PERSPECTIVE

Perspective: Human Milk Composition and Related Data for National Health and Nutrition Monitoring and Related Research

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ABSTRACT

National health and nutrition monitoring is an important federal effort in the United States and Canada, and the basis for many of their nutrition and health policies. Understanding of child exposures through human milk (HM) remains out of reach due to lack of current and representative data on HM's composition and intake volume. This article provides an overview of the current national health and nutrition monitoring activities for HM-fed children, HM composition (HMC) and volume data used for exposure assessment, categories of potential measures in HM, and associated variability factors. In this Perspective, we advocate for a framework for collection and reporting of HMC data for national health and nutrition monitoring and programmatic needs, including a shared vision for a publicly available Human Milk Composition Data Repository (HMCD-R) to include essential metadata associated with HMC. HMCD-R can provide a central, integrated platform for researchers and public health officials for compiling, evaluating, and sharing HMC data. The compiled compositional and metadata in HMCD-R would provide pertinent measures of central tendency and variability and allow use of modeling techniques to approximate compositional profiles for subgroups, providing more accurate exposure assessments for purposes of monitoring and surveillance. HMC and related metadata could facilitate understanding the complexity and variability of HM composition, provide crucial data for assessment of infant and maternal nutritional needs, and inform public health policies, food and nutrition programs, and clinical practice guidelines. *Adv Nutr* 2022;13:2098–2114.

Statement of Significance: Our understanding of exposures through human milk (HM) in the United States and Canada remains out of reach due to lack of current and representative data on the composition and intake volume of HM. This article provides an overview of the current national monitoring activities for HM-fed children, including HM composition (HMC) data. In this Perspective, the authors advocate for a framework for collection and reporting of HM composition data for national monitoring and programmatic needs, including a shared vision for a publicly available Human Milk Composition Data Repository (HMCD-R) to include essential metadata associated with HMC.

Keywords: human milk, intake volume, breast milk, B-24 population, variability in human milk, national monitoring, environmental chemicals in human milk, bioactives in human milk, nutrients in human milk, human milk composition data repository (HMCD-R)

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Introduction

Human milk (HM) is a complex biological fluid made up of many structurally and functionally diverse constituents. The impact of HM on infant growth and development goes beyond its nutritional value (1–5); however, its complexity, composition, and variability are not fully understood (5, 6). Christian et al. (7) have recommended studying HM as a biological system to better understand the interactions of factors that impact HM composition (HMC), intake volume, and the functionality of its diverse constituents for optimizing maternal and infant health. Accurate estimates of dietary exposures for HM-fed children require up-to-date HMC and intake volume data; however, these estimates remain out of reach due to lack of current and nationally representative data for both HMC and intake volume in the United States and Canada (8).

A joint United States-Canada federal government undertaking, the Human Milk Composition Initiative, was initiated in 2018 to articulate how HMC-related data are relevant to both countries' federal programs, policies, and regulations (9). This article provides an overview of the current national monitoring activities for HM-fed children, potential measures in HM, food composition databases used for exposure assessments, and variability factors associated with HMC. In this Perspective, we advocate for a framework for collection and reporting of HMC data for public health and nutrition monitoring, research, and programmatic needs including a shared vision for a Human Milk Composition Data-Repository (HMCD-R), to include essential metadata associated with HMC.

Monitoring Health, Nutrition, and Environmental Chemicals in the United States and Canada

National health and nutrition monitoring programs by the federal governments in the United States and Canada are essential surveillance efforts of their populations' health. They provide nutrient intake estimates from foods, beverages, and dietary supplements and prevalence and trends of selected diseases, risk factors, behaviors, and environmental exposures (10). Linking these data to anthropometric, laboratory, and clinical evaluation data and health outcomes, if available, allows for examination of cross-sectional associations at the national and large subgroup population levels (11). These data are used to inform, guide, and monitor government programs and policies, including the DRIs (12), Dietary Guidelines for Americans (13, 14), Canada's Food Guide (15), food-fortification policies (e.g., iron, vitamin D, and iodine), and food labels (16), among others.

Table 1 summarizes the major federal health and nutrition monitoring efforts in the 2 countries. They vary in their objectives, degree of details, and target populations. NHANES (17–19) monitors the health and nutritional status of the US population of all ages, through interviews and physical examinations. For children aged birth to 24 mo (B-24), it collects frequency of HM consumption through 24-h dietary recalls and questions on infant feeding practices, including breastfeeding, timing of introduction/stoppage of infant formula or complementary foods and beverages, and mode of feeding HM. The Canadian Community Health Survey (CCHS) is an annual general health survey of ~65,000 Canadians aged >12 y (17, 20) with periodic focused surveys on topics of interest. The B-24 population is excluded from CCHS-Annual Component; however, a nutrition-focused survey conducted in 2015 (21), provided data on number of times HM is consumed for children aged 12–24 mo (n = 404) (22). Similarly, the Canadian Health Measures Survey does not include children aged <3 y (23, 24). HM samples, its composition, or volume are not collected in any of the above national surveys. However, these surveys can be potentially important vehicles in future, given their representative sampling methodologies and detailed collection of demographic and health-related data.

Environmental chemicals

Monitoring exposures of environmental chemicals during infancy is important, because it can be the period of largest exposure. Many of these chemicals accumulate within the mothers' tissues and have the potential to pass directly into HM, which can function as an excretion pathway (25, 26), as in polychlorinated biphenyls (27). HM is not included in the 2 US national surveys that monitor exposure to environmental chemicals—the FDA's Total Diet Study (28) and NHANES (18, 29).

Two programs in United States/Canada—Environmental influences on Child Health Outcomes (ECHO) and Maternal-Infant Research on Environmental Chemicals (MIREC)—were established with a focus on environmental chemicals, including in HM (Table 1). The ECHO cohort is made up of >70 individual cohorts of mothers and children from ongoing research projects, forming a massive "cohort of cohorts" of >50,000 children across the United States. HM is an ECHO "recommended data element" (i.e., ECHO strongly encourages, but does not require cohorts to collect), and ~225 samples have been collected since 2016. For the cohorts that do collect, HM is collected and

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Abbreviations used: CANLINE, Canadian Laboratory Information Network; CCHS, Canadian Community Health Survey; ECHO, Environmental influences on Child Health Outcomes; HM, human milk; HMC, human milk composition; HMCD-R, Human Milk Composition Data Repository; MIREC, Maternal-Infant Research on Environmental Chemicals; NASEM, National Academies of Sciences, Engineering, and Medicine; SR, standard reference; TEQ, toxicity equivalent factor.

TABLE 1 Major efforts for monitoring health, nutrition, and environmental chemicals in the United States and Canada[1]

	Objectives	Target population	Sample size		Selected B-24 relevant measurements
			Total	Birth to 24 mo (B-24)	
NHANES, an ongoing nationally representative survey in the USA, since 1971; continuous since 1999 (17, 18)	Continuous monitoring and data collection on health and nutritional status	Noninstitutionalized, civilian US population of all ages	~10,000/2-y cycle	~600–900/2-y cycle	Food, beverage, and nutrient intake, dietary supplements, anthropometric measurements, and laboratory tests. Includes data on human milk (HM) consumption (frequency) collected using 24-h dietary recalls and questions on breastfeeding history and infant feeding practices
The Canadian Community Health Survey (CCHS), a cross-sectional survey conducted since 2000 (196) the phrase	Gathers health-related data (information on determinants of health, health status, and use of the health system) at community level	Population aged ≥12 y	~65,000	Not applicable	Questions on prenatal exposures (e.g., smoking, alcohol consumption, folic acid supplementation) and infant feeding behaviors (e.g., breastfeeding initiation and related behaviors/beliefs, introduction of complementary food and beverages, vitamin D supplementation, and gestational weight gain) are administered to female respondents (ages 15–55 y, who gave birth in previous 5 y)
Canadian Health Measures Survey (CHMS)—ongoing (23)	Gathers health-related information to help improve the prevention, diagnosis, and treatment of illnesses and to promote health and wellness	3–79 y	>5000 including ~1000 women aged 15–49 y every 2 y	Not applicable	Includes anthropometric, sociodemographic, nutrition, environmental exposure, and biomarker information, among other data
Environmental influences on Child Health Outcomes (ECHO) 2016–2022 (197)	Understanding the effects of a broad array of early environmental exposures on children's development and health outcomes	>70 individual cohorts of mothers and children from ongoing research projects	>50,000 children	~10,000	Standardized data elements such as sociodemographics, pregnancy and family history, maternal diet, caregiver environment, child's physical and neurodevelopmental health, among others. Questions related to breastfeeding include initiation of breastfeeding, duration, and whether the HM consumed was from the breast, pumped, or both. About 225 HM samples have been collected so far; none have been analyzed

(*Continued*)

TABLE 1 (*Continued*)

	Objectives	Target population	Sample size		Selected B-24 relevant measurements
			Total	Birth to 24 mo (B-24)	
Maternal-Infant Research on Environmental Chemicals (MIREC), a longitudinal Canadian pregnancy cohort study (2008–2011) (198)	To examine potential adverse health associations on maternal and infant health with exposure to priority environmental chemicals, including those in HM	Pregnant women from 10 cities across Canada	2001	525	Maternal biospecimens were collected throughout pregnancy, along with questionnaire and clinical data, and HM after delivery. HM samples (n = 1017) in MIREC were analyzed for 188 unique environmental chemicals, in addition to several nutritional constituents

[1]B-24, birth to 24 mo; HM, human milk.

stored under standardized conditions and is diverse in terms of factors, for example, urban and rural settings, ethnicity, gestational age at delivery (30). MIREC is a longitudinal Canadian pregnancy cohort study conducted between 2008 and 2011. Over 1000 HM samples were collected, along with information on the date and time, hand/pump expression, right/left breast, and timing of sampling within a feed (4).

To summarize, there is no nationwide monitoring of HM in the United States or Canada. HM samples were collected for study of environmental chemicals over a decade ago in MIREC, and limited HM samples are being collected in the ECHO program, to be useful for current national estimates. However, these 2 programs can provide insights for developing a framework for collection of data on HMC for public health nutrition research and informing policies.

Human Milk Volume

Quantifying HM intake volumes is crucial to determining exposure estimates. Researchers have mainly relied on estimating volume based on a child's age, exclusive or partially fed HM, and volume of other types of milk or infant formula consumed. These calculations (**Table 2**) were first developed for the Feeding Infants and Toddlers Study (31) and have since been used for NHANES (17, 32, 33), and DRIs (25). Problematically, these assumptions and calculations are based on limited data from 3 studies from the 1980s and 1990s, with a limited number of subjects (n = 8–46) from the United States and Australia (34–36). Furthermore, the approach of imputing standard volumes does not consider variability in intake volume. Many health outcomes of HM intake could be dose-dependent (37); the range of intake is large and potentially affected by several physiological and lifestyle factors related to the mother and child (discussed later). Hence, there is a demonstrable need for measurement of HM intake volumes of US and Canadian children to determine accurate exposure estimates. Furthermore, many studies estimate HM intake by weighing infants before and after feeding, a method susceptible to human and mechanical errors, and requiring further corrections to account for insensible fluid loss (38). The use of the deuterium oxide (^2H_2O) technique for quantifying HM intake has also been used and was reviewed by da Costa et al. (39). In pooled data from 12 countries, they reported an average daily HM intake of ∼600 mL/d during the first month of life, rising to ∼820 mL/d at 3–4 mo before declining gradually at 8–9 mo—higher than in previous reports, including those used for NHANES and DRIs (39).

Food Composition Databases and Human Milk Composition Data in the United States and Canada

Food composition databases can contain traditional nutritive (macronutrients, vitamins, minerals, fatty acids, and amino acids) and bioactive components (e.g., carotenoids, flavonoids, glucosinolates), among others. They provide the foundation for food and nutrition research, dietary practice, and analysis of dietary studies, essential to the development

TABLE 2 Human milk intake volume estimates for exclusively HM-fed infants (43–48) currently used for exposure assessment in the United States and Canada[1]

	Infants (0–6 mo)	Infants (>6 mo)	Young children (12–24 mo)
Daily intake (mean)	780 mL	600 mL	N/A (varies)
Intake per feed	—	—	89 mL (12–17 mo); 59 mL (18–24 mo)

[1]For infants partially fed HM, the volume of infant formula plus "other" milk reported consumed on the recall day are subtracted from the age-specific reference volume to estimate HM consumed.

of food and nutrition policies, guidelines, regulations, food and nutrition programs, and risk assessments (29, 40–42).

The USDA Nutrient Database for Standard Reference (SR) (a legacy database since 2018) has served as the primary source of food composition data for most databases in the United States, including for NHANES. The USDA FoodData Central, launched in 2019, is an integrated data system that includes SR and other USDA food composition databases and adds new databases—"Foundation Foods" and "Experimental Foods"—that have nutrient information and extensive underlying metadata that will help users understand nutrient variability (43). However, the limited existing data for HM are from the late 1970s (original data source unknown and no information on sampling, storage, or laboratory analysis). It is recommended by USDA that these data are not used and should be replaced (44).

The Canadian Nutrient File was initiated in 1979 to support nutrition surveys and other needs of Canadian public health agencies, the food industry, hospitals, and universities (45). The public version of the Canadian Nutrient File (2015) reports only 1 nutrient profile for HM, which is primarily sourced from SR with a few *trans*-fatty acids analyzed in Canada. A separate searchable food data repository, the Canadian Laboratory Information Network (CANLINE) (46), contains chemical, nutritional, and microbial surveillance data, food and collection metadata but no data on HM.

In 2017, USDA conducted a review of the existing literature to summarize current knowledge on macro- and micronutrients and update the HMC data in its database (47). They identified 28 studies conducted in the United States/Canada on macro- and micronutrient content of HM over 37 y (1980–2017); most were published before 1990 with relatively small sample sizes and limited generalizability due to different sampling, storage, and analytic methods. The reviewers underlined the need for comprehensive studies to provide current and complete nutrient information on HM in the United States and to fully understand the magnitude of variability in composition. A joint federal workshop sponsored by the NIH in 2017 on "Human Milk Composition – Biological, Environmental, Nutritional, and Methodological Considerations" also emphasized the need to fill these gaps in availability of information on HMC, factors that influence HMC, and their relation to maternal and infant health (9). In 2020, the National Academies of Sciences, Engineering, and Medicine (NASEM), scanned the existing literature and identified ~126 studies on HMC and volume to inform DRIs. The expert group noted the lack of data for several nutritive components and HMC after 6 mo postpartum, and inconsistency in sampling, HM collection, and analytical methods (5).

Potential Measures in Human Milk

Potential measures in HM can be categorized into several groups, based on structural similarity (**Figure 1** lists examples, and **Supplemental Figure 1** provides details on sources used). A brief overview of some of the major categories follows.

Carbohydrates

Potential measures of carbohydrate components of HM include several types of saccharides. Lactose is the predominant sugar, followed by bioactive HM oligosaccharides (nondigestible sugars) (48). Over 200 HM oligosaccharides have been elucidated (49).

Protein and nonprotein nitrogen compounds

The major protein-related categories include amino acids, glycoproteins, and other proteins/peptides (excluding glycoproteins) (50–56). HM glycoproteins are the most abundant proteins or important bioactive proteins. Proteomic studies have reported ~3000 proteins in HM (57, 58). Immunoglobulins, cytokines, chemokines, and growth factors contribute to the infant's immune protection and development (59), and gastrointestinal regulatory peptides help with regulation of infant appetite and feeding patterns (60). Small nitrogen-containing compounds, for example, nucleotides and melatonin, are associated with infant sleep regulation (60, 61).

Lipids

Triacylglycerides comprise ~98% of the lipid fraction (8). About 200 fatty acids were found in HM (62, 63) as were phospholipids, building blocks forming the membrane of the HM fat globules (64, 65). Steroids in HM, especially cholesterol and its precursors, and hormones, can influence the growth and behavior of the infant, but the studies of their presence in HM are limited (66–71). Other lipids of interest include lipid mediators, such as prostaglandins, resolvins, lipoxins, and leukotrienes (72–74).

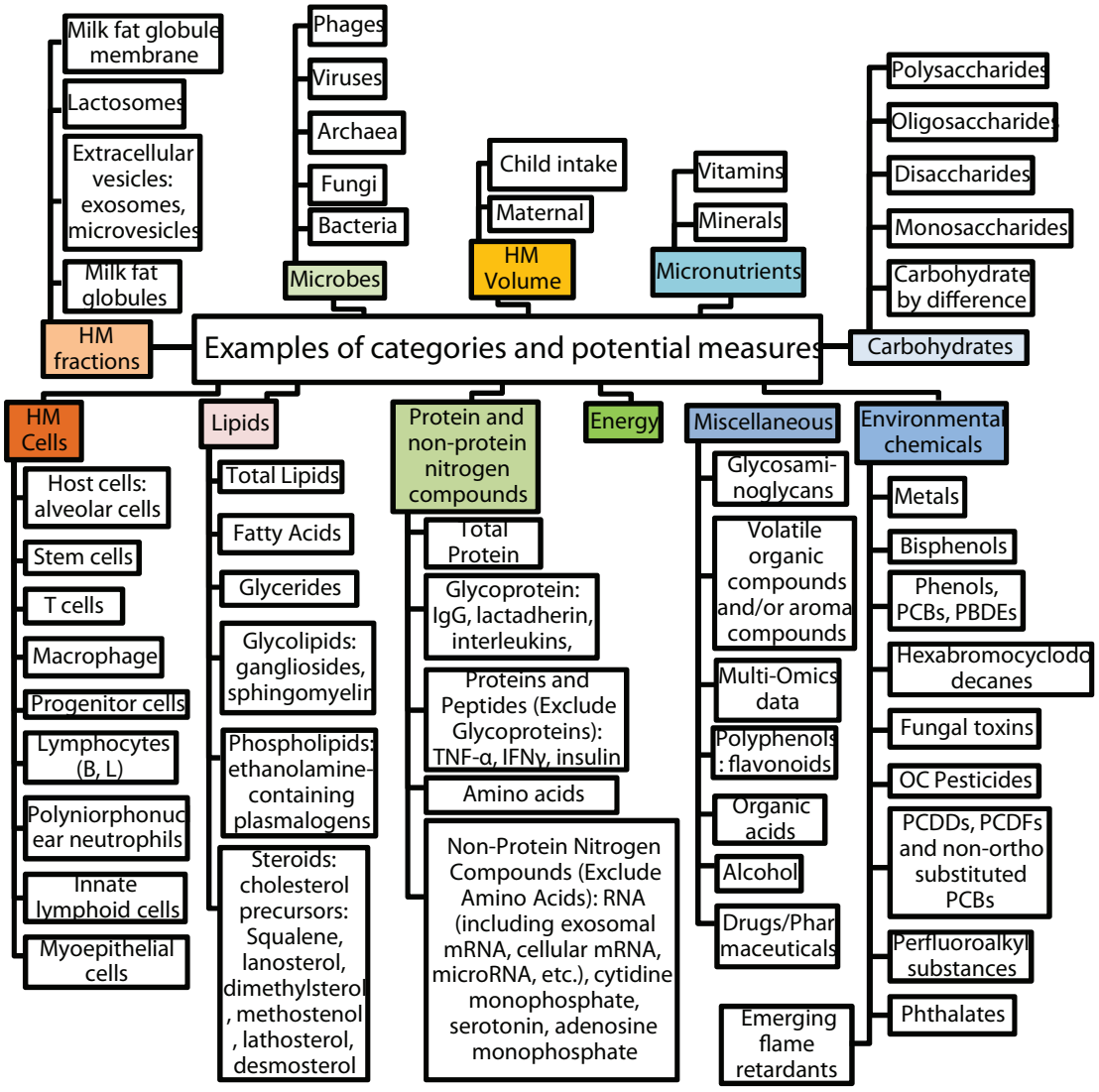

FIGURE 1 Categories of potential components to measure in human milk. HM, human milk; OC, organochlorine; PBDE, Polybrominated diphenyl ethers; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran.

Minerals and vitamins

HM includes major minerals (e.g., calcium and phosphorus), electrolytes (e.g., sodium and potassium), and trace minerals (e.g., iron, copper, and iodine) (75, 76). Vitamins include water- and fat-soluble vitamins. Dror and Allen (77) summarized the current knowledge of nutrients in HM.

Bioactives

HM is rich in a range of bioactives with diverse functions in infant health including antimicrobial factors, immune proteins, growth factors, enzymes, antioxidants, and endocannabinoids. Their presence can partially explain positive health outcomes of HM-fed infants compared with formula-fed counterparts. Many bioactives are proteins (or peptides) with biological activities or carbohydrates like HM oligosaccharides. Bioactives also include living cells like maternal leukocytes and macrophages, or carotenoids, polyphenols, and vitamins (listed under different categories in Figure 1) (53, 62, 78, 79).

Microbes

Bacteria, viruses, fungi, and yeasts are present in HM and associated with the infant's present and future health (80–82). Microbiomes in HM and infant feces are related to each other, but vary by culture/location (83), lactation stage (84), and storage conditions (85). HM transmission of maternal viral infection is well established for cytomegalovirus, HIV-l, and human T-cell lymphotropic viruses (86–89). Transmission of microbes was recently reviewed by Selma-Royo et al. (90).

Environmental chemicals

Women are exposed to a complex mixture of chemicals in their daily lives through their environment, product use, house dust, food, drinking water, air, and workplace exposures; many can partition into HM (25, 26). Figure 1 lists the categories of environmental chemicals analyzed in Methods Advancement for Milk Analysis (US) and MIREC studies (91).

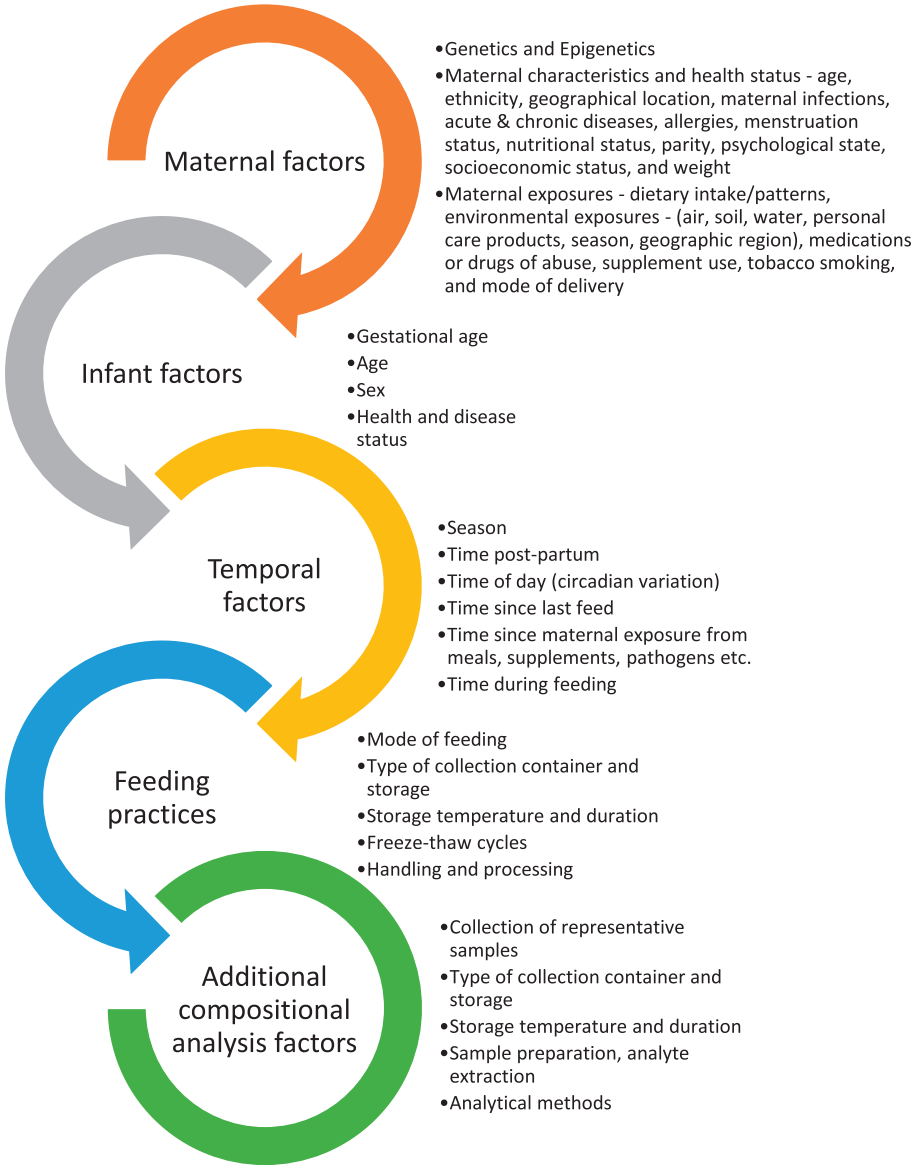

FIGURE 2 Examples of variability factors associated with human milk composition and/or volume.

Prioritizing components to measure

Given the multitude of components in HM, prioritization of the components to analyze is needed. For nutrition monitoring and related research, parameters include:

- Uncertainty or knowledge gap: Data do not exist in the United States/Canada or do exist but with limited generalizability to the US/Canadian population, for example, the HM microbiome.
- Substantial public health concern: Data would address an existing or undercharacterized public health concern and/or are important to monitor in a national sample, for example, per- and polyfluoroalkyl substances (25, 47, 92).
- Potential to inform federal programs: Data are essential to fill gaps in guidance or recommendations to help promote health and prevent chronic disease in the United States/Canada, including but not limited to DRIs, dietary guidance, clinical practice guidelines, and regulations and standards for food and supplement manufacturers, for example, minerals and vitamins.

Variability in Human Milk Composition

HM is unique to each infant-mother dyad, characterized by variability in its composition. HMC varies both between subjects, associated with maternal and infant factors (e.g., genetics, diet, and gestational age at birth) and within subjects by factors (e.g., time postdelivery and time of day). The variability factors can generally be categorized into 4 types (**Figure 2**), examples of which are briefly discussed below, to emphasize the need for collection of metadata and

characterizing factors that can impact HMC and volume to further understand their variability. There is some research on impact of variability factors on HMC of macronutrients. More research is needed to identify factors that contribute to the variation in micronutrients and bioactive components.

Maternal factors

There is a growing body of literature suggesting that several maternal characteristics relate to HMC. Genetic differences can directly impact HMC and alter relations between maternal intake/exposure and HMC (93–98). A systematic review and meta-regression of data from 66 studies reported a positive association (β: 0.56 g/L; 95% CI: 0.034, 1.1) between maternal BMI and HM fat content, but no association with total protein, lactose, or energy (99). Associations have also been reported between individual HM components and various other maternal factors, for example, maternal age, parity, mode of delivery, socioeconomic status, maternal infections, acute and chronic diseases, and allergies, albeit inconsistently (6, 100–102). More research is needed to understand the impact of these factors on HMC. A systematic review found an association between maternal chronic disease prevalence (obesity, cardiovascular disease, and type 2 diabetes mellitus) and HMC in most studies and milk volume in some (103). Hence, documenting medical histories, past and current treatments, and relevant biomarkers of prevalent chronic diseases could provide important metadata for understanding variability.

Maternal nutritional status, dietary intakes, food and behavior choices, food fortification, and supplement use can have varied influences on HMC, especially on micronutrients (53). For example, HMC of vitamin B-12 correlates with maternal intake and status and is lower in women with depleted vitamin B-12 status. In contrast, other than in an overt folate deficiency, HM folate concentration is fairly constant and independent of mother's folate status/intake (6, 77, 104). A systematic review by Bravi et al. (105) of 36 publications found consistent evidence of association only for maternal fish consumption with HM DHA content, and for maternal vitamin C intake with HM vitamin C content. Adhikari et al. (106) reported inconsistent associations between HM macronutrients and energy and maternal dietary intake and methodologies of included studies.

Infant factors

HMC varies according to gestational age at delivery (i.e., preterm compared with term) (53). A systematic review of 26 preterm studies and 30 term studies reported higher protein concentration (+7% to +35%) and lower lactose concentration (−2% to −10%) in preterm compared with term HM in the first weeks of life, with no significant differences after weeks 10–12 of lactation. It also reported significantly higher concentrations of fat, lactose, and energy (+93%, +16%, and +16%, respectively) and lower concentrations of protein (−52%) in term mature milk relative to colostrum (107). Differences in selected vitamins and minerals have been shown in individual studies (108, 109). Documenting infant-associated factors, for example, sex (110), age (111), and medical and health status (112), could help with better characterization of HMC.

Temporal factors

HMC changes as milk matures over the course of lactation. A systematic review and meta-analysis of HM macronutrient content identified significantly higher concentrations of fat, lactose, and energy (+93%, +16%, and +16%, respectively) and lower concentrations of protein (−52%) in term mature milk relative to colostrum (107). Smaller changes in fat and protein content can continue over the course of extended lactation (113, 114). A systematic review of circadian variation in HM reported diurnal changes in concentrations of amino acids, fat, iron, and some hormones (115), and B-vitamins (116). Timing of sampling within a feed (foremilk compared with hindmilk) and time elapsed since the last feed can affect HMC, particularly fat concentrations (113, 114, 117). The time of day when HM is sampled is also important in relation to the time of maternal (and in some cases infant) exposures, for example, to foods and beverages, supplements, alcohol, medications or drugs of abuse, pathogens, tobacco use, and many environmental chemicals. Feeding frequency and duration—for example, number of feeds per 24 h, number of minutes per feed, and amount of time between feeds—are important. Time of collection within a feed has shown that foremilk and hindmilk composition varies (53, 118).

Feeding practices

This category includes mode of feeding and handling methods that can influence HMC. Breastfeeding, pumping then feeding HM by bottle, and cold storage followed by reheating of HM (freeze-thaw cycles) can lead to additions (e.g., bacterial contamination from a breast pump) or losses (e.g., degradation of nutrients after extended freezing or overheating) (6, 53, 119–125).

Several of these factors have been related to bioactives and microbes too. For example, because most HM bioactives are proteins, these are often sensitive to changes in temperature (e.g., freeze-thaw cycles and pasteurization) that induce denaturation and consequent loss of biological activity (126), or cellular components such as maternal leukocytes are highly susceptible to death/inactivation upon prolonged storage and changes in ambient temperature (127). The diet during pregnancy has a stronger impact on the microbiome compared with the diet during the first month of lactation (128). It is important to understand the factors that influence HM microbiota composition, because that is the most probable way of vertical transfer of bacteria and microbiota establishment in the infant gut.

Environmental chemicals

Maternal age (e.g., older mothers have higher lifetime exposure and bioaccumulation of environmental chemicals, especially those phased out of use/production), parity, lactation history, and duration of breastfeeding (e.g., number of months, exclusive or partial HM feeding) have been

associated with HMC of environmental chemicals (25, 47). Maternal exposures to air, soil, water, food, clothes, personal care products (e.g., triclosan in toothpaste), dental amalgam (mercury), electronics, and furniture are part of the maternal environment and potential exposure sources. Geographical location (e.g., living near a point source of environmental chemicals) can lead to differential exposure (47, 129, 130). A recent systematic review based on 20 studies concluded that smoking was inversely associated with HM lipids, energy, and proteins (131). Changes in maternal exposures have the potential to change HMC over time, even daily, especially for chemicals with short half-lives; thus, studies that take repeated measurements of HM give a better characterization of exposure. In addition, as a chemical is phased out of commercial use, for example, per- and polyfluoroalkyl substances—perfluorooctanoate and perfluorooctane sulfonate—by major US producers (132), its concentration in environmental media can decrease (133–135). However, replacement biosimilar environmental chemicals (e.g., new polyfluorinated chemicals) often emerge. Nutritional status and dietary supplementation can impact environmental chemical exposure (136–141). HMC following exposure to environmental chemicals can differ in mature and transitional milk (142–144).

The impact of the above factors is potentially complex and current knowledge is generally limited to impact on a single component/factor. A 2020 systematic review of collection techniques for HM research in macronutrient composition recommended that studies report and standardize details of the collection procedure (time of day of collection, collection method, collection breast, time since last expression), stage of lactation, infant gestational age, and mode of feeding (exclusive or partial breast feeding) (117). The authors noted lack of detail in the reviewed studies, limiting their use for public health purposes. Collection of metadata associated with the composition values can provide insights into each HMC data point and enhance our understanding of HM variability.

Additional Considerations for Compositional Analyses of Human Milk

A recent book (145) and several recent reviews (12, 117, 146) have provided details of best practices for collection and analysis of HM for composition research. Broadly, collection, handling, storage, and analytical protocols should be optimized for the components to be measured.

Sample collection

Study design for HMC research should accommodate HMC variability factors (summarized in Figure 2) and seek to control unintended influences on HMC. There is no consensus on the most reliable method for collecting a "representative" HM sample from the lactating mother. Collection and sampling from all HM expressed over 24 h, with multiple samples collected over time from each participant, is considered a "gold standard" method for providing representative HM samples in longitudinal, multianalyte HMC studies (53, 147, 148). This protocol minimizes within-subject variation due to time of day of sampling or time of within-feed sampling, but can be impractical for large population studies (149). In a recent systematic review of HM sampling methodologies, Leghi et al. (117) reported that weighted pooling of pre- and postfeed samples collected over 24 h provides similar concentrations of total fat, protein, and lactose in HM, and hence an acceptable alternative to the "gold standard." This sampling technique was accepted as an alternative to the 24-h full breast expression method for fat- and fat-soluble components in the 2020 NASEM evidence scan of HMC research (5). The NASEM committee accepted any milk type (fore-, mid-, or hindmilk) for protein and lactose, provided collection conditions were standardized, and any milk type for elements provided trace element–free supplies were used. Afternoon and evening samples are preferred for B-vitamins, because these are reported to best reflect total daily vitamin concentrations (12, 116). In addition, it is recommended that participants in studies of B-vitamins in HM should not be fasting, and that collection should not occur <4 h after supplement use. For large cohort studies, where standardization of protocols is difficult to implement, detailed data on HM collection and storage conditions can be recorded and included in statistical models to facilitate the identification of variables that influence HMC of specific analytes (150).

Handling and storage

Sample storage container type can influence HMC, either by binding analytes of interest or by introducing exogenous substances (151–153), and can differ based on the constituent (154). Fat- and fat-soluble components can adhere to the sides of storage containers, leading to loss of components with aliquoting or container changes (155–157), hence they should be minimized and care should be taken in homogenizing or aliquoting HM samples to limit component loss (158). Trace element–free supplies are recommended for HM mineral analysis to reduce the potential for contamination (12). Many components in HM are light-sensitive, for example, carotenoids, vitamin C, riboflavin, vitamin B-6, folate, and vitamin B-12 (146, 159); opaque or amber storage vials, and processing samples under limited or yellow light can avoid sample degradation (146, 160). Some HM components, for example, vitamin C, long-chain PUFAs, and riboflavin, can also undergo oxidation and degradation during storage, particularly at room temperature or in a standard refrigerator (161); hence ultra-low-temperature freezers (−70°C) are recommended for long-term HM storage (146). Storage conditions and length of storage affect the integrity of expressed HM, and are a logistical consideration in planning HMC studies (162, 163). For example, HM intended for vitamin C analysis is recommended to be stored for <1 mo because degradation can occur even at ultra-low temperatures (161, 164). Other components, for example, tocopherols, total protein, lactose, and minerals, are considered more stable over long-term storage (6, 158). Multiple freeze-thaw cycles reduce total

lipids in HM, increase concentrations of lipolysis products, and change HMC of lipids (6, 165). HM samples should be aliquoted after collection to avoid unnecessary freeze-thaw cycles, particularly for quantification of lipids. Because HM is nonsterile, incubations at room or body temperature can lead to bacterial metabolism and changes in the HM microbiome, and in substrates used by or metabolites produced by bacteria (166). Protocols for handling and storing donor HM such as pasteurization reduce pathogen load but alter the HMC of multiple constituents (163), curtailing the applicability of compositional analyses of donor milk to the general population. HM collection, handling, and storage are most challenging for large, multianalyte HMC studies, but ultimately logistics must be optimized for priority analytes (or groups).

Analytical methods

HM is not a homogeneous mixture, but consists of several compartments, including true solutions, colloids (e.g., casein micelles), membranes, membrane-bound globules, and live cells (167, 168). Therefore, the sample preparation, analyte extraction, and analytical methods must be carefully selected based upon the physicochemical properties of the specific component, how they are present in HM, and their concentrations. Generally, the method that can provide the most accurate measurement of the component in its original form is desired. Wu et al. (8) discussed various analytical methods available for several macro- and micronutrients and their pros and cons, and Hampel et al. (146) listed preferred methods for micronutrient analysis. Both articles emphasized that to achieve optimal results, the entire analytical procedure must be thoroughly validated for HM (8). Furthermore, use of a certified reference material, well-characterized, consensus, or in-house material for method validation and quality assurance, allows comparison of analytical data conducted at different time periods, instruments, and laboratories (146, 169).

Analytical methodologies have evolved with the development of new technology and instrumentation. For instance, microbiological methods were used to analyze B-vitamins, but recently chromatographic methods coupled with UV, fluorometric, or MS detection have become the preferred methods (159). There are still many gaps in the availability of analytical methods for certain components, especially bioactives. For example, despite tremendous efforts in recent years to understand HM oligosaccharides, there is still no consensus method for quantifying them (170). Furthermore, structure-function relations exist for HM oligosaccharides, necessitating the need to quantify them separately (171).

Use of "-omics" approaches for assessing HM can add significantly to our knowledge about specific components and their variability (172, 173). Proteomic and metabolomic approaches are used extensively to characterize various HM components, such as proteins found in casein and whey fractions, HM oligosaccharides and lipids (174–177), and the microbiome (178). Similar approaches could be applied in the characterization of hormones, growth factors, cytokines/chemokines, exosomes (179), or other molecules of interest. The various "-omes" present a high degree of interindividual, geographic, and time-dependent variation, and likely interact, affecting interpretation of results (180). This approach provides details on the type of metadata that could underlie our understanding of variability (173).

Microbes

Next-generation sequencing methods provide data on the relative 16S ribosomal RNA sequence composition but selection of primer pairs is important (4). Sequence data can be used to estimate species composition by comparison with known ribosomal RNA operon copy numbers of the nearest related species (181); limitations of the method have been noted (4, 182). Bacterial strain identification is not possible at present. Estimates of total bacterial load can be determined by qPCR using "universal" primers although primer bias and species-related DNA extraction efficiencies need to be considered (4, 183). Labor-intensive culture-based methods are useful for studying environmental sources of milk-culturable bacteria, but not to estimate total bacterial load or species distributions (184).

Environmental chemicals

Because environmental chemicals are ubiquitous, similar strategies for sample collection, handling, storage, and sample analysis are needed. Often for environmental chemicals, metabolites are the biomarker of exposure and not the parent compound. For example, di-2-ethylhexyl phthalate, the parent chemical in plastics, is metabolized in the body to mono-(2-ethylhexyl) phthalate and other monoester phthalates (185). Analytical methods used include HPLC (186) and MS and nontargeted analyses to identify novel environmental chemicals.

Traditionally, some chemicals, for example, dioxins and dioxin-like compounds, have factors associated with them to define their potential toxicity [toxicity equivalent factors (TEQs)]. TEQs are based on the known effects of a parent compound of a defined toxicity, namely the toxicity of the potent dioxin 2,3,7,8-tetrachlorodibenzodioxin. The TEQ can be summed across hundreds of congeners to give a total TEQ from HM exposure (27).

The above body of information underscores the immense variability reported in HMC and the importance of collecting metadata in conjunction with HM samples. Furthermore, improvement and harmonization of methods for sample collection, handling and storage, and chemical analysis is crucial, allowing compilation of data and comparisons across studies and populations over time. Several recent reviews have also emphasized the need for standardization of methodology for assessment of the variability factors (6, 102, 117).

Potential Next Steps for Human Milk Composition Data

Comprehensive and current data on HMC are lacking in the United States and Canada. Given the variability of

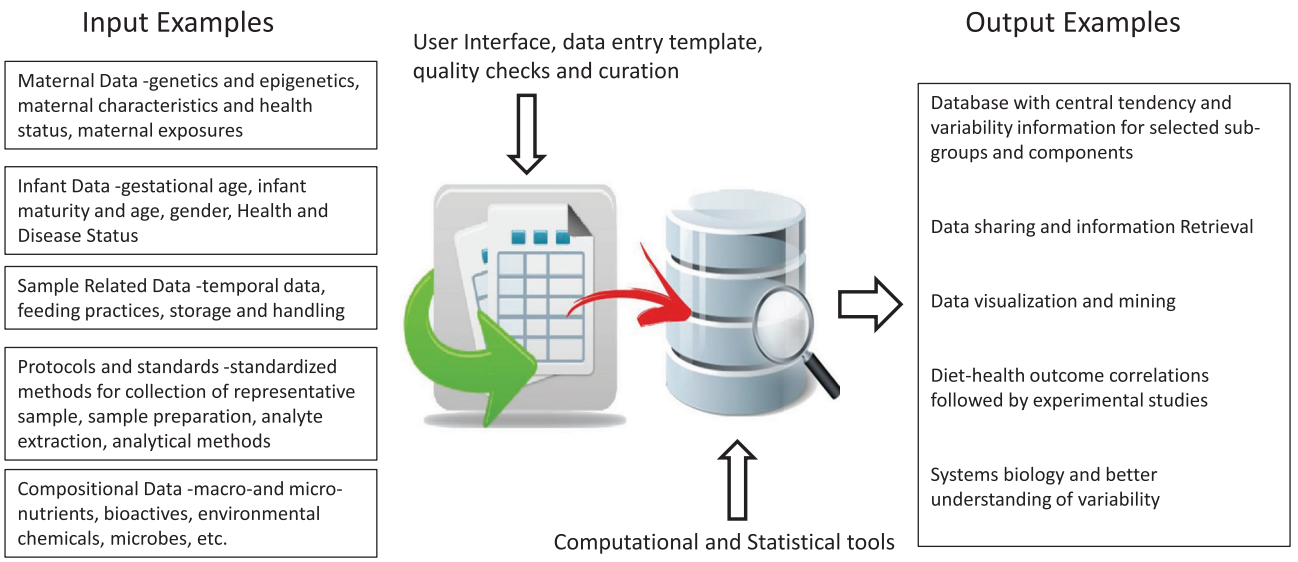

FIGURE 3 Schematic representation of the Human Milk Composition Data Repository (HMCD-R).

HMC, the use of out-of-date, single nutrient values for exposure assessments is inappropriate. FoodData Central and CANLINE in the United States and Canada, respectively, generally have the potential to incorporate metadata for food samples. However, given the uniqueness of HM and its variability factors and potential uses of the data, we articulate a vision for a publicly available HMCD-R. It can provide a central, integrated platform for national and international researchers to submit new or older HMC data that meet established protocols for sampling, collection, storage, analytical methods, and quality control, etc. HMCD-R would enable compiling, evaluating, comparing, tracking, and sharing HMC data, thus facilitating the understanding of the complexity and biological variability of HMC.

Figure 3 provides a schematic representation of a potential HMCD-R (as of now such a data repository does not exist). Some potential features and capabilities envisioned for HMCD-R are:

- Includes known and novel components (Figure 1).
- Contains well-characterized supporting metadata using standardized vocabulary.
- Uses a user-interface and a flexible data-entry template, allowing researchers from heterogeneous research studies to submit data.
- Includes submitted data that have undergone quality control, review, and curation to ensure data quality, integrity, and validity.
- Serves as a portal for standards/protocols for HMC analyses (sample preparation, handling, storage, and analytical methodologies), data sharing, reporting (including minimal metadata reporting standards) and statistical analyses.
- Provides computational, data mining/visualization, and statistical tools for compiling and integrating data from different studies, comparative analyses, and determining central tendency, variability, and other statistical measures.
- Integrates FAIR (Findability, Accessibility, Interoperability, and Reusability) guiding principles for digital datasets (187).
- A structure that allows for incorporation or linkages to other data systems such as the Canadian Nutrient File (45), CANLINE (46), USDA FoodData Central (43), and NIH Metabolomics Workbench (188).
- Ensures data confidentiality by following Federal Health Insurance Privacy and Portability Act (HIPAA) privacy rules.

The vision and the considerations for the HMCD-R will be shaped by funding availability in the future and determined collaboratively by US and Canadian agencies.

Future Considerations

National health and nutrition monitoring is a cornerstone for many federal and state policies. Robust estimates of exposure assessments through HM are lacking to inform infant and toddler nutrition and health research, policy, and programmatic needs. The assumptions historically used to impute HM volume and the use of out-of-date, single average compositional data for all infants are outdated. Expansion is needed of sampling strategy in population surveys to recruit a larger sample of infants from mothers to capture diverse factors that influence HMC, collection of HM samples, and related metadata, additional focus on the components of public health interest, and statistical approaches to account for HMC variability to provide more robust consumption estimates from HM. More research is needed to understand the impact of variability factors on HMC, particularly from large studies that can adjust for

multiple factors to identify independent associations (189–191). HMC studies should report detailed characteristics that could contribute to variation between and within subjects and be adequately powered to estimate the central tendency and variability of nutrient concentrations.

Federal and nonprofit agencies have increased emphasis on HMC (9, 7, 192). The 2020 Dietary Guidelines Advisory Committee recommended updating HMC data in USDA databases (14). Several efforts, for example, BEGIN (Breastmilk Ecology: Genesis of Infant Nutrition) meeting series to describe in detail the variability factors by NIH (193), and the Mothers, Infants and Lactation Quality (MILQ) (194) and CHILD cohort studies (195) will likely provide crucial understanding to inform the development of HMCD-R. Projects such as MIREC and ECHO could be used as models to develop future North American surveillance and biomonitoring studies on HMC. HMC initiatives will continue to positively impact the pace of research by providing a collaboration platform whereby diverse disciplines related to HMC research in the United States/Canada can collaborate and synchronize their efforts to better understand HM through mechanisms to achieve the vision for HMCD-R.

The compiled compositional and metadata in HMCD-R would provide pertinent measures of central tendency and variability and allow use of statistical modeling techniques to approximate compositional profiles for subgroups, providing more accurate exposure assessments for purposes of monitoring and surveillance and development of recommendations to inform public health policies and food and nutrition programs. This work goes beyond nutrition to clarify potentially detrimental exposures that could occur through HM (e.g., chemicals, alcohol, drugs). Furthermore, it could improve understanding of the complexity and variability of HM (a key mediator of the diet-health-disease outcome correlations) and infant and maternal nutritional needs; allow researchers to study the impact of maternal and environmental factors and HM as a mother-milk-infant triad (192); and guide management of infant feeding, formulation of infant formulas, improved clinical practice guidelines, and personalized interventions/guidance to positively influence exposures and improve health outcomes for both the mother and child.

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