Abstract
Fatty acids (FAs) and fat-soluble vitamins are vital components of the human milk lipid fraction. About two-thirds of the human milk FA fraction consist of oleic, linoleic, and palmitic FAs, but the precise composition depends on maternal geography, diet, and genetics. Mothers with high fish consumption have more docosahexaenoic acid (DHA) and other ω-3 FAs in their milk, while mothers with high dairy consumption have more branched-chain FAs in their milk. Vitamins A and E are the most common fat-soluble vitamins, but milk concentrations vary, depending on maternal diet and body stores. Vitamin D is typically low or undetectable in mother’s milk and typically fails to meet the infant needs. However, trial data indicate that high maternal supplementation (6,400 IU/day) safely provides nutritionally adequate amounts of vitamin D in her milk. FA and fat-soluble vitamin levels in mother’s milk can significantly influence infant health; for example, in preterm infants, low endogenous stores of DHA paired with low levels in maternal milk may influence the risk of chronic lung disease and other inflammatory conditions. Greater attention is warranted to the variation in FA and fat-soluble vitamin content of human milk in relation to infant health.

Introduction
Fatty acids (FAs) and fat-soluble vitamins are key components of the lipid fraction of human milk. Lipid is the second-most abundant solid constituent of human milk after lactose, but it is also the most highly variable macronutrient of
Milk expressed late in a feed or pumping episode contains as much as 2–8 times more fat than at the start of the feed [1, 2]. In addition, lipid content is also reportedly lower in night and morning than afternoon or evening feeds [1, 3]. Given this high variability, accurate study of lipid-associated components requires accounting for sample lipid content.

Human milk FA composition has a core similarity (Fig. 1) but differs across populations in the abundance of many FAs, influenced by maternal diet and genetics (Table 1). High interindividual and population variability in docosahexaenoic acid (DHA) and other n-3 FAs of human milk is often observed [4–7]. Other FAs that differ between populations include the trans-FAs, and the n-6/n-3 ratio of polyunsaturated FAs (PUFAs) [6]. We have also reported that the branched-chain FAs (BCFAs) of human milk differ between populations [7]. Evidence indicates that the FA dietary profile of infants influences the risk of inflammatory conditions and neurodevelopment, and is relevant to later-life cardiovascular health [8].

The fat-soluble vitamins are also important contributors to the lipid fraction of human milk. Human milk typically has adequate levels of fat-soluble vitamins A and E to meet infant needs (Table 2), though there is variation between populations. However, human milk is typically low in vitamins D and K. Vitamin D supplementation of 400 IU/day for all infants is a current global consensus recommendation by 11 international scientific organizations for the prevention and management of nutritional rickets [9]. Fat-soluble vitamins perform important health functions and can be stored in the liver and fat tissue until required. Because they are fat soluble, these vitamins are absorbed from the diet through the small intestine along with dietary fat and are readily stored for use. Below, we briefly review the FAs, followed by the fat-soluble vitamins of human milk.

**Human Milk Fatty Acids**

**Description**

FAs are carboxylic acids with long aliphatic chains. In human milk, the FAs are found in saturated, monounsaturated, polyunsaturated, and branched forms. The preponderance of human milk FAs are long-chain FAs, which include tails of 13–21 carbons, but, human milk also includes medium-length FAs, including 8–12 carbon tails, and very-long-chain FAs, with tails of 22 or more carbons. Compared to cow’s milk, human milk contains a higher proportion of PUFAs and long-chain PUFAs (LCPUFAs) [1]. Most human milk FAs are unbranched, but human milk also contains forms of BCFAs ranging from 14 to 18 carbon
chains [7]. About two-thirds of human milk fat is composed of 3 major FAs: oleic (c18:1 n-9, a monounsaturated FA); palmitic (c16:0, a saturated FA); and linoleic (c18:2 n-6, a PUFA). While these 3 FAs are consistently dominant, the exact FA quantities and profile of human milk otherwise varies significantly between mothers and populations (Fig. 1) [6, 7].

### Table 1. Dietary factors associated with varying concentrations of fatty acids (FAs) in human milk

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Associated factor(s)</th>
<th>First author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHA</td>
<td>Increased with intake of fish and other DHA-rich foods; differs by population Lower in milk of mothers who deliver preterm</td>
<td>Martin [6], 2012</td>
</tr>
<tr>
<td>Branched-chain FAs</td>
<td>Dairy and beef consumption associated with higher levels of specific branched-chain FAs</td>
<td>Dingess [7], 2017</td>
</tr>
<tr>
<td>Trans-FAs</td>
<td>Higher in westernized populations</td>
<td>Martin [6], 2012</td>
</tr>
<tr>
<td>n-6:n-3 ratio</td>
<td>Higher in westernized populations</td>
<td>Martin [6], 2012</td>
</tr>
</tbody>
</table>

C/T, ratio of Cincinnati to Tsimane values. Bolded ratios indicate those that are 0.5-fold or lower (increased in Tsimane) or nearly 2-fold or higher (increased in Cincinnati).
Factors Affecting Varied Concentrations

Many FAs of human milk vary between populations, but some vary more than others. DHA is one of the most-well-studied FAs of human milk. DHA (C22:6) is a critical n-3 PUFA, and its contribution to human milk content is significantly lower in populations with low DHA dietary intake. Martin et al. [6] reported twofold greater levels of DHA and other n-3 FAs in the milk of the Tsimane, a Bolivian forager population, compared to mothers residing in Cincinnati, OH, an urban, midwestern US city. Consistent with known differences in diet, the milk of Cincinnati mothers had a significantly higher ratio of n-6/n-3 FAs, and twofold increased linoleic acid and total trans-FAs compared to Tsimane mothers. Differences in FA composition have been observed within the United States. A comparison of donor human milk from 6 milk banks across the US found a trend towards linoleic and other FA profile differences in individual milk samples donated from different regions of the United States [5]. In a study of human milk FA composition in the US over nearly 60 years, Ailhaud et al. [10] reported a threefold rise in linoleic acid between about 1945 and 2005. Thus, some of the differences now observed between populations may be due to relatively recent changes in dietary fat sources.

Table 2. Reported human milk vitamin A and E values in global studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Populations</th>
<th>Human milk vitamin A (retinol) concentration</th>
<th>Human milk vitamin E (α-tocopherol) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campos [23], 2007</td>
<td>Brazilian primiparous and multiparous women</td>
<td>1,730 μg/L</td>
<td>6,990 μg/L</td>
</tr>
<tr>
<td></td>
<td>n = 18</td>
<td></td>
<td></td>
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<tr>
<td>Kim [31], 2017</td>
<td>Korean women</td>
<td>396 + 196 μg/L</td>
<td>2,300±1,300 μg/L</td>
</tr>
<tr>
<td>Mature milk</td>
<td>n = 334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samano [29], 2017</td>
<td>Mexican women</td>
<td>430 μg/L</td>
<td>1,750 μg/L</td>
</tr>
<tr>
<td>Preterm vs. term pregnancy, n = 95</td>
<td>(IQR: 360–660)</td>
<td>(IQR: 1,030–4,500)</td>
<td></td>
</tr>
<tr>
<td>Mature milk</td>
<td>520 μg/L</td>
<td>2,850 μg/L</td>
<td></td>
</tr>
<tr>
<td>(IQR: 370–720)</td>
<td>(IQR: 4,540–8,660)</td>
<td>Term pregnancy (p = 0.03)</td>
<td></td>
</tr>
<tr>
<td>Engle-Stone [25], 2014</td>
<td>Cameroon women</td>
<td>2.4 μmol/L – lowest 30th percentile Intake of vitamin A in previous day</td>
<td>4.7 μmol/L – highest 30th percentile Intake of vitamin A in previous day (p &lt; 0.05)</td>
</tr>
<tr>
<td>n = 440</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olafsdottir [24], 2001</td>
<td>Icelandic women</td>
<td>584 μg/L</td>
<td>4,070 μg/L</td>
</tr>
<tr>
<td>Milk from mature milk</td>
<td>(IQR: 416–732)</td>
<td>(IQR: 2,791–5,147)</td>
<td></td>
</tr>
<tr>
<td>WHO recommendation, daily intake</td>
<td>375 μg/L</td>
<td>2,700 μg/L</td>
<td></td>
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</tbody>
</table>
We compared FAs of milk from mothers in Shanghai, China; Mexico City, Mexico and Cincinnati, OH, USA and identified another intriguing population level difference: The BCFA content was highest in women residing in Cincinnati, followed by women in Mexico, and lowest among women in Shanghai. Higher dietary intake of dairy foods was significantly associated with higher levels of the BCFAs iso C14:0, anteiso C15:0, and iso C16:0. Higher beef intake was associated with significantly higher levels of the BCFA iso C16:0 in human milk.

In addition to diet, the LCPUFA composition of human milk can also be influenced by polymorphism in maternal FA desaturase (FADS) genes, which are involved in FA elongation. In humans, FADS1 and FADS2 genes influence the ability to synthesize LCPUFAs; thereby, they modify the concentration of LCPUFA in human milk, and, specifically, the impact of fish intake on the DHA composition of human milk.

It is noteworthy that proteomic analysis of samples from 3 global populations found that FA synthesis proteins consistently increase over the course of lactation. This finding suggests that early in lactation, the FAs found in human milk...
milk may be derived more from direct blood influx (dietary sources), while late in lactation, human milk FAs may be derived more from de novo mammary synthesis (which may indicate a greater potential genetic influence).

**Physiological Effects**

Human milk FA composition may have a powerful influence on infant health. FAs regulate intracellular signaling and affect inflammatory response, cardiovascular development, and central nervous system development and function [14]. Thus, it is intriguing that infants worldwide can be exposed to significantly different FA profiles in their mother’s milk. The current Western diet may be reasonably represented by Cincinnati mothers, who have high BCFA, linoleic acid, and trans-FA contents, and low ω-3 and monounsaturated FA contents in their milk, than the milk of women in Tsimane, who represent the traditional dietary pattern. The physiological or metabolic impact these differences remains to be determined.

There is strong and growing evidence, however, that the FA content of mother’s own milk and donor milk are insufficient to meet recommendations for the health and nutrition in preterm infants. Several randomized, controlled trials have reported that supplementation of pregnant mothers with DHA contributes to longer gestation and greater infant birth weight [14]. Consistent with that finding, DHA levels are low in the milk of preterm infants [4, 15]. Further, it was found that the level of DHA measured in donor milk was also too low to support the nutritional needs of preterm infants. Preterm infants are at further risk of DHA deficiency because the accretion of DHA occurs in utero predominantly during the last trimester of pregnancy, a period that preterm infants have missed. The lack of endogenous DHA stores and low DHA levels in maternal or donor milk appear to place preterm infants at high risk of adverse outcomes during their hospitalization. In a cohort of preterm infants <30 weeks gestation, low DHA levels and increased linoleic acid/DHA ratios were associated with chronic lung disease and late onset of sepsis [15]. These and other findings provide a strong argument for attending to maternal and infant FA nutrition, including the FA composition of human milk.

**Fat-Soluble Vitamins of Human Milk**

**Description**

The fat-soluble vitamins – vitamins A, D, E, and K – are critical to infant health. Fat-soluble vitamins are absorbed from the diet through the small intestine along with dietary fat. They are readily stored for use and tend to persist in the body. Levels of fat-soluble vitamins in human milk are thus typically stable. The
quantities of vitamins A and E in human milk appear typically adequate to meet infant needs, though limited in some vitamin-A-deficient mothers (Table 2). However, vitamin D is typically absent in human milk [16], though recent data indicate that vitamin D supplementation of lactating women with 6,400 IU/day can safely produce adequate levels of milk vitamin D to satisfy the requirements of nursing infants [17, 18]. Like Vitamin D, vitamin K is also low in human milk and typically provided directly to newborns.

Vitamin A
Vitamin A refers to a set of related compounds that include preformed vitamin A and provitamin A carotenoids. Once consumed, these forms are converted and stored as retinol, which is used as the measure of vitamin A equivalence. Preformed vitamin A is predominantly obtained from the liver, fish oil, milk, and eggs. The provitamin A carotenoids are dietary vitamin A precursors obtained from plant foods. The most important provitamin A carotenoid is β-carotene. α-Carotene and β-cryptoxanthin also contribute some provitamin A activity.

Vitamin A plays a key role in vision, bone growth, reproduction, immunity, cell development, and skin health. Retinol and its metabolites regulate many functions in the body, including maintenance of epithelial cell integrity [19]; expression of genes that encode structural proteins, enzymes, extracellular matrix proteins, and retinol-binding proteins and receptors; and maintenance of immune function [20]. In the eye, these molecules are responsible for the differentiation of the cornea and conjunctiva, for the activity of retinal photoreceptor cells, and for changing light to neural signals for vision. Retinol and its metabolites are especially critical in early development.

Vitamin A deficiency remains a major health problem of low-resource countries and results in impaired resistance to infection, xerophthalmia, blindness, and increased risk of mortality (Table 3). As many as 190 million preschool-aged children and 19 million pregnant women suffer from vitamin A deficiency according to WHO estimates [21, 22]. Based on observations of breastfed infants in communities in which good nutrition is the norm, WHO set the recommended dietary intake for infants <6 months as 375 μg retinol equivalents (RE) per day. For an exclusively breastfed infant consuming between 650 and 750 mL per day, meeting the target intake could require a milk concentration as high as 500–600 μg/L RE/day. In healthy women with adequate vitamin A nutrition, these levels may be exceeded (Table 2) [23, 24], while in some populations, reported concentrations appear to be just meeting or modestly below the WHO-recommended intake (Table 2). In some low-resource regions, as reported in Cameroon [25], concentrations of retinol may be low among women with lim-
ited dietary sources of vitamin A. Despite finding vitamin A levels in the milk of a vitamin-A-deficient mother to be less than ideal in such populations, they are considered adequate to help reduce the risk of xerophthalmia in the infant [20]. Prenatal vitamin supplementation is effective in increasing maternal serum and breast milk concentrations [3]. In populations at high risk of vitamin A deficiency, maternal supplementation programs have focused on pregnant mothers and infants after 6 months of age. In preterm infants in high-resource countries, vitamin A supplementation of the infant during hospitalization is a priority, as preterm infants are typically born with low vitamin A stores.

The vitamin A content of human milk varies also in relation to the dietary sources of vitamin A. Analysis of provitamin A carotenoids in milk samples from China, the US, and Mexico found that the most abundant provitamin A carotenoids was β-carotene, followed by β-cryptoxanthin and α-carotene [26]. Chinese mothers had significantly higher levels of carotenoids in their milk than US and Mexican mothers, likely due to maternal dietary differences (Table 3). However, while these carotenoids contribute to the total retinol activity of human milk, they are far less abundant and efficient than retinol to support the retinol activity of human milk.

**Vitamin D**

Worldwide, studies of vitamin D in human milk have found concentrations to be below detectable levels. Inadequate vitamin D nutrition results in poor bone health and increased risk of infection. Given the absence of vitamin D in breast milk, breastfed infants are at increased risk of vitamin D deficiency, with occurrence of its most severe form – rickets – in many populations. Thus, the global public health recommendation has been to provide breastfed infants with vitamin D supplements after birth to prevent vitamin D deficiency and provide essential support for calcium absorption and bone growth [9]. Recent studies provide an alternative strategy. In a randomized, controlled trial, mothers given 6,400 IU of vitamin D during lactation achieved clinically adequate amounts of vitamin D in their milk to satisfy infant needs during early infancy (Table 3) [17]. Nevertheless, the recommended public health strategy at this time remains direct supplementation of the breastfed infant with vitamin D.

**Vitamin E**

It is comprised of 8 isoforms, including 4 tocopherol isoforms: α-, γ-, β-, and δ-tocopherol. Of these, α-tocopherol is the dominant form of vitamin E in human milk, followed by γ-tocopherol. Vitamin E is a potent antioxidant that protects against free radicals, molecules that cause cellular damage. Vitamin E is also reported to benefit immune health and serves to reduce the risk of blood clot-
Fatty Acids and Fat-Soluble Vitamins in Breast Milk

α- and γ-tocopherol differ by 1 methyl group and have a similar capacity to scavenge reactive oxygen species, but γ-tocopherol may serve as a more potent antioxidant due to its capability to react with reactive nitrogen species. However, α-tocopherol is found at higher concentrations in milk and tissues than γ-tocopherol, likely due to the preferential transfer of α-tocopherol to lipid particles by liver α-tocopherol transfer protein [27].

WHO recommends an infant intake of vitamin E of 2,700 μg/day. Typically, concentrations of vitamin E in human milk from different populations meet this recommendation such that mothers are able to provide this quantity to their exclusively breastfed infants per day (Table 2). However, vitamin E levels have been reported to be considerably higher than the WHO recommendation in some populations [23, 24]. In other populations, e.g., in mothers who have delivered a preterm infant, vitamin E levels may be somewhat lower than recommended (Table 3). The possible impact of lower vitamin E levels on infant health outcomes is understudied.

**Vitamin K**

Vitamin K is responsible for the carboxylation of proteins that bind calcium, which is required for normal coagulation. Thus, vitamin K deficiency can be dangerous and result in delayed coagulation and vitamin K deficiency bleeding. For exclusively breastfed infants, the two sources of vitamin K are mother’s milk and their own endogenous gut bacteria. Human milk is a poor source of vitamin K, containing only 1–4 μg/L. The recommended dietary intake of vitamin K in infancy is 1 μg/kg body weight/day, which translates to a daily requirement of 5–10 μg/day, a requirement rarely met by human milk consumption. A single placebo-controlled trial showed that supplementing lactating mothers with high-dose vitamin K (5 mg/day) increases the level in their breast milk and is associated with an improved protein carboxylation profile (Table 3) [28–30]. Nevertheless, to assure prevention of early vitamin K deficiency bleeding of the newborn, administration of vitamin K to the newborn is the standard of care.

**Conclusion**

Exclusively breastfed infants rely on mother’s milk to meet their needs. The study of diverse populations has elucidated the compositional description of human milk components and basic understanding of factors that influence human milk composition. In relation to the FAs, 3 FAs consistently form the major part of the FA fraction (oleic, palmitic, and linoleic acids). Nevertheless, considerable variation is seen between populations in the quantity of specific FAs. This varia-
tion is largely due to maternal dietary differences, though genetic polymorphisms can contribute when dietary intakes of specific LCPUFAs are limited. In relation to the fat-soluble vitamins, vitamins A and E are typically present in robust quantities in human milk, though some high-risk mothers (e.g., those who deliver preterm or live in resource-poor countries) have lower than recommended levels. However, vitamins D and K are typically low in human milk, and infant supplementation is the recommended strategy for assuring adequate infant nutrient status. Our review of the literature suggests that the most critical scientific questions are: Do variable levels of FAs in human milk impact the health or development of infants? This question is also pertinent for some of the fat-soluble vitamins of human milk. Greater attention is also warranted to maternal supplementation as an approach to modifying FA levels and fat-soluble vitamins in human milk, particularly in relation to DHA and vitamin D.

Acknowledgment

We gratefully acknowledge Donna Wuest for her expert support in the preparation of this paper.

Disclosure Statement

The authors have no conflicts to disclose.

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