

Human Milk Nutrient Composition in the United States: Current Knowledge, Challenges, and Research Needs

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Abstract

Human milk is considered to be the ideal food for infants. Accurate, representative, and up-to-date nutrient composition data of human milk are crucial for the management of infant feeding, assessment of infant and maternal nutritional needs, and as a guide for developing infant formula. Currently in the United States, the nutrient profiles of human milk can be found in the USDA National Nutrient Database for Standard Reference, and in books or review articles. Nonetheless, these resources all suffer major drawbacks, such as being outdated, incomplete profiles, limited sources of data, and uncertain data quality. Furthermore, no nutrient profile was developed specifically for the US population. The purposes of this review were to summarize the current knowledge of human milk nutrient composition from studies conducted in the United States and Canada, and to identify the knowledge gaps and research needs. The literature review was conducted to cover the years 1980–2017, and 28 research papers were found containing original data on macronutrients and micronutrients. Most of these 28 studies were published before 1990 and mainly examined samples from small groups of generally healthy lactating women. The experimental designs, including sampling, storage, and analytic methods, varied substantially between the different studies. Data of several components from these 28 studies showed some consistency for 1–6 mo postpartum, especially for protein, fat, lactose, energy, and certain minerals (e.g., calcium). The data for 7–12 mo postpartum and for other nutrients are very scarce. Comprehensive studies are required to provide current and complete nutrient information on human milk in the United States. *Curr Dev Nutr* 2018;2:nzy025.

Introduction

Human milk is considered to be the ideal source of nutrition for infants. Given the documented short- and long-term medical and neurodevelopmental advantages of breastfeeding, the American Academy of Pediatrics affirmed its recommendation: “exclusive breastfeeding for about 6 months, followed by continued breastfeeding as complementary foods are introduced, with continuation of breastfeeding for 1 year or longer as mutually desired by mother and infant” (1). This recommendation was in agreement with the US Surgeon General’s Call to Action, the Centers for Disease Control and Prevention, and The Joint Commission in the US (1), and was also supported by the WHO (2).

Breastfeeding confers unique nutritional and nonnutritional benefits to both the infant and the lactating mother (3). In addition to supporting normal growth and development, human milk offers numerous physiologic advantages through various functional components (4). Breastfeeding has also been associated with higher scores on tests of neurodevelopment and cognition in later life (5, 6).

Recently, the US Department of Health and Human Services Office of Disease Prevention and Health Promotion and the USDA Center for Nutrition Policy and Promotion initiated a project entitled “Evaluating the evidence base to support the inclusion of infants and children from birth to 24 months of age in the Dietary Guidelines for Americans—the B-24 Project.”



Keywords: human milk, composition, nutrient, database, USDA Food Data System

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Abbreviations used: AAS, atomic absorption spectrometry; AES, atomic emission spectrometry; HMO, human milk oligosaccharide; ICP, inductively coupled plasma-atomic emission spectrometry; NPN, nonprotein nitrogen; SR, USDA National Nutrient Database for Standard Reference.

This project intended to respond to the challenge of developing and implementing US-driven, evidence-based health and nutrition promotion programs for this age group (7). Of the research priorities identified by the B-24 research committee, the first one is “Human milk composition: need for up-to-date analyses of human milk across populations including nutrients and bioactive components of human milk, and the factors influencing human milk composition” (7).

From a nutritional perspective, infancy is a critical and vulnerable period. Because of immaturity in tissues and organs involved in nutrient metabolism, infants display a narrow tolerance to deviations in nutrient intakes (8). Unlike commercial infant formula, which is standardized and tightly regulated, human milk is a very dynamic, constantly changing biological fluid. Therefore, accurate and up-to-date nutrient composition data and the factors that influence them are especially critical for the management of infant feeding and assessment of infant nutritional needs.

The composition of human milk changes constantly throughout the entire lactation period (2, 9). The colostrum and transitional milk in early lactation change rapidly and are distinct in many ways from mature milk. Mature milk remains relatively similar in composition with subtle changes over the course of lactation. The nutrient composition is also different between term and preterm human milk (10). Ethnicity, diet, and environment are known to be important factors influencing human milk composition. Some micronutrients can vary with nutritional status; and environmental toxins would differ based on the exposure of region-specific environmental chemicals. Therefore, understanding the human milk composition in one country or region, rather than combining international data, would be more relevant and provide useful information in assessing the infant and maternal nutritional need and supporting policy making of that specific country or region. For this current review, the efforts were restricted to term mature milk. The purposes of this review were to summarize the current knowledge of human milk nutrient composition studies conducted in the United States and Canada and to support an update to the USDA Food Data System for human milk with accurate, up-to-date, representative, and complete data gathered via valid analytic techniques. Although the actual data were not included, the studies on non-US/Canadian populations provided valuable information when discussing the influential factors and analytic methodology. This review is also intended to identify the research questions and provide information for developing a robust experimental design for the proposed sampling and analysis of human milk.

Current status

Currently in the United States, the nutrient profile of mature human milk can be found in the USDA National Nutrient Database for Standard Reference (SR) (11), and in the future, the USDA Food Data System, as well as in books and review articles (12–16). All these profiles share some major drawbacks, the first of which is their outdated sources. For instance, the nutrient profile in USDA SR was created in the 1970s, without being substantially updated since. Yet, it has still been widely used for various purposes and cited even in the most recently published books (17). In 1 book, the nutrient profiles of human milk were generated from data published between 1946 and 1980 (12). Second, the available data used to create the nutrient profiles came from very limited sources, or the sources were not clearly

described (13). In some cases, values of certain nutrients came from only one or very few references (12). Third, there was no data quality evaluation. The data used to develop the aforementioned nutrient profiles were generated by different researchers, over a long period of time, for different populations and with different experimental designs and analytic methods. All of these factors would undoubtedly affect the quality of the data. Fourth, no statistical analyses of variability (e.g., mean, variance, and SD) were presented in any of the 5 profiles. Therefore, the sample variability could not be determined. Finally, none of the profiles were developed specifically for the US population. To create a US-specific human-milk nutrient profile, the data must be carefully examined to ensure they represent the US population.

Several excellent reviews have addressed human milk composition over the last 2 decades (3, 8–10, 13, 18–20). Nonetheless, very few of them summarized the traditional nutrient composition data and discussed the quality of these data. More recent data have focused mainly on so-called “bioactive components” and their potential health benefits.

Factors affecting data quality

Many factors need to be considered when discussing the quality of food composition databases. The USDA has maintained tables of food composition for >124 y (21). To ensure data quality, the USDA Nutrient Data Laboratory (NDL) has developed a comprehensive data quality evaluation system (22, 23). Five categories (sampling plan, number of samples, sample handling, analytic method, and analytic quality control) must be carefully assessed to ensure data quality for the food composition database development. Although these data quality criteria were initially developed for foods and food products, the same principles can be applied to determine the quality of human milk nutrient data. However, owing to the uniqueness of human milk, the 5 major categories are examined next specifically.

Sampling plan

One of the most important characteristics of a good food composition database is that the data effectively represent the foods consumed by the population that the database intends to cover. The sampling plan should be designed to fulfill this purpose. For designing a good sampling plan, the key is to understand the influential factors affecting the nutrient composition of a given food/food product. The composition of human milk is influenced by many factors. The main factors are discussed here to provide a basis for evaluating the existing data and designing appropriate sampling plans for future studies.

Maternal factors. Many maternal factors are known to influence human milk nutrient composition including stage of lactation, genetic background of the mother, parity, age, and health status.

Stage of lactation. Human lactation stage can be divided into 3 major phases: colostrum, transitional milk, and mature milk. Colostrum is rich in immunologic components and contains relatively low concentrations of lactose but higher protein content, suggesting its important functions to be immunologic and trophic besides nutritional (24). Transitional milk shares some of the characteristics of colostrum but represents a period of accelerated milk production to support the infant’s nutritional and developmental needs for rapid growth. Mature

milk is relatively similar to transitional milk, but the changes are not as remarkable as in the early weeks (9, 13). The influence of lactation stage differs for different nutrients. For example, total protein and lipids show a gradual decrease during the first 6 mo of lactation. Whereas the lactose is initially low in colostrum and transitional milk, it then increases in mature milk and remains at the same levels for up to 6 mo (25). In addition to the alterations of total protein, protein composition also changes. Milks mainly contain 2 types of protein: caseins and whey proteins. The ratio of whey to casein in human milk can vary from ~80:20 in early lactation to ~50:50 in late lactation (26). Because the amino acid compositions differ between caseins and whey proteins, the type and content of the amino acids of human milk in turn vary during lactation.

Maternal factors and infant status. Genetic factors of lactating women have been shown to influence human milk composition, which at least partly explains the considerable interindividual variations in milk composition. All physiologic and biochemical events that influence the composition of plasma may potentially affect the composition of milk. Hormones or other factors that are capable of influencing biosynthetic processes in the mammary gland can also modify the milk composition (14). For example, genetic variants of fatty acid desaturase were associated with variability of 20:4n-6, 20:5n-3, and DHA in human milk (27). Another good example is the array of human milk oligosaccharides (HMOs), which are believed to be genetically determined. Different profiles of HMOs occur as a result of specific transferase enzymes expressed in the mammary epithelial cells (18). For instance, human milk fucosylated oligosaccharide synthesis is controlled by the same fucosyltransferase genes (*FUT2* and *FUT3*) that control secretor and Lewis blood group types (28). As few as 23 and as many as 130 different oligosaccharides were found in the milk from randomly selected mothers (29).

Infant need could be a driving force of the quantity and quality of human milk, as its composition changes in response to the infant's age and other characteristics. Human milk from women delivering prematurely supplies more protein and higher levels of many bioactive molecules compared with milk from women delivering at term (30, 31). Another important factor is the infant's gender. In a recent study, Fujita et al. (32) found that infant's gender and socioeconomic status interacted in their relation with milk fat concentration. In Northern Kenya, the economically sufficient mothers produced more milk fat for sons than for daughters (2.8 compared with 0.6 g/dL), whereas poor mothers produced more milk fat for daughters than for sons (2.6 compared with 2.3 g/dL). This finding was later confirmed by a study in other mammals, i.e., dairy cows (33). These studies may indicate a possible "programming" of mammary function by offspring in utero. However, how the programming is regulated, and how it affects human milk nutrient composition, are yet to be determined.

Milk volume. A key element defining lactation performance is the total amount of milk produced. The volume of milk transferred to the infant affects the infant's nutrient intake and the mother's nutrient requirements (34). The milk volume could also affect the milk composition. For example, milk protein concentration was negatively related to milk volume at 6 and 9 mo postpartum, whereas milk lactose concentration was positively related to milk volume at 6 and 9 mo

postpartum (35). In another study, an association between weaning and significant changes in milk composition was observed. When milk volume fell below 300 mL/d, the protein and sodium increased whereas lactose, calcium, and zinc decreased (36).

Parity, age, and other characteristics. Human milk composition may be influenced by the parity and age of lactating women. Protein concentration was found to be the highest in the milk of mothers 20–30 y old (13). In a study to investigate the influence of diet and maternal parity on the fatty acid composition of mature milk in rural Gambian mothers, the authors found that the proportion of endogenous fatty acids was markedly reduced in the milk of mothers of very high parity. It was hypothesized that this represented an impairment of the ability to synthesize milk fatty acids de novo in these mothers (37). A more recent study investigated the effects of smoking, mother's age, BMI, and parity on the lipids and proteins of human milk. The study found that smoking was associated with lower milk lipid and protein concentrations, the increase of parity number led to an increase in lipid concentration, and the overweight mothers showed lower milk protein content (38). Carbohydrate content in mature milk was significantly higher in the older mothers group, and carbohydrates in mature milk correlated positively with maternal age (39).

Health conditions. The mother's health may also affect human milk composition, but this factor has not been well studied. One of the most common health problems for Americans is the prevalence of obesity (40). The prepregnancy obesity rate increased by an average of 0.5%/y, from 17.6% to 20.5%, in 2003–2009 (41). A recent study compared many nutritional components of human milk between obese and lean lactating women. The results showed that the composition of human milk from obese women differed in fatty acids, certain vitamins, and carotenoid composition from that of the lean women. Specifically, human milk from obese mothers contained lower DHA, vitamin D, and lutein + zeaxanthin content (42). Differences in the lipid composition of human milk have long been described in maternal diseases known to affect fat metabolism such as diabetes, cystic fibrosis, hypobetalipoproteinemia, and type I hyperlipoproteinemia (43). Other diseases, such as allergic disease, also alter fatty acid profiles and eicosanoids (44). Changes of fatty acid composition in human milk have also been observed in lactating mothers with cold-like syndrome (45). Cholesterol content is generally tightly regulated and is not affected by maternal diet. However, a 16-fold elevation was noted in milk of women with familial hypercholesterolemia (46).

Environmental factors. Environmental factors, especially maternal diet, play important roles in human milk nutrient composition. Other factors such as season, region, and socioeconomic status are suggested to be more or less related to maternal diet.

Maternal diet. Maternal diet has a profound influence on the composition of human milk for some nutrients, depending on the nutrition status of the lactating women (27, 47, 48). Maternal diet has been shown to have little effect on total protein, carbohydrates, and certain minerals, but affects fatty acids, certain vitamins, zinc, calcium, selenium, iodine, and fluorine (13, 48, 49). The maternal nutrition could have an impact on human milk composition through 3 aspects: current

dietary intake, nutrient stores, and alterations in nutrient utilization. For instance, the nature of the fat consumed by the mother influences the fatty acid composition of milk (14, 50). The vitamin content of human milk is influenced by the mother's vitamin status. It was suggested that when maternal intakes of a vitamin are chronically low, its levels in human milk are also low (14). Some major minerals such as calcium, phosphorus, and magnesium are shown to be tightly regulated in maternal serum. However, the concentrations of minor minerals (e.g., iron and copper) in human milk may be influenced largely by maternal nutrition (51).

Nutritional requirements to support lactation are among the highest priorities in human development. According to the theory of systems biology, robustness is a ubiquitously observed property of biological systems and is considered to be a fundamental feature of complex evolvable systems. Robustness enables the system to maintain its functionalities against external and internal swings in concentration (52). The more important the function, the more robustly the system will ensure self-regulation. Genetic buffering is one of the fundamental mechanisms that provides robustness. The likely reason why many studies did not find maternal diet to influence human milk nutrient composition is probably this buffering mechanism. Although maternal intake influences the provision of nutrients to the nursing infant, maternal hormonal adjustments and nutritional stores serve to buffer daily fluctuations, so that excesses or moderate dietary deficiencies do not always alter nutrient transfer to the infant (46). When maternal nutrition is continuously compromised but the nutrient content and the milk volume remain unchanged, the nutrients could be synthesized from the maternal stores or body tissues (14). However, any buffer system has its own capacity and range. Beyond its capacity or range, the buffer system will lose its function to absorb perturbations. If severe malnutrition has persisted for some time, the ability of the lactating mother to maintain the macronutrient in the human milk will reduce or even collapse. Two possible reasons may lead to a buffering function reduction: 1) low dietary intake and depletion of nutrient stores, because the nutrients in human milk ultimately come from the mother's diet; and 2) malnutrition or disease conditions causing the mother's body to be less capable of managing the buffer system. More research is needed to fully understand the buffer system, how it is regulated, and, more importantly, to understand its capacity and range. There are also no studies specifically designed to evaluate the consequences when prenatal stores become depleted (2).

Another important aspect is the use of dietary supplements. Because nutrient requirements increase during periods of lactation, many clinicians recommend dietary supplements during these important periods of the life cycle (53). Data suggest that in the United States, most (73%) lactating women take a multivitamin preparation, with fewer reporting use of specific supplements of calcium (11%), folic acid (7%), or iron (4%) (54). Some supplements, such as DHA, showed a strong and dose-dependent increase in human milk (55). However, not all nutrients found in human milk respond positively to supplementation. For instance, a dietary folate intake of $\sim 380 \mu\text{g/g}$ did not appear to prevent mobilization of maternal folate stores during lactation (56).

Region, season, and other environmental factors. Some distinct regional differences are evident, particularly in concentrations of certain protein components, minerals, vitamins, and trace elements. Season

could be an influential factor for human milk nutrient composition. The influence may be more or less related to maternal diet, breastfeeding behavior, and sun exposure (13). In a recent study also conducted in China, significant differences among 3 distinct regions were observed in regard to total MUFAs and PUFAs in human milk. Different dietary habits were indicated as the main drivers behind the different fatty acid profiles between the 3 regions (57). HMO concentrations and profiles have also been shown to vary geographically in healthy women, which may be determined by a combination of genetic, sociocultural, behavioral, and environmental factors (58).

Sample size

Assessment of the number of samples analyzed is critical in developing the nutrient composition database of a given food. The sample size should be large enough to cover major variations, such as geographic and demographic factors, to allow valid statistical analyses. The analysis of an inadequate number of independent samples limits the ability to estimate the mean and variability. On the other hand, analyses of too many samples (either individual or composite samples) will add substantial unnecessary cost. USDA NDL has extensive experience in handling mainstream food products (22, 59). However, human milk is very different from other foods, and very few studies have discussed appropriate sampling quantities. In a recent study to develop an online database for human milk composition in China, the number of samples was estimated based on the variation and SD of the studied nutrients (60).

Sample collection and handling

Human milk composition can vary during the day and from the beginning to the end of a feeding. This variation is most pronounced for zinc, fat, and fat-soluble components such as vitamin A (61, 62). Human milk nutrient concentrations are also influenced by handling and storage conditions, including also the freeze-thaw cycles and whether or not pasteurization is used (9). Therefore, to obtain high-quality data, sample handling methods must be carefully examined, validated, and standardized.

Sample collection methods. Certain milk nutrients are known to be altered by diurnal variation. A study of milk from 71 mothers over a 24-h period found that the milk fat content was significantly lower in night and morning feedings compared with afternoon or evening feedings (63). In another study, the energy concentration of human milk was measured for samples collected at 4 different time periods: 1) 0000–0600; 2) 0600–1200; 3) 1200–1800; and 4) 1800–0000. The variability of the energy concentration of milk throughout the day was estimated to be 14% at month 1 and 22% at month 4 (64). The stage of the nursing process is probably responsible for some of the largest variabilities in milk composition. For example, the fat content gradually increases from the beginning to the end of a feed, but there were no significant changes for either lactose or protein (65). The gold standard of milk collection involves multiple collections from the same individuals over a 24-h period (9). Alternatively, the milk can be collected at a specific time of day (e.g., morning) by emptying the entire breast. But collection should be avoided from a breast that was used for nursing within the past 2–3 h (9).

Sample handling and storage. In most published studies, especially those with large sample numbers or with samples collected far from the analytic facilities, the milk samples were stored, shipped, and in many cases involved in multiple freeze-thaw cycles for aliquots to analyze different nutrients. The samples could be taken by the collectors or lactating women themselves, and were temporarily stored under different conditions. If not handled properly, varying degrees of nutrient loss occur, depending on the nutrient and the storage methods. Products of lipolysis, including diacylglycerols, monoacylglycerols, and FFAs, were found in human milk unless new milk was extracted and analyzed immediately after sample collection (50). Storage temperature at -20°C is not low enough to preserve the integrity of the milk lipid, for it results in hydrolysis of TGs and the appearance of FFAs (66). The amounts of hydrolytic substances increase as storage lengthens at all temperatures except -70°C (67); lipases in milk are inactive at this temperature (50). Freeze-thaw cycles should be minimized because thawing and warming change the integrity of previously frozen human milk (68).

Many published studies on human milk composition used samples from a milk bank. The milk bank receives milk from the milk donors. After being collected, donated milk is pooled and pasteurized to prevent the risk of transmitting pathogens. A variety of protocols have been developed for pasteurization of donor milk (69). Heat treatment has been shown to reduce the concentration and functionality of certain nutrients, particularly functional proteins (70). Moreover, the variability is lost by pooling, and the process method may significantly alter the nutrient profile and their concentrations. Therefore, donor milk from a milk bank is not appropriate to be used to generate data for a human milk composition database.

Analytic methods

Valid and meticulously applied analytic methodology is critical for obtaining accurate nutrient data. In some cases, it is analytic inaccuracies, rather than true biological variances, that result in the large variations of many milk constituents. Therefore, the entire analytic procedure, from pretreatment to extraction, separation, analysis, and quantification, must be carefully validated for optimal results. The methods to be used should be determined by the chemo-physical properties of nutrients, how they present in human milk, and their concentrations.

Sample preparation and extraction. Before the nutrients can be analyzed, they must be extracted from the milk. Human milk is a heterogeneous mixture, which consists of true solutions, colloids, membranes, membrane-bound globules, live cells, etc. (46). For analysis of some nutrients, pretreatment is necessary to either separate them from similar components or release them from binding to other compounds. For instance, the Kjeldahl method is the most commonly used method to determine total nitrogen, which can then be used to calculate total protein by a conversion factor. However, in human milk, about 20–25% of total nitrogen is nonprotein nitrogen (NPN). For this reason, the classic Kjeldahl method will considerably overestimate milk protein. A more accurate approach known as “corrected Kjeldahl” is to first measure the total nitrogen, and then NPN after precipitating protein by trichloroacetic acid. The total protein nitrogen is obtained by subtracting the NPN value from the total nitrogen (71).

Another seldom-mentioned but important aspect is the sample aliquot. Jensen reported that sampling milk and obtaining an aliquot that contains the true amount of lipid is critical for measuring total lipid. The heterogeneously sized lipid globules, with a density of 0.9, rise at different rates and must be shaken at $\geq 38^{\circ}\text{C}$ to achieve a random distribution (50).

Nutrient analysis. In general, more than one method can be used to analyze a given nutrient, and each has its own advantages and disadvantages. For developing values for a database, the method that provides the most accurate measurement of the nutrient in its original form in the milk is the most desirable. In addition, analytic methods evolve with the development of new analytic technology and instrumentation. For instance, methods used in the past for analysis of B-vitamins were microbiological, but in recent years, spectroscopic and chromatographic methods coupled with UV, fluorometric, or MS detection have been developed (72). The most recently developed method, which is ultra-performance liquid chromatography—tandem MS (UPLC-MS/MS), has offered improved resolution, speed, and sensitivity for analytic determinations of B-vitamins (73, 74). Another issue is that the analytic methods designed to study constituents in bovine milk or other biological fluids have been directly applied in the analysis of human milk, thereby providing inaccurate information (14). To obtain accurate results, the methods must be validated or modified for human milk (e.g., through the use of standards from human milk rather than from bovine milk). Analytic methods will be discussed in detail in the following sections.

Analytic quality control

Analytic quality control ensures accuracy and precision in the day-to-day performance of the analytic method. It also serves as the foundation to compare analytic data that were conducted at different dates, in different laboratories, or by different instruments. A certified reference material, such as the standard material from the National Institute of Standards and Technology (NIST), is preferred, when possible. Consensus or in-house material, when carefully characterized, can also be used (23).

Methods

Literature search

PubMed and Google Scholar were selected as primary search engines for the literature review. The following keywords were used in different combinations: breast milk, human milk, composition, nutrient, protein, amino acid, lipid, fatty acid, carbohydrate, lactose, oligosaccharide, vitamin, and mineral/element/electrolyte. The search was conducted for the period from 1 January 1980 to 31 December 2017. Reference lists of the retrieved research and review articles were reviewed to identify references not found using electronic search engines. Only data in original research articles were included; secondary data in review articles or books were excluded. The detailed inclusive criteria are listed as follows:

- 1) The subjects were stated as living in the United States or Canada, or there was clear indication that the subjects were in the

United States or Canada (e.g., the protocol was approved by a US institutional review board). Data from Canada were included because of the similarity in terms of ethnicity, diet, and environment between the United States and Canada.

- 2) Information on lactation stage, sample collection, and storage was provided. Therefore, the data from pooled samples or milk banks could not be included.
- 3) Only the data on traditional nutrients were included. In addition, considering the current interest in oligosaccharides and their quantities in human milk, total HMO was also included. The nutrient list is presented in the USDA SR (11).
- 4) Only the data of milk for full-term infants were included. Full-term was determined by gestation (>37 wk) (10, 75) or specified in the references.
- 5) Only the data for mature milk in the first year of lactation were included. There is no consensus on defining milk as mature milk. In this review, the definition of the Institute of Medicine was adopted, in which milk after 3 wk or 21 d lactation is considered mature milk (14). Therefore, the data from the lactation stage of 21–365 d were included.
- 6) From the papers that aimed to study the factors or influential effects of certain treatments/supplements, only the data from control or placebo groups were included.

Data extraction

Data extracted from studies were converted to units of gram, milligram, or microgram per 100 g basis. The human milk specific gravity of 1.031 g/mL was used for the conversion (76). For protein content analysis using the Kjeldahl method, protein nitrogen was converted to total protein with 6.38 as the conversion factor (77, 78). When other conversion factors (i.e., generic Jones factor 6.25) were used to estimate the true protein content, the data were recalculated with 6.38 as the conversion factor. Fatty acids, presented as percentage of total lipid, were calculated to grams per 100 g human milk based on the value of the total lipid.

As mentioned earlier, human milk composition constantly changes over the entire lactation. Currently, only 1 nutrient profile for mature human milk is present in the USDA food composition databases and other sources (11–16). It does not reflect the variation of human milk composition during the entire lactation. On the other hand, it is not necessary to present the values for every week or month of postpartum. To comply with the recommendation of the American Academy of Pediatrics which states “exclusive breastfeeding for about 6 months, followed by continued breastfeeding as complementary foods are introduced, with continuation of breastfeeding for 1 year or longer,” it is reasonable to us that at least 2 nutrient profiles should be developed, representing 1–6 mo and 7–12 mo postpartum, respectively. Considering the recommendation “exclusive breastfeeding for about 6 months,” it is more critical to understand the nutrient profile of this period. The nutrient profile for 7–12 mo postpartum may be less critical because supplemental foods are typically added to the infant’s diet during that time, although data for the nutrient profile of human milk for 7–12 mo postpartum are important for guiding the type and quantity of supplemental foods to be added.

For individual nutrients analyzed for milk from these 2 time periods, the data were pooled from different time points. Each nutrient value was

calculated as a mean and the SD was calculated as a pooled SD via the following equation:

$$S_{\text{pooled}} = \sqrt{\frac{S_1^2 + S_2^2 + \dots + S_k^2}{k}} \quad (1)$$

A *t* test was run in Excel (Excel 2013, Microsoft) to calculate the *P* values of the 2 paired sample groups.

Results

Overview of the publications

After several sequential stages of screening, 52 papers were found that met all of the aforementioned criteria. Among them, data from 28 papers were extracted for this review (Table 1). The data in the other 24 papers were excluded after assessing the subjects and data quality, if ≥ 1 of the following issues were present:

- 1) The data were presented only in graph format with no numerical data included (79–82). Unless the raw data were obtained through personal communication, the data could not be extracted.
- 2) Data represented only a portion or were not complete for a given nutrient. For instance, only phospholipid and fatty acids of phospholipids were presented (83) and because they are only a part of total fat and total fatty acids, the data were not included. For protein, if only total nitrogen was measured, the data were not included because of the considerable amount of nonprotein nitrogen in human milk (84).
- 3) Subjects were following a special diet. For example, in a study to examine the effect of folate supplements on human milk folate, in addition to the treatment group receiving a folic acid supplement, all participants received a folate-free daily multivitamin and mineral supplement (85). However, those studies that include the special diet or dietary supplements into experimental designs were still included.

Overall, the number of studies on human milk has steadily increased. Nonetheless, the most recent studies on human milk composition have focused on nonnutritional bioactive components, including cells, antimicrobial factors, cytokines and anti-inflammatory agents, hormones, growth factors, prebiotics, and digestive enzymes (13, 86). Of the 28 studies whose data we included in this review, most were published in the 1980s or early 1990s, possibly due to the assumption that not much new research was needed for traditional nutrients (Figure 1). Even for these 28 studies, not 1 was designed to develop a dedicated nutrient database for human milk. These studies were primarily designed for various other purposes, such as the influential factors on human milk composition, comparison between term and preterm milk, impact of certain diet/supplements on milk nutrients, human milk and infant growth, or sampling/analytic method development. Therefore, the experimental design generally did not take into full consideration the factors discussed earlier, so some key information was missing or not presented in the papers.

The number of the subjects in these 28 studies was generally small, with most of them <40. Only 2 papers (apparently from the same study) included >100 subjects (Figure 1). The ethnicity of the subjects was

TABLE 1 Summary of the studies that contained the original nutrient composition data for populations in the United States and Canada (1980–2017)¹

Ref	Year	Subjects	Location	Lactation stage, d	Subject number	Sample collection	Storage	Protein		Fat		Carbohydrates			Mineral	Energy
								Total protein	Amino acids	Total fat	Fatty acids	Lactose	HMO	Others		
Gross et al. (92)	1980	NA	USA	21–28	18	Morning	Lyophilized and stored –20°C	X		X		X				X
Clark et al. (88)	1982	NA	CT, MA	42–112	10	Morning and afternoon	Transport on dry ice, stored –70°C		X	X			X			
Garza et al. (125)	1983	NA	USA	180	6	Between 0800 and 1200	Transport at 4°C, stored –20°C	X		X				X		X
Clark et al. (126)	1983	NA	USA	42–112	10	Morning and afternoon	Transport on dry ice, stored –70°C		X		X					
Feeley et al. (94)	1983	Caucasian, black, oriental	Athens, GA	30–45	105	Morning and afternoon	Stored –20°C							X		
Feeley et al. (127)	1983	Caucasian, black, oriental	Athens, GA	30–45	105	Morning and afternoon	Stored –20°C							X		
Dewey et al. (36)	1984	NA	Davis, CA and northern CA	120–330	12	2nd feeding, morning	Stored –20°C	X		X		X		X		
Butte et al. (90)	1984	NA	Houston, TX	28–84	13	Between 0800 and 1200	Transport at 4°C, stored –20°C	X		X				X		X
Butte et al. (128)	1984	NA	Houston, TX	30–120	41	24 h	Transport on ice, stored temp NA	X		X		X				X
Casey et al. (93)	1985	White	USA	28	9	Midfeed, midmorning	Stored –70°C									X
Janas and Picciano (129)	1986	NA	IL, USA	28–56	10	Foremilk and hind milk, morning	Stored –20°C		X							
Garza et al. (64)	1986	NA	USA	30–120	20	24 h	Transport on ice, stored temp NA									X
Janas et al. (89)	1987	NA	Urbana-Champaign, IL	28–56	40	Foremilk and hind milk, time NA	Freezing, temp NA		X							
Ferris et al. (130)	1988	NA	USA	42–112	12	Morning and afternoon	Transport on dry ice, stored –70°C			X		X				X
Dewey and Lommerdal (91)	1983	NA	Davis, CA	30–180	20	2nd feeding, morning	Freezing, temp NA	X		X		X				X

(Continued)

TABLE 1 (Continued)

Ref	Year	Subjects	Location	Lactation stage, d	Subject number	Sample collection	Storage	Protein			Fat			Carbohydrates			
								Total protein	Amino acids	Total fat	Fatty acids	Others	Lactose	HMO	Others	Vitamin	Mineral
Casey et al. (87)	1989	Caucasian, 1 Native American, 1 Hispanic	Denver, CO	4–360	22	Midfeed, midmorning	Stored –70°C										X
Stuff and Nichols (131)	1989	NA	Houston, TX	112–252	58	24 h	NA	X	X								
Butte et al. (111)	1990	NA	TX, USA	30–120	20	24 h	Freezing, temp NA	X	X								X
Allen et al. (79)	1991	Caucasian	Denver, CO	21–180	13	Midmorning	Transport on ice, stored –70°C							X			X
Nommensen et al. (35)	1991	NA	CA, USA	90–360	21–58	24 h	Freezing, temp NA	X	X								X
Wack et al. (132)	1997	NA	USA	0–360	30	Between 1000 and 1400	Stored –35°C						X				X
Friel et al. (95)	1999	NA	Newfoundland, Canada	7–84	17	Between 1000 and 1400	Transport in cooler, stored –20°C										X
Glew et al. (96)	2008	NA	NM, USA	30–180	29	Between 0700 and 1000	Stored –70°C	X	X								
Hannan et al. (97)	2009	Mexican-American	Rio Grande Valley, south TX	30–90	17	NA	Stored –20°C										X
Tijerina-Saenz et al. (123)	2009	Caucasian	Vancouver, Canada	30	60	Hindmilk	Transport frozen, stored –80°C				X						X
Glew et al. (122)	2011	Hispanic and non-Hispanic white	NMn, USA	30–180	19	Between 0830 and 1030	Stored –70°C	X	X								
Alderete et al. (133)	2015	NA	OK, USA	30–180	25	Morning, 0800–1000	Stored –80°C						X				
Perrin et al. (98)	2016	NA	Raleigh, NC	330–360	19	1st or 2nd feeding, morning	In subject's freezer until study completed, then –80°C	X	X				X				X

¹NA, not available; Ref, reference.

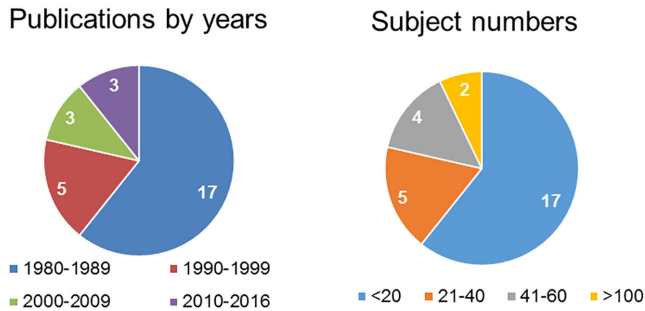


FIGURE 1 Summary of publication years and sample sizes of studies on human milk nutrient composition.

typically not mentioned. For those that were mentioned, the subjects were mostly Caucasian and generally healthy, hence the data from these 28 studies may not well represent the US population. However, in terms of geographic variation, the subjects came from diverse regions of the United States and Canada.

Discussion

Influential factors

Lactation stage is a key factor influencing human milk composition. Most of the data from these 28 studies reflected 4 mo postpartum (<120 d). Only 5 studies provided data for lactation at 7–12 mo (181–360 d).

Diurnal variation has been shown to dramatically influence human milk composition. In a recent study, circadian variation in concentration was significant for all vitamins, with riboflavin showing the greatest range (62–220%) from the daily median (73). Among these 28 studies, only 5 employed 24-h collections. The rest of the studies collected samples using different protocols: 1) sampling at the midmorning, midfeed from both breasts (87); 2) sampling at morning and afternoon from 1 breast (88); 3) sampling foremilk and/or hindmilk (89); 4) second feeding in the morning from 1 breast (36); and 5) sampling over a 2–4 h period ≥ 2 h after a feeding and emptying 1 breast (90). There are limited studies that indicate the methods which represent a 24-h mean or minimize the influence of diurnal variation, such as midmorning, midfeed from both breasts (81), and second feeding in the morning from 1 breast (91). However, these studies only analyzed and compared certain nutrients, not the complete nutrient profile. Again, the impact of diurnal variation on specific nutrients may be quite different. For instance, the highest correlation for energy between single samples and the 24-h value was from the samples collected between 0000 and 0600 (64). More studies are required to evaluate the influences of sampling time and to determine the best alternative sampling procedure should 24-h collections be unsuitable or not feasible.

Sample handling and storage is another important factor that may influence certain unstable or heat-sensitive nutrients but not others (e.g., minerals). In many studies, milk samples were collected by lactating women at their own homes; therefore, optimized transportation conditions of the sample shipment to the laboratory should be considered. Different transportation procedures were described in these 28 studies, including keeping the milk in ice, dry ice, a cooler, or at 4°C without further detail. Depending on the ways that the samples

were transported, these different methods could lead to deterioration of certain nutrients. In terms of storage, -70°C storage is preferable, especially for the long term. However, milk samples were stored at -20°C in over half of the 28 studies. Interestingly, in 1 study, samples were lyophilized and stored at -20°C (92). There was no evaluation of how this method affected the nutrient composition of the human milk.

Method validation and quality control are keys to ensuring data accuracy. However, of the 28 studies, only 8 studies applied some kind of method validation or quality control procedures (79, 87, 93–98). Of these, 6 used standard reference materials (87, 93, 94, 96–98).

Nutrients

Protein. The protein content of mature human milk is ~ 8 – 10 g/L (2). Several methods have been developed over the years to determine the total protein content of human milk and each may yield different results. Among them, amino acid analysis is the most accurate direct method for determining true protein content. However, this method is rather time consuming and costly, so it has not been routinely used to quantify total protein. In future, because the amino acid profile is an important part of the food nutrient profile, amino acid analysis is recommended as the standard method for quantifying total protein in human milk. Another direct method of analysis is the classic Kjeldahl method. This method measures total nitrogen and the total protein is further calculated by a conversion factor (77). Total protein can also be measured by indirect colorimetric assays, based on the proteins' functional groups, such as the Biuret method (peptide bond), the bicinchoninic acid (BCA) method (dye-binding sites), and the Lowry method (tyrosine and phenylalanine content) (2). However, overestimation of ~ 25 – 40% is possible if the human milk protein content is measured by colorimetry (99). The accuracy of indirect measurements depends on the standard, calibration of the individual machines, and the technique. The protein content of mature human milk was reported as ~ 9 g/L by the Kjeldahl method, and 12–14 g/L by the Lowry method. In the Lowry method, bovine serum albumin is commonly used as the standard. However, BSA has fewer aromatic amino acids than human milk, which explains the 25% higher values (2).

Of the 28 studies, 10 reported total protein or total protein nitrogen (Table 2). The total protein nitrogen was converted to total protein by the Jones factor of 6.38 for milk (77). Among these 10 studies, no study reported total protein by summing up total amino acids, 6 used the Kjeldahl method to calculate total protein nitrogen, and the other 4 studies used colorimetric assays. The protein contents from the Kjeldahl method, except for the data from 1 study (92), were generally lower than from colorimetric assays. Statistical analysis of the values from these 2 types of method across all studies indicated that the protein contents calculated by the Kjeldahl method were significantly lower ($P = 0.007$) (Figure 2A). These findings are in accordance with a previous report (14). Considering the potential drawbacks of colorimetric assays, and that the evidence showing the true protein contents determined by the “corrected Kjeldahl” method and by amino acid analysis has agreed closely (26), the “corrected Kjeldahl” method is accepted for database development should total amino acids analysis not be available.

Lipids. Lipids are the main source of energy in human milk, contributing 40–55% of the total energy depending on state of lactation.

TABLE 2 Total protein contents in human milk for populations in the United States and Canada (1980–2017)¹

Ref	Protein				Method
	1–6 mo		7–12 mo		
	Mean	SD	Mean	SD	
Perrin et al. (98)	—	—	1.55	0.19	BCA
Dewey et al. (36)	1.29	0.37	1.35	0.37	Lowry assay
Nommsen et al. (35)	1.14	0.15	1.16	0.16	Lowry assay
Butte et al. (111)	0.92	0.21	—	—	Kjeldahl method for protein N
Stuff and Nichols (131)	0.79	0.13	0.78	0.13	Kjeldahl method for protein N
Gross et al. (92)	1.40	0.18	—	—	Kjeldahl method for protein N
Butte et al. (90)	0.95	0.15	—	—	Kjeldahl method for protein N
Dewey and Lonnerdal (91)	1.28	0.22	—	—	Dye-binding assay
Butte et al. (128)	0.89	0.12	—	—	Kjeldahl method for protein N
Garza et al. (125)	0.82	0.04	—	—	Kjeldahl method for protein N

¹BCA, bicinchoninic acid assay; Ref, reference.

In addition, they provide essential nutrients such as PUFAs and lipid-soluble vitamins. Lipids are present as an emulsion in human milk. The vast majority of lipids are TGs, contributing about 98% of the lipid fraction, followed by phospholipids (0.8%), cholesterol (0.5%), and others. Lipid components are packaged into milk fat lipid globules, with the phospholipids forming the bulk of the membrane and the triacylglycerols found in the core (18, 25, 50).

The method most widely used is solvent extraction followed by gravimetry. Common methods for determining total lipid concentration include the Folch and Rose-Gottlieb methods, which both use solvent extraction followed by gravimetric determination. Folch originally employed 2:1 chloroform:methanol but later modified by substituting chloroform with dichloromethane for lower toxicity (50). The Rose-Gottlieb and its modified methods (e.g., Mojonnier method) use a hexane-ethyl ether-petroleum ether-ethyl alcohol-ammonia solvent system (100, 101). In general, solvent in the Folch method is more polar than that in the Rose-Gottlieb method, therefore total lipid measured by the Folch method tends to be higher because more polar lipids such as phospholipids can be recovered. The Creamatocrit procedure is a simple and quick method to measure lipid content of human milk (102). It was widely used for samples from field studies but is not accurate for

database development. In addition, other methods such as turbidimetry, colorimetry, densitometry, and infrared spectrophotometry have also been used to measure total lipids. Nonetheless, owing to their inherent drawbacks, they are not commonly used at present (50, 101, 103). Finally, total lipid can be estimated by summing up individual fatty acids analyzed by GC or GC-MS, based on the fact that over 98% of milk fat is TGs. Again, this method is very time consuming, costly, and requires good chromatographic separation and standards to identify and quantify individual fatty acids. For this method to be most accurate, theoretically, a conversion factor needs to be determined in order to include the glycerol parts, which provides the added advantage of obtaining a fatty acid profile at the same time.

Fifteen studies contained total lipid data, of which Folch or Rose-Gottlieb-based methods were found to be the predominant methods, with each being used in 6 studies (Table 3). Surprisingly, the degree of fluctuation of total lipid was quite consistent across different studies, methods, and lactation stages. The CV was 15.2% for lactation 1–6 mo and 5.9% for 7–12 mo. In accordance with the previous discussion, total lipids measured by the Folch method usually showed significantly higher values than the Rose-Gottlieb methods ($P = 0.047$) (Figure 2B). However, in some cases, the Folch method gave low values

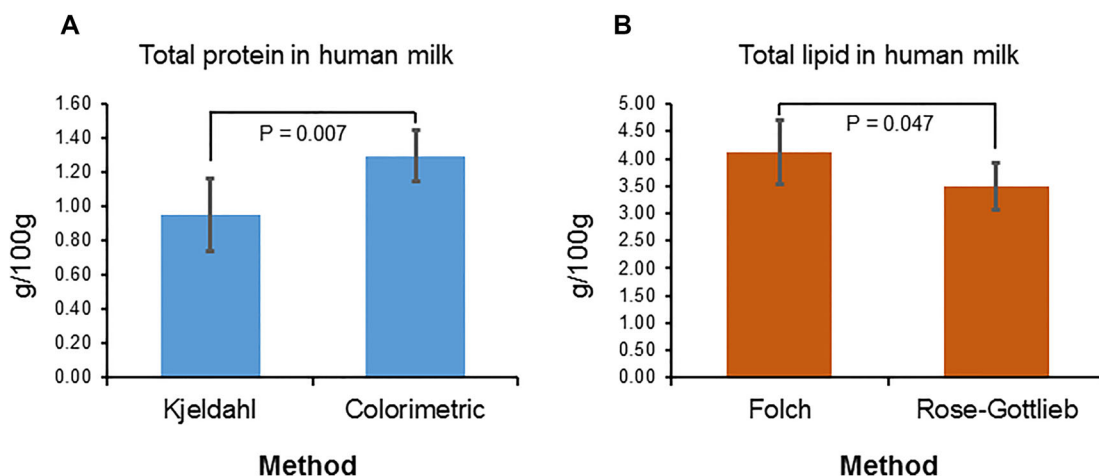
**FIGURE 2** Comparisons between the methods for total protein (A) and total lipid (B) (data were expressed as means \pm SDs).

TABLE 3 Total lipid contents in human milk for populations in the United States and Canada (1980–2017)

Ref ¹	Lipid				Method
	1–6 mo		7–12 mo		
	Mean	SD	Mean	SD	
Clark et al. (88)	4.49	0.53	—	—	Folch procedure
Perrin et al. (98)	—	—	3.83	1.94	Moisture/fat analyzer
Dewey et al. (36)	4.09	2.02	3.63	2.21	Colorimetric
Nommsen et al. (35)	3.58	0.82	3.65	0.95	Folch procedure
Butte et al. (111)	3.08	0.81	—	—	Roese-Gottlieb mixed ethers
Stuff and Nichols (131)	3.04	0.83	3.32	1.15	Roese-Gottlieb mixed ethers
Gross et al. (92)	3.83	1.37	—	—	Roese-Gottlieb mixed ethers
Butte et al. (90)	4.26	1.14	—	—	Roese-Gottlieb mixed ethers
Ferris et al. (130)	4.78	1.11	—	—	Folch procedure
Dewey and Lonnerdal (91)	4.42	1.53	—	—	Colorimetric
Butte et al. (128)	3.44	0.84	—	—	Roese-Gottlieb mixed ethers
Garza et al. (125)	3.49	0.78	—	—	Roese-Gottlieb mixed ethers
Clark et al. (126)	4.49	0.55	—	—	Folch procedure
Glew et al. (96)	3.27	2.27	—	—	Folch procedure
Glew et al. (122)	4.53	1.84	—	—	Folch procedure

¹Ref, reference.

(3.27 ± 2.27 g/100 g) while the Roese-Gottlieb method yielded higher values (4.26 ± 1.14 g/100 g). In addition to the possible variations due to differences in samples and solvents, detailed experimental procedures, such as solvent to sample ratio, extraction times, and separation between lipid phase and aqueous phase, could also contribute to the difference. Based on the foregoing discussion, the Folch or Roese-Gottlieb-based methods are easy to conduct and could generate agreeable data. However, in the future, analyzing fatty acids could provide a good estimate of total lipids. Whereas total lipids remain stable, the fatty acid profile of human milk may vary in relation to maternal diet and health conditions. For instance, with long-chain PUFA intake in the Western world shifting toward ω -6 fatty acids, the DHA composition of human milk is particularly low in North American populations (9). The human milk inflammatory factors and fatty acid composition might be related. A possible association was observed between TGF- β 2 and the fatty acids in human milk (44).

Carbohydrates. The principal carbohydrate of human milk is lactose, a β -disaccharide consisting of glucose and galactose. Of all mammals, lactose is present in the highest concentration in the milk of humans, corresponding to the high energy demands of the human brain (18). In addition, human milk is comprised of a complex mixture of HMOs that differ in size, charge, and abundance. In mature milk, the concentration of HMOs was found to be 5–20 g/L (104). HMOs are composed of both neutral and anionic species with building blocks of 5 monosaccharides: D-glucose, D-galactose, N-acetylglucosamine, L-fucose, and N-acetylneuraminic acid. The basic structure of HMOs includes a lactose core at the reducing end and they are elongated by N-acetylglucosamine units, with greater structural diversity provided by extensive fucosylation and/or sialylation wherein fucose and sialic acid residues are added at the terminal positions. Milks from randomly selected mothers contain 23–130 different HMOs (29). Although research on HMOs started in the early 1900s on the function of HMOs for breastfed neonates related to their high quantity and diversity, their indigestibility has only been revealed since the 1980s (29, 105). HMOs have now been widely recognized as

prebiotics—indigestible carbohydrates that stimulate the colonization of beneficial gut microflora (86). Most recent studies have suggested that their functions may go beyond prebiotic effects, including acting as signaling molecules, immune-modulators, and possible brain function developers (106).

As for other macronutrients, various methods based on different analytic principles were developed to analyze lactose in milk or milk products (107). Among them, the most commonly used one is based on the enzyme hydrolysis of lactose. The lactose is hydrolyzed by β -galactosidase or acid/base into glucose and galactose, then the glucose or galactose is quantified colorimetrically by reacting with an enzyme (e.g., glucose oxidase) and a color reagent (108–110). The assay can be performed manually or by a more automated biochemistry analyzer (111). However, chromatography-based methods, especially HPLC and LC-MS, are superior to enzymatic methods in many different ways. First, no reactions are involved and thus possible artificial products are not produced. Second, they are direct methods that can analyze different mono- and disaccharides and quantify them with a single run. Third, a much smaller sample size is required for analysis, because usually a milliliter-level quantity is enough. In recent years, mid-infrared spectroscopy has been used to analyze macronutrients in human milk (112–114). Although shown to be a quick alternative technology with acceptable accuracy in field studies (115), it behaved poorly for quantifying lactose because measurement is confounded by the presence of oligosaccharides (113).

Tremendous efforts have been made in recent years to understand the compositional profile of HMOs in human milk (116). However, in regard to quantifying total HMOs, there is still no consensus method available. Accurate quantification of individual and total HMOs remains a challenge (117). Traditionally, quantification of HMOs has involved multiple steps. The oligosaccharide fraction of milk is usually obtained by precipitating protein and then the HMOs are separated from the lactose. The weight of the oligosaccharide fraction could be used as a rough estimate of total HMOs. Analysis and quantification of individual HMOs are accomplished by chromatographic technology such as HPLC and high pH anion exchange chromatography with

TABLE 4 Lactose contents in human milk for populations in the United States and Canada (1980–2017)

Ref ¹	Lactose				Method
	1–6 mo		7–12 mo		
	Mean	SD	Mean	SD	
Perrin et al. (98)	—	—	5.67	0.73	LC-MS
Dewey et al. (36)	7.56	0.29	7.49	0.51	Enzymatic colorimetric (Dahlqvist)
Nommsen et al. (35)	7.22	0.17	7.15	0.27	Enzymatic colorimetric (Dahlqvist)
Butte et al. (111)	6.51	0.20	—	—	Colorimetric (Biochemistry analyzer)
Gross et al. (92)	6.97	0.61	—	—	Enzymatic colorimetric
Ferris et al. (130)	6.67	0.67	—	—	Enzymatic colorimetric
Dewey and Lonnerdal (91)	7.17	0.51	—	—	Enzymatic colorimetric
Butte et al. (128)	6.59	0.24	—	—	Colorimetric (Biochemistry analyzer)

¹Ref, reference.

pulsed amperometric detection (HPAEC-PAD) and, more recently, mass spectrometric technology (118).

Eight studies provided data for lactose, of which 7 were for the lactation stage 1–6 mo (Table 4). Except for 1 recent study using HPLC-MS (98), all other papers used enzymatic methods. The data from 7 studies for the lactation stage 1–6 mo yielded quite consistent data, with the CV only 5.6%. This is in accordance with the previous finding that the concentration of lactose in human milk is the least variable of the macronutrients (35). The reason is probably due to the critical role lactose plays in addition to being a major source of energy. Lactose exerts 60–70% of the total osmotic pressure of human milk. Compared with glucose, lactose provides nearly twice the energy value per molecule (14). Notably, the lactose analyzed by LC-MS yielded a much lower value compared with enzymatic methods. However, because the result is from only 1 study, no statistical analysis could be done, nor do we know the cause for the variability. More studies are needed to compare and evaluate the 2 types of methods. Despite the extensive research on HMOs in recent years, only 2 studies reported total HMOs for the US population.

Energy. Energy in human milk can either be measured by bomb calorimetry, or be calculated from proximate composition (protein, fat, and carbohydrate) by applying energy conversion factors (119). Bomb calorimetry directly measures energy and thus is considered an accurate method. However, a lot of researchers still use calculated methods owing to their simplicity and generally satisfactory results. The general energy conversion factor was originally developed by WO Atwater and his

colleagues at USDA (120), and it is recognized as the Atwater general factor. It uses a single factor for each of the energy-yielding substances, with the values 4, 9, and 4 kcal/g for protein, fat, and carbohydrate, respectively (120). After the Atwater general factor was developed, a more extensive general factor system was developed for specific foods. Over the years, different researchers have used slightly different energy conversion factors to calculate energy in human milk, which obviously affected the values obtained. They are superior to the original Atwater factors because they take into consideration the differences of different energy sources (77), although there is still no consensus on which energy conversion factors should be used. The UN's FAO published energy conversion factors for milk and milk products as 4.27, 8.79, and 3.87 kcal/g for protein, fat, and carbohydrate, respectively (77). A comparison between measured and calculated energy contents of milk was conducted in a recent review, which showed measured values were –6 to 10 kcal/dL (–9 to 13%) greater than the calculated values (10).

Nine studies provided data on energy of human milk (Table 5). Of them, 5 used bomb calorimetry and 4 used the calculated method. Interestingly, 4 different energy conversion factors were used in the calculations, including general Atwater factors and 3 food-specific modified Atwater factors. Despite this difference, the values were actually quite consistent, with the CV only 8.6%.

Minerals. Minerals in human milk can be divided into 3 major categories: 1) major minerals, such as calcium, phosphorus, and magnesium, which are tightly regulated in maternal serum; 2) electrolytes, including sodium, potassium, and chloride, which in milk are

TABLE 5 Energy in human milk for populations in the United States and Canada (1980–2017)

Ref ¹	Energy				Method
	1–6 mo		7–12 mo		
	Mean	SD	Mean	SD	
Nommsen et al. (35)	68.09	7.81	68.62	9.09	Calculated 5.65, 3.95, 9.25 kcal/g
Butte et al. (111)	64.00	7.07	—	—	Bomb calorimeter
Gross et al. (92)	67.07	11.74	—	—	Calculated 4.27, 3.87, 8.87 kcal/g
Butte et al. (90)	65.62	9.89	—	—	Bomb calorimeter
Ferris et al. (130)	75.43	10.71	—	—	Calculated 4.27, 4.27, 8.87 kcal/g
Garza and Butte (64) ²	70.47	9.35	—	—	Bomb calorimeter
Dewey and Lonnerdal (91)	73.59	14.04	—	—	Calculated 4, 4, 9 kcal/g
Butte et al. (128)	64.50	8.79	—	—	Bomb calorimeter
Garza et al. (125)	55.87	3.98	—	—	Bomb calorimeter

¹Ref, reference.

²24-h collection.

TABLE 6 Major minerals in human milk for populations in the United States and Canada (1980–2017)¹

Ref	Calcium				Magnesium				Phosphorus		Method
	1–6 mo		7–12 mo		1–6 mo		7–12 mo		1–6 mo		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Allen et al. (79)	28.07	2.01	22.95	3.19	3.87	0.24	3.98	0.48	—	—	Ion-selective electrodes and colorimetry
Perrin et al. (98)	—	—	19.40	2.63	—	—	—	—	—	—	ICP-MS
Dewey et al. (36)	22.94	4.01	22.36	4.27	3.12	0.56	3.06	0.53	—	—	AAS and AES
Gross et al. (92)	24.98	5.98	—	—	2.62	0.70	—	—	15.08	3.71	AAS and colorimetric
Butte et al. (90)	25.38	3.16	—	—	3.46	0.83	—	—	14.44	2.75	AAS and colorimetric
Dewey and Lonnerdal (91)	25.30	4.54	—	—	3.17	0.55	—	—	—	—	AAS
Garza et al. (125)	21.34	1.16	—	—	—	—	—	—	—	—	AAS
Friel et al. (95)	27.96	3.69	—	—	2.91	0.51	—	—	—	—	ICP-MS
Feeley et al. (127)	26.20	6.14	—	—	5.00	1.26	—	—	13.30	3.76	ICP

¹AAS, atomic absorption spectrometry; AES, atomic emission spectrometry; ICP, inductively coupled plasma-atomic emission spectrometry; Ref, reference.

determined by an electrical potential gradient in the secretory cell; and 3) trace elements, such as iron, copper, and zinc, which exist at very low concentrations in human milk and may be influenced to a large extent by maternal diet (14).

When analyzing minerals, electrolytes, and trace elements in human milk, it is important to remember that the samples must be collected in acid-washed, mineral-free containers to eliminate contamination. Whole milk (not defatted or deproteinized milk) should be used because considerable amounts of certain minerals may be present in these removed fractions (109). Analytic methods for measuring minerals in milk and milk products were reviewed in a recent paper (121). Historically, a number of techniques were used to determine minerals and elements in milk, including colorimetry, titrimetry, and ion-selective electrodes. However, they have been largely replaced since the 1980s with atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES) with proper digestion procedures. AAS was firstly introduced with a flame atomization source (FAAS) and later a graphite atomizer. The AES technique can be used either as flame emission spectrometry or as inductively coupled plasma-atomic emission spectrometry (ICP). ICP coupled with a mass spectrometer (ICP-MS) after high-pressure digestion is currently the best technology for mineral analysis (121).

Data from 14 studies are summarized in Tables 6–8. Again, most of the data were generated for 1–6 mo postpartum. The majority of papers used either AAS or AES, and only 3 early papers used colorimetry or ion-selective electrodes. Calcium, magnesium, sodium, potassium, zinc, copper, and iron are among the most measured minerals; each was presented in >3 studies. In general, the levels for major minerals from different times, researchers, and by different analytic methods are fairly consistent. For instance, the CV of calcium for 1–6 mo is 9.1%, and for 7–12 mo, 8.8%.

Other nutrients. There are scattered data on other nutrients, including fatty acids, cholesterol, glucose, vitamin E, and retinol. Four studies provided data on fatty acids (Table 9). Although over 200 fatty acids have been found in human milk (18), about 20 fatty acids are major fatty acids with 7 of them (12:0, 14:0, 16:0, 18:0, 16:1, 18:1, and 18:2) in amounts >1% (100). Data from the 4 studies largely agreed with previous findings. Fatty acids must be converted to methyl esters prior to analysis by GC or GC-MS to increase volatility. Methylation of fatty

acids is usually done by reactions with sodium hydroxide-methanol or boron trifluoride-methanol (100). There have been recommendations to use the boron trifluoride method for human milk and the sodium methoxide method for formulas and bovine milk (50). Of the 4 studies, 1 used the sodium methoxide method (88) and 2 used the sodium methoxide/boron trifluoride method (96, 122). Acetyl chloride in methanol-benzene was used in the fourth paper (123), which may lead to incomplete methylation, thus partly explaining the low total fatty acids reported (Table 10).

However, for other nutrients, because the numbers of studies are too few (≤ 2), no statistical analysis could be conducted and the data quality cannot be evaluated (Table 10).

Closing remarks and future directions

This review focused on summarizing the current knowledge and identifying knowledge gaps in human milk nutrient composition. Based on the foregoing discussions, several conclusions can be drawn with suggestions for future research directions:

- 1) Human milk nutrient composition is fundamental information for the management of infant breastfeeding, and serves as the gold standard for developing infant formulas. Despite the great need for more accurate, representative, and up-to-date data, the current review found that in the last 37 y, at least for the research published for the US and Canadian populations, the data are quite limited. Only 28 studies containing original data were found.
- 2) Of the 28 studies, most of them were published before 1990. Problems associated with these old data could be: 1) the analytic methods used are outdated, thus data quality could be questionable; and 2) the data may not reflect the current population. Rapid diet and demographic changes over the past few decades may affect human milk composition. For instance, there is a continual trend to replace saturated fats with polyunsaturated fats, which may in turn change the human milk fatty acid composition. In an Australian study, the linoleic acid content in human milk increased during 1981–2000, whereas DHA decreased at the same time (124).
- 3) In general, the study designs of these 28 studies were not comprehensive. The subject numbers in most of these studies were small (<50) and the subjects were largely healthy Caucasians, so

TABLE 9 Fatty acids in human milk for populations in the United States and Canada (1980–2017)

Fatty acid	Clark et al. (88)		Tijerina-Saenz et al. (123)		Glew et al. (96)		Glew et al. (122)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4:0	—	—	—	—	—	—	0.000	0.000
6:0	—	—	—	—	0.001	0.001	0.001	0.001
8:0	—	—	—	—	0.003	0.001	0.004	0.002
10:0	0.043	0.003	0.026	0.009	0.036	0.009	0.054	0.014
12:0	0.191	0.023	0.148	0.056	0.140	0.042	0.250	0.113
14:0	0.256	0.019	0.188	0.059	0.163	0.050	0.285	0.116
15:0	—	—	—	—	0.009	0.003	0.011	0.003
16:0	0.976	0.089	0.615	0.089	0.615	0.069	1.004	0.105
17:0	—	—	—	—	0.010	0.002	0.015	0.005
18:0	0.342	0.062	0.194	0.047	0.208	0.039	0.340	0.043
20:0	0.009	0.001	—	—	0.006	0.001	0.009	0.002
22:0	0.008	0.001	—	—	0.004	0.002	0.009	0.002
24:0	—	—	—	—	0.001	0.000	0.002	0.017
16:1	0.162	0.012	0.082	0.021	0.072	0.019	—	—
18:1	1.685	0.256	1.070	0.131	1.060	0.111	1.452	0.163
20:1	—	—	—	—	0.012	0.003	—	—
22:1	—	—	—	—	0.003	0.001	0.003	0.003
24:1	—	—	—	—	0.001	0.002	0.002	0.002
18:2	0.680	0.080	0.406	0.096	0.651	0.150	0.834	0.151
18:3	0.068 ¹	0.009	0.045	0.016	0.044	0.001	0.061	0.024
20:2	—	—	—	—	0.020	0.005	—	—
20:3	0.014	0.001	—	—	0.012	0.003	0.019	0.016
20:4	0.019	0.001	0.013	0.002	0.014	0.003	0.014	0.004
20:5	—	—	0.003	0.002	0.003	0.001	0.002	0.004
22:2	—	—	—	—	0.001	0.000	—	—
22:4	—	—	—	—	0.003	0.001	0.004	0.002
22:5	—	—	—	—	0.005	0.000	0.003	0.001
22:6 <i>trans</i>	—	—	0.009	0.005	0.004	0.001	0.005	0.002
16:1 <i>trans</i>	—	—	—	—	0.005	0.001	0.019	0.004
18:1 <i>trans</i>	—	—	—	—	0.133	0.018	0.222	0.002
18:2 <i>trans</i>	—	—	—	—	0.022	0.001	—	—
Total	4.453	—	2.799	—	3.259	—	4.623	—

¹18:3 + 20:1.

the study populations did not represent the diversity of the current American population. Because these studies were published over a 37-y period, the experimental designs, including sampling, storage, and analytic methods, varied substantially between the different studies. All of these factors make data quality evaluation and comparison very difficult.

- 4) From the standpoint of updating the data in the USDA Food Data System, data for several nutrients from these 28 studies showed some consistency for 1–6 mo, especially for protein, fat, lactose, energy, and certain minerals (e.g., calcium). The data for

7–12 mo and for other nutrients are very scarce, and definitely need additional research.

- 5) Human milk is recommended as the sole source of nutrition for young infants, which contributes to normal physiologic and cognitive development during infancy and in later life. Country-specific human milk nutrient information is the foundation for the management of infant feeding. Nevertheless, based on this current review, knowledge on human milk nutrient composition in the United States is still very limited. More comprehensive studies or a set of studies on human milk nutrient composition,

TABLE 10 Other nutrients in human milk for populations in the United States and Canada (1980–2017)¹

Ref	Cholesterol		Glucose				HMO				Vitamin E		Retinol		Method
	1–6 mo		1–6 mo		7–12 mo		1–6 mo		7–12 mo		1–6 mo		1–6 mo		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Clark et al. (88)	9.83	0.37	—	—	—	—	—	—	—	—	—	—	—	—	GC-MS
Allen et al. (79)	—	—	27.70	2.55	24.33	5.50	—	—	—	—	—	—	—	—	Analyzer
Perrin et al. (98)	—	—	—	—	—	—	—	—	0.70	0.19	—	—	—	—	HPLC
Alderete et al. (133)	—	—	—	—	—	—	1.05	0.21	—	—	—	—	—	—	HPLC
Tijerina-Saenz et al. (123)	—	—	—	—	—	—	—	—	—	—	280.31	—	7.76	7.51	HPLC

¹HMO, human milk oligosaccharide; Ref, reference.

representative of the current US population, are necessary to provide accurate, representative, current data, and data variability. The factors that affect the nutrient content of milk and data quality must be considered in experimental design: stage of lactation, mother's diet and body nutritional needs, mother's genetic background and health condition, time of day, region, sample collection, sample storage methods, and nutrient analytic methods.

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