Boron Concentrations in Milk from Mothers of Exclusively Breast-Fed Healthy Full-Term Infants Are Stable during the First Four Months of Lactation1,2

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ABSTRACT Because boron is a bioactive element that satisfies several of the criteria for essentiality in humans, the aim of the present work was to determine the profile of boron metabolism in human milk during the first 4 mo of lactation. The concentration of boron and other minerals was determined in archived milk collected (1980–84) 1 time/mo for 4 mo from lactating mothers of full-term, exclusively breast-fed infants living in Houston, TX. A linear model (treating month as a continuous variable) indicated that B concentrations were stable (P = 0.14) between mo 1 [3.88 ± 0.6 μmol (42 ± 6.5 μg)/L milk] and 4 [3.24 ± 0.6 μmol (35 ± 6.5 μg)/L milk, mean ± SEM]. Mg concentrations increased slightly over time (1.18 ± 0.09 to 1.36 ± 0.09 mmol/L, P < 0.0001), whereas Ca concentrations decreased slightly (7.01 ± 0.29 to 6.88 ± 0.29 mmol/L milk, P < 0.02) and Zn decreased substantially (0.04 ± 0.004 to 0.02 ± 0.004 mmol/L milk, P < 0.0001). Similarities in findings reported here and earlier (from samples collected in St. John’s, Newfoundland) provide further evidence that boron may be metabolically regulated. Future investigations of boron regulatory mechanisms should focus on metabolism of bone as the major storage site of B and renal excretion, the major excretory route for B. J. Nutr. 135: 2383–2386, 2005.

KEY WORDS: • boron • human milk • lactation • calcium • magnesium • zinc

Boron is a low-weight element (atomic weight = 10.81) that is required by all vascular plants (1). It is recognized as a constituent of various biomolecules including a bacterial quorum-sensing signal (2), dimers of rhamnogalacturanan-II in higher plants, and several antibiotics (i.e., tartrolon B) (3). Furthermore, embryological development in fish (4) and frogs (5) does not proceed normally in the absence of extracellular boron. In addition, there is some evidence that dietary boron may be beneficial to humans and/or higher animal models for bone health (6–12), glucose metabolism (13), and immune function (14–16).

Evidence suggests that boron may be under metabolic control. A borate transporter was described recently that appears essential for cellular boron uptake and cell growth in mammalian cells (17). Human blood boron concentration was found to be insensitive to changes in dietary boron intake (18). The concentration of boron in expressed human milk samples also appears stable at least during the first 12 wk of lactation (19) despite the fact that dietary intake of boron typically varies widely with food intake patterns and drinking water sources (20,21).

Because evidence of conservation of boron concentrations in human milk has direct implications for its metabolism including its metabolic regulation, we determined the boron concentrations in milk samples from mothers of exclusively breast-fed infants living in an area not previously studied, i.e., Houston, TX.

SUBJECTS AND METHODS

Subjects. In a study reported earlier (22–24), human milk samples were collected (1980–84) from consenting mothers living in Houston, TX, during the first 4 mo of lactation to determine the macro- and trace-mineral intakes of exclusively breast-fed infants and the milk production, dietary intake, and body composition of the mothers. Residual milk collections from that study were archived and utilized in the current study as described below. The study was longitudinal in design and consisted of stages of screening, postpartum counseling, and observational points at birth, 1, 2, 3, and 4 mo postpartum. Potential subjects were recruited prenatally through the Baylor Milk Bank Program. Participants were required to meet the following selection criteria: healthy, nonsmoking, aged 18–36 yr, no chronic medication regimen, parity of 1 or 2, and the intent to breast-feed exclusively for at least 4 mo. Forty-five women participated with a mean maternal age of 28.0 ± 3.1 yr and a distribution of racial backgrounds (41 Caucasian, 2 Hispanic, 1 Asian, and 1 West Indian).


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Infants were required to be healthy, term, and appropriate size for gestational age. The mean birthweight of the infants (27 boys; 18 girls) was 3.58 ± 0.45 kg (range 2.56–4.57 kg). Within 3 days postpartum, a breast-feeding consultant visited the mother to review basic breast-feeding skills. At ~2 wk postpartum, explicit directions were given on the collection and freezing of human milk.

**Milk collection.** The measurements of milk production were made at 35.4 ± 4.6, 65.8 ± 3.4, 91.3 ± 3.8, and 119.0 ± 5.7 d postpartum (mean ± SD). The composition of the milk samples was considered “mature” because all collections occurred >10 d after birth (25). Mothers were instructed not to alter their usual feeding routine during collection of breast milk for compositional studies. At each feeding throughout a 24-h period, the infant was offered 1 breast and the contents of the contralateral breast were expressed using an Egnell electrical breast pump. Breasts were alternated for feeding and pumping with successive feeds. If necessary, infants were supplemented with human milk that had been collected and frozen in advance.

Milk samples were refrigerated separately in sterile, acid-washed polypropylene bottles for a maximum of 24 h and transported on ice to the laboratory where volumes of each feeding were measured and a 24-h pooled milk sample was composed. The pooled milk samples were stored in acid-washed polypropylene bottles with screw caps (Nalge Nunc International).

**Mineral analyses.** Our method (26) for the simultaneous analyses of low amounts [>1 μg/g (>1 ppm)] of boron and other mineral elements in biological materials was modified significantly (19) to be applicable for human milk samples. Briefly, aliquots of breast milk from individual study subjects were thawed as a single batch and resuspended. Triplicate samples (5 g) were transferred to sample polytetrafluoroethylene tubes that were injected with 16.0 mol high-quality (HQ)4 HNO3/L (“Baker Analyzed” grade [J.T. Baker Chemical]) purified in a subboiling point quartz still), heated to 110°C, and refluxed for 72 h until nearly dry. The acid addition, refluxing, and evaporation steps were repeated once. Subsequently, sample and blank tubes were injected with 30% H2O2 (Ashland Chemical) and re-refluxed. Triplicate samples (5 g) were transferred to sample polypropylene tubes (Becton Dickinson Labware) and diluted to 5 mL with demineralized, deionized water (~18 MΩ·cm; Millipore System, Super-Q, Millipore).

Inductively coupled argon plasma optical emission spectroscopy (Optima 3300DV; PerkinElmer Instruments) was used to determine sample concentrations of boron [detection wavelength (DW) = 208.959 nm; working detection limit (WDL) = 8.0 μg B/L], calcium (DW = 405.79 nm; WDL = 50 μg Ca/L), magnesium (DW = 279.277 nm; WDL = 150 μg Mg/L), and zinc (DW = 213.856 nm; WDL = 1.0 μg Zn/L).

To monitor batch-to-batch variability, duplicate samples of each of 2 different standards were analyzed for the relevant elements in each batch. No boron standards of appropriate matrix are available. Accordingly, for the bovine liver standard [1577b; U.S. Department of Commerce, National Institute of Standards and Technology (NIST)] the average respective analyzed (mean ± SD) and certified (mean with range in parentheses) mineral values (μg/g) for boron were 0.6 ± 0.1; calcium: 115 ± 3, 116 (112–120); magnesium: 581 ± 10, 601 (573–629); and zinc: 119 ± 5, 127 (111–143). For the wheat flour standard (1567a; NIST) the average respective analyzed and certified mineral values (μg/g) for boron were 0.41 ± 0.02; calcium: 190 ± 3, 191 (195–187); magnesium: 385 ± 8, 400 (380–420); and zinc: 11.3 ± 0.2, 11.6 (11.2–12.0).

**Validation of sample integrity.** At the time of sample collection, practical methodology for the detection of low concentrations of boron in biological tissues was not available. Thus, considerable time elapsed between sample collection (1983) and boron analysis (2001–2004). Furthermore, in April 1997, all samples were unintentionally thawed and maintained at room temperature for ~10 d (due to local catastrophic overland flooding and subsequent electrical power outage) before being refrozen. Thus, milk samples were examined for evidence of contamination and/or dilution or evaporation by making statistical comparisons between the original average mineral values [as determined by one of the investigators (N.F.B.) by atomic absorption spectroscopy after a dry-ashing procedure (22)] with the current average mineral values. Results indicated that after an interval of ~18 y, average magnesium values of the archived milk samples had not changed (P = 0.64). Values were slightly lower for calcium (274 vs. 294 mg/L; P < 0.006), and were slightly higher for zinc (1.78 vs. 1.40 mg/L; P < 0.0001). It is likely that these changes reflect slight differences in analytical techniques rather than changes in sample integrity (i.e., evaporation).

**Statistical analysis.** Results are shown as means ± SEM. Box-whisker plots generated for element values revealed that no data point qualified for elimination as an outlier. The data were analyzed by using a mixed linear model in which time (month of lactation) was treated as a continuous variable using SAS version 8.02 (SAS Institute) (27). This model took into consideration repeated measures on individuals.

**Ethical considerations.** The original study was designed to examine the measurement of milk volume and milk composition and was approved by the Institutional Human Experimentation Committee (Houston, TX). The measurements of milk production were made at 35.4 ± 4.6, 65.8 ± 3.4, 91.3 ± 3.8, and 119.0 ± 5.7 d postpartum (mean ± SD). The composition of the milk samples was considered “mature” because all collections occurred >10 d after birth (25). Mothers were instructed not to alter their usual feeding routine during collection of breast milk for compositional studies.

**RESULTS**

**Boron.** The concentration of boron in milk of mothers of healthy full-term infants did not change significantly during the first 4 mo of lactation [mean at mo 1: 3.88 ± 0.6 μmol (42 ± 6.5 μg/L); mean at mo 4: 3.24 ± 0.6 μmol (35 ± 6.5 μg/L)], but it tended to decrease slightly (Fig. 1; P = 0.14; slope of 1.8 ± 1.2 U decline/mo).

For all mothers in this study, the boron concentration in any milk sample for any measured time interval did not exceed 88 μg (8.14 μmol)/L during the first 4 mo of lactation. For all mothers, only 2 values were at or below the working detection limit for boron [8 μg (0.74 μmol)/L]. At the 75th percentile, all values were <55.0 μg (5.09 μmol)/L.

**Calcium, magnesium, and zinc.** Milk calcium concentrations decreased slightly but significantly (slope = −4.5 ± 1.9 mg/mo, P < 0.02) during the first 4 mo of lactation [mean at

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4 Abbreviations used: DW, detection wavelength; HQ, high-quality; WDL, working detection limit.

**FIGURE 1** Mean boron concentrations estimated in breast milk from mothers of full term infants (n = 36) in Houston, TX. To convert μg boron/L milk to μmol boron/L milk, divide by 10.81.
mo 1: 281 ± 11.5 mg (7.01 ± 0.29 mmol)/L; mean at mo 4: 268 ± 11.5 mg (6.69 ± 0.29 mmol)/L]. There was a wide range of individual milk calcium concentrations at each measured time point [i.e., at 1 mo postpartum: highest, 358 mg (8.93 mmol) Ca/L; lowest, 214 mg (5.34 mmol) Ca/L]. Milk zinc concentrations decreased very substantially to less than half (slope = −0.43 ± 0.04 mg/mo, P < 0.0001) the beginning mean value during the first 4 mo of lactation [mean at mo 1: 2.3 ± 0.26 mg (0.035 ± 0.004 mmol)/L]; mean at mo 4: 1.0 ± 0.26 mg (0.015 ± 0.004 mmol)/L]. Individual milk zinc concentrations at a given measured time point varied considerably [i.e., at 1 mo postpartum: highest, 4.29 mg (0.07 mmol)/L; lowest, 0.83 mg (0.01 mmol)/L]. Magnesium concentrations increased slightly (slope = 1.5 ± 0.3 mg/mo, P < 0.0001) during the same time period [mean at mo 1: 28.6 mg ± 2.2 (1.18 ± 0.09 mmol)/L; mean at mo 4: 33.0 mg ± 2.2 (1.36 ± 0.09 mmol)/L]. Similar to calcium and zinc values, there was a relatively wide range of individual milk magnesium concentrations at a given measured time point [i.e., at 1 mo postpartum: highest, 36.1 mg (1.49 mmol)/L; lowest, 19.5 mg (0.80 mmol)/L].

**DISCUSSION**

We demonstrated earlier (19) that concentrations of boron in human milk from women in St. John’s, Newfoundland, did not vary widely and were stable over time. The data for the present study were collected from a separate population, Houston, TX, and agree with those of our previous study. For example, the highest concentration of boron in any milk sample during the first 4 mo of lactation [88 μg (8.14 μmol)/L] was similar to the highest value determined in our previous study [100 μg (9.25 μmol)/L]. Also, the mean concentration [39 μg (3.61 μmol)/L] of boron in milk samples determined in this study is similar to the mean concentration reported earlier [29 μg (2.68 μmol)/L]. Furthermore, the concentration of boron milk in the 2 populations did not change over time. The consistent range in milk boron concentration coupled with a distinct pattern of no change over time (i.e., stable) in 2 separate populations suggest that lactating mothers who self-select diets may maintain homeostatic control over milk boron concentrations.

The mean boron intake of lactating mothers is estimated to be 1.39 ± 0.16 mg/d (mean ± SE) and the 10th and 90th percentiles of boron intake are 0.67 ± 0.13 and 2.26 ± 0.31 mg/d, respectively (28). This range of dietary boron intakes arises from a variety of factors. For example, compared with animal-based food products, plant-based products are much richer sources of dietary boron (20). Furthermore, the concentration of boron in plant materials varies with soil type, length of exposure, rate of transpiration, and different agricultural practices (29). Finally, most plant species within the subclass Dicotyledoneae, which includes fruits, nuts, vegetables, tubers, and legumes, have much higher concentrations of boron than do species in the subclass Monocotyledoneae, which includes the grasses (e.g., corn, rice, and wheat) (20). For this reason, experimental human diets that provide only 0.36 mg B/2000 kcal (8.37 MJ) (and are otherwise nutritionally replete with minor supplements) are easily prepared by avoiding nuts, fruits, and most vegetables (18). In our studies, mean concentrations of boron in milk were remarkably similar between test populations despite probable differences in dietary boron intake.

Two other lines of evidence also support the possibility of boron homeostasis. First, boron is present in tissues of different animals at comparable concentrations. Despite the fact that boron, at least in its inorganic form, is nearly quantitatively absorbed and rapidly excreted (18), tissue boron concentrations are very similar across species (30,31). Similar concentrations of boron were reported in the plasma of humans [34–95 μg (3.14–8.79 μmol)/L], rats [55 μg (5.09 μmol)/L] and chicks [77–152 μg (7.12–14.1 μmol)/L]; in the livers (dry weight) of humans, 2.25 mg (0.21 mmol)/kg; rats, 0.51–1.17 mg (0.05–0.11 mmol)/kg; and chicks, 1.01–1.35 mg (0.09–0.12 mmol)/kg; in the brains of humans, 0.87 mg (0.08 mmol)/kg; rats, 0.64 mg (0.06 mmol)/kg; and chicks, 0.74–0.75 mg (0.07–0.07 mmol)/kg. The apparent lack of boron accumulation in response to increases in dietary boron supports the possibility of boron homeostasis.

The second line of evidence supporting boron homeostasis is that plasma boron concentrations are resistant to change over a range of physiological intakes. For example, plasma boron concentrations in postmenopausal women fed a diet low in boron content increased only 0.5-fold (8.79 ± 5.18 compared with 5.92 ± 4.16 μmol B/L) when the subjects received a 13-fold increase in boron as a dietary supplement (3.23 compared with 0.23 mg B/d) (18). Other investigators also reported a remarkably narrow range of boron concentrations in whole blood from subjects with unknown dietary histories (32).

Possible mechanisms for homeostatic control of boron in human milk most likely involve the active transport of boron across physiological membranes. In studies with yearling beef heifers, the percentage of filtered boron that was reabsorbed decreased as the amount of filtered boron increased (33). In another animal study, female rats consuming water very high in boron (100 mg/L) for 21 d had increased plasma boron concentrations without accumulating boron in liver and brain (34). Our group found boron to be taken up by RAW264.7 and HL60 cells against a boron concentration gradient (35). This may be explained on the basis of a boron transporter, such as that recently described (17).

As before, our subject pool for milk analysis was apparently not different from the general population that gives birth to full-term healthy infants; the concentrations of minerals in the mature milk samples analyzed in this study were within the ranges of those commonly reported: calcium, 250–300 mg/L throughout lactation (36); zinc, 4–5 mg/L during early lactation and −0.5 mg/L after 6 mo of lactation (36); magnesium, 28 mg/L during mo 1 of lactation and 34 mg/L after 6 mo of lactation (37).

The patterns of change in milk concentrations of calcium, magnesium, and zinc over time were similar to those reported elsewhere. For example, findings from several longitudinal studies indicate that the calcium concentrations in breast milk are stable (36,38) or exhibit a transient decrease (39) during the first 3 mo of lactation. Similarly, it has been reported repeatedly that concentrations of magnesium in breast milk remain constant (36,40,41) or increase over that time frame (42,43). The zinc concentration of breast milk falls precipitously during the lactation period (36). For each of these elements, the range in their concentrations in milk across individuals per time period was relatively wide. This characteristic suggests a relatively wide diversity in individual metabolic “set points” for these metabolically controlled elements. The data reported here and earlier (19) suggest that this is also a characteristic of boron metabolism in milk.

Although further research is warranted to establish boron homeostasis, the current evidence, including the distinct pattern of milk boron concentrations over time, points in that
direction. Further investigations of boron metabolism should focus on metabolism of bone as the major storage site and kidney excretion as the major excretory route.

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LITERATURE CITED