Nitrate and Nitrite Content of Human, Formula, Bovine, and Soy Milks: Implications for Dietary Nitrite and Nitrate Recommendations

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Abstract

Background: Estimation of nitrate and nitrite concentrations of milk sources may provide insight into potential health risks and benefits of these food sources for infants, children, and adults. The World Health Organization and American Academy of Pediatrics recommends exclusive consumption of human milk for the first 6 months of life. Human milk is known to confer significant nutritional and immunological benefits for the infant. Consumption of formula, cow's, and soy milk may be used as alternatives to human milk for infants.

Methods: We sought to estimate potential exposure to nitrate and nitrite in human, formula, bovine, and soy milk to inform total dietary exposure estimates and recommendations. Using sensitive quantitative methodologies, nitrite and nitrate were analyzed in different samples of milk.

Results: Human milk concentrations of colostrum (expressed days 1–3 postpartum; $n=12$), transition milk (expressed days 3–7 postpartum; $n=17$), and mature milk (expressed >7 days postpartum; $n=50$) were 0.08 mg/100 mL nitrite and 0.19 mg/100 mL nitrate, 0.001 mg/100 mL nitrite and 0.52 mg/100 mL nitrate, and 0.001 mg/100 mL nitrite and 0.3 mg/100 mL nitrate, respectively, revealing that the absolute amounts of these anions change as the composition of milk changes. When expressed as a percentage of the World Health Organization's Acceptable Daily Intake limits, Silk® Soy Vanilla (WhiteWave Foods, Broomfield, CO) intake could result in high nitrate intakes (104% of this standard), while intake of Bright Beginnings Soy Pediatric® formula (PBM Nutritionals, Georgia, VT) could result in the highest nitrite intakes (383% of this standard).

Conclusions: The temporal relationship between the provision of nitrite in human milk and the development of commensal microbiota capable of reducing dietary nitrate to nitrite supports a hypothesis that humans are adapted to provide nitrite to the gastrointestinal tract from birth. These data support the hypothesis that the high concentrations of breastmilk nitrite and nitrate are evidence for a physiologic requirement to support gastrointestinal and immune homeostasis in the neonate.

Introduction

THE RELEVANCE OF DIETARY sources of nitrites and nitrates for human health is in its ascendancy. Historically, health risks due to nitrates in groundwater have been associated with risk of methemoglobinemia (blue-baby syndrome) in children;¹ consumption of nitrites and nitrates in processed meats, used to enhance flavor and prevent microbial growth, is associated with a modest increased risk of gastrointestinal cancer and chronic obstructive pulmonary disease.^{2–4} The health risks due to excessive nitrite and nitrate consumption in these specific population subgroups led to regulatory limits

on the permissible concentration of nitrate in drinking water (50 mg of nitrate/L in the European Union, 44 mg/L in the United States) in accordance with World Health Organization (WHO) recommendations first established in 1970 and reaffirmed in 2004.⁵ The Joint Food and Agricultural Organization/WHO has set the Acceptable Daily Intake (ADI) for the nitrate ion at 3.7 mg/kg of body weight and for the nitrite ion at 0.06 mg/kg of body weight.⁶

Exposure estimates from national dietary surveys reveal average nitrate intakes in the United States and Europe of 31–185 mg/day and 40–100 mg/day, respectively.^{7,8} Nitrite intake varies from 0 to 20 mg/day.⁹ Approximately 80% of

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dietary nitrate is derived from vegetable intake. Making high-nitrate compared to low-nitrate food choices within a healthful dietary pattern such as the Dietary Approaches to Stop Hypertension (DASH) diet¹⁰ can yield differences from 174 to 1,222 mg of nitrate;¹¹ this high-nitrate DASH dietary pattern exceeds the WHO's ADI for nitrate by 550% for a 60-kg adult.¹¹

In humans, dietary nitrate and nitrite sources have been demonstrated to lower blood pressure^{12–14} and decrease oxygen consumption during submaximal and maximal aerobic exercise.^{15,16} In animal models, nitrite has been demonstrated to enhance mucosal blood flow and serve antimicrobial functions,¹⁷ protect against heart attack¹⁸ and stroke,¹⁹ and reverse vascular inflammation from a high fat diet.²⁰ Gastroprotective and blood pressure-lowering effects of dietary nitrate are abolished by frequent spitting after nitrate consumption or by antiseptic mouthwash.^{13,21} These manipulations abolish the reduction of nitrate to nitrite by bacteria on the lingual surface made possible by enterosalivary circulation of nitrate. Physiologically, dietary sources of nitrate and nitrite have been estimated to serve as substrates for as much as 50% of nitric oxide (NO) production in the human body.¹¹ This provides proof of concept that dietary sources of nitrite and nitrate have very important and essential physiological functions. The demonstration of physiological functions to nitrate and nitrite has warranted the proposition that nitrates and nitrites be considered nutrients in humans.¹¹

Breastmilk or formula milk serves as a primary source of calories and nutrients in infants and children and may take several forms. Human breastmilk is recommended to serve as the exclusive food for the first 6 months of life and continue, along with safe, nutritious complementary foods, up to 2 years.^{22,23} Breastmilk is nature's most perfect food. In fact, the U.S. Centers for Disease Control and Prevention in 2010 stated breastmilk is widely acknowledged as the most complete form of nutrition for infants, with a range of benefits for infants' health, growth, immunity, and development. Breastmilk is a unique nutritional source for infants that cannot adequately be replaced by any other food, including infant formula. It remains superior to infant formula from the perspective of the overall health of both mother and child. Human milk is known to confer significant nutritional and immunological benefits for the infant.^{24–26} According to the Centers for Disease Control and Prevention's National Immunization Survey in 2006, 74% of infants were breastfed in early postpartum, 43% at 6 months, and 23% at 12 months of age; among infants born in 2006, only 33% and 14% were exclusively breastfed through 3 months and 6 months of age, respectively.²⁷ These data also suggest that approximately 25% of breastfeeding infants will receive formula supplementation in the first 2 days of life. Nearly 38% of breastfeeding infant have supplement by 3 months, and 44.7% have formula supplement before 6 months.²⁷ These data suggest that, in addition to human milk, other forms of milk or complementary foods may be significant nutrient sources for infants. Therefore, it is critical to estimate the potential dietary exposures to nitrate and nitrite from fluid milk sources to assess potential health benefits and risks associated with their consumption.

This study provides additional data for dietary exposure estimates by using sensitive, quantitative methodologies to estimate nitrite and nitrate concentrations in samples of

human, formula, cow's, and soy milk. These data, considered together with nitrite and nitrate exposure estimates from foods, demonstrate that humans are exposed from birth to dietary sources of nitrites and nitrates. As with other food sources of nitrite and nitrate,¹¹ exposures in infants and children, particularly from breastmilk, routinely exceed WHO ADI levels for both nitrate and nitrite.

Subjects and Methods

This study was reviewed by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston (Institutional Review Board-approved protocol HSC-IMM-08-0203). All study participants provided informed consent. Study participants were admitted to Memorial Hermann Hospital (Houston) for childbirth or were new mothers visiting the University of Texas Clinic. Mothers who smoked or had hypertension were excluded from the study. All investigators completed and passed an institutional course on the Health Insurance Portability and Accountability Act.

Sample preparation

Upon consent, colostrum or milk was collected from nursing mothers using a conventional breast pump. Roughly 0.5 mL of colostrum and 1.0 mL of milk was collected from each patient. Colostrum (day 1–3 postpartum) was collected from nursing mothers at Memorial Hermann Hospital. Milk was collected from patients in the clinic at Hermann Professional Building. The samples were collected and immediately taken on ice to the laboratory for biochemical analysis. All samples were de-identified and assigned a patient number.

Nitrite and nitrate determination

All samples were held on ice from collection until analysis. Ice-cold methanol was added 1:1 (vol/vol) to each sample, vortex-mixed, and immediately centrifuged to precipitate protein. The supernatant was collected and analyzed in triplicate within 30 minutes of collection. Complete analytical procedures have been previously described by Bryan and Grisham.²⁸ A dedicated ENO-20 high-performance liquid chromatography system (Eicom Corp., San Diego, CA) was used for nitrate/ nitrite analysis. To ensure no degradation of nitrite and nitrate in our milk samples during transport, pilot tests were conducted by spiking known concentrations of nitrite and nitrate in refrigerated milk samples and tested over time for up to 1 week. There was very little consumption of either anion during this time (data not shown), illustrating the integrity of the nitrite and nitrate in our samples were maintained during transport. Standard curves were performed using known molar concentrations of both nitrite and nitrate. The data are reported as both molar concentrations and as mg/100 mL. Conversions from molar concentrations to mg/100 mL were calculated using molecular weight of each anion (62 mg/mmol for nitrate and 46 mg/mmol for nitrite).

Statistical analysis

Comparisons were made using a two-tailed *t* test. Significance was considered a *p* value of <0.05. The software package used was Stata (College Station, TX) Statistical Software, release 10 (2007).

TABLE 1. NITRATE AND NITRITE CONCENTRATIONS IN HUMAN MILK SAMPLES BY STAGE OF PRODUCTION

Stage	In mg/100 mL		In mg/750 mL	
	Nitrate	Nitrite	Nitrate	Nitrite
Colostrum	0.19 ± 0.03	0.08 ± 0.02	1.43 ± 0.24	0.60 ± 0.15
Transition	0.52 ± 0.10	0.001 ± 0.001	3.90 ± 0.75	0.008 ± 0.008
Mature	0.31 ± 0.02	0.001 ± 0.001	2.32 ± 0.21	0.008 ± 0.008

Sample means are based on 12–50 individual samples in triplicate.

Results

Nitrate and nitrite concentrations in milk samples

Table 1 shows the mean concentrations of nitrite and nitrate from human milk samples obtained in our study population. Mean concentrations of nitrite and nitrate are expressed as mg/100 mL. This is a commonly used reference volume based on the average daily intake of infants (750 mL) during the first year of life as expressed by the U.S. Food and Nutrition Board, Institute of Medicine, National Academy of Science.²⁹ Human breastmilk nitrite and nitrate concentrations are expressed by phase of milk production: colostrum (expressed days 1–3 postpartum; $n = 12$), transition milk (expressed days 3–7 postpartum; $n = 17$), and mature milk (expressed >7 days postpartum; $n = 50$). Human milk nitrite and nitrate concentrations were 0.08 mg/100 mL nitrite and 0.19 mg/100 mL nitrate (or $16.8 \pm 4.5 \mu\text{M}$ nitrite and $30 \pm 5 \mu\text{M}$ nitrate) in colostrum, 0.001 mg/100 mL nitrite and 0.52 mg/100 mL nitrate (or $0.3 \pm 0.1 \mu\text{M}$ nitrite and $84.9 \pm 16 \mu\text{M}$ nitrate) in transition milk, and 0.001 mg/100 mL nitrite and 0.31 mg/100 mL nitrate (or $0.3 \pm 0.1 \mu\text{M}$ nitrite and $48.9 \pm 4.6 \mu\text{M}$ nitrate) in mature milk. Figure 1A illustrates the difference in nitrite and nitrate content in different phases of human breastmilk. Colostrum has significantly higher concentrations of nitrite and significantly lower concentrations of nitrate than both transition and mature milk. In order to corroborate the results above from a single donor in a longitudinal study, we consented two mothers day 2 after birth and collected breastmilk daily over a period of 16 days. As shown in Figure 1B, the

amounts of nitrite and nitrate in the milk from individual mothers followed the same trend as the group data from above. Human breastmilk contains high concentrations of nitrite in the early postpartum period, and the relative concentrations of the two anions change from colostrum to transition milk to mature milk. These data demonstrate that the values recorded from multiple donors at different time points hold true for changes in a single donor and are likely not a result of dietary or health differences of individual donors.

Table 2 gives nitrate and nitrite concentrations in a representative sample of artificial formulas for infants and children. Among infant formulas, nitrite and nitrate concentrations were 0.00005 mg/100 mL nitrite and 0.05 mg/100 mL nitrate (or $0.01 \mu\text{M}$ nitrite and $7.72 \mu\text{M}$ nitrate) for Similac® (Abbott Laboratories, North Chicago, IL), 0.00005 mg/100 mL nitrite and 0.12 mg/100 mL nitrate (or $0.01 \mu\text{M}$ nitrite and $19.49 \mu\text{M}$ nitrate) for Pregestimil® (Mead Johnson Nutritionals, Evansville, IN), and 0.00005 mg/100 mL nitrite and 0.10 mg/100 mL nitrate (or $0.01 \mu\text{M}$ nitrite and $15.43 \mu\text{M}$ nitrate) for Enfamil® (Mead Johnson Nutritionals). Two supplemental formulas for children, Boost Kids Essential® Lactose Free (Nestlé HealthCare Nutrition, Minneapolis, MN) and Bright Beginnings Soy Pediatric® (PBM Nutritionals, Georgia, VT), yielded average concentrations of 0.007 mg/100 mL nitrite and 0.26 mg/100 mL nitrate (or $1.49 \mu\text{M}$ nitrite and $41.83 \mu\text{M}$ nitrate) and 0.03 mg/100 mL nitrite and 0.16 mg/100 mL nitrate (or $6.80 \mu\text{M}$ nitrite and $25.36 \mu\text{M}$ nitrate), respectively. TwoCal HN® (with fructooligosaccharide) (Abbott Laboratories), a

FIG. 1. (A) Nitrite and nitrate concentrations from the three stages of human breastmilk. Data are average $\pm$ SEM values from 12 colostrum samples, 17 transition samples, and 50 mature milk samples. * $p < 0.001$ compared to colostrum; # $p < 0.05$ compared to colostrum. (B) A longitudinal study in the same nursing mothers from multiple collections over a 16-day period. Data are averages of single donations from two nursing mothers for 16 days.

TABLE 2. NITRATE AND NITRITE CONCENTRATIONS FROM INFANT AND PEDIATRIC FORMULA MILK PRODUCTS

Brand	In mg/100 mL		In mg/750 mL	
	Nitrate	Nitrite	Nitrate	Nitrite
Boost Kid Essentials Lactose Free	0.26 ± 0.01	0.007 ± 0.001	1.95 ± 0.08	0.05 ± 0.008
Bright Beginnings Soy Pediatric	0.16 ± 0.01	0.03 ± 0.001	1.20 ± 0.08	0.23 ± 0.008
Similac	0.05 ± 0.001	<0.0001	0.36 ± 0.008	<0.0003
Pregestimil—protein hydrolysate	0.12 ± 0.01	<0.0001	0.91 ± 0.08	<0.0003
Enfamil EnfaCare	0.10 ± 0.01	<0.0001	0.72 ± 0.08	<0.0003
Nestlé Carnation Instant Breakfast Plus (lactose free)	0.08 ± 0.01	<0.0001	0.60 ± 0.08	<0.0003
TwoCal HN with FOS-milk-based hydrolysate	0.18 ± 0.02	<0.0001	1.32 ± 0.16	<0.0003
Nutramigen AA Elemental	0.02 ± 0.001	<0.0001	0.14 ± 0.008	<0.0003

Limit of detection was approximately 20 nM or 0.0001 mg/100 mL.
FOS, fructooligosaccharide.

high-calorie enteral supplement with prebiotic fructooligosaccharide, had 0.00005 mg/100 mL nitrite and 0.18 mg/100 mL nitrate (or 0.01 μ M nitrite and 28.31 μ M nitrate). Nutramigen® AA Elemental (Mead Johnson Nutritionals), for those requiring predigested sources of macronutrients, had 0.00005 mg/100 mL nitrite and 0.02 mg/100 mL nitrate (or 0.01 μ M nitrite and 3.12 μ M nitrate). A popular milk supplement, Nestlé Carnation Instant Breakfast Plus®, had 0.00005 mg/100 mL nitrite and 0.08 mg/100 mL nitrate (or 0.01 μ M nitrite and 12.93 μ M nitrate).

Table 3 represents nitrate and nitrite concentrations in bovine and soy milk samples. Cow's milk (2% milkfat) samples had 0.0002 mg/100 mL nitrite and 0.23 mg/100 mL nitrate (or 0.04 μ M nitrite and 37.45 μ M nitrate), and the organic cow's milk (2% milkfat) sample had 0.0003 mg/100 mL nitrite and 0.16 mg/100 mL nitrate (or 0.07 μ M nitrite and 25.38 μ M nitrate). Silk® Soy Vanilla Milk (WhiteWave Foods, Broomfield, CO) had 0.0036 mg/100 mL nitrite and 3.48 mg/100 mL nitrate (or 0.78 μ M nitrite and 561.9 μ M nitrate), and Silk Soy Egg Nog contained 0.013 mg/100 mL nitrite and 0.34 mg/100 mL nitrate (or 2.74 μ M nitrite and 55.08 μ M nitrate), showing a large difference in nitrite and nitrate content in different types of milk.

Nitrate and nitrite concentrations relative to WHO ADI levels for representative samples of human, bovine, and soy milk samples

Table 4 represents the nitrate and nitrite concentrations consumed by a hypothetical 6.8-kg infant consuming the reference 750 mL/day. The exception to this is for colostrum, which is estimated based upon a 3.2-kg (7-pound) infant consuming 100 mL/day. In addition, nitrate and nitrite intakes are expressed as a percentage of the WHO ADI standards.⁵ Among the milk samples analyzed, nitrate intakes

were highest from pediatric formulas (Boost Kids Essential Lactose Free and Bright Beginnings Soy Pediatric) and lowest from infant formulas (Similac and Pregestimil). The highest nitrate exposure of the milk samples tested, relative to the WHO ADI standard, is from Silk Soy Vanilla intake, which could result in intakes of 104% of this standard. Boost Kids Essential Lactose Free intake could result in 83% of the WHO ADI for nitrate. Regarding potential nitrite exposures, Bright Beginnings Soy Pediatric intake could result in 383%, and Boost Kids Essential Lactose Free intake could result in 83%, of the WHO ADI standard. Human milk colostrum samples had the third highest nitrite concentration (42% of WHO ADI at 100 mL intake).

Discussion

The fear of fatal infantile methemoglobinemia due to nitrite and nitrate contamination has led to stringent regulations of the amount of nitrite and nitrate in our food supply and drinking water. However, several research studies have previously demonstrated relatively high concentrations of nitrite and nitrate in human breastmilk. Ohta et al.³⁰ found high concentrations of nitrite and nitrate (166–1,246 μ M [or 1–7 mg/100 mL]) in the breastmilk of Japanese mothers from days 1 to 8. Cekmen et al.³¹ found extremely high concentrations of nitrite in breastmilk of healthy mothers, but levels were reduced in preeclampsia patients. Total nitrite levels were 56.09 ± 11.18 μ M (0.23 mg/100 mL) versus 82.20 ± 12.01 μ M (0.37 mg/100 mL) ($p < 0.05$) in colostrum of those with preeclampsia and controls, respectively, consistent with our results from colostrum. Whereas nitrate and nitrite composition of milk has been previously reported, research with the goal of estimating exposure from these important food sources for infants, children, and adults using WHO ADI regulatory limits has not been performed. Scientific study

TABLE 3. NITRATE AND NITRITE EXPOSURE ESTIMATES FROM BOVINE MILK AND SOY MILK SAMPLES

Milk type	In mg/100 mL		In mg/750 mL	
	Nitrate	Nitrite	Nitrate	Nitrite
Market Pantry 2% cow's milk	0.23 ± 0.06	0.0002 ± 0.0001	1.74 ± 0.45	0.0013 ± 0.0003
Organic 2% cow's milk	0.16 ± 0.03	0.0003 ± 0.0001	1.18 ± 0.22	0.0023 ± 0.0003
Silk Soy Vanilla Milk	3.48 ± 0.61	0.0036 ± 0.00051	26.1 ± 4.6	0.027 ± 0.0004
Silk Soy Egg Nog	0.34 ± 0.04	0.013 ± 0.0001	2.56 ± 0.3	0.094 ± 0.0003

TABLE 4. AVERAGE NITRATE AND NITRITE EXPOSURE IN A HYPOTHETICAL 15-POUND (6.8-KG) INFANT CONSUMING 750 mL OF MILK/DAY

Milk type	Exposure (mg/kg)		% WHO ADI for ^a	
	Nitrite	Nitrate	Nitrite	Nitrate
Human (colostrum phase) ^b	0.025	0.059	42	1.60
Human (mature phase)	0.0120	0.34	2.7	9.10
Bovine (2% milkfat)	0.0013	1.74	2.1	47.0
Soya (Silk Soy Vanilla flavor)	0.004	3.84	6.7	104
Infant formulas				
Similac	0.0003	0.36	0.5	9.70
Pregestimil	0.0003	0.91	0.5	24.6
Pediatric formulas				
Boost Kid Essentials Lactose-Free	0.05	1.95	83	52.7
Bright Beginnings Soy Pediatric	0.23	1.20	383	32.4

^aThe Joint Food and Agricultural Organization/World Health Organization (WHO) acceptable daily intake (ADI) for the nitrate ion is 3.7 mg/kg of body weight and for the nitrite ion is 0.06 mg/kg of body weight.

^bIntake is estimated using the nitrite and nitrate concentrations derived in Table 1 and calculated for a 3.2-kg (7-pound) infant consuming 100 mL of colostrum.

regarding changes of these anions in different stages of milk has also not been previously reported.

The composition of human breastmilk is adapted to fit the nutritional needs of the infant and provide immunologic protection during maturation of the immune system.²⁵ The quality and characteristics of breastmilk change over time. This change in composition is thought to be due partly to the changing intestinal microflora in the baby and the changing metabolic demands as the baby grows. Colostrum, the early milk made by maternal breasts, is usually present after the fifth or sixth month of pregnancy. Once the baby is born, it is present in small amounts for the first 3 days to match the small size of your baby's stomach. This precious colostrum should be given to the newborn as soon as possible after giving birth. It is this precious colostrum that contains the highest amount of nitrite of any of the milk products tested. Colostrum is designed to meet a newborn's special needs. Breastmilk will change and increase in quantity about 48–72 hours after giving birth.³² Our data suggest that the ratio of nitrite and nitrate also changes in order to meet the changing metabolic demands on the infant.

Indeed, the pattern of nitrate and nitrite composition of human milk suggests that, as observed by others, these components also serve immunomodulatory and gastroprotective roles. Therefore, it is reasonable to surmise that nitrite must be supplied through human milk to the newborn in order to derive the resulting vascular, immunologic, and gastroprotective benefits. The beneficial effects of NO in adult stomachs on gastric mucosal integrity and blood flow from the reduction of nitrate to nitrite to NO are known. At birth, the gastrointestinal tract of the infant is sterile, and it is then rapidly colonized by bacteria originating from the mother and the environment.^{33,34} The first colonizing bacteria are aerobic bacteria, such as staphylococci, enterococci, and enterobacteria.³⁵ Then, anaerobic bacteria, such as *Bacteroides*, *Bifidobacterium*, and *Clostridium* species, gradually colonize the gastrointestinal tract.³⁵ The infant microbiota are also established from bacteria such as *Bifidobacterium* and *Lactobacillus* found in human milk.^{36,37} The high concentration of galactooligosaccharides in human milk provides optimal growth conditions for bifidobacteria species, which are

prevalent microbes in the infant's intestinal microbiome.^{38,39} We now appreciate that reduction of nitrate to nitrite requires the commensal bacteria that normally reside in our body. However, in newborn infants this pathway has not developed. After the establishment of the infant's oral microbiome on the lingual surface as well as intestinal colonization by bacteria, the infant, like the adult, supplies the gastrointestinal tract with a constant source of nitrite by enterosalivary circulation of nitrate. Therefore breastmilk high in nitrite relative to nitrate overcomes the natural deficiency early in life. At later stages of development nitrate becomes the predominant anion when a symbiosis exists with the colonized bacteria. There appears to be a complementary system whereby the nitrite in breastmilk can be reduced to NO even before the colonization of bacteria in the infant.

Our data indicate that the substitution of human milk in the diet of infants with formula, bovine, or soy milk will result in the provision of significant levels of nitrate and nitrite. This becomes interesting in terms of levels of exposure based on nitrite ingestion relative to body weight in the infant (over 1 mg/kg). The Joint Food and Agricultural Organization/WHO has set the ADI for the nitrate ion at 3.7 mg/kg of body weight and for the nitrite ion at 0.06 mg/kg of body weight.⁶ Normal daily infant breastmilk intake is 2–3 ounces/pound/day or 200 mL/kg. That translates into 14–21 ounces or 400–630 mL of milk for a typical 7-pound (3-kg) infant or more simply ~200 mL/kg. Taking an average of 50 μ mol/L concentration of nitrite or 2,300 μ g/L, total daily nitrite exposure to nursing infants is roughly 1.2 mg/kg, or 20 times higher than the acceptable daily intake for nitrite. The ability to exceed WHO ADI limits with usual intake levels of single foods, such as breastmilk, spinach, or a desiccated vegetable supplement¹¹ indicates that these regulatory limits may not have a rational basis when applied to food sources of nitrate and nitrite for children and adults.

The nitrate and nitrite content of milk products demonstrates that these foods are significant contributors to total dietary nitrate and nitrite intakes. We hypothesize that human milk provides a dietary source for nitrite prior to the establishment of lingual and gastrointestinal microbiota. Once the microbiota are established, these commensal

organisms are capable of reducing dietary nitrate, via enterosalivary circulation, to nitrite and support gastrointestinal, immune, and cardiovascular health. The significant concentration of nitrate and nitrite in bovine milk demonstrated here indicates that these conclusions may be applicable across mammalian species. When we compare freshly expressed human breastmilk at day 3 to Enfamil baby formula, human breastmilk contains almost 20 times more nitrite. Studies have demonstrated a number of important health benefits to breastfeeding.²⁶ It provides several health advantages beginning at birth and continuing throughout a child's life. A large number of the health problems today's children face might be decreased, or even prevented, by breastfeeding the infant exclusively for at least the first 6 months of life. The longer the mother breastfeeds, the more likely her child will get the health benefits of breastfeeding. Many studies have shown that breastfeeding strengthens the immune system. Infants who are breastfed exclusively for 6 months have a more developed immune system than those who are not exclusively breastfed.^{40,41} Breastfeeding has also been shown to reduce the risks of the infant developing asthma and allergies⁴² as well as childhood leukemia.⁴³ Cardiovascular disease risk is also reduced through the reduction of obesity, blood pressure, and cholesterol.⁴⁴ Breastfeeding as an infant also provides benefit later in life. Breastfed children are less likely to contract a number of diseases later in life, including juvenile diabetes, multiple sclerosis, heart disease, and cancer before the age of 15 years.⁴⁵ In fact, breastfed babies have been shown to have a small reduction in blood pressure later in life.⁴⁶ Breastfeeding during infancy is also associated with a reduction in risk of ischemic cardiovascular disease later in life.⁴⁷ The reduction in adult-onset diseases may be due to the early influence of the nitrite/nitrate composition of breastmilk.

The origins of nitrate and nitrite in human milk are not known. Maternal nitrate and nitrite intakes are not reflected in nitrate and nitrite composition of human milk.¹ The data supporting this conclusion are sparse. One study published on this topic demonstrated that women who consumed water with a nitrate concentration <100 mg/L did not produce milk with elevated nitrate levels.⁴⁸ Physiologic production of nitrate and nitrite in tissues is dynamic. Other work has demonstrated an important role for xanthine oxidase in milk in the generation of NO_x in milk. In the early postpartum stage, nitrite may exert critical antimicrobial activity. The presence of high levels of xanthine oxidoreductase in breastmilk, along with the high nitrite levels and low oxygen tension, allows for the generation of peroxynitrite, a potent bactericidal agent, from NO.⁴⁹ These proposed antimicrobial actions of nitrite and peroxynitrite may help fight infections established in utero and/or peripartum.

The presence of nitrate and nitrite in human milk provides evidence for a physiologic benefit of dietary nitrite for the protection of the gastrointestinal tract in the neonate prior to the establishment of commensal bacteria in the mouth and intestine. The temporal relationship between the provision of nitrite in human milk and the development of commensal microbiota capable of reducing dietary nitrate to nitrite supports a hypothesis that milk nitrite may supply this component in the immediate term after birth. The most reasonable conclusion that can be made from these exposure estimates is that humans are adapted to receive dietary nitrite and nitrate from birth and therefore may not pose significant risks at

levels naturally found in certain foods. In fact, we believe that the absence of nitrite—recently proposed as a nutrient¹¹—in baby formulas may contribute to many of the health disparities in formula-fed babies, including necrotizing enterocolitis, infections, poor nutrient absorption, and even increased health risks later in life.

Acknowledgments

This work was supported by National Scientist Development grant 0735042N to N.S.B. by the American Heart Association.

Disclosure Statement

N.S.B. has a financial interest in NeoGenis Inc. His financial and research conflicts of interest are managed by the University of Texas Health Science Center at Houston Conflicts of Interest Management Plans, developed from and reviewed by the Research Conflicts of Interest Committee and approved by the Executive Vice President for Research at the University of Texas Health Science Center at Houston. None of the other authors report a conflict of interest.

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