±3.4 (550) a	13.8±3.7 (481) c	13.0±2.5 (39) c
K (mg/100 mL)	72.3±12.7 (21) a	70.9±22.8 (38) a	63.9±10.4 (40) b	46.6±8.3 (550) c	43.4±10.3 (478) d	43.2±7.0 (39) d
Ca (mg/100 mL)	29.3±7.2 (21) a	31.0±9.7 (38) a	30.4±4.1 (40) a	25.7±6.3 (550) b	23.0±7.4 (481) c	26.0±5.4 (39) b
Cr (μg/100 mL)	1.7±1.0 (20) a	3.5±5.4 (38) b	4.5±5.3 (40) b	5.0±3.3 (553) c	7.6±5.4 (475) d	2.5±1.7 (39) b
Mn (μg/100 mL)	1.2±0.8 (18) a	1.8±5.3 (38) a	2.5±6.6 (40) a	0.8±2.2 (555) b	1.2±1.1 (476) a	0.9±1.1 (39) c
Fe (μg/100 mL)	110±54 (20) a	96±70 (38) ab	136±83 (39) a	180±327 (542) ab	52±143 (476) c	85±66 (39) b
Cu (μg/100 mL)	37±15 (20) a	48±10 (38) b	46±10 (40) b	34±19 (555) a	36±25 (476) a	16±5 (39) c
Zn (μg/100 mL)	475±248 (20) a	384±139 (38) a	337±89 (40) b	177±108 (551) c	67±80 (476) d	65±43 (39) d
Se (μg/100 mL)	2.5±0.7 (10) a	2.4±0.6 (10) a	2.7±0.8 (10) a	1.8±0.4 (129) b	1.5±0.6 (134) c	1.3±0.4 (10) c

Values with different letters differ significantly: $p<0.05$.
*Average±SD (n).

Table 3. Amino acid composition of pooled milk samples (mg/100 mL)*

	Day					
	1–5	6–10	11–20	21–89	90–180	181–365
Isoleucine	77.4±7.7 (9) a	84.6±17.3 (10) a	70.3±4.2 (10) b	57.9±4.1 (40) c	48.6±3.3 (40) d	47.0±2.4 (10) d
Leucine	169.4±21.8 (9) ab	182.9±50.1 (10) ab	151.5±17.5 (10) b	114.6±9.8 (40) c	96.1±9.6 (40) d	93.0±5.1 (10) d
Lysine	118.2±14.5 (9) a	130.3±39.3 (10) a	104.1±10.3 (10) b	79.3±6.7 (40) c	66.4±3.7 (40) d	64.4±5.0 (10) d
Methionine	27.0±3.3 (9) a	29.0±7.8 (10) a	23.1±2.2 (10) b	17.7±1.6 (40) c	14.6±1.5 (40) d	13.8±0.6 (10) d
Cystine	44.6±7.9 (9) a	49.1±17.8 (10) a	32.2±3.0 (10) b	25.8±2.6 (40) c	21.8±3.3 (40) d	17.9±2.2 (10) e
Phenylalanine	80.8±12.2 (9) a	87.5±31.2 (10) a	62.0±6.1 (10) b	46.9±4.5 (40) c	38.9±3.7 (40) d	38.6±3.5 (10) d
Tyrosine	178.0±35.6 (9) a	211.3±36.0 (10) a	124.4±26.2 (10) b	90.2±16.8 (40) c	62.1±12.3 (40) d	55.4±7.5 (10) d
Threonine	89.9±13.8 (9) a	96.7±36.2 (10) a	67.6±7.3 (10) b	51.5±5.2 (40) c	42.8±2.7 (40) d	41.5±2.5 (10) d
Tryptophan	37.4±7.2 (9) a	37.1±13.5 (10) a	26.6±2.8 (10) b	19.7±1.9 (40) c	16.7±1.5 (40) d	15.5±2.0 (10) e
Valine	96.4±11.2 (9) a	98.8±42.7 (10) a	77.1±5.9 (10) b	61.7±5.9 (40) c	53.1±4.0 (40) d	52.6±3.1 (10) d
Arginine	76.6±11.8 (9) a	81.8±37.1 (10) a	53.0±6.8 (10) b	37.9±4.8 (40) c	32.0±3.0 (40) d	31.2±3.5 (10) d
Histidine	55.6±9.6 (9) a	60.9±17.5 (10) a	44.7±4.4 (10) b	35.7±3.8 (40) c	29.1±3.5 (40) d	28.3±4.2 (10) d
Alanine	73.4±10.8 (9) a	81.0±33.6 (10) a	53.8±6.4 (10) b	42.6±4.8 (40) c	35.9±3.5 (40) d	35.6±3.3 (10) d
Aspartic acid	179.0±23.7 (9) a	193.4±63.6 (10) a	142.1±14.3 (10) b	105.6±9.0 (40) c	87.8±5.2 (40) d	84.2±4.9 (10) d
Glutamic acid	308.1±28.0 (9) a	339.5±72.6 (10) a	256.4±22.0 (10) b	214.6±12.3 (40) c	189.0±12.6 (40) d	182.5±8.2 (10) d
Glycine	50.2±7.5 (9) a	53.8±25.7 (10) a	38.1±4.6 (10) b	27.2±3.3 (40) c	22.8±1.5 (40) d	22.0±2.1 (10) d
Proline	145.1±17.8 (9) ab	155.2±28.5 (10) ab	130.4±11.8 (10) b	103.3±17.8 (40) c	96.3±6.8 (40) d	92.4±3.1 (10) d
Serine	96.3±16.2 (9) a	103.2±36.1 (10) a	69.2±8.2 (10) b	51.7±5.7 (40) c	42.5±3.3 (40) d	41.4±2.8 (10) d
Total	1903.9±240.5 (9) a	2077.1±574.9 (10) a	1526.6±133.1 (10) b	1183.8±91.3 (40) c	996.1±59.8 (40) d	957.5±47.4 (10) d

Values with different letters differ significantly: $p<0.05$.
*Average±SD (number of pooled samples).

Table 4. Free amino acid composition of pooled milk samples (mg/100 mL)*

	Day					
	1–5	6–10	11–20	21–89	90–180	181–365
Phosphoserine	1.83±0.32 (9) a	1.47±0.35 (10) b	0.87±0.17 (10) c	0.66±0.13 (40) d	0.48±0.06 (40) e	0.44±0.05 (10) f
Taurine	7.00±1.02 (9) a	6.95±1.64 (10) a	7.10±0.61 (10) a	6.56±0.85 (40) b	4.83±0.81 (40) c	5.05±0.78 (10) c
Urea	19.23±1.92 (9) a	22.06±3.66 (10) a	25.00±2.53 (10) b	25.31±3.61 (40) b	28.89±1.98 (40) c	27.90±2.26 (10) c
Aspartic acid	0.76±0.27 (9) a	0.85±0.22 (10) a	0.57±0.24 (10) ab	0.65±0.26 (40) ab	0.86±0.25 (40) a	0.92±0.22 (10) a
Threonine	0.90±0.26 (9) a	0.92±0.23 (10) a	0.75±0.21 (10) ab	0.92±0.17 (40) a	1.03±0.14 (40) ac	1.00±0.11 (10) ac
Serine	0.72±0.20 (9) a	1.02±0.50 (10) a	0.83±0.26 (10) a	1.17±0.18 (40) b	1.30±0.17 (40) c	1.22±0.12 (10) bc
Glutamic acid	10.51±3.80 (9) a	15.88±4.90 (10) b	17.03±5.69 (10) b	21.09±3.70 (40) c	21.87±2.04 (40) c	21.18±1.64 (10) c
Proline	0.77±0.49 (9) a	0.78±0.79 (10) a	0.28±0.10 (10) b	0.24±0.25 (40) c	0.21±0.04 (40) c	0.19±0.03 (10) c
Glycine	0.37±0.09 (9) a	0.54±0.27 (10) b	0.56±0.16 (10) b	0.81±0.16 (40) c	0.92±0.10 (40) d	0.91±0.09 (10) d
Alanine	1.30±0.44 (9) a	1.61±0.36 (10) ab	1.70±0.49 (10) bc	1.88±0.25 (40) bc	1.93±0.24 (40) c	1.84±0.14 (10) bc
Citrulline	0.08±0.03 (9) a	0.19±0.33 (10) a	0.07±0.02 (10) a	0.22±0.40 (40) b	0.33±0.05 (40) c	0.25±0.13 (10) bc
α-Amino-*n*-butyric acid	0.05±0.02 (9) a	0.08±0.04 (10) ab	0.08±0.02 (10) b	0.11±0.11 (40) b	0.13±0.03 (40) c	0.22±0.13 (10) c
Valine	0.68±0.43 (9) a	0.70±0.39 (10) ab	0.55±0.11 (10) abc	0.56±0.21 (40) abc	0.50±0.07 (40) abd	0.41±0.06 (10) ae
Cystine	0.57±0.16 (9) a	0.60±0.10 (10) a	0.63±0.05 (10) a	0.77±0.48 (40) b	0.77±0.09 (40) c	0.73±0.12 (10) bc
Methionine	0.18±0.12 (9) a	0.14±0.12 (10) ab	0.08±0.01 (10) b	0.14±0.35 (40) b	0.07±0.02 (40) b	0.08±0.01 (10) b
Isoleucine	0.28±0.19 (9) a	0.26±0.24 (10) ab	0.13±0.04 (10) b	0.16±0.30 (40) c	0.11±0.03 (40) cd	0.09±0.02 (10) d
Leucine	0.76±0.60 (9) a	0.69±0.65 (10) ab	0.38±0.12 (10) b	0.40±0.27 (40) b	0.35±0.06 (40) b	0.29±0.04 (10) bc
Tyrosine	0.42±0.25 (9) a	0.36±0.27 (10) ab	0.23±0.04 (10) b	0.24±0.41 (40) c	0.16±0.05 (40) d	0.13±0.05 (10) d
Phenylalanine	0.34±0.24 (9) a	0.29±0.16 (10) ab	0.22±0.07 (10) ab	0.29±0.36 (40) ab	0.24±0.08 (40) ab	0.19±0.04 (10) bc
Ornithine	0.12±0.09 (9) a	0.09±0.03 (10) a	0.06±0.01 (10) b	0.10±0.31 (40) c	0.04±0.01 (40) d	0.05±0.01 (10) cd
Lysine	1.88±0.63 (9) a	0.97±0.73 (10) b	0.48±0.20 (10) c	0.38±0.31 (40) cd	0.31±0.06 (40) d	0.29±0.06 (10) d
Histidine	0.32±0.09 (9) a	0.42±0.20 (10) ab	0.45±0.19 (10) bc	0.41±0.32 (40) ab	0.32±0.08 (40) ad	0.27±0.02 (10) ae
Arginine	0.86±0.28 (9) a	0.54±0.33 (10) b	0.25±0.09 (10) c	0.21±0.38 (40) d	0.13±0.04 (40) e	0.12±0.02 (10) e

Values with different letters differ significantly: $p<0.05$.
*Average ±SD (number of pooled samples).

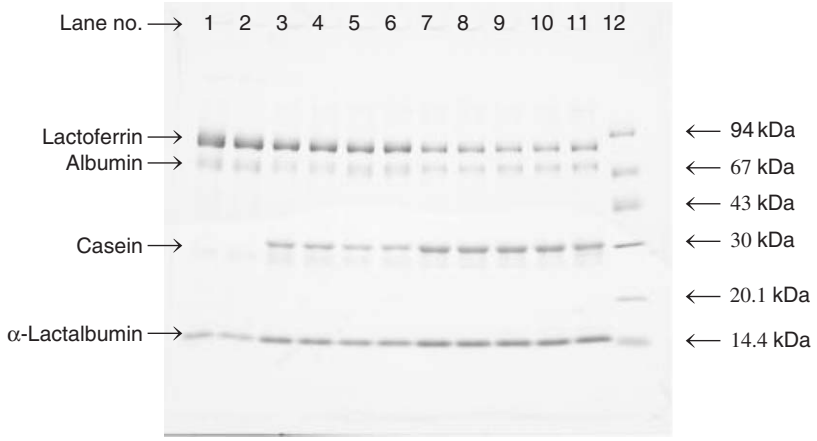

Fig. 1. SDS-PAGE profile of pooled samples of human milk from different lactation stages. Lanes 1 and 2, days 1–5; lanes 3 and 4, days 6–10, lanes 5 and 6, days 11–20, lanes 7 and 8, days 21–89; lanes 9 and 10, days 90–180; lane 11, days 181–365; lane 12, molecular weight markers. Experiments were repeated 17 times and this figure is representative of the typical experimental outcome. Each protein was identified from its molecular weight.

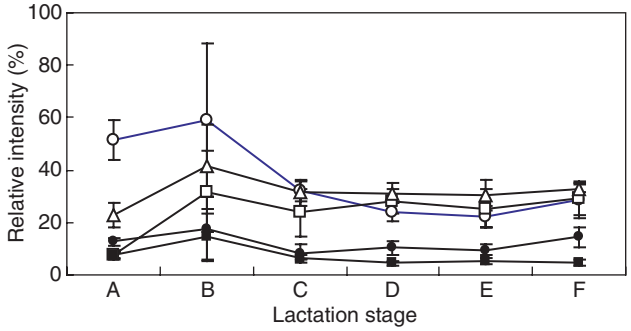

Fig. 2. The values represent the densitometric quantification of each band expressed as a percentage of total intensity. The bar graph shows the densitometric analysis of the gels. Bars represent means ± SD ($n = 17$). Each protein was identified from its molecular weight: ○, lactoferrin; ●, albumin; □, β-casein; ■, κ-casein; △, α-lactalbumin. Lactation stages were as follows: A, days 1–5; B, days 6–10; C, days 11–20; D, days 21–89; E, days 90–180; F, days 181–365.

studies that made measurements of mineral and trace element components in human milk [28–32], our results indicated that the mean concentrations of various minerals and trace elements, including Cr, Mn, Fe, Cu, Zn, and Se, showed a wide range of standard variation.

Unfortunately, we could not get information about the trace element concentration in the serum of the mothers who participated in our study, but it is well known that the concentration of trace elements in human serum may vary considerably depending on the geographical region or the type of consumed food, and hence it is supposed that the concentration of micronutrients in milk may result from their content in the local food products consumed by the mothers.

It is well known that trace element concentrations of breast milk vary considerably among individuals and decrease as the stage of lactation progresses. Wasowicz et al. [24] reported that Se and Zn concentrations in breast milk decreased rapidly as the stage of lactation progressed. We are convinced from the results of the present study that the Se and Zn content in the breast milk of Japanese mothers is relatively high compared with reported values of milk from other populations, and that the content decreases with increasing time of lactation.

There have been a few reports about Se content in human breast milk [33–36], and at one time Se was considered to be only of toxicological significance. However, since the discovery that it is an essential element for animals, and the discovery that it protects cells against oxidative damage by peroxides, etc., strong evidence has been obtained indicating that it is also essential for humans. Rockens et al. [33] reported almost the same concentration in the breast milk of Belgian mothers as that observed in the present study and pointed out that food consumption habits or other factors regulate the milk Se content.

The Se content of breast milk is known to be affected by maternal dietary Se intake, which depends on the food Se content reflecting geographically different soils [34]. In some countries, the geographic distribution of Se has been elaborated into a "Se map" [37]. In Japan, Mizutani et al. [38] reported a Se map based on analysis of Se in soil from 150 points. Our results showed that the Se concentration of the breast milk of mothers who lived in eastern Japan was significantly higher than that of mothers who lived in western Japan, but we could not demonstrate a correlation between the Se concentration in human milk and the distribution of Se content in

Japan according to the Se map. No satisfactory explanation has been proposed about why the higher concentrations of Se and Zn are observed in particular regions, but we think that there must be a large regional difference of Se content in the soil among the various prefectures in Japan. Furthermore, since the distribution of agricultural products in Japan covers a large area, the Se map may not necessarily be correlated with a mother's resident environment. We believe the concentrations of micronutrients, including Se, in milk depend on many factors, but the maternal dietary intake is the crucial one.

Because we could not acquire information about the milk volume of each sample in our research, the quantity of nutrients which the infants did take in remains unknown. Moreover, neither non-protein nitrogen nor oligosaccharides were examined. We hope to study these questions in the future.

Changes continue to be made to infant formulas, and these changes generally result in products with compositions and functions closer to those of human milk. At present we can only suggest that investigators be aware that the potential for variation in milk composition depending on the lactation period exists.

Conclusion

In summary, our results agree on the whole with previous reports about the composition of human milk, but it became clear that there is a large standard variation in the composition regarding free amino acids and trace elements such as Cr, Mn, Fe, Cu, Zn and Se. We confirmed that the composition of human milk is affected by factors such as stage of lactation, and varies depending on the individual.

Acknowledgements

We gratefully thank all the co-workers who participated actively in this research: Yuka Terasawa, B.A., Haruna Yamamoto, B.A., Kaori Akabane, B.A., Yuki "Gappy" Yamada, B.A., Kaoru Kusakari, B.A., and Yasufumi Fujiwara, M.S., of the Nutrition Research Institute, Meiji Dairies Corporation. Without their skill and devotion, this study could not have been performed.

References

[1] Lo CW. Human milk: nutritional properties. In: Walker WA, Watkins JB, editors. Nutrition in pediatrics. 2nd ed. Hamilton, London: BC Decker Inc.; 1997.

[2] Picciano MF. Nutrient composition of human milk. Pediatr Clin North Am 2001;48:53–67.

[3] Packard VS. Human milk and infant formula. New York, London, Toronto, Sydney, San Francisco: Academic press; 1982.

[4] Jonas A. Human milk at different stages of lactation. In: Freier S, Eidelman AL, editors. Human milk, its biological and social value. Amsterdam, Oxford, Princeton: Excerptamedica; 1980.

[5] Anderson GH, Atkinson SA, Bryan MH. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am J Clin Nutr 1981;34:258–65.

[6] Saint L, Smith M, Hartmann PE. The yield and nutrient content of colostrum and milk of women from giving birth to 1 month post-partum. Br J Nutr 1984;52:87–95.

[7] Neville MC, Keller RP, Seacat J, Casey CE, Allen JC, Archer P. Studies on human lactation. 1. Within-feed and between-breast variation in selected components of human milk. Am J Clin Nutr 1984;40:635–46.

[8] Ferris AM, Dotts MA, Clark RM, Ezrin M, Jensen RG. Macronutrients in human milk at 2, 12, and 16 weeks postpartum. J Am Diet Assoc 1988;88:694–7.

[9] Butte NF, Garza C, Smith EO. Variability of macronutrient concentrations in human milk. Eur J Clin Nutr 1988;42:345–9.

[10] Nagra SA. Longitudinal study in biochemical composition of human milk during first year of lactation. J Trop Pediatr 1989;35:126–8.

[11] Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 months of lactation: the DARLING Study. Am J Clin Nutr 1991;53:457–65.

[12] Allen JC, Keller RP, Archer P, Neville MC. Studies in human lactation: milk composition and daily secretion rates of macronutrients in the first year of lactation. Am J Clin Nutr 1991;54:69–80.

[13] Neville MC, Allen JC, Archer PC, Casey CE, Seacat J, Keller RP, et al. Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. Am J Clin Nutr 1991;54:81–92.

[14] Chulei R, Xiaofang L, Hongsheng M, Xiulan M, Guizheng L, Defrancesco CA, et al. Milk composition in women from five different regions of China: the great diversity of milk fatty acids. J Nutr 1995;125:2993–8.

[15] Paul VK, Singh M, Srivastava LM, Arora NK, Deorari AK. Macronutrient and energy content of breast milk of mothers delivering prematurely. Indian J Pediatr 1997;64:379–82.

[16] Maas YGH, Gerritsen J, Hart AAM, Hadders-Algra M, Ruijter JM, Tamminga P, et al. Development of macronutrient composition of very preterm human milk. Br J Nutr 1998;80:35–40.

[17] Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr 2002;88:29–37.

[18] Yamamoto Y, Yonekubo A, Iida K, Takahashi S, Tsuchiya F. The composition of Japanese human milk. 1 Macro-nutrient and mineral composition. Shoni-Hoken-Kenkyu (in Japanese) 1981;40:468–551.

[19] Idota T, Sakurai T, Ishiyama Y, Muramaki Y, Kubota J, Ii N, et al. The latest survey for the composition of human milk obtained from Japanese mothers. Part 1. The contents of gross components and minerals. Nihon-Shoni-Shokakibyo-Gakkai-Shi (in Japanese) 1991;5: 145–58.

[20] Idota T, Sakurai T, Sugawara M, Ishiyama Y, Murakami Y, Maeda T, et al. The latest survey for the composition of human milk obtained from Japanese mothers. Part 3. Composition of amino acids and related nitrogenous compounds. Nihon-Shoni-Shokakibyo-Gakkai-Shi (in Japanese) 1991;5:209–19.

[21] Picciano MF, Guthrie HA. Copper, iron, and zinc contents of mature human milk. Am J Clin Nutr 1976; 29:242–54.

[22] Lin T, Jong Y, Chiang C, Yang M. Longitudinal changes in Ca, Mg, Fe, Cu, and Zn in breast milk of women in Taiwan over a lactation period of one year. Biol Trace Elem Res 1998;62:31–41.

[23] Friel JK, Andrews WL, Jackson SE, Longerich HP, Mercer C, McDonald A, et al. Elemental composition of human milk from mothers of premature and full-term infants during the first 3 months of lactation. Biol Trace Elem Res 1999;67:225–47.

[24] Wasowicz W, Gromadzinska J, Szram K, Rydzynski K, Cieslak J, Pietrzak Z. Selenium, zinc, and copper concentrations in the blood and milk of lactating women. Biol Trace Elem Res 2001;79:221–33.

[25] Kojima T, Asoh M, Yamawaki N, Kanno T, Hasegawa H, Yonekubo A. Vitamin K concentrations in maternal milk of Japanese women. Acta Paediatr 2004;93:457–63.

[26] de Kanashiro HC, Brown KH, de Romana GL, Lopez T, Black RE. Consumption of food and nutrients by infants in Huascar (Lima), Peru. Am J Clin Nutr 1990;52:995–1004.

[27] Nielsen HK, Hurrell RF. Tryptophan determination of food proteins by HPLC after alkaline hydrolysis. J Sci Food Agric 1985;36:893–907.

[28] Karra MV, Kirksey A, Galal O, Bassily NS, Harrison GG, Jerome NW. Zinc, calcium, and magnesium concentrations in milk from American and Egyptian women throughout the first 6 months of lactation. Am J Clin Nutr 1988;47:642–8.

[29] Kent JC, Arthur PG, Retallack RW, Hartmann PE. Calcium, phosphate and citrate in human milk at initiation of lactation. J Dairy Res 1992;59:161–7.

[30] Balogun FA, Akanle OA, Spyrou NM, Owa JA. A comparative study of elemental composition of human breast milk and infant milk substitutes. Biol Trace Elem Res 1994;43-45:471–9.

[31] Dorea J. Iron and copper in human milk. Nutrition 2000;16:209–20.

[32] Silvestre D, Martinez-Costa C, Lagarda MJ, Brines J, Farre R, Clemente G. Copper, iron, and zinc contents in human milk during the first three months of lactation. A longitudinal study. Biol Trace Elem Res 2001;80:1–11.

[33] Roekens E, Deelstra H, Robberecht H. Trace elements in human milk, selenium: a case study. Sci Total Environ 1985;42:91–108.

[34] Tamari Y, Chayama K, Tsuji H. Longitudinal study on selenium content in human milk particularly during early lactation compared to that in infant formulas and cow's milk in Japan. J Trace Elem Med Biol 1995;9:34–9.

[35] Bratter P, de Bratter VEN, Recknagel S, Brunetto R. Maternal selenium status influences the concentration and binding pattern of zinc in human milk. J Trace Elem Med Biol 1997;11:203–9.

[36] Dorea JG. Selenium and breast-feeding. Br J Nutr 2002 ;88:443–61.

[37] Zachara BA, Pilecki A. Selenium concentration in the milk of breast-feeding mothers and its geographic distribution. Environ Health Perspect 2000;108:1043–6.

[38] Mizutani T, Kanaya K, Osaka T. Map of selenium content in soil in Japan. J Health Sci 2001;47(4): 407–13.