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Review

Human Milk Nutrient Composition Data is Critically Lacking in the United States and Canada: Results from a Systematic Scoping Review of 2017–2022

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A B S T R A C T

Characterization of the nutrients in human milk is important to understand the dietary and developmental requirements of infants. The objective of this review was to summarize the state-of-the-science on the nutrient composition of human milk in the United States and Canada published from 2017 to 2022. Four databases were searched for randomized controlled studies and others given the scoping nature of this review. We limited type to mature milk collected 21 d postpartum and beyond from lactating individuals in the United States and Canada who gave birth at 37-wk gestation or later (full-term). Outcomes of interest included traditional macro- and micronutrients, including human milk oligosaccharides (HMOs), and milk volume. The publication date range was selected as January 1, 2017, to the day the literature search was performed. A total of 32 articles were included in the scoping review from primarily longitudinal cohort or cross-sectional designs. The most prevalent sample collection method was full-breast expression ($n = 20$) with most studies ($n = 26$) collecting samples from a single timepoint. Carbohydrates (HMOs [$n = 12$], glucose [$n = 8$], and lactose [$n = 6$]) and protein ($n = 5$) were the most frequently assessed nutrients in this body of work, with consensus among studies that glucose is present in limited concentrations compared to lactose (24–64 mg/dL compared with 6–7 g/dL) and that HMOs are influenced by temporality and secretor status. Included studies displayed an overall level of heterogeneity and sparsity paralleling previous reports and nutrient data in the USDA FoodData Central system. Much of the data extracted from retained articles generally provided analysis of a specific nutrient or group of nutrients. Moreover, many studies did not use the preferred analytical methods as outlined by the Human Milk Composition Initiative to increase measurement confidence. Up-to-date nutrient composition data of human milk is still greatly needed as it is paramount for the management of infant feeding, assessment of infant and maternal nutritional and health needs, and as a reference for infant formula development.

Keywords: breast milk, nutrition, pediatrics, maternal health, USDA Food Data System, vitamins, minerals

Statement of Significance

The current body of research profiling the nutrient content of human milk cannot be used to update the USDA's FoodData Central based on the scarcity of reliable data derived from preferred analytical and sampling methods. There is a critical need for nutrient composition data, and priorities must be placed on addressing the methodological deficiencies described in this review to enable the development of evidence-based reference values for human milk composition across the course of lactation.

Introduction

Human milk is the universally recognized gold standard of nutrition for infants in the first 6 mo of life and is recommended for up to 2 y and beyond, in combination with solid foods, by the WHO [1]. Exclusive breastfeeding is endorsed on a global scale because human milk confers a constellation of health benefits to human milk-fed infants [1,2]. These health benefits have largely been attributed to the developmentally appropriate nutritional composition and individual constituents of human milk, which

Abbreviations: 2'-FL, 2'-fucosyllactose; AOAC, Association of Official Analytical Collaboration; BCA, bicinchoninic acid; HMCI, Human Milk Composition Initiative; HMO, human milk oligosaccharide; LC-MS, liquid chromatography–mass spectrometry.

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in general consist of water, protein, fat, carbohydrate, and many vitamins and minerals, although emerging evidence reveals the importance of nonnutritive bioactive factors as well [3]. Given that the composition of human milk is incredibly variable and complex [4–7], the profile of human milk is still poorly defined. Many current estimates for standard references of constitutional nutrients are derived from data dating back 4 decades [8]. Characterization of the nutrients and bioactive components in human milk is important to understand the dietary and developmental requirements of infants and to address potential gaps in nutritional needs among infants who do not consume human milk.

Biological and environmental influences may contribute to variations in the composition of human milk. In contrast to commercial infant formula, which is relatively consistent in nutrient composition, human milk composition is dynamic and changes over the course of a single feeding at the breast, over the course of a day, and over the course of lactation [9,10]. The nutrient profile of human milk is also a function of infant (e.g., preterm birth, health status, etc.) [11] and maternal factors including those attributed to differences in geographic location [12], genetic characteristics (e.g., secretor status) [13], and nutritional and metabolic status [14]. Moreover, research design and methodology introduce additional obstacles in characterizing human milk composition, as studies vary in the timing and method of sample collection, characteristics in study populations, and the analytical tools and techniques used to assess individual nutrients [15,16]. Given these considerations, it is prudent to use a systematic approach when evaluating the state-of-the-science on the nutrient composition of human milk. The evidence must be reviewed and synthesized using a standard set of criteria to allow for "apples to apples" comparisons and an overall interpretation of findings without dismissing important evidence and limiting knowledge on the composition of human milk that needs to advance the field further.

Two recent reviews have considered the composition of human milk. A systematic review by Wu et al. [8] focused on the composition of human milk from the United States and Canada to provide up-to-date data to inform the USDA Food Data System. The authors identified 28 papers published between 1980 and 2017 that were conducted in relatively small cohorts of apparently healthy females using a variety of experimental designs and analytical methods. The review concluded that, *1*) concentrations of macronutrients, energy, and certain minerals were relatively consistent from 1 to 6 mo postpartum; *2*) information on other micronutrients and findings beyond 6 mo postpartum were scarce; and *3*) results may not reflect the current American population and their dietary practices given that most studies were conducted before 1990 [8]. A global review by Leghi et al. [17] included 101 studies and reported that fat and protein composition differed by sample collection technique, emphasizing the importance of considering sampling techniques when synthesizing the literature.

As human milk reference values are still lacking in the United States and Canada, the purpose of this review was to summarize the state-of-the-science on the nutrient composition of human milk (i.e., mature human milk fed to infants aged 3 wk and beyond) in the United States and Canada published since the Wu et al. systematic review, with an emphasis on sample collection processes and analytical methods. Ultimately, findings from this review may be used to determine the feasibility of performing a systematic review and meta-analysis, identify gaps in research and funding opportunities for investigators, provide up-to-date data on the nutrient composition of human milk to the USDA FoodData Central system, and guide the development of human milk repositories, databases, and infant formula composition with the overall goal of understanding and enhancing the nutritional status, health and development of all infants.

Methods

This scoping review was conducted according to the requirements of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for Scoping Reviews [18]. The protocol was registered with the Open Science Framework on August 17, 2022 (osf.io/e5bf8). Articles eligible for inclusion and evaluation in this review were required to meet the pre-established inclusion and exclusion criteria (Supplemental Table 1) developed from a pre-established analytical framework (Figure 1). Specifically, the framework and data-charting forms used in this review were developed by the Human Milk Composition Initiative (HMCI) Scientific Steering Committee, which is led by the Pediatric Growth and Nutrition Branch within the National Institute of Child Health and Human Development. The HMCI framework was adapted by content experts (JMM, MTP, and PKB) and reviewers from Academy of Nutrition and Dietetics' Evidence Analysis Center (AEM, KES, and DH) for practical implementation to determine which study characteristics and methodology components to extract. Due to the relevance of the analytical method used to assess a given human milk nutrient, a list of preferred analytical techniques was developed by experts on the HMCI Scientific Steering Committee based on the techniques used to inform nutrient values in the USDA FoodData Central system (Supplemental Table 2) [19–22]. Importantly, no studies were excluded from this review for using alternative analytical techniques.

Eligibility criteria

Study design inclusion extended beyond randomized controlled studies based on the scoping nature of this review (e.g., prospective cohort studies, case-control, and cross-sectional, etc.), though case studies and reviews were excluded. In relation to the human milk sample, we limited the type to mature milk collected 21 d postpartum and beyond from lactating individuals in the United States and Canada who gave birth at 37-wk gestation or later (full-term). In addition, any studies designed to examine the effects of a nutritional intervention (e.g., dietary supplements) were excluded unless data from control or placebo groups were included in an extractable presentation (i.e., numerical data). Outcomes of interest included traditional nutrients (e.g., macronutrients, amino acids, fatty acids, fiber – e.g., human milk oligosaccharides [HMOs], vitamins, minerals) as well as milk volume. The lens for this review was nutritional reference values for food databases; therefore, specific proteins or nonnutritive compounds that might have biological function were not included. The publication date range was selected as 1 January, 2017 to the day the literature search was performed. We selected this more recent starting date to provide an update to the review by Wu et al. [8] (search performed from 1 January, 1980 to 31 December,

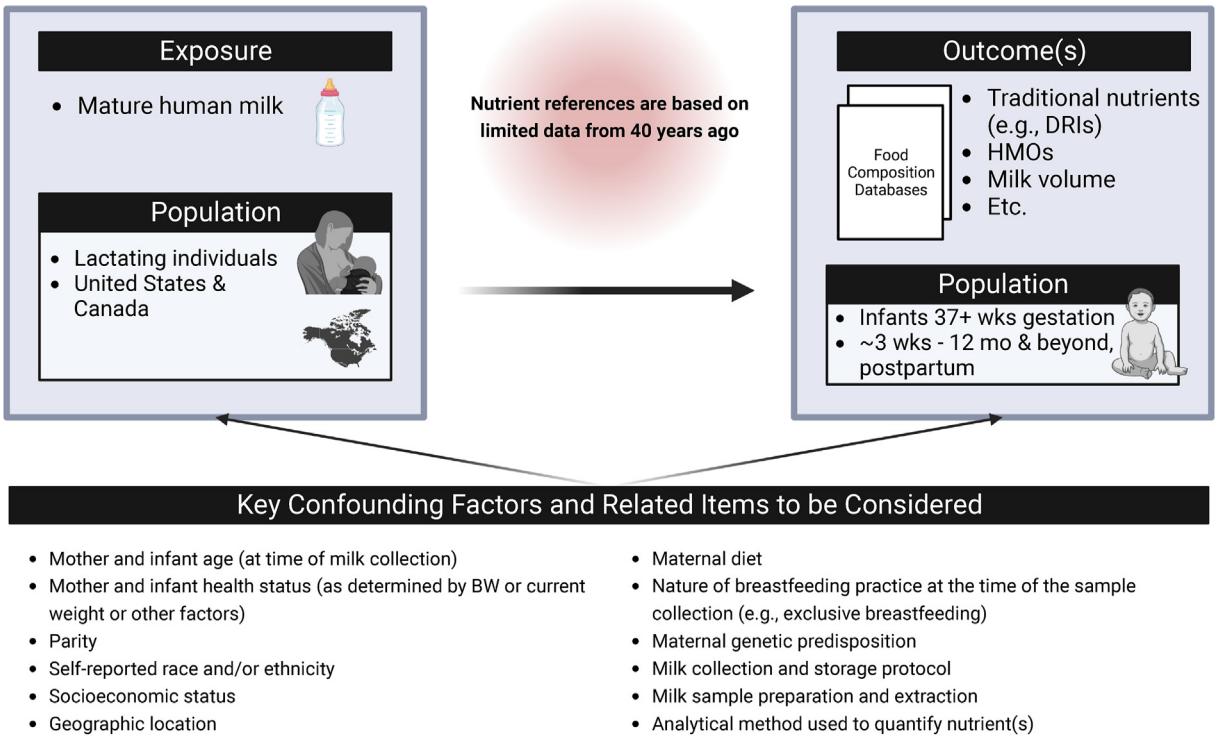

FIGURE 1. Analytical framework used to guide the scoping review of nutrient composition of human milk in the United States and Canada from studies published from 2017 to 2022. Abbreviations: DRI, Dietary Reference Intake; HMO, human milk oligosaccharide.

2017). We allowed for overlap in 2017 to capture potential articles that were not extracted by Wu et al. (e.g., articles ahead of print). Only peer-reviewed articles with research conducted in the United States and Canada and published in English were considered (Supplemental Table 1).

Search strategy

The literature search was performed on 15 April, 2022, using the electronic databases MEDLINE (Ovid), CINAHL (Ebsco), the Agricultural & Environmental Science Collection (ProQuest), and Food Science and Technology Abstracts (Web of Science) to capture as many relevant articles as possible (for full search strategy see Supplemental Table 3). Articles captured from the database searches were then uploaded into Rayyan, a software program for title/abstract screening [23], and reviewed independently (AEM, KES, and DH). Included articles were then full-text screened, again independently, by reviewers (AEM and KES). The screening process involved 2 passes based on the volume of articles and the specifications for milk sample collection characteristics and nutrient analysis techniques. During the first pass, articles were included if the following requirements were all satisfied: *1*) The study was conducted in the United States and/or Canada; *2*) The participants (mother and infant) had the appropriate health status (i.e., healthy mothers who were lactating with infants born full-term [≥37 wk and 0/7 d gestational age]); *3*) The study had the appropriate intervention and/or exposure (i.e., mature human milk collected at approximately 3-wk or 21-d lactation and beyond); *4*) The study focused on nutrient composition and not only on analytical method development; *5*) Relevant outcomes were assessed; and *6*) The data were presented in numeric form, appropriate for extraction and subsequent synthesis. Articles that met all requirements were then assessed for study methodology using the following: *1*) Were the study participants described in the appropriate detail (e.g., nature of breastfeeding practice [e.g., exclusive breastfeeding, predominant, partial, or how the authors defined], etc.)? *2*) Was the preferred sample collection method used (e.g., full-breast expression; fore- and hindmilk) and were other sample collection characteristics documented (e.g., milk expression technique; time of day sample was collected; interval of time since last feed; breast used for collection (left, right, or both)?; and *3*) Was the HMCI's preferred analytical technique used for the nutrient assessed (e.g., protein assessed via the modified Kjeldahl method with removal of nonprotein nitrogen by acid precipitation) [24]? Any discrepancies were discussed and resolved to reach consensus. Data from articles included in the full-text review were extracted to standardized templates (Supplemental Table 2 and Supplemental Table 4) developed from the pre-established analytical framework and data-charting form.

Data-charting process

Data were independently charted, results discussed, and the data-charting form updated continuously in an iterative process. Data items included bibliographic information, participant characteristics (e.g., age, sex, race/ethnicity), intervention description (e.g., intervention duration and follow-up duration), study methodology, comparator description, outcomes reported, drop-out rate, and adverse events. To present a summary of the findings, the number of extracted articles, participants, and milk samples were summed for each nutrient overall and for those using the preferred methodology. In addition, where possible, the articles from Wu et al. (2018) [8] were tabulated in the same fashion and compared to the body of literature in this scoping review.

Results

Study selection

The initial literature search yielded 12,322 records from MEDLINE (Ovid), CINAHL (Ebsco), the Agricultural & Environmental Science Collection (ProQuest), and Food Science and Technology Abstracts (Web of Science). After removing duplicate records, 6232 unique records were screened for eligibility by title and abstract. Of these, 5766 were excluded as they did not meet the defined inclusion criteria. Next, the full texts of the remaining 466 articles were retrieved and assessed for inclusion via 2 levels of screening. Ultimately, a total of 32 articles were included in the scoping review, representing 0.51% of the initial unique records identified (Figure 2).

Study characteristics

Individual characteristics of the extracted articles including study design, study duration, postpartum time of sampling, and maternal/infant characteristics are presented in Supplemental Table 5. Briefly, most of the studies were longitudinal cohort designs [13,14,25–45] or cross-sectional studies [46–51]. Only a few reported the use of parallel designs [52,53] and crossover [54]. In addition, 28 were conducted within the United States, with only 5 conducted in Canada [13,33,40,47,53], though several of the studies conducted in Canada were multisite cohort studies with participants from 4 or more provinces [13,33]. Thus, when assessing the total sample pool from each country, Canadian studies included 1079 participants and United States studies included 2525 participants. Overall, most studies had less than 100 participants, though there were many exceptions [13,33,35,37,38,40–45,53]. The study duration and number of milk samples collected was more variable, with multiple studies assessing 2 or more time points [13,14,25–32,37,41,42,44,52]. Only 3 studies explored the composition of human milk beyond 6 mo postpartum [26,27,42]. In relation to maternal characteristics, the mean age of most participants was approximately 30 y. Some earlier studies did not report race/ethnicity, yet most of the later studies did. Greater representation of diverse populations was apparent with several studies, including some that exclusively investigated Hispanic individuals [34,35,37,42]. Other characteristics such as health status were less defined,

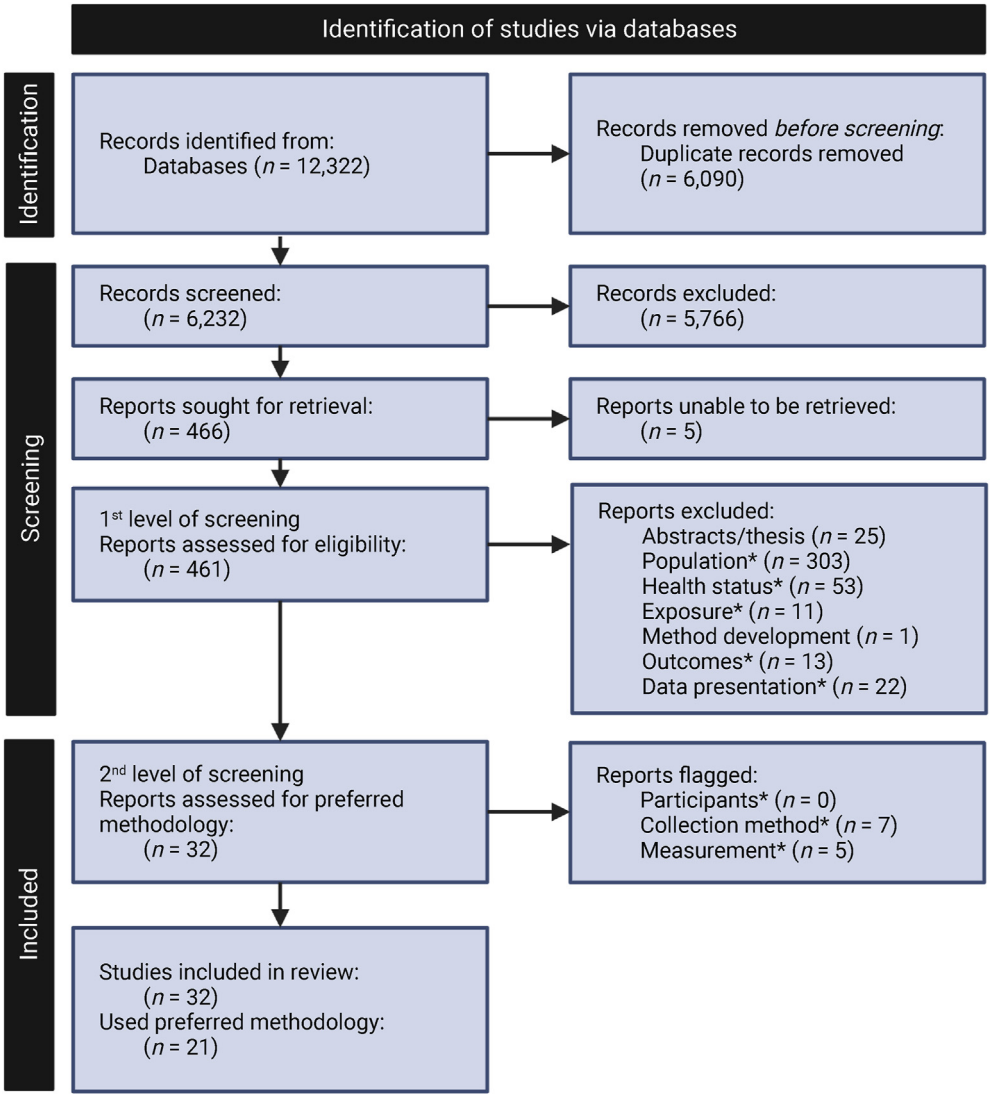

FIGURE 2. Flow diagram of study identification and screening process according to the 2020 PRISMA guidelines. *Note*: *Denotes studies that did not use preferred methods.

though when reported, BMI was generally within the normal/-overweight range. Finally, all infants were healthy full-term (>37 wk) as specified in our inclusion criteria, and infant sex, when reported, was relatively evenly distributed (for details see Supplemental Table 5).

Milk sample collection characteristics

Human milk sample collection characteristics are outlined in Supplemental Table 6. The most prevalent sample collection method was full-breast expression (study $n = 20$), followed by fore- and hindmilk ($n = 4$) [13,33,37,39] and midmilk ($n = 2$) [29,47]. Several studies did not describe the sample collection method ($n = 7$) [28,31,32,48,51,52]. Most samples were obtained from a single timepoint ($n = 26$). There were also samples taken over a 24-h period ($n = 2$) [13,33] and 1 over a 3-h period [50]. Three articles did not report whether or not multiple milk samples were pooled [28,48,52]. Most milk samples were expressed via an electric breast pump ($n = 17$) [14,25,30,31,34–37,40–42,44–46,50,53,54]. Others included samples expressed by hand or with a hand pump ($n = 7$) [13,26,33,39,45,47,49] or did not describe the milk expression technique [27,28,32,38,43,48,51,52]. Most articles reported that samples were from one breast ($n = 20$), though 3 were mixed [36,40,50], and 9 did not describe this information [13,28,29,32,33,47,48,51,52]. The time since the last milk expression or feeding was not described in 15 of the included studies [13,14,25,28,30,31,33,36,46,48,50–54], though when reported, 9 were collected approximately 1.5 to 2 h since the last feed/expression [26,34,35,37,40–42,44,49], 7 were collected during the feeding period [27,29,32,38,43,45,47], and 1 collected milk before and after the participants breastfed their infant for 6 to 10 min in accordance with the participant's perceived milk availability and the infant's feeding rate [39]. The time of day that most samples were collected was described as the morning [25,27,30,31,34,38,39,43,45,49,50,54], morning and afternoon [41,44], over a 24-h period [13,33,48], and around breakfast, lunch, and dinner [47]. As with many of the collection characteristics, multiple studies did not describe the collection time of day [14,26,28,29,32,35–37,40,42,46,51–53].

Human milk characteristics and nutrients

Our literature search revealed a greater number of publications with each advancing year (Figure 3A). Broadly assessing the number of studies that examined a characteristic and/or nutrients, we noted that carbohydrates (HMOs, glucose, and lactose) and protein were most frequent in this body of work (Figure 3B). The studies included in the present scoping review displayed an overall level of sparsity compared to nutrients included in the Standard Reference database (Figure 3C).

Energy density

Of the extracted articles, only 3 reported energy (kcals) values, and none described accounting for the metabolizable energy of fermentable carbohydrates (e.g., HMOs), which the FAO of the United Nations recommends as the preferred method for reporting energy in foods [21,22]. In 2 studies, caloric density was measured indirectly by calculating the summation of fat, protein, and lactose assuming 9 kcal/g, 4 kcal/g, and 4 kcal/g, respectively [29,32], while in the other study, the methods determining energy were not described [36] (Table 1). Importantly, the methods for collecting the sample and/or the methods for analyzing the underlying macronutrients in these studies were not based on preferred methods. Therefore, energy values reported by these studies (57 to 68 kcal/dL) should be interpreted with caution. When studied longitudinally at 1, 2, 3, and 4 mo postpartum, energy was not significantly different between time points (range: 64.1 ± 14.4 kcals/100 mL to 68±17 kcal/100 mL) and did not vary between normal weight and obese mothers [29].

Milk volume

Milk volume was only assessed in 4 studies, with 2 studies reporting on the volume of milk collected at a single feed [27,41] and 2 studies reporting on the volume of milk produced across a 24-h period [48,50]. Studies that reported 24-h milk volume used the test weighing methods [48,50]. Carrega et al. [48] reported 24-h milk volumes of 602 ± 214 mL at 1 mo postpartum, and Roznowski et al. [50] reported 24-h milk volumes of 717±119 g at approximately 2 mo postpartum. When studied longitudinally using a sample collected from a single full-breast expression at each time point, Perrin et al. [27] described a decrease from 11 to 17 mo postpartum (59±28 mL compared with 35±19 mL). As volume decreased, the nutrient concentration increased (total protein, fat, potassium, iron, and sodium), except for lactose, calcium, zinc, and HMOs. More proximal to birth, Nagel et al. [41] examined milk volume 2 h after feeding from a single complete breast expression at 1 and 3 mo postpartum. They found no significant difference between the 2 time points (1-mo: 69.3±2.34 mL; 3-mo: 72.5±2.67 mL). When volume was assessed longitudinally within a 24-h period at approximately 2 mo postpartum, Roznowski et al. [50] reported a decline in milk production rates from time point 1 (both breasts: 60±26 g/h) to time point 3 (43±13 g/h).

Carbohydrate

As a nutrient class, carbohydrates were profiled more than any other outcome in the included articles. Lactose, the most abundant carbohydrate in human milk, was presented in 6 articles [25,27,29,31,32,54] (Table 1). Lactose values were not consistent between studies using liquid chromatography–mass spectrometry (LC-MS) and ranged from 5.5 g/dL to 7.8 g/dL, which may be explained by differences in lactation stage and/or by methodological differences. When studied longitudinally, lactose concentrations were generally stable between 1 and 6 mo postpartum, and between 11 and 17 mo postpartum [25,27,32,54]. One exception was Young et al. [29], who noted an increase in lactose from 1 to 4 mo postpartum.

HMOs were the most studied carbohydrate, with concentrations assessed in 12 studies [13,27,28,31,33–35,37,38,40,42,43]. All studies used the preferred method of chromatography to separate HMOs, followed by a variety of detection techniques including fluorescence [13,33–35,37,38,40,42,43], mass spectrometry, and pulsed amperometric detection. Although concentrations were variable across studies, they were generally within range of previously established figures of 5 to 20 g/L in mature milk [4,55]. Total HMO concentrations were higher in secretors compared to nonsecretors [13,42,43]. When studied longitudinally, 3 studies reported that total HMOs and specific HMOs declined between 1 and 6 mo postpartum [28,34,37]. Between 6 and 24 mo postpartum, results were less clear, with Plows et al. [42] reporting differences by secretor status

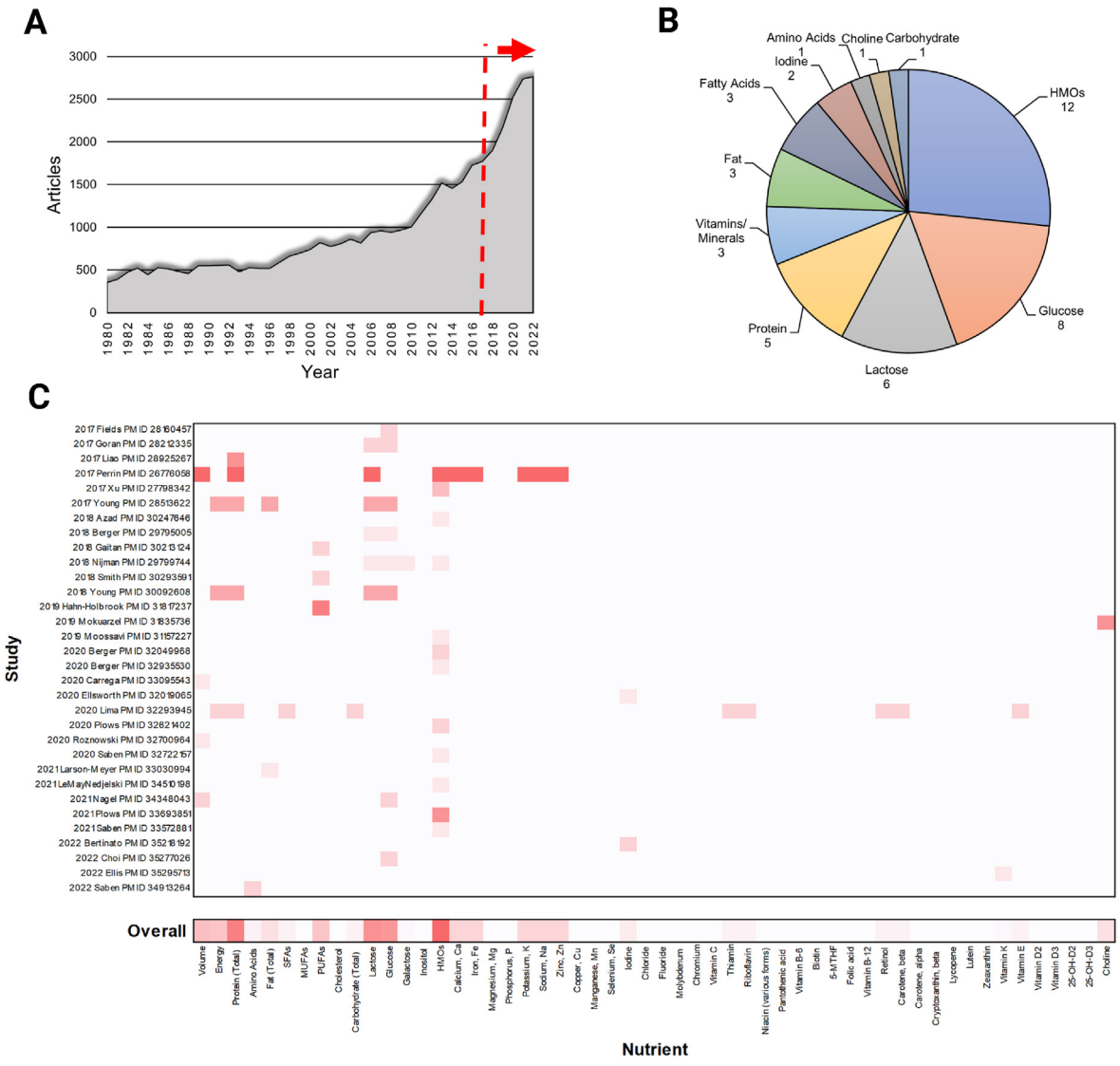

FIGURE 3. (A) Number of articles published from 1980 to 2022 using the search term 'human milk' (PubMed, 1 January, 1980, to 31 December, 2022). (B) Number of articles from the included studies that reported on a particular nutrient. (C) Heatmap of nutrients assessed by each included study. Intensity of cell color is based on number of sample collections. Abbreviation: HMO, human milk oligosaccharide.

(declining total HMOs in secretors and relatively stable total HMOs in nonsecretors), whereas Perrin et al. [27] reported an increase in total HMOs between 11 and 17 mo postpartum.

Glucose was the most frequently studied monosaccharide, with 8 studies reporting glucose concentrations [14,25,29,31,32,41,44,54] and 2 of these studies using the preferred analytical technique (i.e., LC-MS or HPLC) [31,54]. Glucose concentrations at approximately 6 wk postpartum were in the range of 24 to 64 mg/dL when assessed using a preferred analytical technique. All other analytical techniques consistently reported glucose concentrations between 25 and 30 mg/dL (Table 1). In studies that reported longitudinal assessments, glucose concentrations were relatively stable between 1 and 6 mo postpartum [25,32,41,44]. Relative to lactose concentrations, glucose concentrations were less than 1% of lactose concentrations (24–64 mg/dL compared with 6–7 g/dL). Galactose [31] and fructose [25] were each assessed in a single study (Table 1).

Protein

For the purposes of this paper, we use the term "overall protein" to refer to all protein in human milk. We have intentionally not used the terms "total protein," "crude protein," and "true protein" as they have unique meanings with some methods of analysis. Specific proteins that may confer biological benefits to an infant (e.g., lactoferrin, lysozyme, hormones) were not included in the scope of this review. Five studies included in this review assessed the overall protein content of human milk [26,27,29,32,36], though only one study employed the preferred analytical technique of a modified Kjeldahl analysis [26], while the remainder implemented the Bradford method [29,32], bicinchoninic acid (BCA) assay [27], or spectrometry [36] (Table 1). Longitudinal studies that included the early postpartum period reported declining overall protein content [26,29], whereas studies in extended lactation reported increasing content beyond 1 y [27]

TABLE 1
Articles published from 2017 to 2022 reporting macronutrient data from human milk samples

Article	Concentration	Analytical technique used by authors	Preferred method employed?
~ Energy ~			
2017 Young [29]	1-mo (n = 34): 64.1±14.4 kcal/100 mL	Indirect calculation	NA
2018 Young [32]	2-mo (n = 41): 66.6±19.1 kcal/100 mL 3-mo (n = 41): 64.6±14.4 kcal/100 mL 4-mo (n = 41): 67.5±16.9 kcal/100 mL		
2020 Lima [36]	>1-mo (n = 13): 57.1±0.7 kcal/100 mL	Not described	NA
~ Glucose ~			
2017 Fields [14]	1-mo (n = 37): 25.4±9.0 mg/dL 6-mo (n = 30): 25.6±7.5 mg/dL	Glucose oxidase method	No
2017 Goran [25]	1-mo (n = 25): 263.6±87.5 μg/mL 6-mo (n = 25): 246.8±76.8 μg/mL	Glucose oxidase method	No
2017 Young [29]	1-mo (n = 34): 24.7±6.9 mg/dL 2-mo (n = 32): 25.2±5.8 mg/dL 3-mo (n = 30): 26.3±5.9 mg/dL 4-mo (n = 40): 26.2±7.4 mg/dL	Hexokinase ultraviolet assay	No
2018 Berger [54]	6-wk (n = 41): 0.64±0.3 mg/mL	LC-MS	Yes
2018 Nijman [31]	42-d (n = 10): 0.236 ± 0.009 g/L	HPAEC−PAD	Yes
2018 Young [32]	1-mo (n = 41): 25.2±6.5 mg/dL 2-mo (n = 41): 25.2±5.8 mg/dL 3-mo (n = 41): 26.3±5.9 mg/dL 4-mo (n = 41): 26.2±7.4 mg/dL	Radioimmunoassay	No
2021 Nagel [41]	1-mo (n = 94): 25.9±1.68 mg/dL (FF) 1-mo (n = 269): 30.7±0.57 mg/dL (FB)	Glucose oxidase method	No
2022 Choi [44]	1-mo (n = 151): 29.66±0.76 mg/dL 3-mo (n = 151): 28.94±0.76 mg/dL	ELISA	No
~ Lactose ~			
2017 Goran [25]	1-mo (n = 25): 7.8±0.8 g/dL 6-mo (n = 25): 7.5±0.7 g/dL	LC-MS	Yes
2017 Perrin [27]	11-mo (n = 19): 5.7±0.7 g/dL 12-mo (n = 19): 6.0±0.8 g/dL 13-mo (n = 19): 5.6±0.7 g/dL 14-mo (n = 19): 5.9±0.8 g/dL 15-mo (n = 18): 5.7±0.8 g/dL 16-mo (n = 18): 5.5±0.5 g/dL 17-mo (n = 18): 5.6±0.9 g/dL	LC-MS	Yes
2017 Young [29]	1-mo (n = 34): 7.4±1.3 g/dL 2-mo (n = 32): 7.5±1.7 g/dL 3-mo (n = 30): 8.1±0.6 g/dL 4-mo (n = 40): 8.1±0.7 g/dL	Enzymatic assay	No
2018 Berger [54]	6-wk (n = 41): 6.83±1.6 g/dL	LC-MS	Yes
2018 Nijman [31]	42-d (n = 10): 56.7±0.92 g/L	HPAEC−PAD	Yes
2018 Young [32]	1-mo (n = 41): 7.6±1.0 g/dL 2-mo (n = 41): 7.7±1.3 g/dL 3-mo (n = 41): 8.1±0.6 g/dL 4-mo (n = 41): 8.1±0.7 g/dL	Enzymatic assay	No
~Galactose~			
2018 Nijman [30]	42-d (n = 10): Trace amounts g/L	HPAEC-PAD	Yes
~Fructose~			
2017 Goran [24]	1-mo (n = 25): 7.2±1.72 μg/mL 6-mo (n = 25): 6.3±1.70 μg/mL	LC-MS	Yes
~ HMOs ~			
2017 Perrin [27]	11-mo (n = 19): 7.0±2.1 mg/mL 12-mo (n = 19): 7.5±1.9 mg/mL 13-mo (n = 19): 7.2±2.6 mg/mL 14-mo (n = 19): 8.3±3.4 mg/mL 15-mo (n = 18): 8.3±2.3 mg/mL 16-mo (n = 18): 8.7±2.9 mg/mL 17-mo (n = 18): 8.8±3.8 mg/mL	HPLC	Yes
2017 Xu [28]	26-d (n = 26): 16.3±2.7 g/L 71-d (n = 31): 10.4±1.4 g/L 120-d (n = 23): 8.64±1.30 g/L	LC-MS	Yes
2018 Azad [13]	Nonsecretor 3- to 4-mo (n = 120): 8.94±1.51 μmol/L Secretor 3- to 4-mo (n = 307): 15.90±2.80 μmol/L	HPLC/FLD	Yes
2018 Nijman [31]	42-d (n = 10): 6.38±0.29 g/L	HPAEC−PAD	Yes
2019 Moossavi [33]	17-wk (n = 393): 10.2±2.1 mg/mL	HPLC	Yes

(*continued on next page*)

TABLE 1 (*continued*)

Article	Concentration				Analytical technique used by authors	Preferred method employed?
2020 Berger [35]	1-mo (n = 50): 11441±1356 μg/mL				HPLC/FLD	Yes
2020 Plows [37]	1-mo (n = 157): 11426.4±1362.9 μg /mL 6-mo (n = 69): 10144.1±1198.0 μg /mL				HPLC	Yes
2020 Saben [38]	2-mo (n = 136): 15008.35 (12668.05, 15657.68 Quartiles 1 and 3) nmol/mL				HPLC/FLD	Yes
2021 LeMayNedjelski [40]	3-mo (n = 107): 11.0 (8.50, 11.8) mg/mL				HPLC	Yes
2021 Plows [42]	Nonsecretor	1-mo (n = 24): 8440 (8060, 9320 IQR (interquartile range)) μg/mL 6-mo (n = 15): 7960 (7870, 8610) μg/mL 12-mo (n = 7): 7610 (7110, 7990) μg/mL 18-mo (n = 5): 7590 (7300, 8280) μg/mL 24-mo (n = 2): 7890 (7840, 7930) μg/mL			HPLC/FLD	Yes
	Secretor	1-mo (n = 183): 12100 (11700, 12400) μg/mL 6-mo (n = 104): 11200 (10400, 11900) μg/mL 12-mo (n = 76): 10600 (9830, 11300) μg/mL 18-mo (n = 54): 10100 (9830, 11000) μg/mL 24-mo (n = 26): 9350 (8980-9930) μg/mL				
2021 Saben [43]		*Normal weight*	*Overweight*	*Obese*	HPLC/FLD	Yes
	Nonsecretor	2-mo (n = 11): 8200±82 μg/mL 2-mo (n = 57):	2-mo (n = 16): 8278±52 μg/mL 2-mo (n = 35):	2-mo (n = 25): 8384±164 μg/mL 2-mo (n = 50):		
	Secretor	11530±57 μg/mL	11511±57 μg/mL	11618±75 μg/mL		
2020 Berger [34]	DSLNT	1-mo (n = 50): 435±181 μg/mL 6-mo (n = 50): 365±207 μg/mL			HPLC/FLD	Yes
	LNH	1-mo (n = 50): 108±56 μg/mL 6-mo (n = 50): 70.0±47 μg/mL				
	FLNH	1-mo (n = 50): 155±93 μg/mL 6-mo (n = 50): 57.8±71 μg/mL				
~Overall Protein~						
2017 Young [29]	1-mo (n = 34): 1.2±0.2 g/dL 2-mo (n = 32): 1.1±0.6 g/dL 3-mo (n = 30): 0.9±0.1 g/dL 4-mo (n = 40): 0.8±0.2 g/dL				Bradford method	No
2018 Young [32]	1-mo (n = 41): 1.1±0.2 g/dL 2-mo (n = 41): 1.1±0.6 g/dL 3-mo (n = 41): 0.9±0.1g/dL 4-mo (n = 41): 0.8±0.2 g/dL				Bradford method	No
2017 Liao [26]	31-60 d (n = 4): 9.7 g/L 61-120 d (n = 4): 8.8 g/L 121-240 d (n = 4): 8.1 g/L 241-365 d (n = 4): 7.3 g/L				Kjeldahl analysis	Yes
2017 Perrin [27]	11-mo (n = 19): 1.6±0.2 g/dL 12-mo (n = 19): 1.6±0.2 g/dL 13-mo (n = 19): 1.7±0.2 g/dL 14-mo (n = 19): 1.8±0.2 g/dL 15-mo (n = 18): 1.7±0.2 g/dL 16-mo (n = 18): 1.7±0.2 g/dL 17-mo (n = 18): 1.8±0.3 g/dL				Bicinchoninic acid assay	No
2020 Lima [36]	>1-mo (n = 13): 16.01±0.22 mg/mL				Spectroscopy	No
~Amino Acids~						
2022 Saben [45]	AA	*Normal weight*		*Obese*	HPLC	Yes
	His	2-mo (n = 66): 33.5±1.7 nmol/mL 6-mo (n = 53): 28.9±1.4 nmol/mL		2-mo (n = 63): 22.7±1.6 nmol/mL 6-mo (n = 40): 17.7±1.7 nmol/mL		
	Ile	2-mo (n = 66): 10.4±0.6 nmol/mL 6-mo (n = 53): 10.3±0.5 nmol/mL		2-mo (n = 63): 16.3±1.0 nmol/mL 6-mo (n = 40): 14.6±1.0 nmol/mL		
	Leu	2-mo (n = 66): 27.5±1.2 nmol/mL 6-mo (n = 53): 29.2±1.1 nmol/mL		2-mo (n = 63): 37.0±2.3 nmol/mL 6-mo (n = 40): 33.5±1.9 nmol/mL		
	Lys	2-mo (n = 66): 21.6±2.2 nmol/mL 6-mo (n = 53): 19.8±1.9 nmol/mL		2-mo (n = 63): 24.9±2.7 nmol/mL 6-mo (n = 40): 21.0±2.2 nmol/mL		
	Phe	2-mo (n = 66): 11.9±0.7 nmol/mL 6-mo (n = 53): 12.3±0.7 nmol/mL		2-mo (n = 63): 14.3±0.8 nmol/mL 6-mo (n = 40): 16.2±1.1 nmol/mL		
	Val	2-mo (n = 66): 47.7±2.2 nmol/mL 6-mo (n = 53): 42.8±2.0 nmol/mL		2-mo (n = 63): 49.6±1.9 nmol/mL 6-mo (n = 40): 44.9±2.5 nmol/mL		

(*continued on next page*)

TABLE 1 (*continued*)

	Ala	2-mo (n = 66): 188.6±8.7 nmol/mL 6-mo (n = 53): 211.1±8.2 nmol/mL	2-mo (n = 63): 209.7±10.6 nmol/mL 6-mo (n = 40): 210.9±11.6 nmol/mL
	Asn	2-mo (n = 66): 19.0±21.7 nmol/mL 6-mo (n = 53): 23.0±2.2 nmol/mL	2-mo (n = 63): 11.6±1.4 nmol/mL 6-mo (n = 40): 14.2±2.2 nmol/mL
	Asp	2-mo (n = 66): 58.2±4.2 nmol/mL 6-mo (n = 53): 81.2±7.0 nmol/mL	2-mo (n = 63): 87.4±5.9 nmol/mL 6-mo (n = 40): 104.3±8.8 nmol/mL
	Glu	2-mo (n = 66): 851.5±26.6 nmol/mL 6-mo (n = 53): 866.2±24.8 nmol/mL	2-mo (n = 63): 743.0±23.5 nmol/mL 6-mo (n = 40): 789.9±25.5 nmol/mL
	Ser	2-mo (n = 66): 147.3±6.0 nmol/mL 6-mo (n = 53): 171.8±7.7 nmol/mL	2-mo (n = 63): 125.1±4.7 nmol/mL 6-mo (n = 40): 132.9±7.0 nmol/mL
	Cys	2-mo (n = 66): 21.6±1.0 nmol/mL 6-mo (n = 53): 20.9±1.1 nmol/mL	2-mo (n = 63): 14.5±0.8 nmol/mL 6-mo (n = 40): 19.7±1.5 nmol/mL
	Gln	2-mo (n = 66): 406.7±21.5 nmol/mL 6-mo (n = 53): 535.1±21.8 nmol/mL	2-mo (n = 63): 286.2±18.7 nmol/mL 6-mo (n = 40): 337.9±26.3 nmol/mL
	Gly	2-mo (n = 66): 105.9±4.9 nmol/mL 6-mo (n = 53): 126.5±5.0 nmol/mL	2-mo (n = 63): 86.5±4.0 nmol/mL 6-mo (n = 40): 98.5±5.6 nmol/mL
	Pro	2-mo (n = 66): 40.7±1.9 nmol/mL 6-mo (n = 53): 36.0±1.4 nmol/mL	2-mo (n = 63): 42.0±2.3 nmol/mL 6-mo (n = 40): 38.6±1.9 nmol/mL
	Tyr	2-mo (n = 66): 12.9±0.9 nmol/mL 6-mo (n = 53): 13.2±0.9 nmol/mL	2-mo (n = 63): 18.9±1.2 nmol/mL 6-mo (n = 40): 17.3±1.2 nmol/mL

~Total Fat~				
2017 Perrin [27]	11-mo (n = 19): 4.0±2.0 % 12-mo (n = 19): 3.9±2.0 % 13-mo (n = 19): 4.6±1.9 % 14-mo (n = 19): 5.4±2.5 % 15-mo (n = 18): 4.3±2.7 % 16-mo (n = 18): 3.9±1.9 % 17-mo (n = 18): 4.6±2.1 %		NMR spectroscopy	No
2017 Young [29]	1-mo (n = 34): 3.3±1.6 g/dL 2-mo (n = 32): 3.5±1.9 g/dL 3-mo (n = 30): 3.2±1.6 g/dL 4-mo (n = 40): 3.5±1.9 g/dL		Creamatocrit analysis	No
2020 Lima [36]	>1-mo (n = 13): 16.5±0.53 mg/mL		Spectroscopy	No
2021 Larson-Meyer [39]	1-mo (n = 22): 7.4±10.8% (Foremilk) 1-mo (n = 22): 13.0±15.1% (Hindmilk)		Creamatocrit analysis	No
~Fatty Acids~				
2018 Smith [52]	22:6n–3	4-wk (n = 13): 0.48±0.39 nmol/mL 7-wk (n = 13): 0.34±0.18 nmol/mL	GLC	Yes
2018 Gaitan [30]	22:6n–3 20:5n–3	1-mo (n = 24): 2384.71±1140.13 ng/mL 1-mo (n = 24): 1362.93±933.24 ng/mL	LC-MS	Yes
2019 Hahn-Holbrook [46]	Total PUFAs 18:2n–6 18:3n–3 20:4n–6 22:6n–3 20:5n–3	3-mo (n = 52): 227.21±83.57 μg/mL 3-mo (n = 52): 0.54±0.06 μg/mL 3-mo (n = 52): 13.80±6.15 μg/mL 3-mo (n = 52): 1.17±0.94 μg/mL 3-mo (n = 52): 2.72±1.91 μg/mL 3-mo (n = 52): 1.09±1.16 μg/mL	GLC	Yes

Note: Preferred analytical technique was defined by established references and committee expertise [19–22].
Abbreviations: AA, amino acid; Ala, alanine; Asn, asparagine; Cys, cysteine; DSLNT, disialyllacto-N-tetraose; ELISA, enzyme-linked immunosorbent assay; FB, full breastfeeding; FF, formula feeding; FLD, fluorescence detector; FLNH, fucosyllacto-N-hexaose; GLC, gas/liquid chromatography; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; HMO, human milk oligosaccharide; HPAEC–PAD, high-performance anion exchange chromatography with pulsed amperometric detection; HPLC, high-performance liquid chromatography; HPLC/FLD, high-performance liquid chromatography with fluorescence detection; Ile, isoleucine; LC-MS, liquid chromatography–mass spectrometry; Leu, leucine; LNH, lacto-se-N-hexaose; Lys, lysine; MS, mass spectrometry; NA, not applicable; NMR, nuclear magnetic resonance; Phe, phenylalanine; Pro, proline; Ser, serine; Tyr, tyrosine; Val, valine.

(Table 1). In all included studies, overall protein concentrations ranged from ~7.3 g/L to 16.0 g/L across the timespan, as compared with the previously reported range of ~8 to 10 g/L [8]. When only considering studies using the preferred analytical method [26], overall protein concentration ranged from 7 to 10 g/L, which is in better agreement with previously reported ranges, highlighting the importance of analytical methods.

A single study evaluated individual amino acid concentrations, and it used the preferred analytical technique of ultra-HPLC [45]. Sixteen free amino acids were detected in human milk at 2 and 6 mo postpartum, and the majority of these differed significantly by maternal weight status. Longitudinal analysis revealed that the majority of amino acids increased over time, with the exception of histidine, lysine, and proline, which significantly decreased.

Lipids

Total fat was assessed in 4 studies, with none of the studies using the preferred technique of solvent extraction with gravimetry [27,29,36,39]. These studies also used a variety of sample collection techniques, which can profoundly influence

whether a representative milk sample has been acquired for measuring fat. Not surprisingly, a wide range of average fat values were reported including 1.7 g/dL by Lima et al. [36] (full-breast expression), 3.2 to 3.5 g/dL by Young et al. [29] (midfeed collection), 3.9% to 5.4% by Perrin et al. [27] (full--breast expression), and 7.4% (foremilk) and 13.0% (hindmilk) by Larson-Meyer et al. [39]. When studied longitudinally, total fat composition was stable between 1 and 4 mo postpartum and between 11 mo and 17 mo postpartum.

Three studies assessed individual fatty acids using gas/liquid chromatography and LC-MS [30,46,52]. Collectively, these studies encompassed a narrow period of lactation (1–3 mo postpartum), did not account for diurnal variations of fat in the sample collection process, and focused on polyunsaturated fatty acids (Table 1). Longitudinal changes were explored in a single study by Smith et al. [52] that investigated the influence of a daily ω-3 supplement (750 mg EPA, 250 mg DHA) on human milk DHA concentrations. For the purposes of this review, only the control data (no DHA supplement) is reported in Table 1. DHA concentrations declined from 4 wk to 7 wk postpartum (0.48 nmol/mL compared with 0.34 nmol/mL).

Vitamins/minerals

Of the extracted articles, only 6 reported limited data on vitamins, minerals, and/or trace elements (Table 2) [27,36,47,49,51,53]. Importantly, all studies collected samples from a single timepoint rather than over a 24-h period. This approach does not capture diurnal changes in micronutrients. Major minerals such as calcium were measured in only 1 study [27], which also assessed electrolytes, including sodium and potassium, and trace elements, iron and zinc, whereas the trace element iodine was measured in 2 studies [49,53]. When considering longitudinal changes, Perrin et al. [27] reported increasing sodium concentrations, decreasing calcium and zinc concentrations, and stable potassium and iron concentrations between 11 and 17 mo postpartum. A secondary analysis of a longitudinal study by Bertinato et al. [53] reported higher median milk iodine concentrations at 1 mo compared with 6 mo of lactation.

There were single studies that reported on a variety of vitamins or vitamin-like compounds (Table 2) including vitamin A (β-carotene and retinol), vitamin E, thiamin, and riboflavin [36]; vitamin K [51]; and choline [47]. None of these studies explored longitudinal changes in vitamin concentrations.

Summary of findings

We identified 32 studies on the composition of human milk in the United States and Canada conducted in the 5-y period from 2017 to 2022, which suggests a rapid increase in human milk research compared to the 28 studies identified by Wu et al. [8] in the 36-y period from 1980 to 2016. Our scoping review adds to the findings of Wu et al. [8] on the composition of human milk in the United States and Canada by summarizing emerging data on glucose and HMOs (Table 3). Notably, our review reports consensus among 8 studies representing over 700 participants that glucose is present in trace amounts compared with lactose concentrations (24–64 mg/dL compared with 6–7 g/dL, respectively). Similarly, the growing body of HMO research highlighted in our review (12 studies representing over 2000 participants) supports differences in HMOs by temporality and secretor status. Findings from both reports demonstrated a lack of studies using the preferred/reference methods as well as a shortage of studies conducted beyond 6 mo postpartum. In addition, multiple nutrients have been poorly characterized, with few studies examining milk volume, total carbohydrate, galactose, amino acid profiles, essential fatty acids, vitamins, and minerals.

Discussion

The objective of this review was to summarize the state-of-the-science on the nutrient composition of human milk in the United States and Canada between 1 January, 2017 and 15 April, 2022. While there has been an increase in human milk research since the review conducted by Wu et al. [8], we observed that recent additions to the literature primarily focused on macronutrient composition rather than micronutrients. Additionally, these studies often provided limited assessments beyond the first 6 mo postpartum, despite evidence suggesting that human milk components undergo changes throughout the 24 mo of lactation [42]. Moreover, several studies did not use preferred analytical techniques or describe the specific approach for human milk sampling. These gaps in research methodology raise potential concerns regarding the reliability of data and highlight the lack of standardization across study protocols. Given these limitations, we are not able to provide recommendations for updating the nutrient profile of human milk on the USDA FoodData Central database beyond what has already been determined. To establish a more accurate nutrient profile of human milk and inform dietary guidelines during infancy, we highlight important considerations and priorities for future research below.

Human milk sampling

Sample collection is a critical consideration in the assessment of human milk composition. The “gold standard” for sample collection requires the serial sampling and pooling of milk from full-breast expressions over a 24-h period [17,56,57]. Although pooling across multiple collections may not always be feasible, it is paramount that studies are transparent and provide a detailed description of the approach. As described by Leghi et al. [17], studies should strive to standardize and report collection procedures, validate analytical methods, and describe milk volume measures.

Consistent with the findings of Wu et al. [8], recent studies often did not specify the collection time of human milk samples. Neglecting to consider circadian variations in these samples may obscure our understanding of several nutrients. For example, lipid concentrations were found to be lower in the morning compared to the afternoon and early evening based on collecting pre- and postfeed samples [58]. In contrast, there were no differences in fat, protein, or lactose concentrations over 24 h when collecting prefeed samples only, highlighting the importance of both sampling time and sample type (e.g., single full-breast expression, pre- and/or postfeeds, 24-h collection) [59]. Circadian variations in the concentrations of vitamins and some macronutrients have also been reported and may be related to timing of meals/supplements [60,61]. Findings reported by Bilston-John et al. [62] suggested that the most accurate approach was collecting larger milk volumes over multiple feeds within a 24-h period. Therefore, future studies should prioritize

TABLE 2
Articles published from 2017 to 2022 reporting vitamin or mineral data from human milk samples

Article	Nutrient	Concentration	Analytical technique used by authors	Preferred method employed?
2017 Perrin [27]	Calcium, Ca	11-mo (n = 16): 200±29 μg/mL 12-mo (n = 16): 200±25 μg/mL 13-mo (n = 16): 190±29 μg/mL 14-mo (n = 16): 190±27 μg/mL 15-mo (n = 16): 190±25 μg/mL 16-mo (n = 16): 180±36 μg/mL 17-mo (n = 14): 180±30 μg/mL	ICP-OES	No
	Iron, Fe	11-mo (n = 16): 180±87 ng/mL 12-mo (n = 16): 210±110 ng/mL 13-mo (n = 16): 170±130 ng/mL 14-mo (n = 16): 200±100 ng/mL 15-mo (n = 16): 180±110 ng/mL 16-mo (n = 16): 190±91 ng/mL 17-mo (n = 14): 260±140 ng/mL	ICP-OES	No
	Potassium, K	11-mo (n = 16): 370±51 μg/mL 12-mo (n = 16): 380±69 μg/mL 13-mo (n = 16): 370±53 μg/mL 14-mo (n = 16): 380±59 μg/mL 15-mo (n = 16): 380±73 μg/mL 16-mo (n = 16): 360±78 μg/mL 17-mo (n = 14): 370±78 μg/mL	ICP-OES	No
	Sodium, Na	11-mo (n = 16): 70±19 μg/mL 12-mo (n = 16): 70±24 μg/mL 13-mo (n = 16): 74±34 μg/mL 14-mo (n = 16): 76±34 μg/mL 15-mo (n = 16): 88±23 μg/mL 16-mo (n = 16): 89±25 μg/mL 17-mo (n = 14): 86±35 μg/mL	ICP-OES	Yes
	Zinc, Zn	11-mo (n = 16): 560±330 ng/mL 12-mo (n = 16): 600±360 ng/mL 13-mo (n = 16): 600±390 ng/mL 14-mo (n = 16): 440±250 ng/mL 15-mo (n = 16): 470±320 ng/mL 16-mo (n = 16): 420±350 ng/mL 17-mo (n = 14): 420±310 ng/mL	ICP-OES	Yes
2019 Moukarzel [47]	Choline	Median (IQR): [T1, n = 20] Total water-soluble choline: 1727 (366) μmol/L, Free choline: 119 (73.5) μmol/L; [T2, n = 20] Total water-soluble choline: 1219 (410) μmol/L, Free choline: 125 (63.7) μmol/L; [T3, n = 20] Total water-soluble choline: 1200 (308) μmol/L, Free choline: 131 (75.4) μmol/L; [T4, n = 20] Total water-soluble choline: 1289 (404) μmol/L, Free choline: 146 (119) μmol/L; [T5, n = 20] Total water-soluble choline: 1230 (344) μmol/L, Free choline: 132 (81.1) μmol/L	ID-LC/MS/MS	Yes
2020 Ellsworth [49]	Iodine	2 mo (n = 32): 86.0 ng/mL (variation not reported)	ICP-MS	Yes
2020 Lima [36]	Thiamin	Light protected (n = 13): 0.27±0.04 mg/L Light exposed (n = 13): 0.23±0.04 mg/L	UHPLC	Yes
	Riboflavin	Protected (n = 13): 99.7±0.66 μg/L Exposed (n = 13): 62.1±0.61 μg/L	UHPLC	Yes
	Retinol	Protected (n = 13): 0.67±0.06 mg/L Exposed (n = 13): 0.62±0.06 mg/L	HPLC	Yes
	Carotene, beta	Protected (n = 13): 29.3±1.14 μg/L Exposed (n = 13): 31.5±2.42 μg/L	HPLC	Yes
	Vitamin E	Protected (n = 13): 5.12±0.19 mg/L Exposed (n = 13): 4.13±0.17 mg/L	HPLC	Yes
2022 Bertinato [53]	Iodine	1 mo (n = 105): 198 μg/kg (IQR, 124–274) 6 mo (n = 78): 109 μg/kg (IQR, 67–168)	ICP-MS	Yes
2022 Ellis [51]	Vitamin K	6 wk (n = 23): 1.3±0.2 ng/mL (2.9±0.5 pmol/mL) phylloquinone; 0.4±0.1 ng/mL (0.9±0.2 pmol/mL) menaquinones	HPLC/FLD	Yes

Note: Preferred analytical technique was defined by established references and committee expertise [19–22].
Abbreviations: ICP-MS, inductively coupled plasma mass spectrometer; ICP-OES, plasma optical emission spectroscopy; ID-LC/MS/MS, isotope dilution liquid chromatography tandem mass spectrometry; HPLC, high-performance liquid chromatography; HPLC/FLD, high-performance liquid chromatography with fluorescence detection; IQR, interquartile range; UHPLC, ultra-high-performance liquid chromatography.

TABLE 3
Summary and comparison of findings to Wu et al. (2018) [8] for human milk nutrient analysis from articles published from 2017 to 2022

Name	Present Scoping Review		Wu et al. (2018) [8]	
	Total studies (participant *n*)	Studies using preferred technique (participant *n*)	Total studies (participant *n*)	Studies using preferred technique (participant *n*)
Milk volume	4 (434)	NA	—	NA
Energy	3 (102)	—	9 (236)	NR
Carbohydrate	1 (13)	—	—	—
Glucose	8 (719)	2 (51)	1 (13)	—
Lactose	6 (184)	4 (95)	8 (200)	1 (19)
Galactose	1 (10)	1 (10)	—	—
HMOs	12 (2109)	12 (2109)	2 (44)	2 (44)
Overall protein	5 (125)	1 (4)	10 (228–265)	6 (156)
Amino acids	1 (222)	1 (222)	2 (50)	2 (50)
Total lipids	4 (102)	-	15 (308–345)	12 (257–294)
Total PUFAs	1 (52)	1 (52)	4 (118)	4 (118)
18:2n–6	1 (52)	1 (52)	4 (118)	4 (118)
18:3n–3	1 (52)	1 (52)	4 (118)	4 (118)
20:4n–6	1 (52)	1 (52)	4 (118)	4 (118)
20:5n–3	2 (76)	2 (76)	3 (108)	3 (108)
22:6n–3	3 (89)	3 (89)	3 (108)	3 (108)
Calcium, Ca	1 (19)	—	9 (223)	2 (36)
Iron, Fe	1 (19)	—	5 (160)	6 (179)
Magnesium, Mg	—	—	7 (198)	2 (122)
Phosphorus, P	—	—	3 (136)	1 (105)
Potassium, K	1 (19)	—	7 (198)	1 (17)
Sodium, Na	1 (19)	1 (19)	3 (136)	-
Chloride, Cl-	—	—	3 (61)	1 (18)
Zinc, Zn	1 (19)	1 (19)	6 (112)	4 (57)
Copper, Cu	—	—	6 (269)	6 (269)
Selenium, Se	—	—	1 (17)	1 (17)
Iodine	2 (189)	2 (132)	8 (131)	6 (88)
Thiamin	1 (13)	1 (13)	10 (324)	8 (126)
Riboflavin	1 (13)	1 (13)	6 (269)	4 (71)
Retinol	1 (13)	1 (13)	1 (17)	1 (17)
Carotene, beta	1 (13)	1 (13)	—	—
Vitamin E	1 (13)	1 (13)	3 (61)	2 (31)
Vitamin K	1 (23)	1 (23)	—	—
Choline	1 (20)	1 (20)	—	—

Note: Preferred analytical technique was defined by the committee expertise.
Abbreviations: HMOs, human milk oligosaccharide; NA, not applicable; NR, not reported; PUFA, polyunsaturated fatty acid.

sampling larger milk volumes over multiple time points to increase the accuracy of findings [19].

Analytical techniques

A key finding of this review was a lack of consistency in the approach used to analyze human milk components, which may contribute to discrepant results and reduce reliability and validity of data. For example, the recommendation by the FAO for estimating the energy content of foods is to report metabolizable energy values, which assigns lower energy values for macronutrients that are not as readily converted into energy (e.g., the indigestible HMOs in human milk) [21]. Reporting metabolizable energy values for human milk represents the energy available to the infant for growth and development [22]. No studies in our review or the review conducted by Wu et al. [8] reported on metabolizable energy values in human milk, highlighting a critical gap in the existing literature. In addition, energy content of human milk is affected by sampling technique and analytical approach, and none of the reported studies used the recommended 24-h sampling protocol or included all energy-yielding nutrients in their assessments. Future studies need to measure metabolizable energy in human milk and ensure proper sampling and reliable analytical techniques.

Carbohydrates are the most abundant component of human milk. Although lactose remains relatively stable over lactation [25,27,29,32], there are discrepancies in reported concentrations among studies that have been attributed to differences in analytical techniques. For example, studies identified in this review reported lactose concentrations at 4 to 6 wk postpartum from 5.7 to 7.8 g/dL, which reflects a 35% difference [25,31,54]. A recent study comparing 4 different analytical methods for measuring lactose across the same set of human milk samples reported similar discrepancies (mean concentrations of 6.3–7.7 g/dL depending on method) [63]. There are 2 Association of Official Analytical Collaboration (AOAC)-approved methods for measuring lactose in bovine milk that have been tested in human milk: HPLC with refractive index detection (AOAC 984.22) and an enzymatic method (AOAC 2006.06) that uses a series of species-specific enzymes [64]. Wu et al. [8] suggested that enzymatic methods were not reliable for measuring lactose in human milk because of theoretical interference from the terminal lactose unit on HMOs. However, a recent methodological study found that enzymatic method AOAC 2006.06 was not

influenced by HMOs and had excellent agreement with AOAC 984.22 ($r > 0.98$) as opposed to other enzymatic methods [63], highlighting the importance of establishing reliable methods within the field. Given the wide range of lactose values reported in human milk that may be due to differences in analytical methods, longitudinal studies to describe the lactose composition of human milk that use AOAC methods 984.22 or 2006.06 are warranted given the current state of disparate findings.

While the sample collection method has a minimal impact on protein composition [19], there is a need to employ the preferred modified Kjeldahl method for overall protein analysis. The modified Kjeldahl method accounts for nonprotein nitrogen fraction, whereas other methods (e.g., BCA method) may overestimate protein values in human milk (e.g., ~25%–30%) [65]. The use of infrared spectroscopy also quantifies total nitrogen, which also leads to an overestimate of protein concentrations due to high levels of nonprotein nitrogen compounds (e.g., 25%–30% of nitrogen in human milk is unrelated to protein) [66,67]. Longitudinal studies using the modified Kjeldahl method (i.e., accounting for the nonprotein nitrogen in human milk) are warranted, with an emphasis on the period beyond 6 mo postpartum. Other research gaps include the shortage of information on the amino acid composition of human milk and the need to develop and evaluate new technologies for quantifying total protein in human milk.

The lipid profile of human milk varies based on time of day and changes over the course of a feeding. To measure lipid concentrations, it is therefore recommended that participants complete a full-breast expression to account for fore-, mid-, and hindmilk over a 24-h period [19]. However, none of the studies that measured total fat used a 24-h sample collection. Further, no studies identified in this review used the preferred analytical technique for measuring fat. Wu et al. [8] previously reported that total fat was the most studied human milk nutrient, and 80% of studies assessed fat using a preferred extraction and gravimetric technique. However, only 5 of the studies reviewed by Wu et al. [8] used a 24-h sample collection [56,68–71]. Overall, longitudinal studies with appropriate sample collection and sample analysis techniques are warranted related to total lipid concentrations in human milk.

Imbalance in assessment of nutrients

The studies identified in this review were largely focused on the macronutrient composition of human milk, with a particular emphasis on HMOs. All studies reporting HMO concentrations used the preferred analytical technique, HPLC. Prospective observational studies were also consistent in their findings that HMO concentrations change over time and as a function of maternal genetics (i.e., secretor status). Specifically, total and individual HMO concentrations (i.e., fucosylated and sialylated HMOs) have been shown to decrease over the short-term (postnatal day 10–120) [28] and long-term (1–24 mo postpartum) [42]. Of the more than 200 HMOs identified to date, only 2 HMOs have consistently been found to increase over lactation, namely 3'-sialyllactose and 3-fucosyllactose [42]. Although changes in HMO composition may have a biological basis, supporting later stages of infant development, the findings lend support to justify the benefits of extended breastfeeding.

Another consistent finding across HMO studies was stratifying samples by maternal secretor status, a genetic polymorphism that affects HMO production and compositional profile. Although there are ongoing efforts to elucidate additional influences of HMO concentrations, maternal secretor status is the most well-established, defined by the presence or near absence of the HMO, 2'-fucosyllactose (2'-FL) [13,34,42]. Overall, studies found that concentrations of almost all individual HMOs differed between maternal secretors and nonsecretors; for example, maternal secretors had higher concentrations of total HMOs and fucosylated HMOs (e.g., 2'-FL and lacto-N-fucopentaose I) compared to nonsecretors [13,34,42]. Larger studies with more balanced sample sizes among the main maternal secretor status groups are needed to better understand variations in HMO exposure among infants of maternal secretors compared to nonsecretors.

Findings from this review also revealed significant knowledge gaps in the vitamin and mineral composition of human milk. Current research using preferred analytical methods is limited, with a small number of micronutrients studied in only a few studies (see Table 3). Given reported diurnal variations in human milk micronutrients [61,62], future studies should use a 24-h collection protocol, if possible, when characterizing vitamins and minerals in human milk.

Priority areas for further investigation and future directions

Despite the increase in studies on human milk nutrients and composition, there is an overall lack of standardization in human milk sampling and analytical techniques that must first be addressed before recommendations can be made to update the human milk profile in the USDA FoodData Central database. There is also a need for studies that extend beyond 6 mo postpartum, based on evidence that human milk composition changes over the course of 24 mo, and extended breastfeeding yields additional benefits for infant growth and development [42,72,73].

While these are the overarching knowledge gaps and areas of priority for future studies related to traditional nutrient composition of human milk, emerging evidence on the importance of other compounds that likely confer health benefits to an infant (e.g., antimicrobial proteins, hormones, enzymes, microbiota, etc.), should also be prioritized, though they were outside the scope of this review. Additionally, detailed reviews of analytical techniques for measuring human milk nutrients and milk volume would help identify important methodological details and research gaps that may further influence findings (e.g., sample preparation, limits of detection, appropriate standards) and are essential for advancing the field. Evidence from this review of wide variations in lactose values from studies that all used preferred analytical methods supports the need for further inquiry into methodologies. The low number of studies reporting milk volume is an important point to highlight as this is a fundamental and seemingly simple measurement. Milk volume is commonly measured by infant test weighing before and after feeding, although this method is prone to similar measurement inaccuracies as infant anthropometric measurements. Volume estimation methods such as the deuterium oxide dose-to-mother technique [74] have been reported to improve accuracy [75]. We acknowledge that there are myriad biological and environmental influences on human milk composition, including maternal factors (e.g., diet, genetics), geography (as geography may be a proxy for measured and unmeasured maternal factors associated

with diet, health that are in turn related to living in different geographic settings such as urban/rural, coastal/inland, higher or lower latitude/longitude, etc.), and feeding practices (e.g., volume, frequency) that were outside of the scope of this review. Advances in systems approaches and machine learning are exciting developments that will allow multiple factors and complex interactions to be considered in future human milk composition research [76–80].

Conclusion

We were unable to provide recommendations for updating the human milk nutrient profile in USDA's FoodData Central based on the scarcity of reliable data derived from preferred analytical methods. Therefore, in most instances, the current nutrient values remain insufficient and are not specific to populations in the United States and Canada. Priorities should be placed on addressing the methodological deficiencies described in this review to enable the development of evidence-based reference values for human milk composition across the course of lactation.

Author contributions

The authors' responsibilities were as follows—all authors: contributed to the conception of the research; AEM, KEM, DH: contributed to the design of the research; AEM, KES: contributed equally to the acquisition of the data; all authors: analyzed and interpreted the data, drafted the manuscript, critically revised the manuscript, and read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.advnut.2023.09.007.

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