

The Nutritional Composition and Energy Content of Donor Human Milk: A Systematic Review

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ABSTRACT

The American Academy of Pediatrics recommends donor human milk (DHM) as the preferred feeding strategy for preterm infants when the milk of the mother is unavailable, based on conclusive evidence of lower rates of necrotizing enterocolitis with DHM feedings compared with preterm infant formula. The nutritional composition of DHM may differ from maternal milk for many reasons including differences in maternal characteristics, milk collection methods, and the impact of donor milk banking practices. The purpose of this systematic review is to examine the literature regarding research on the fat, protein, carbohydrate, vitamin, and mineral composition of DHM obtained through nonprofit milk banks or commercial entities. PubMed, CINAHL, and Scopus databases were searched for articles published between 1985 and 30 April, 2019. In total, 164 abstracts were screened independently by 2 investigators, and 14 studies met all inclusion criteria. Studies were predominantly small (<50 samples) and measured macronutrients. Few studies assessed vitamins and minerals. Information bias was prevalent due to the use of a variety of analytical methods which influence accuracy and cross-study comparisons. Other sources of information bias included missing information regarding methods for protein and calorie assessment. Despite these limitations, existing research suggests the potential for 2-fold and greater differences in the fat, protein, and energy composition of DHM, with mean values for energy and fat often below clinical reference values expected for human milk. Further research is warranted regarding the nutritional composition of DHM, with a prioritization on measuring macronutrients and micronutrients using established reference methods. *Adv Nutr* 2020;11:960–970.

Keywords: preterm, composition, milk banking, donor milk, human milk, breastmilk

Introduction

The American Academy of Pediatrics (AAP) recommends donor human milk (DHM) as the preferred feeding strategy for preterm infants when the milk of the mother is unavailable, based on conclusive evidence of lower rates of necrotizing enterocolitis with use of DHM feedings compared with preterm infant formula (1–4). In its most recent assessment of maternity hospital practices, the CDC found that over 65% of neonatal intensive care units in the USA use

DHM, suggesting wide adoption of DHM recommendations (5). Unfortunately, DHM use is also associated with inferior in-hospital preterm infant growth compared with preterm formula and maternal milk, raising concerns about long-term outcomes (3, 6, 7).

Poorer growth with DHM feedings may be related to the inadequate nutrient composition of DHM compared with other feeding options. While there are multiple reviews on the composition of maternal milk (8, 9), a 2017 working group of the NIH concluded that “the limited scope of human milk research initiatives has led to a lack of robust estimates of the composition and volume of human milk consumed and, consequently, missed opportunities to improve maternal and infant health” (10). Even with improved knowledge regarding the nutrient composition of maternal milk, new findings may not be generalizable to DHM due to additional sources of variation in DHM (Figure 1) including: a wide variety of donors (e.g., by gestation stage, lactation stage,

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Abbreviations used: AAP, American Academy of Pediatrics; BCA, bicinchoninic acid; DHM, donor human milk; HMBANA, Human Milk Banking Association of North America; HMO, human milk oligosaccharides.

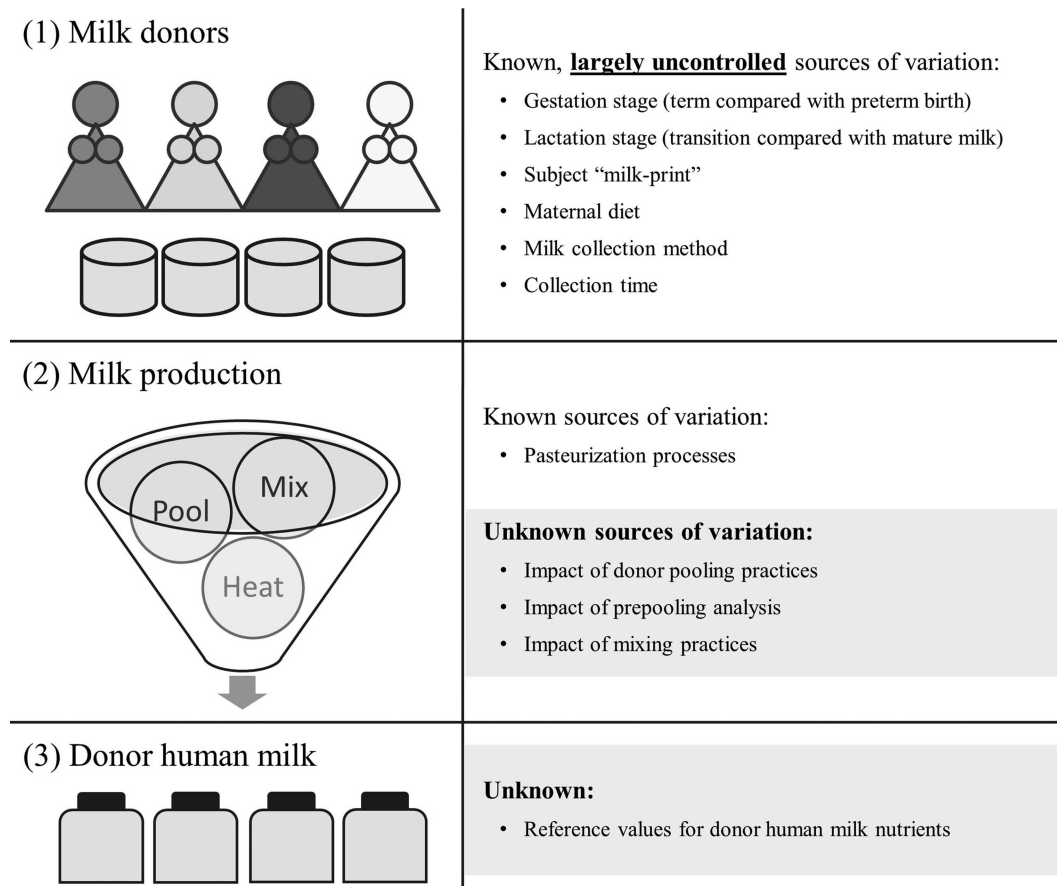


FIGURE 1 Overview of milk bank processes and potential sources of variation compared with milk from the mother that may influence nutritional reference values for donor human milk. Milk-print, a larger subject effect than temporal effect on many of the nutrients in human milk.

maternal diet); inconsistency in milk collection methods (including incomplete breast expression leading to fat loss); evidence of a human “milk-print,” with who the milk was collected from as a better predictor of nutrient composition than the stage of lactation (11); additional impact of milk banking processes (e.g., pooling, mixing, multiple container transfers) that may influence nutrient distribution and retention in DHM; as well as the documented loss of some bioactive factors and nutrients during pasteurization and storage (12).

Information regarding the nutrient composition of DHM may enable the development of improved preterm infant feeding protocols including the formulation of DHM-specific fortifiers or modification of existing fortification strategies. It may also help inform quality improvement initiatives and future research within donor milk banking practices. Therefore, the purpose of this review was to examine the literature regarding research on the nutrient composition of DHM obtained through milk banks and commercial entities. The goal of the review was to characterize the current evidence regarding DHM composition and identify gaps in knowledge to inform future research.

Methods

We conducted a systematic search of original peer-reviewed research published between 1 January, 1985 (founding year of the Human Milk Banking Association of North America; HMBANA) and 30 April, 2019 to identify studies providing quantitative data on the nutritional composition of DHM obtained from milk banks or commercial entities worldwide. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist was used to guide the review process. Electronic searches of PubMed, Scopus, and CINHAL databases were performed using the following search term: (“donor milk” OR “donor human milk” OR “milk banks” OR “milk bank” OR (Donat* AND milk)) AND (composition OR nutrients) NOT review. Advanced filters included articles that were based on human subjects only and articles that were published in English.

The abstracts for all studies identified in the initial search were independently assessed by 2 researchers (EAB and MTP). Abstracts were excluded from further review if they did not mention DHM or if they did not assess the nutritional composition of DHM. Abstracts that passed the initial review were subject to a full article review by

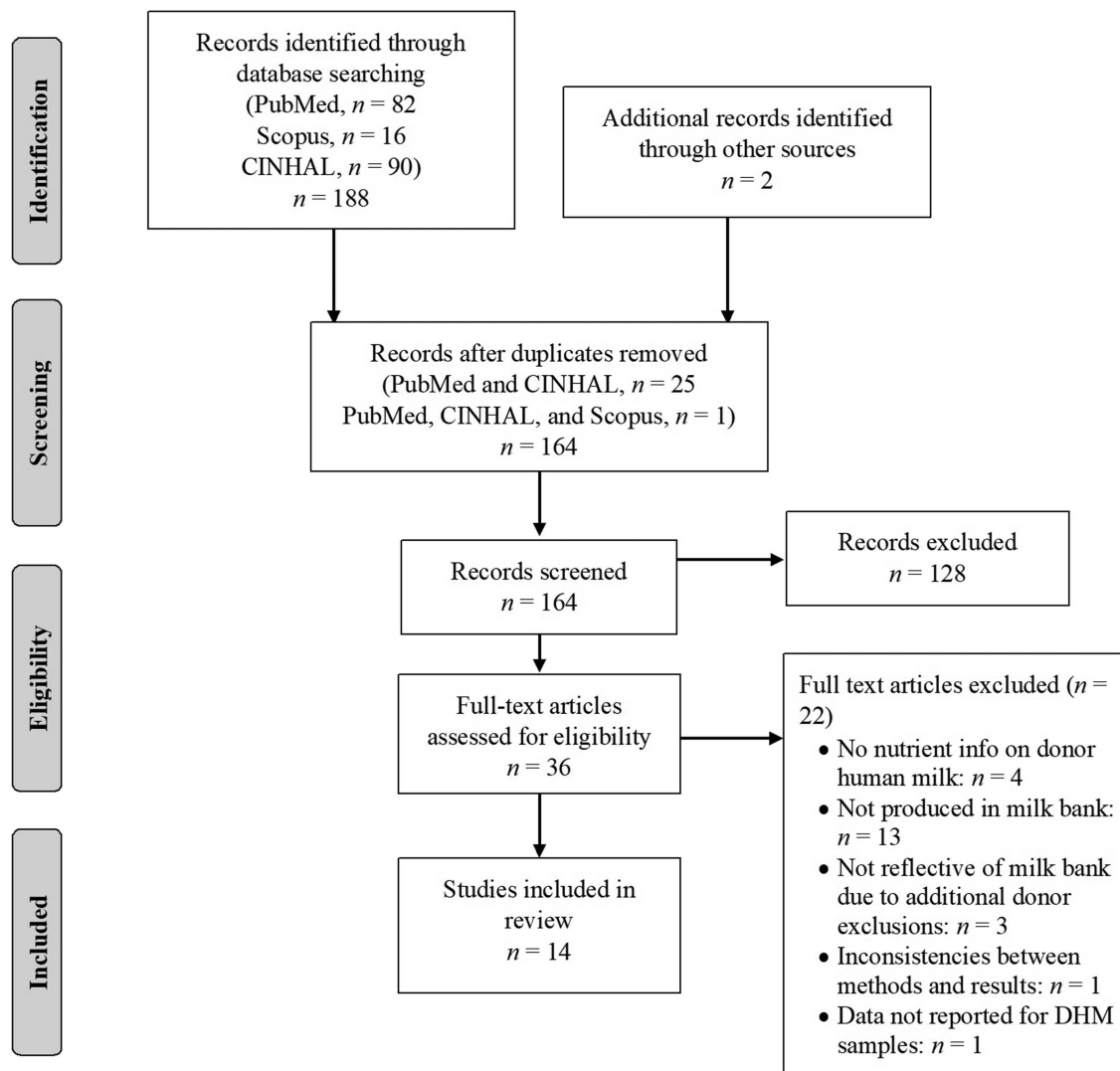


FIGURE 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of the literature search process to identify studies addressing nutrient composition of donor human milk obtained through milk bank or commercial entities. DHM, donor human milk.

2 independent reviewers (EAB and MTP). Studies were excluded for the following reasons: donor milk was created in a laboratory versus a milk bank or commercial setting; only nonnutritional factors were assessed (e.g., hormones, cytokines); studies did not reflect normal milk banking processes (e.g., use of additional donor exclusion criteria); inconsistencies between methods and results (e.g., inability to distinguish data that represented mother versus donor milk); and data not reported for DHM samples. Where information was lacking to assess exclusion criteria, we contacted the primary study author to request the relevant information. Hand searches of bibliographies were also conducted to identify additional potential studies for review.

Studies that met the inclusion criteria were abstracted by 2 reviewers (EAB and MTP) for the following information: source of donor milk, milk processing method, number of samples, donor characteristics, number of donors per

sample, nutrients assessed, analytical methods, descriptive statistics, and funding source. In instances where reviewers did not agree, differences were resolved by discussion with a third reviewer (LT). Potential sources of bias were identified by 2 reviewers (EAB, MTP) and feedback was obtained from all coauthors. The following sources of bias were considered qualitatively: information bias (did analytical methods influence findings or was key information omitted); selection bias (did source of DHM samples influence findings); and funding bias (did funders have financial interest in the topic and were they involved in study design or data interpretation).

Results

An overview of the search and review process is described in [Figure 2](#). The initial search identified 162 studies after the removal of duplicates, with 2 additional studies identified

TABLE 1 Summary of studies included in the review of composition of donor human milk obtained from milk banks or commercial entities

Author	Year	No. of samples	Milk sources	Outcome variables	Funding source
Luukkainen (13)	1995	48	Finland MB	Fatty acids	Yrjo Jahnsson Foundation; The Foundation for Pediatric Research
Goes (14)	2002	60	Brazil MB	Calcium, copper, fat, lactose, iron, phosphorus, protein, vitamin A, zinc	CNFq, FAPERJ, FINEP, and FUJB
Valentine (15)	2010	16	HMBANA (1 MB)	Amino acids, LCPUFAs, total protein	Research Institute at Nationwide Children's Hospital
de Halleux (16)	2013	376	Belgium MB	Carbohydrates, energy, fat, protein	None
Radmacher (17)	2013	6	HMBANA (1 MB)	Carbohydrates, energy, total fat, total protein	None
Marx (18)	2014	31	HMBANA (1 MB)	Total HMOs	German Academic Exchange Service Research Fellowship
Perrella (19)	2015	15	Australia MB	Energy, fat, lactose, protein	Medela research grant
Hanson (20)	2016	1	HMBANA (1 MB)	Vitamins – retinol, α -tocopherol, β -carotene	University of Nebraska Medical Center
Barbarska (21)	2017	179	Poland MB	Carbohydrates, crude protein, energy, fat, true protein	None
Perrin (22)	2016	33	HMBANA (2 MB)	Carbohydrates, minerals, total fat, total HMOs, total protein	North Carolina State University; American Society of Nutrition predoctoral fellowship; research gifts from HMBANA milk banks
Donovan (23)	2017	37	HMBANA (1 MB)	Carbohydrates, energy, total fat, total protein	Gerber Foundation
Moukarzel (24)	2017	30	HMBANA (1 MB)	Total fat, total protein, water-soluble choline	None
Meredith-Dennis (25)	2018	9	HMBANA (1 MB), Medolac, Prolacta	Carbohydrates, energy, total fat, total HMOs, total protein	NIH; University of California, Davis
John (26)	2019	1111	HMBANA (1 MB)	Total fat, total protein	National Science Foundation

CNPq, Brazilian Council for Scientific and Technological Development; FAPERJ, Carols Chagas Foundation for Research Support of the State of Rio de Janeiro; FINEP, Funding Authority for Studies and Projects; FUJB, Jose Bonifacio University Foundation; HMBANA, Human Milk Banking Association of North America; HMO, human milk oligosaccharides; LCPUFA, long chain PUFAs; MB, milk bank.

through a hand search of bibliographies. After review of the abstracts, a total of 128 studies were excluded because they were not about donor milk or related to donor milk composition, leaving 36 studies for full review. Twenty-two studies were excluded after a full article review leaving 14 studies in this systematic review of DHM composition. Included studies were conducted between 1995 and 2019 and mostly reflected DHM that was produced within the HMBANA milk bank network (Table 1) (13–26). Only 1 study examined milk that was produced by a commercial entity (25) and only 4 contained >50 DHM samples. The nutrient concentrations, milk characteristics, and analytical methods reported in the included studies are summarized by nutrient in Table 2.

Energy

Six studies described the calorie composition of DHM ($n = 3$ to 224) with mean values between 49.3 kcal/dL and 69.3 kcal/dL (16, 17, 19, 21, 23, 25). Five studies measured energy using infrared analysis. The largest study ($n = 179$) that provided information on ranges reported a minimum of 46.0 kcal/dL and a maximum of 86.0 kcal/dL,

which represents an almost 2-fold difference in energy (21). Four studies identified the energy conversion factors that were used to compute energy values from macronutrients (16, 17, 19, 25). While all 4 studies reported using the Atwater conversion factors of 4 kcal/g protein, 4 kcal/g carbohydrate, and 9 kcal/g fat, 2 of the studies applied the conversion factor to lactose values and 2 applied the conversion factors to total carbohydrate values.

Carbohydrates

Four studies assessed lactose in DHM ($n = 6$ to 60 samples), with mean values between 5.6 g/dL and 7.3 g/dL (14, 17, 19, 22). The largest study providing information on ranges ($n = 15$) reported a minimum lactose concentration of 5.7 g/dL and a maximum of 8.6 g/dL, which represents a 1.5-fold difference in lactose (19). Studies that reported lactose values described using a variety of analytical methods including picric acid, enzymatic, chromatography coupled with tandem MS, and midinfrared. Four studies assessed total carbohydrates ($n = 3$ to 224), with mean values between 6.5 and 7.4 g/dL (16, 21, 23, 25). Only 1 study reported ranges for total carbohydrates ($n = 3$), and the difference between

TABLE 2 Nutrient concentrations in donor human milk obtained from milk banks or commercial entities

Nutrient (citation)	N	Mean ± SD	Range	Treatment	Donor type	Donors/pool	Analytical method
Carbohydrates							
Lactose, g/dL (14)	60	7.3 ± 1.6	—	Holder	n/a	1	Picric acid method
Lactose, g/dL (17)	6	6.1 ± 0.4	5.5–6.7	Holder	n/a	n/a	Mid-IR
Lactose, g/dL (19)	15	6.6 ± 0.8	5.7–8.6	Holder	n/a	n/a	Enzymatic
Lactose, g/dL (22)	33	5.6 ± 0.7	—	Raw	n/a	n/a	LC-MS/MS
Total carbohydrates, g/dL (16)	138	6.9 ± 0.4	—	Holder	n/a	1	FT Mid-IR
Total carbohydrates, g/dL (16)	224	6.8 ± 0.2	—	Holder	n/a	Multiple	FT Mid-IR
Total carbohydrates, g/dL (16)	14	6.5 ± 0.1	—	Holder	Colostrum	n/a	FT Mid-IR
Total carbohydrates, g/dL (21)	179	7.4 ± 0.3	—	Raw	n/a	1	Mid-IR
Total carbohydrates, g/dL (23)	16	6.8 ± 0.2	—	Holder	Preterm	n/a	FT Mid-IR
Total carbohydrates, g/dL (23)	21	6.7 ± 0.2	—	Holder	Term	n/a	FT Mid-IR
Total carbohydrates, g/dL (25)	3	—	7.2–7.3	Holder	11% preterm	2	FT Mid-IR
Total carbohydrates, g/dL (25)	3	—	7.0–7.2	Vat Holder	n/a	250	FT Mid-IR
Total carbohydrates, g/dL (25)	3	—	7.0–7.2	Retort	n/a	200	FT Mid-IR
Total HMOs, g/L (18)	31	—	4–16*	Holder	n/a	3	HPLC
Total HMOs, g/L (22)	33	9.3 ± 2.0	—	Raw	n/a	n/a	LC-MS/MS
Total HMOs, g/L (25)	3	12.6 ± 0.8	12.0–13.5	Holder	11% preterm	2	UPLC-MRM/MS
Total HMOs, g/L (25)	3	8.2 ± 0.1	8.1–8.4	Vat Holder	n/a	250	UPLC-MRM/MS
Total HMOs, g/L (25)	3	6.6 ± 0.8	5.7–7.2	Retort	n/a	200	UPLC-MRM/MS
Energy, kcal/dL (16)	138	64.1 ± 5.9	—	Holder	n/a	1	FT Mid-IR + Atwater
Energy, kcal/dL (16)	224	63.6 ± 4.5	—	Holder	n/a	Multiple	FT Mid-IR + Atwater
Energy, kcal/dL (16)	14	60.3 ± 3.5	—	Holder	n/a	n/a	FT Mid-IR + Atwater
Energy, kcal/dL (17)	6	49.3 ± 4.7	44.3–56.1	Holder	Colostrum	n/a	FT Mid-IR + Atwater
Energy, kcal/dL (19)	15	63.9 ± 12.2	42.6–84.8	Holder	n/a	n/a	Mid-IR + Atwater
Energy, kcal/dL (21)	179	61.7 ± 6.5	46.0–86.0	Raw	n/a	n/a	Proximate + Atwater
Energy, kcal/dL (23)	21	64.9	—	Holder	Term	1	Mid-IR
Energy, kcal/dL (23)	16	69.3	—	Holder	Preterm	n/a	FT Mid-IR
Energy, kcal/dL (25)	3	64.6 ± 8.5	58.1–74.0	Holder	11% preterm	n/a	FT Mid-IR
Energy, kcal/dL (25)	3	69.0 ± 1.4	67.6–70.3	Vat Holder	n/a	250	FT Mid-IR + Atwater
Energy, kcal/dL (25)	3	58.8 ± 5.7	52.4–62.9	Retort	n/a	200	FT Mid-IR + Atwater
Fat							
LCPUFA							
α-Linolenic acid, % wt (13)	48	0.9	0.6–1.6	Raw	Transition	1–3	GC
α-Linolenic acid, nmol/mL (15)	16	8.3 ± 1.2	—	Holder	Term/preterm	3–4	GC
Arachidonic acid, % wt (13)	48	0.5	0.2–0.8	Raw	Transition	1–3	GC
Arachidonic acid, nmol/mL (15)	16	2.7 ± 0.4	—	Holder	Term/preterm	3–4	GC
DHA, % wt (13)	48	0.5	0.2–0.8	Raw	Transition	1–3	GC
DHA, nmol/mL (15)	16	0.7 ± 0.2	—	Holder	Term/preterm	3–4	GC

(Continued)

TABLE 2 (Continued)

Nutrient (citation)	N	Mean ± SD	Range	Treatment	Donor type	Donors/pool	Analytical method
Linoleic acid, % wt (13)	48	9.1	6.3–13.4	Raw	Transition	1–3	GC
Linoleic acid, nmol/mL (15)	16	118 ± 18	—	Holder	Term/preterm	3–4	GC
Monounsaturated, % wt (13)	48	42.2	37.1–48.4	Raw	Transition	1–3	GC
Saturated, % wt (13)	48	46.4	37.7–50.1	Raw	Transition	1–3	GC
Total fat, g/dL (14)	60	1.8 ± 1.1	—	Holder	n/a	1	Creamatocrit
Total fat, g/dL (16)	138	3.5 ± 0.6	—	Holder	n/a	1	FT Mid-IR
Total fat, g/dL (16)	224	3.4 ± 0.5	—	Holder	n/a	Multiple	FT Mid-IR
Total fat, g/dL (16)	14	2.9 ± 0.4	—	Holder	Colostrum	n/a	FT Mid-IR
Total fat, g/dL (17)	6	2.5 ± 0.3	2.2–3.0	Holder	n/a	n/a	Mid-IR
Total fat, g/dL (19)	15	3.5 ± 1.3	1.4–6.0	Holder	n/a	n/a	Creamatocrit
Total fat, g/dL (21)	179	3.1 ± 0.8	1.1–7.4	Raw	n/a	1	Mid-IR
Total fat, g/dL (22)	33	3.5 ± 1.7	—	Raw	n/a	n/a	NMR
Total fat, g/dL (23)	16	3.9 ± 0.6	—	Holder	Preterm	n/a	FT Mid-IR
Total fat, g/dL (23)	21	3.7 ± 0.4	—	Holder	Term	n/a	FT Mid-IR
Total fat, g/dL (24)	30	2.8 ± 1.0	1.1–4.8	Holder	Term	n/a	Creamatocrit
Total fat, g/dL (25)	3	3.4 ± 0.9	2.8–4.6	Holder	11% preterm	2	FT Mid-IR
Total fat, g/dL (25)	3	4.1 ± 0.2	3.9–4.6	Vat Holder	n/a	250	FT Mid-IR
Total fat, g/dL (25)	3	3.0 ± 0.6	2.3–3.3	Retort	n/a	200	FT Mid-IR
Total fat, g/dL (26)	1111	—	2.7–5.9	Holder	n/a	Target	FT Mid-IR
Minerals							
Calcium, mg/L (14)	60	237 ± 53	—	Holder	n/a	1	Methylthymol blue
Calcium, mg/L (22)	33	220 ± 49	—	Raw	n/a	n/a	ICP-OES
Copper, mg/L (14)	60	0.5 ± 0.1	—	Holder	n/a	1	AA
Iron, µg/L (14)	60	660 ± 31	—	Holder	n/a	1	AA
Iron, µg/L (22)	33	230 ± 64	—	Raw	n/a	n/a	ICP-OES
Phosphorus, mg/L (14)	60	132 ± 32	—	Holder	n/a	1	AA
Potassium, mg/L (22)	33	390 ± 75	—	Raw	n/a	n/a	ICP-OES
Sodium, mg/L (22)	33	83 ± 31	—	Raw	n/a	n/a	ICP-OES
Zinc, mg/L (14)	60	1.7 ± 1.0	—	Holder	n/a	1	AA
Zinc, mg/L (22)	33	1.2 ± 0.7	—	Raw	n/a	n/a	ICP-OES
Protein							
Amino acids, µmol/L							
Alanine (15)	16	224.6 ± 45.2	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Arginine (15)	16	12.3 ± 4.2	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Aspartate (15)	16	59.1 ± 16.8	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Glutamate (15)	16	1415 ± 269	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Glycine (15)	16	95.5 ± 23.2	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Histidine (15)	16	22.7 ± 5.9	—	Holder	Term/preterm	3–4	Ion exchange HPLC

(Continued)

TABLE 2 (Continued)

Nutrient (citation)	N	Mean ± SD	Range	Treatment	Donor type	Donors/pool	Analytical method
Isoleucine (15)	16	7.4 ± 2.4	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Leucine (15)	16	28.7 ± 7.1	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Lysine (15)	16	17.5 ± 7.0	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Methionine (15)	16	4.4 ± 1.6	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Phenylalanine (15)	16	13.5 ± 2.5	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Proline (15)	16	26.2 ± 6.6	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Serine (15)	16	95.4 ± 27.7	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Taurine (15)	16	248.1 ± 54.7	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Threonine (15)	16	71.2 ± 30.2	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Tyrosine (15)	16	8.4 ± 3.1	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Valine (15)	16	50.6 ± 9.9	—	Holder	Term/preterm	3–4	Ion exchange HPLC
Crude protein, g/dL (21)	179	0.8 ± 0.2	0.4–1.5	Raw	n/a	1	Mid-IR
Total protein, g/dL (14)	60	1.2 ± 0.3	—	Holder	n/a	1	Lowry
Total protein, g/dL (15)	16	0.9	—	Holder	n/a	3–4	MilkOScope
Total protein, g/dL (16)	138	1.3 ± 0.4	—	Holder	n/a	1	FT Mid-IR
Total protein, g/dL (16)	224	1.5 ± 0.2	—	Holder	n/a	Multiple	FT Mid-IR
Total protein, g/dL (16)	14	2.0 ± 0.1	—	Holder	Colostrum	n/a	FT Mid-IR
Total protein, g/dL (17)	6	1.0 ± 0.1	0.8–1.1	Holder	n/a	n/a	Mid-IR
Total protein, g/dL (19)	15	1.3 ± 0.2	1.0–1.6	Holder	n/a	n/a	Bradford
Total protein, g/dL (22)	33	1.5 ± 0.2	—	Raw	n/a	n/a	Bicinchoninic acid
Total protein, g/dL (23)	16	1.4 ± 0.2	—	Holder	Preterm	n/a	FT Mid-IR
Total protein, g/dL (23)	21	1.1 ± 0.1	—	Holder	Term	n/a	FT Mid-IR
Total protein, g/dL (24)	30	3.2 ± 0.4	—	Holder	Term	n/a	Bradford
Total protein, g/dL (25)	3	1.0 ± 0.1	0.9–1.1	Holder	11% preterm	2	FT Mid-IR
Total protein, g/dL (25)	3	0.8 ± 0.0	0.8–0.8	Vat Holder	n/a	250	FT Mid-IR
Total protein, g/dL (25)	3	0.8 ± 0.0	0.7–0.8	Retort	n/a	200	FT Mid-IR
Total protein, g/dL (26)	1111	—	0.8–2.2	Holder	n/a	Target	FT Mid-R
True protein, g/dL (21)	179	0.7 ± 0.2	0.3–1.2	Raw	n/a	1	Mid-IR
Vitamins							
Free choline, $\mu\text{mol/L}$ (24)	30	170 ± 86	16–297	Holder	Term	n/a	LC-MS/MS
GPC, $\mu\text{mol/L}$ (24)	30	383 ± 195	61–772	Holder	Term	n/a	LC-MS/MS
PhosC, $\mu\text{mol/L}$ (24)	30	722 ± 255	51–1223	Holder	Term	n/a	LC-MS/MS
WS choline, $\mu\text{mol/L}$ (24)	30	1275 ± 414	128–1934	Holder	Term	n/a	LC-MS/MS
Retinol, $\mu\text{g/L}$ (14)	60	432 ± 266	—	Holder	n/a	1	HPLC
Retinol, $\mu\text{g/L}$ (20)	1	185.8 [†]	—	Holder	n/a	n/a	HPLC
α -tocopherol, $\mu\text{g/L}$ (20)	1	1381.9 [†]	—	Holder	n/a	n/a	HPLC
β -carotene, $\mu\text{g/L}$ (20)	1	13.7 [†]	—	Holder	n/a	n/a	HPLC

*Values interpreted from graphs.

[†]N = 1 observation reported; data represent actual amounts, not measurements of central tendency and dispersion.

AA, atomic absorption spectrometry; FT, Fourier transformed; GPC, glycerophosphocholine; HMO, human milk oligosaccharide; ICP-OES, inductive coupled plasma atomic emission spectroscopy; IR, infrared; LCPUFA, long chain PUFA; n/a, not available; MRM, multiple reaction monitoring; MS, mass spectroscopy; PhosC, phosphocholine; Target, selection of donors for pool based on macronutrient analysis of each donor; Transition, milk from 1–8 wk postpartum; UPLC, ultra-performance LC; WS, water-soluble.

minimum and maximum total carbohydrate concentrations was <5% (25). Studies that reported total carbohydrate values all used infrared analysis as the analytical method. Three studies assessed total human milk oligosaccharides (HMOs) ($n = 3$ to 33 samples) with mean values between 6.6 and 12.6 g/L (18, 22, 25). The largest study that included ranges for HMOs ($n = 31$) reported minimum values of 4 g/L and maximum values of 16 g/L, which represents a 4-fold difference (18). All studies measuring HMOs used LC and tandem MS.

Fat

Total fat was assessed in 10 studies ($n = 3$ to 1111 samples), with mean values between 1.8 and 4.1 g/dL (14, 16, 17, 19, 21–26). Two studies that contained over 100 samples reported ranges for fat of 1.1 to 7.4 g/dL ($n = 179$) and 2.7 to 5.9 g/dL ($n = 1111$), which represents a 5.7-fold and 2.2-fold difference, respectively (21, 26). Total fat was assessed using a variety of analytical methods including midinfrared, Fourier-transformed midinfrared, NMR, and creatocrit. Two studies ($n = 16$ and 48) assessed long chain polyunsaturated acids, and both used GC (13, 15).

Protein

Ten studies reported that they assessed protein ($n = 3$ to 1111 samples), with mean values between 0.8 g/dL and 3.2 g/dL (14–17, 19, 22–26). The largest study to include information on ranges ($n = 1111$) reported a minimum protein content of 0.8 g/dL and a maximum of 2.2 g/dL, which represents an almost 3-fold difference (26). Protein was assessed using a variety of methods including Bradford, bicinchoninic acid assay (BCA), Lowry, midinfrared, Fourier-transformed midinfrared, and ultrasound. One study ($n = 179$) used midinfrared analysis and reported crude protein values (min = 0.4 g/dL and max = 1.5 g/dL; 3.8-fold difference) and true protein values (min = 0.3 g/dL and max = 1.2 g/dL; 4-fold difference). A single study containing 16 samples assessed amino acids using ion exchange chromatography (15).

Micronutrients

Two studies ($n = 33$ and 60 samples) assessed minerals including calcium, copper, iron, phosphorus, potassium, sodium, and zinc. Methods of measuring minerals included atomic absorption, inductive coupled plasma optical emission spectrometry (ICP-OES), and methylthymol blue (calcium only) (14, 22). The only vitamins assessed were water-soluble choline compounds ($n = 30$), vitamin A ($n = 1$ and 60 samples), and vitamin E ($n = 1$ sample), and all vitamin studies used LC methods for assessment (14, 20, 24).

Discussion

In this review of the global literature on the nutrient composition of DHM we report that the protein, fat, and calorie composition of DHM can differ by 2-fold or more

and that there is a dearth of information on the vitamin and mineral composition of DHM. Clinical implications of the demonstrated wide ranges of protein and energy strengthens the case for point-of-care analysis and targeted fortification of DHM. This research also suggests opportunities within donor milk banks to reduce the potential low nutritional content of DHM through nutrient analysis and targeted pooling (26).

This review highlights the limited information available on the nutritional composition of DHM from commercial entities. We located only a single study reporting on donor milk obtained through 2 commercial entities that operate in the USA (3 samples from each company). In the USA, there are currently several commercial sources of donor milk (e.g., Prolacta, Medolac, Ni-Q). Information regarding processing methods and donor recruitment is often not available for review and may lead to different nutritional profiles than findings from our review, which predominantly represent DHM produced in a milk bank setting.

Macronutrient composition of DHM

Carbohydrates, fat, and protein were the most frequently studied nutrients in DHM. For studies that reported minimum and maximum values, the magnitude of differences in the lactose and total carbohydrate concentrations in DHM was relatively small (<1.5-fold). The variability in protein was also <1.5-fold in all studies except 1, which reported an almost 3-fold difference. Conversely, 4 studies showed a 2-fold or greater difference in fat, with 3 of the studies showing 4-fold or greater differences. This observation is consistent with the current literature on human milk composition which suggests that fat composition is highly variable between and within women and is influenced by a variety of factors including maternal diet and how the sample was collected (complete versus partial breast expression) (22, 27–31). Protein composition is strongly influenced by preterm birth and early lactation stage (32), which may explain some of the protein variability observed in this review; however, information on donor pregnancy term and lactation stage was not available for most of the included studies. It is difficult to draw meaningful conclusions on actual nutrient values from existing studies due to the use of a variety of analytical methods, many of which are not considered reference methods. While this limits the ability to draw comparisons between studies, an assessment of the results within studies provides insights into the potential range of nutrients found in DHM. **Table 3** summarizes findings from this review and compares the published clinical references for human milk composition (33, 34). Importantly, the nutrition guidebook of the AAP reports reference calorie ranges for human milk of 65–70 kcal/dL (33). Nine of the 11 values we found in the literature for mean energy in DHM were below these AAP reference values. Similarly, 8 of 14 mean fat values reported were below clinical reference values of 3.5 g/dL, suggesting the need for updated clinical reference values that are specific to DHM.

TABLE 3 Comparison of published clinical reference values for human milk composition compared with reviewed literature for donor human milk

Nutrient	AAP early ^a	AAP mature ^a	AND preterm ^b	AND DHM ^b	Review of DHM ^c
Protein, g/dL	1.6	0.9	2.1	1.2	0.8–2.2
Fat, g/dL	2.0	3.5	4.5	3.2	1.1–7.4
Carbohydrates, g/dL	0	—	7.5	7.8	7.0–7.3
Lactose, g/dL	2.0–3.0	6.7	—	—	5.5–8.6
Energy, kcal/dL	—	65–70	77	65	43–86

AAP, American Academy of Pediatrics; AND, Academy of Nutrition and Dietetics; DHM, donor human milk.

^aFrom the American Academy of Pediatrics' Pediatric Nutrition, 7th Edition (33).

^bFrom the Academy of Nutrition and Dietetics' Infant and Pediatric Feedings, 3rd Edition (34).

^cRepresents the collective ranges reported in reviewed studies.

Micronutrient composition of DHM

This review highlights the lack of available information on the vitamin and mineral composition of DHM, with only a few small studies reporting on limited numbers of micronutrients. This is an important area for future research.

Analytical methods

A recent review on human milk composition conducted by the USDA's Nutrient Data Laboratory suggested the following standard reference methods for accurately measuring macronutrients in human milk: protein can be accurately measured using the sum of amino acids or Kjeldahl method, accounting for nonprotein nitrogen; total fat can be accurately measured via solvent extraction and gravimetric methods (e.g., Roese-Gottlieb, Folch) or by summing individual fatty acids measured by GC; lactose can be accurately measured by chromatography (8). These methods are also supported by Jensen and Neville in their handbook on human milk laboratory methods (35). The most common analytical method used for measuring macronutrients in the studies reviewed was infrared analysis. A growing body of evidence suggests that infrared analysis can reliably measure total nitrogen (e.g., crude protein) and fat in human milk, with appropriate sample handling and instrument calibration, though measurements of lactose were less accurate, and the measurement of total carbohydrates in human milk has not been validated (36–42). None of the reviewed studies used reference methods suggested by the USDA (8) to measure total protein or total fat, and only 1 study used reference methods for measuring lactose, which limits the ability to draw conclusions about actual nutrient ranges. For example, while the Lowry and BCA method have high correlations with reference methods for measuring protein ($R^2 \geq 0.94$) they may overreport actual protein values by 0.3 to 0.5 g/dL (43). Similarly, while creatinocrit was reported as highly correlated with fat measured by gravimetric reference methods ($R^2 = 0.99$), it tended to underreport fat content by 0.3 to 0.6 g/dL (44). Finally, only 1 of the 14 studies provided information on how DHM was mixed prior to collecting the sample for analysis. Given that fat separates during storage, inadequate mixing could influence values obtained for the fat content of DHM.

Impact of milk banking processes

Included studies were inconsistent in reporting the milk banking processes that may impact nutritional composition, such as the number of donors per pool, and if targeted pooling was used to strategically combine donors based on the macronutrient analysis of the milk of the donor. A simulation of donor nutrient profiles from the Mother's Milk Bank of North Texas found that targeted pooling was more effective at reducing the fat variability in DHM than randomly pooling ≤ 5 donors per pool (26). Valentine et al. created pools of DHM using 3–4 donors per pool collected from 5 different HMBANA milk banks and found that the amino acid and fatty acid profiles did not significantly differ between pools, but was influenced by lactation stage (45). Future studies should collect milk bank pooling information and donor characteristics (e.g., lactation stage and pregnancy term) to identify potential process improvement opportunities within donor milk banking.

Sources of bias and limitation

In addition to different analytical methods and limited use of reference methods as a source of information bias, there was also missing information that contributed to information bias. For example, infrared analyzers typically measure crude protein based on total nitrogen and then estimate true protein by accounting for the nonnitrogen fraction in human milk. The nonprotein nitrogen fraction of human milk averages between 20–25% of the total nitrogen in human milk and can be $\leq 50\%$ (37, 46, 47). Thus, if nonprotein nitrogen is not accounted for, protein values may be overestimated. Only 1 of the studies that measured protein with infrared analysis described whether they were reporting crude protein values or true protein values. Calorie information from this review is also subject to information bias as not all studies reported on the conversion factors used to compute energy from macronutrient values. Further, some studies used lactose to derive calorie values, while other studies used total carbohydrates. Previous studies have suggested that there is a 10–15% difference in the metabolizable compared with the gross energy in human milk, likely due to the HMOs and the nonprotein nitrogen fraction, which are theoretically unavailable sources of energy for the neonate (48, 49); therefore different methods of computing energy

may bias results. This highlights the need to establish a standard approach to ensure uniformity in future studies and for translation to clinical nutrition delivery. There is selection bias present in these findings as most studies reflect DHM procured from a single milk bank in the HMBANA network. While these milk banks follow similar guidelines for DHM processing, regional differences in donors and milk-bank-specific practices may influence the nutritional composition of DHM. There was limited evidence of funding bias, with only 1 study partially funded by a milk bank and 2 studies funded by commercial entities. In these studies, there was no evidence that the funder played a role in study design or data interpretation. Additional limitations to this review include the small sample sizes within the included studies, and lack of any information on most vitamins and minerals. Finally, given that the reviewed studies were descriptive in nature and that the analytical methods were not consistent among studies, we elected to conduct a systematic review rather than a meta-analysis.

Conclusion

These findings highlight the significant gap in the literature regarding the nutrient composition of DHM, with current evidence limited by both information and selection biases. Findings from the review suggest the potential for large variations in the fat, protein, and calorie composition of DHM based on small studies from a limited number of milk banks. Data on the vitamin and mineral composition of DHM was scarce.

Recommendations

Further research is needed into the nutritional composition of DHM, with prioritization on measuring macronutrients and micronutrients using established reference methods as summarized by Wu et al. (8). Detailed descriptions of sample storage and handling practices must be included, as these factors may influence findings. To increase generalizability and allow for comparison among studies, future research should include more samples from multiple nonprofit and commercial milk banks. Further, collection of milk banking information (e.g., donors per pool, lactation stage, use of macronutrient analyzer), may provide insights into methods for improving DHM nutrient composition.

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