Boron concentrations in milk from mothers of full-term and premature infants

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ABSTRACT
Background: Boron is a bioactive element that satisfies several of the criteria for essentiality in humans.
Objective: The objective was to establish the profile of boron metabolism in human milk.
Design: Lactating mothers of premature (PRT; n = 10, <2000 g birth weight, <37 wk gestation) and full-term (FT; n = 10, >2500 g, 39–41 wk gestation) infants living in St John’s, Canada, collected milk samples once a week for 12 wk. Samples were analyzed for boron, copper, iron, selenium, and zinc by atomic emission or absorption spectrometry after wet-ash digestion with nitric acid and hydrogen peroxide in polytetrafluoroethylene tubes.
Results: A mixed-model analysis of variance indicated that boron concentrations were stable in full-term (30 and 28 μg/L milk; P = 0.5) but not in preterm (37 and 27 μg/L; P = 0.01) milk between weeks 1 and 12, respectively. As expected, there were reductions in the concentrations of copper (FT: 651 to 360 μg/L, P < 0.0001; PRT: 542 to 425, P = 0.051), iron (FT: 355 to 225 μg/L, P = 0.0003; PRT: 406 to 287, P = 0.002), selenium (FT: 26.9 to 18.6 μg/L, P < 0.0001; PRT: 28.7 to 20.4, P < 0.0001), and zinc (FT: 4060 to 1190 μg/L, P < 0.0001; PRT: 5970 to 1270, P < 0.0001) over time.
Conclusions: The stable milk boron concentrations over time suggest that boron may be under homeostatic control. The patterns of change in copper, iron, selenium, and zinc concentrations in milk differ from those of boron. Further research is needed to elucidate the mechanism of milk boron secretion.

KEY WORDS Boron, copper, iron, selenium, zinc, human milk, lactation, prematurity, full-term infants, development

INTRODUCTION
Boron is a bioactive element that is essential for all vascular plants (1) and has been proposed as being essential for animals and humans. American postmenopausal women consume 1.34 ± 0.02 (± SE) mg B/d (1st percentile: 0.31; 99th percentile: 3.34 mg/d) (2). Yet, diets that provide 0.36 mg B/2000 kcal (and are otherwise nutritionally replete with minor supplements) result in a negative boron balance (3). Boron appears to be required for reproduction in lower vertebrates; embryologic development in fish (4) and frogs (5) does not proceed normally in the absence of extracellular boron. Four lines of evidence, derived in large part from animal model research, indicate that dietary boron can have beneficial effects on humans:

In amounts typically found in human and animal diets, boron improved bone health (independent of vitamin D status) by increasing bone development in frogs (6); bone breaking strength in pigs (7), broilers (8), and growing pullets (9); and bone calcium concentrations in chicks (10). Boron concentrates with specific steroid hormones; it increased circulating concentrations of 25-hydroxycholecalciferol in humans (11) and chicks (12) and counteracted the deleterious effects of dietary vitamin D deficiency on body growth in chicks (12) and growth plate morphology in embryonic (13) or hatched (10, 14) chicks. In addition, boron increased the circulating concentrations of 17β-estradiol in humans (15, 16), and, together with injections of 17β-estradiol, increased trabecular bone surfaces in ovariectomized rats (17).

Physiologic amounts of boron apparently reduced the amount of insulin required to maintain plasma glucose in rats (18). Borate or borate analogues can inhibit the in vitro activities of several enzymes in the eicosanoid pathway related to inflammation and immune function (19–22). If boron were to have an essential nutritional role, one might expect to find a significant amount of the element in human milk and its concentration in milk should, as for other essential minerals, follow predictable patterns over time (23). Therefore, we hypothesized that boron is present in human milk and that the boron content in milk decreases with the length of lactation, the pattern established for the essential trace elements copper, iron, selenium, and zinc.

SUBJECTS AND METHODS
Subjects
This study was approved by the Memorial University of Newfoundland and Health Care Corporation Ethics Committees and the Institutional Review Board of the University of North

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3 The US Department or Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.
4 Address reprint requests to CD Hunt, US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034. E-mail: chunt@gfhnrc.ars.usda.gov. Received February 11, 2004. Accepted for publication June 10, 2004.
In a study reported earlier (24), human milk samples were collected from each of 45 consenting mothers during the first 12 wk of lactation to determine the antioxidant properties of human milk over time. Residual milk collections from that study were archived and used in the current study as described below. In the original study, 45 lactating mothers delivered either full-term (FT; 39 ± 1 wk of gestation, 3753 ± 528 g weight at birth, n = 28) or premature (PT; 32 ± 3 wk of gestation, 1810 ± 691 g weight at birth, n = 17) infants. Our study represents the archived collection sets that were complete (an aliquot was available from each of the 12 wk of collection from a study mother). Ten complete collection sets were available from the group of mothers who delivered FT infants (39 ± 1 wk of gestation, 3920 ± 540 g weight at birth). For comparison, 10 complete collection sets from the group of mothers who delivered PRT infants (30 ± 3 wk of gestation, 1473 ± 500 g weight at birth) were selected at random from those available.

The infants were born at the Janeway Child Health Center (St John’s, Canada) between December 1997 and April 1999. For our study, all mothers were of European origin, and those who delivered PRT or FT infants were aged 31 ± 5 y and 31 ± 4 y, respectively. FT infants began direct breastfeeding within 1 d after birth; PRT infants began direct breastfeeding within 4 wk after birth on average. Before direct suckling of PRT infants, mothers collected milk (by pump or hand expression) in volumes intended to supply most of the foodstuffs. Assessment of maternal dietary history was not attempted. The composition of the milk is transitional and is considered “mature” at ~10 d after birth (25). Therefore, the first 2 collection times in this study represent milk with a transitional composition.

Milk collection

Milk samples were collected by the lactating mothers once weekly between 1000 and 1400 h for 12 wk. For mothers of FT infants, collections occurred during a regular feeding of the child. Week 1 samples were collected as soon as possible after birth between days 0 and 4, week 2 samples were collected between days 6 and 8, and week 3 through 12 samples midweek (eg, between days 70 and 74). A 15-mL aliquot was retained from a whole right breast collection that was conducted according to a strict protocol. The mother first cleaned the nipple and surrounding area with soft Kimwipes EX-L tissue (Fischer Scientific, Toronto) and ultrapure water (≈18 MΩ · cm; Barnstead Co, Boston) while wearing disposable talc-free trace element–free gloves (26). Each sample was collected by manual pump (Holister, Libertyville, IL), electric pump (Medela Inc, McHenry, IL), or by hand expression into the container-supplied cups with the pumping device. The milk collection kit was acid-washed before the sampling by repeated washing in 5% distilled HNO₃. Immediately after collection, the milk was transferred into 20 mL acid-washed snap-top vials (Corning “Snap-seal;” Wilmad-Labglass, Buena, NJ), placed in a styrofoam cooler on ice for delivery to the laboratory, and stored there at −20 °C after the vials were wrapped tightly in plastic. On the basis of the availability of complete or nearly complete collections, sample sets from 10 PRT and 10 FT infants were shipped to the US Department of Agriculture, Grand Forks Human Nutrition Research Center (Grand Forks, ND), on dry ice and stored at −20 °C until processed for mineral analysis. Before the publication of data, all subjects were randomly assigned new identification numbers.

Mineral analyses

Boron, copper, iron, and zinc analyses

For determination of boron, copper, iron, and zinc concentrations in milk samples (27, 28), aliquots from an individual study subject were thawed simultaneously at room temperature as a single batch and then gently resuspended by repeated inversion of the vial by hand to avoid foaming. The samples were mixed further by gentle manipulation with a plastic disposable pipette and transferred as 5.0-g triplicates to acid-washed, preweighed, polytetrafluoroethylene tubes (28.5 × 104 mm, 50 mL; Nalge Co, Rochester, NY). Sample tubes and blanks in triplicate were injected with 10.0 mL of 16.0 mol high-quality HNO₃/L (Baker Analyzed grade, purified in a subboiling point quartz still and stored in polytetrafluoroethylene bottles; JT Baker Chemical Co, Phillipsburg, NJ) and stored for a minimum of 24 h before heating to near dryness; the entire process was repeated once. Subsequently, 10 mL of 30% H₂O₂ (clean room grade and obtained in plastic containers; Ashland Chemical, Dublin, OH) and 3 mL of high-quality 16.0 mol HNO₃/L were added sequentially and allowed to sit for a minimum of 12 h before heated refluxing. After refluxing for 72 h, the samples were brought again to near dryness. After digestion, the tubes sat overnight with 0.5 mL of 10 mol HCl/L (Instra-analyzed grade, purified in a subboiling point quartz still and stored in polytetrafluoroethylene bottles; JT Baker Chemical Co). All samples and blanks were then quantitatively transferred (with plastic, 3.0-mL transfer pipettes, minimum of 3 washes; BA Supply Co, San Antonio, TX) to preweighed polypropylene tubes (Becton Dickinson Labware, Lincoln, Park, NJ). Samples were diluted (1:5, by wt) with de-mineralized, deionized water (~18 MΩ · cm, Millipore System, Super-Q; Millipore Corp, Bedford, MA).

Inductively coupled argon plasma optical emission spectroscopy (Optima 3300DV; Perkin-Elmer Instruments, Shelton, CT) was used to determine sample concentrations of boron [detection wavelength (DW) = 208.995 nm; working detection limit (WDL) = 8.0 µg B/L], copper (DW = 324.754 nm; WDL = 1.0 µg Cu/L), iron (DW = 259.940 nm; WDL = 10 µg Fe/L), and zinc (DW = 213.856 nm; WDL = 1.0 µg Zn/L).

Selenium analysis

The remaining milk samples were transferred to 7.5-mL polypropylene screw cap vials, refrozen, and then thawed for selenium analysis (29, 30). Aliquots (average: 3.00 mL; minimum: 0.20 mL; maximum: 5.0 mL) of the remaining milk samples, when available, were transferred to 100-mL glass beakers with 10 mL HNO₃ (reagent grade; JT Baker Chemical Co), 10 mL 40% (wt:vol) Mg(NO₃)₂ · 6H₂O (American Chemical Society grade; Alphaesar, Ward Hill, MA), and 2 mL of 12 mol HCl/L (reagent grade; JT Baker Chemical Co). The mixtures (covered with watch glasses) were refluxed (110 °C) overnight, evaporated to dryness, dry-ashed at 500 °C in a muffle furnace for 16 h, boiled with 4.0 mL of 12.1 mol HCl/L (reagent grade; JT Baker Chemical Co), diluted to a final volume of 25 mL, and stored in 50-mL conical bottom polypropylene tubes (Falcon tube; Becton Dickinson, Franklin Lakes, NJ). Samples were processed by using hydride generation (from sodium borohydride)

Dakota. Informed consent and experimental procedures were consistent with the Declaration of Helsinki.
in a flow-injection mercury system (FAS-100; Perkin-Elmer Instruments) attached to an atomic absorption spectrometer with a selenium EDL lamp (selenium DW = 196.000 nm, WDL = 0.1 μg Se/L, 5100PC; Perkin-Elmer Instruments) equipped with an electrically heated quartz cell.

For each batch, duplicate samples of each of 3 different standards were analyzed for the relevant elements. No standards of appropriate matrix are certified for boron. Accordingly, the average respective analyzed (±SD) and certified (mean with range in parentheses) values (μg/g) for boron, copper, iron, and zinc for bovine liver [1577b; US Department of Commerce, National Institute of Standards and Technology (NIST), Gaithersburg, MD] were as follows: 0.629 ± 0.097; 154 ± 7, 160 (152–168); 185 ± 10, 184 (168–209); and 117 ± 4, 127 (111–143). For wheat flour (1567a; NIST), the respective values were as follows: 0.419 ± 0.158; 143). For nonfat milk powder (1549; NIST), the respective values were as follows: 0.13; 0.55 (0.60–0.80); 1.65 ± 0.29, 1.78 (1.68–1.88); and 43.4 ± 2.5, 46.1 (43.9–48.3). A normal human serum standard (66816; UTAK Laboratories, Valencia, CA) was analyzed for selenium (122 g Se/L, 5100PC; Perkin-Elmer Instruments) equipped with an electrically heated quartz cell.

Statistical analysis

Outliers

For subjects 3 (FT) and 20 (PRT), all analytic data for boron were removed because of analytic errors. Subsequently, box-whisker plots were generated for the boron, copper, iron, selenium, and zinc values for each group (PRT and FT), and extreme values in these plots were eliminated as outliers.

Model

Zinc values were logarithmically transformed before statistical analysis because their variances were heterogeneous. The data were analyzed by using a mixed linear model in which time (week of lactation) was treated as a continuous variable by using SAS version 8.02 (SAS Institute, Cary, NC). The covariance structure was modeled by using an autoregressive model with a random effect for each subject (31).

RESULTS

Boron

For all mothers in this study, the boron concentration in any FT or PRT milk sample for any measured time interval did not exceed 100 μg (9.25 μmol/L) during the first 12 wk of lactation (Figure 1). For all mothers, only 13 values were at or below the working detection limit for boron [8 μg (0.74 μmol/L)]. For all subjects in the FT group (Figure 1), milk boron concentrations were <51 μg (4.72 μmol/L) at all measured time intervals except for all of those of one subject (subject 5: weeks 1–12). The PRT group (Figure 1) showed a similar pattern in that all boron concentrations were <60 μg (5.55 μmol/L), except for all values for one subject (subject 17: weeks 1–12) and for one value each for 2 other subjects (subject 16: week 3; subject 14: week 4).

The mean concentration of boron in milk was stable (P = 0.5) in the FT group during the first 12 wk of lactation [week 1: 30 μg (2.78 μmol/L); week 12: 28 μg (2.59 μmol/L)] (Figure 2). On the other hand, the concentration of boron in milk decreased linearly over time (P = 0.01) in the PRT group [week 1: 37 μg (3.42 μmol/L); week 12: 27 μg (2.50 μmol/L)].

Copper

In the FT group, milk copper concentrations (Figure 3) decreased significantly (P < 0.0001) during the first 12 wk of lactation [week 1: 651 μg (10.24 μmol/L); week 12: 360 μg (5.67 μmol/L)]. Likewise, there was a significant linear decrease (P < 0.05) in PRT copper milk concentrations over time [week 1: 542 μg (8.53 μmol/L); week 12: 425 μg (6.69 μmol/L)]. Gestational age affected the rate of decrease in milk copper concentrations;
it occurred at a significantly slower rate \((P < 0.02)\) in the PRT than in the FT group.

Iron

Milk iron concentrations (Figure 4) decreased significantly \((P < 0.0003)\) during the first 12 wk of lactation in the FT group [week 1: 355 \(\mu g\) (6.36 \(\mu mol\)/L; week 12: 225 \(\mu g\) (4.03 \(\mu mol\)/L)]. Likewise, there was a significant linear decrease \((P < 0.002)\) in PRT iron milk concentrations over time [week 1: 406 \(\mu g\) (7.27 \(\mu mol\)/L; week 12: 287 \(\mu g\) (5.14 \(\mu mol\)/L)]. For both the FT and PRT groups, the rate of decrease in iron concentrations over time was not different \((P < 0.8)\).

Selenium

Milk selenium concentrations (Figure 5) decreased significantly \((P < 0.0001)\) during the first 12 wk of lactation in the FT group [week 1: 26.9 \(\mu g\) (0.34 \(\mu mol\)/L; week 12: 18.6 \(\mu g\) (0.24 \(\mu mol\)/L)]. Likewise, there was a significant linear decrease \((P < 0.0001)\) in PRT selenium milk concentrations over time [week 1: 28.7 \(\mu g\) (0.36 \(\mu mol\)/L; week 12: 20.4 \(\mu g\) (0.26 \(\mu mol\)/L)]. As for iron, the rates of decrease in selenium concentrations over time for both the FT and PRT groups were not different \((P < 0.8)\).

Zinc

Milk zinc concentrations (Figure 6) decreased significantly \((P < 0.0001)\) during the first 12 wk of lactation in the FT group [week 1: 4060 \(\mu g\) (62.1 \(\mu mol\)/L; week 12: 1190 \(\mu g\) (18.2 \(\mu mol\)/L; means back-transformed from natural log transformation]. Likewise, there was a significant linear decrease \((P < 0.0001)\) in PRT zinc milk concentrations over time [week 1: 5960 \(\mu g\) (91.2 \(\mu mol\)/L; week 12: 1270 \(\mu g\) (19.4 \(\mu mol\)/L)]. Gestational age affected the rate of decrease in milk zinc concentrations; the rate of decrease was significantly slower in the FT than in the PRT group \((P < 0.05)\).

DISCUSSION

The data presented here are the first to characterize the profile of boron in human milk and include a range of concentrations, patterns of change over time, and perturbations induced by pre-maturity or low birth weight. Our subject pool for milk analysis was apparently not different from the general population that gives birth to full-term healthy infants because the milk concentrations of the minerals analyzed in this study were fully within the ranges of those commonly reported (32) for copper (colostrum: 400–600 \(\mu g\)/L; mature milk: 200–300 \(\mu g\)/L), iron (colostrum: \(\approx\)400–800 \(\mu g\)/L; mature milk: 200–400 \(\mu g\)/L), selenium (\(\approx\)15 \(\mu g\)/L; dependent on maternal dietary selenium...
intakes), and zinc (early lactation: 4000–5000 μg/L; 6 mo: ≈500 μg/L).

The data from this study indicate that boron is present in human milk within a well-defined range (1–10 μmol/L milk) when lactating mothers self-select their own diets. It is generally accepted that human milk should serve as the "gold standard" when foodstuffs for infants are compared and when dietary recommendations are made (32). In this respect, the findings of our study indicated that the mean concentration of boron in human milk over the first 12 wk of lactation (28 μg/L) from the mothers who delivered FT infants was much less than that in ready-to-eat infant formulas with and without supplemental iron (120 μg B/L) or in bovine whole milk (280 μg B/L; fluid, 3.3% milk fat) (33). Assuming that full-term, breastfed, healthy infants consume milk at a daily rate of ≈0.74 L (average of selected reported values; 23, 34), their total daily boron intake during the first 12 wk of lactation is estimated to be 22.3 μg (2.06 μmol). Even so, at a slightly older age (6–11 mo), American infants are estimated to consume 50.9 μmol (550 μg) B/d from all dietary sources (33).

Little is known about the speciation or bioavailability of boron in natural foodstuffs. However, boron transport molecules in breast milk are probably associated with the soluble, instead of the fat, fraction because the boron contents of bovine whole milk (3.3% milk fat) and skim milk (0.08% milk fat) are not different: 280 and 310 μg/L, respectively (33). The biomolecules in animals and humans known to have high boron-binding affinities [5'-adenosylmethionine and the diadenosine polyphosphates (Ap₃A, Ap₄A, Ap₅A, and Ap₆A)] (35) do not appear to be candidate molecules for boron transport because of the unfavorable molar ratios. Lactoferrin, a main constitutive protein in human milk (25), may be an ideal candidate for investigation. This compound is thought to be a serine protease (36), and boron forms stable but reversible complexes with all tested serine proteases at the catalytic site of the enzyme (37). The molar ratio between lactoferrin and boron also seems to be advantageous toward complex formation; the concentration of lactoferrin in human milk (40 μmol/L; week 2 of lactation) (38, 39) is ≈14 times that of boron (2.78 μmol/L at week 2 of lactation in this study). In general, it seems prudent to determine the affinity of boron for lactoferrin.

Within the intestinal tract, most ingested boron is probably converted to B(OH)₃, the normal end product of hydrolysis of most boron compounds (40), and is available for absorption. Therefore, the bioavailability of boron in human milk is likely to be high. It is known that low amounts of naturally occurring dietary boron [0.36 mg (0.033 mmol) B/d] as well as supplemental inorganic forms [2.87 mg (0.265 mmol) B/d; as orthoboric acid] are absorbed almost completely and excreted in the urine in postmenopausal women (3). Because boron can be bioactive, it seems important that future studies determine the bioavailability of the element in infant foods, especially human milk substitutes.

Contrary to our hypothesis, we report here that the boron content in human milk does not follow the pattern established for the essential trace elements copper, iron, selenium, and zinc (25, 32). Instead, our data indicate that the concentration of boron in milk from mothers of full-term healthy infants is highly conserved across time. This pattern is similar to that established for calcium during the first 3 mo of lactation. Reports from several longitudinal studies indicate that the calcium concentrations in human milk are stable (23, 32, 41) or decrease only temporarily (42) during the first 3 mo of lactation. Likewise, it has been reported repeatedly that concentrations of magnesium in human milk remain constant (32, 43, 44) or increase over that time frame (23, 45, 46).

It seems highly unlikely that the consistency in milk boron concentrations over time is a simple reflection of dietary boron intakes over 12 wk. Animal food products contain very low concentrations of boron compared with plant-based foods, and plant species within the subclass Dicotyledoneae (eg, fruit, vegetables, tubers, nuts, and legumes) are, without exception, much richer sources of dietary boron than are edible species in the subclass Monocotyledoneae (eg, corn, rice, rye, oats, and wheat) (33). Thus, it is highly unlikely that boron intakes were constant over a 12-wk period of time because of the unequal distribution of boron in foodstuffs. Furthermore, the maternal diet typically does not affect the concentrations of minerals in human milk (23), except for those of a few elements, including selenium (47) and iodine (48).

The conservation of boron concentrations in human milk over time suggests homeostatic control of the element. Other evidence supports the concept of the metabolic control of boron in humans (3, 49) and other mammals (50–52). The mechanism that explains the apparent precise homeostatic control of boron in milk from mothers of full-term infants needs to be investigated. Such studies may concurrently elucidate biomolecules that interact strongly with boron.

The findings from this study indicate that prematurity perturbs the rate of change in the boron content of human milk early in lactation. Within the first 12 wk of lactation, milk boron concentrations decreased linearly over time in the PRT group but remained stable in the FT group. Similarly, milk zinc concentrations fell faster in the PRT group than in the FT group. Similar to boron and copper, the rates of decrease in zinc concentrations over time differed between the FT and PRT groups, but, in contrast, the rate of decrease was significantly slower in the FT than in the PRT group.

Prematurity did not affect milk mineral concentrations per se. It is possible that we did not have the statistical power to detect differences in mineral milk concentrations induced by prematurity. However, other reports indicate that prematurity did not affect the milk concentrations of copper (42), iron (53), selenium (54), and zinc (42, 45, 55), even though the concentrations of those elements decreased over time.

In summary, the findings of the current study indicate that boron is present in the milk of mothers of full-term, healthy infants who consume self-selected diets. The stable concentration of boron in milk over time in the FT group suggests that the element is under homeostatic control and, as such, in a manner similar to that established by others for calcium and magnesium. Prematurity appears to perturb that control. The patterns of change in copper, iron, selenium, and zinc concentrations in human milk over time differ from those of boron.

We thank the members of the Grand Forks Human Nutrition Research Center who helped make this study possible, especially Terrance Shuler (mineral analyses) and Joseph Ido and Kay Keehr (technical support); the members of the Neonatology Unit at the Janeway Child Health Center (St John’s, Canada), especially Wayne Andrews and Khalid Aziz (attending physicians), Claude Mercer (technical support), and Allison McDonald (staff nurse in charge of milk collections). We are deeply indebted to the study participants.
CDH conceived, designed, and supervised the portion of the study related to mineral analysis; analyzed and interpreted the data; and wrote the manuscript. JKF conceived and designed the portion of the study related to subject mineral analysis; analyzed and interpreted the data; and wrote the manuscript. LKJ conducted the statistical and mathematical modeling analyses. The authors had no conflicts of interest.

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