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Maternal dietary intake, nutritional status and macronutrient composition of human breast milk: systematic review

Shiksha Adhikari[1]*, Urszula Kudla[1], Jean Nyakayiru[1] and Elske M. Brouwer-Brolsma[2]

[1]*FrieslandCampina, Amersfoort, the Netherlands*
[2]*Division of Human Nutrition and Health, Wageningen University & Research, Wageningen, the Netherlands*

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Abstract

Human breast milk (BM) is the best source of nutrition in early life, particularly during the first 6 months. Nevertheless, human BM composition is variable, and more insight in the exact factors contributing to this variability is warranted. In this review, we explored the impact of maternal dietary intake and nutritional status (e.g. anthropometric measures, BMI, bioimpedance) on human milk macronutrient composition. PubMed, Scopus and Cochrane were systematically searched till November 2019. In total, 4946 publications underwent title–abstract screening; 101 publications underwent full-text screening. Eventually, fifty publications were included in this review, investigating either associations between maternal dietary intake (*n* 29) and/or maternal nutritional status (*n* 29), and macronutrient composition of human BM. Reported energy composition ranged from 213 to 301 kJ/100 ml, and 67 % and 54 % of the studies reported associations between with maternal nutritional intake and status, respectively. Protein content ranged from 0·8 to 3·3 g/100 ml, and four studies suggested a negative association with nutritional status. Fat content ranged from 2·1 to 9·8 g/100 ml, and 68 % of the studies reported positive associations with nutritional status. Carbohydrate content ranged from 5·8 to 7·5 g/100 ml, and 67 % of the included studies did not report an association between intake and status. Literature investigating associations of maternal dietary intake and nutrition status with BM composition of macronutrients and energy content is diversified, both in terms of used methodology and results. Further studies using well-defined and standard parameters are essential to aid the formulation of scientific recommendations.

Key words: **Human breast milk: Dietary intake: Nutritional status: Milk composition: Macronutrients**

Current recommendations by the WHO indicate that human breast milk (BM) is the preferable source of nutrients during early life, particularly during the first 6 months of life[1], which is supported by a variety of studies indicating associations between breast-feeding and reduced risks of acute otitis media, non-specific gastroenteritis, severe lower respiratory tract infections, atopic dermatitis, asthma, obesity, type 1 and 2 diabetes, childhood leukaemia and sudden infant death syndrome[2]. Continued breast-feeding – along with complementary feeding – is recommended from 6 to 24 months of life based on associations indicating reduced risks of infectious morbidity and mortality, and obesity and diabetes in later life[3].

These health effects of human BM may be related to immunological and metabolic properties of, for example, BM oligosaccharides (hMOS), Ig (particularly sIgA), lactoferrin and cytokines[4], but literature is scarce and inconclusive. Consequently, research on the exact benefits of human BM and its underlying mechanisms is still ongoing. As scientific data suggest variations in human BM composition depending on maternal and child characteristics – for example, ethnic background, maternal age[5], parity, gestational age, infant sex, time of day, stage of lactation[6,7], breast-feeding pattern[8] and maternal dietary intake and status[5,9–11] – insight in the impact of these characteristics may help to further elucidate the suggested benefits of breast-feeding in the development, growth and health of infants.

Analyses of mature BM samples of mothers having full-term babies show average macronutrient concentrations between 6·7–7·8 g/100 ml for carbohydrate, 3·2–3·6 g/100 ml for fat and 0·9–1·2 g/100 ml for protein[12]. Various – but not all[13] – observational studies established that variations in macronutrient concentrations of BM may be related to maternal intake of fat[14] and protein[15,16]. Carbohydrate composition of BM appears to be rather independent of maternal dietary intake. In terms of micronutrients, concentrations of thiamine, riboflavin, vitamin B_6, vitamin B_{12}, choline, vitamin A, vitamin C, vitamin D, Se, Zn and iodine are suggested to be rapidly secreted into milk or substantially reduced by maternal

Abbreviation: BM, breast milk.

* **Corresponding author:** Shiksha Adhikari, email shikshadhikari@gmail.com

depletion, whereas concentrations of Ca, Fe and Mg tend to be relatively unaffected by variations in maternal intake[5]. However, the available information on this topic is scarce and diverse, where the most comprehensive body of evidence, in terms of number of articles, comes from only three studies supporting an association between maternal fish consumption and high DHA in human BM[5]. Similar conclusions were reached by another review conducted to summarise current literature on the association of maternal dietary intake with human BM composition[9]. Thus far, limited attention has been paid to the influence of maternal nutritional status – defined by various parameters such as BMI and body composition – on human BM composition[17,18]. In this systematic review, we provide an overview of studies exploring associations between maternal dietary intake and nutritional status, and macronutrient composition of human BM composition.

Methods

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[19]. To realise a comprehensive identification of relevant articles, three databases were consulted, that is, Medline via PubMed, Scopus and Cochrane. The search string included combinations of various search terms and MeSH terms with Boolean logic to identify the relevant publications, for example, 'human milk', 'breast milk', 'composition', 'macronutrients', 'amino acid', 'BMI', 'nutritional status', 'nutrition intake' and 'maternal diet'. The search string and number hits per databases can be found in supplementary data. The selection process started with a title and abstract screening. Human intervention and observational studies investigating potential relations between maternal dietary intake and/or maternal nutritional status in relation to macronutrient composition of BM, published in English language, were ordered as full text. During full-text screening, articles were included when providing (1) data on maternal dietary intake and/or nutritional status (i.e. on anthropometric measurements, BMI or body composition) and (2) data on BM composition of macronutrients. Articles were excluded when: (1) studies focused on animal or pre-clinical data; (2) studies focused on micronutrient composition of human BM instead of macronutrient composition; (3) studies only included mothers of pre-term infants; (4) methodology used to determine human BM composition was not clearly explained and/or (5) published as conference papers, book chapters, letters or editorials. Further, studies that included colostrum or transition BM only were not included in this study. Studies that included both mature and transition BM were included however, only considered for the observations for mature BM. As no search strategy can guarantee completeness, additional hand searches were conducted to identify studies that were not retrieved by the systematic search, for example, by screening reference lists of identified original articles as well as related reviews.

Data extraction

All identified publications were exported to Excel; author S. A. screened all identified articles (n 4942) and author J. N. screened 5 % of the identified publication: both during title/abstract screening and full-text screening. To control the bias of selective reporting, differences identified were solved after discussion. Data were extracted using an extraction form that was developed based on the Cochrane form[20], including items on study characteristics, participant characteristics, milk characteristics, milk analysis method used, statistical analysis method used, anthropometric measurement used to determine maternal nutrition status, dietary intake assessment method, major findings of the study and main discussion point of the publication. A separate form was developed and used for the quantitative data collection, including items on concentration of macronutrients in BM, maternal nutritional status based on anthropometric measurements (i.e. undernourished, normal, over-nourished or obese), dietary intake of energy, macronutrients, micronutrients and amino acid composition of mother's diet. Quantitative information on associations between maternal factors and BM composition was also recorded. Principal outcome reported in this review is mean and standard deviations. When available, regression coefficient (β) and coefficient of determination (R^2) are also reported as outcome. Unless stated otherwise, the term significant in this review refers to results with P-values $\leq 0{\cdot}05$. Outcomes are presented respective to their studies, and data of different studies were not combined.

Quality assessment

The quality of all full articles included in this review was evaluated by means of the Study Quality Assessment Tool, which is developed by the National Heart, Lung, and Blood Institute. (https://www.nhlbi.nih.gov/sites/default/files/media/docs/risk-assessment.pdf assessed on 28 October 2019). As specified in the document, different tools for observational studies and intervention studies were used. A maximum of fourteen points could be achieved, and no further qualitative assessment was done.

Results

Articles included in this study were published between 1954 and 2019 (Fig. 1), of which only 14 (24 %) articles were published within the past 5 years[18,21–32]. The majority of the studies were conducted among Asian participants (30 %, n 15)[17,18,21,23–25,27,31,33–39], followed by studies among Europeans (20 %, n 10)[13,28–30,40–45], Africans (10 %, n 5)[46–50], North-Americans (24 %, n 12)[14,22,32,51–59], South-Americans (14 %, n 7)[54,60–65] and Australians (2 %, n 1)[26] (Table 1 and Table 2). The age of the mothers included in the studies ranged from 14 to 43 years. Four (8 %) articles included both transition and mature milk[17,27,36,41]. All other 46 (92 %) articles used mature human BM for their study. Eleven (22 %) articles included breast-feeding mothers irrespective of the postpartum age, that is (0–≥ 6 months)[21,24,25,30,37,40,53,54,56,59,60]; all other studies used more restricted postpartum age ranges. Only 14 % of the studies used milk samples collected from 24-h milk sampling by complete emptying of a breast. In 40 % (n 20) of the studies, milk sampling was done in the morning[13,17,22–24,26,32,34,35,37,39,40,44,45,51,54–56,64,65] and 6 % (n 3)

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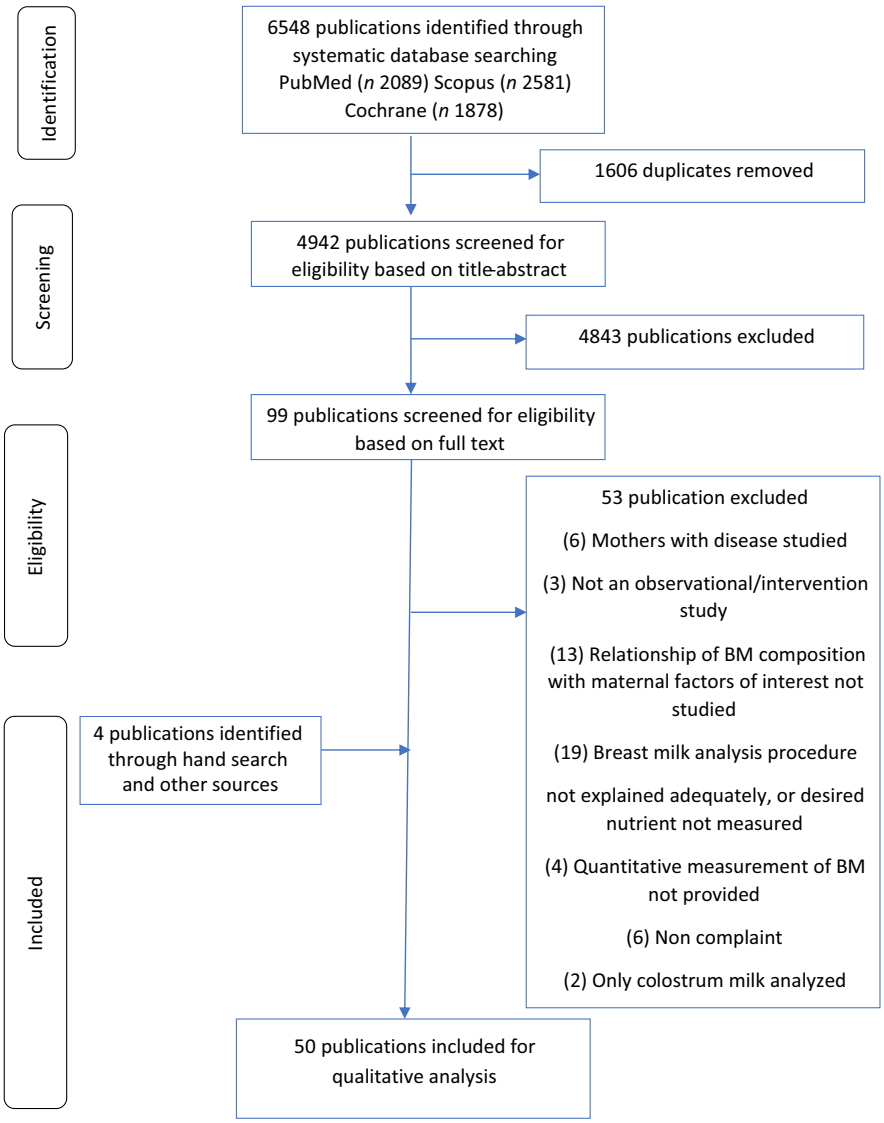

Fig. 1. Publication selection procedure.

of the studies sampled in the afternoon[18,53,59]. Eight percentage (*n* 4) of the studies collected milk samples at three time points (morning, afternoon and evening) during the day[30,54,61,62], 6 % (*n* 3) of the studies collected milk samples at two time points (morning and afternoon) during the day[27,46,49], 14 % (*n* 7) of the studies collected milk samples at multiple times throughout the day[14,28,29,34,43,51,63] and 25 % of the studies (*n* 13) did not distinguish based on point of time of the day[21,25,49,64] or the information was not available[31,37,39,40,42,44,48,58,60]. Whereas 19 (38 %) studies collected whole BM samples[17,21,22,25,27,32,34,51,53,55,56,58–63,65,66], 5 studies (10 %) collected any milk samples provided by the mothers[31,40,47,48,50]. Others specifically reported a focus on fore milk (18 %, *n* 9)[23,24,34–36,38,41,63,65], hind milk (8 %, *n* 4)[13,30,38,45], both fore and hind milk (20 %, *n* 10)[18,26,28,29,41,43,44,46,52,57], middle milk (2 %, *n* 1)[54], all fore, middle and hind milk (2 %, *n* 1)[14] or middle and hind milk (2 %, *n* 1)[49].

Across the identified studies, nine different parameters were used to define maternal nutritional status, including BMI (*n* 25), skin fold thickness at various measuring sites (*n* 8), mid-upper arm circumference (*n* 3), weight or weight difference between pre-pregnancy and postpartum weight (*n* 4), weight for height (*n* 3), % ideal body weight (*n* 1), fat mass (*n* 5), fat-free mass (*n* 2) and total body water (*n* 1) calculated based on various measurements such as bioelectric impedance analysis, skin fold thickness, dual isotope method or dual-energy x-ray absorptiometry. While nineteen studies used single parameters to define maternal nutritional status, four studies used two parameters, other four studies used three parameters and two studies used more than three parameters. With respect to maternal dietary intake, the most frequently used dietary intake assessment method was the food record (*n* 11) for 1[51,65], 2[58], 3[25,29,38,42,53,56,59] or 7[43] d, 24-h recall (*n* 6) for 1 d[17,48,51,54,61], and 3[35] d. Other methods used were the

Table 1. Studies investigating maternal nutrition intake and breast milk (BM) composition of macronutrients

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Observational studies					
Wurtman *et al.*, 1979[54]	5	20 American and 19 Guatemalan mothers at 1–37 months postpartum	American mothers: mature middle milk (after 6 min into feeding) collected in the morning, midday and evening at the same day; Guatemalan mothers: mature fore milk (3 min into feeding) was collected either in the morning or evening twice a week. Milk samples were frozen and thawed before analysing protein (Lowry *et al.*), and lipid content (chloroform–methanol extraction method)	American mothers: one face-to-face 24-h recall day before sample collection; Guatemalan mothers: one face-to-face 24-h recall one of the days in the week of sample collection; Guatemalan mothers had significantly lower intake of protein and fat however significantly higher intake of carbohydrate	Significant difference ($P < 0{\cdot}01$) for protein content with 1·4 (0·05) g/100 ml *v.* 1·1 (0·09) g/100 ml among American *v.* Guatemalan mothers. BM fat content did not significantly differ.
Vuori *et al.*, 1982[43]	7	20 Finnish mothers at 6–8 weeks postpartum (study 1); 13 of the 20 mothers were also included in study 2 at 17–22 weeks postpartum.	16 ml mature foremilk (8 ml) and hind milk (8 ml) collected during each feeding for 24 h; all samples were pooled. Samples were frozen and thawed before quantifying fat content (Rose Gottlieb method).	Self-reported 7-d food record during the week of sample collection	Maternal nutritional intake and BM fat content were not sign correlated
Finley *et al.*, 1985[55]	7	57 (172 samples) American mothers aged 29 years (range: 22–37); BM sampling at 3–4 weeks and 20 months postpartum	15 to >100 ml mature milk by whole breast emptying collected in the morning by hand expression or using a pump; samples were frozen and thawed before analysing for fat content (gravimetrically following procedures by Erickson and Dunkley).	Self-reported 24-h recall and 4–2-d diet record; only maternal protein intake was significantly different between group	No statistical significance was observed between BM fat content of vegetarian and non-vegetarian group
Speckers *et al.*,1987[56]	8	19 American mothers aged 30 years (range: 22–35 years); median postpartum age for vegetarian (5 months) *v.* non vegetarian (2 months)	Mature milk by whole breast emptying was collected in the morning by hand expression or breast pump; no pre-treatment was done before analysing for fat content by Folch method	Self-reported 3-d dietary record; Vegetarian (*n* 12) *v.* Omnivores (*n* 7): had significantly different percentage contribution from fat and carbohydrate to total energy between group but same % contribution by protein	No statistical significance was observed between BM fat content in vegetarian (3·1 (1·5) g/100 ml) and non-vegetarian (4·2 (2·4) g/100 ml) participants
Nommsen *et al.*, 1991[51]	8	73 mothers from DARLING cohort study USA with age 30·4 (4·6) years were followed for 12 months; milk samples were collected at 3, 6, 9 and 12 months postpartum; 36 % of the mothers included were primiparous	Whole breast emptying by hand expression from alternate breast was done for 24 h to collect mature milk sample; no pre-treatment; protein content by Lowry, fat content by Folch, lactose content by colorimetry and energy by Atwater specific factor	Self-reported food record was collected	BM fat ($r = 0{\cdot}49$, P-value $= 0{\cdot}013$) and energy content was significantly correlated with maternal protein intake; no other significant correlation of BM fat, protein, lactose and energy content was observed with maternal intake of nutrients
Villalpando *et al.*, 1992[61]	8	30 Mexican mothers of average age 26 (6·1) years (range 18–35 years) with postpartum age of 4 months (*n* 15) or 6 months (*n* 15); measurements were taken on day of enrolment (day 1) and at day 15	Mature milk; whole breast emptying using a breast pump from one breast at 10.00, 14.00 and 18.00 hours; no pre-treatment; individual sample used for BM fat content by Jeejebhoy, BM lactose content by YSI automated enzyme, and BM energy by bomb calorimetry method	24-h recall was collected by face-to-face interview to collect current dietary intake; staple food (tortilla) was collected and analysed for nutrient composition	No significant association between maternal intake of macronutrients and BM composition of fat and lactose at 4 month postpartum and 6 months postpartum was observed

Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Ronayne De Ferrer *et al.*, 1993[65]	6	38 mothers residing in Argentina at postpartum age 14–30 d	Mature whole BM collected at 10.00–12.00 h by hand expression; no pre-treatment; BM protein content analysed by Kjeldahl method ($n \times 6{\cdot}25$)	One-day food record	No significant association between maternal intake of protein and BM protein content
Boniglia *et al.*, 2003[13]	9	117 Italian mothers with average age of 31·2 (4·5) years; approximately 1 month postpartum	10 ml mature post-feed hind milk was collected from second or third feeding in the morning; samples were frozen before analysing for protein content by Kjeldahl method ($n \times 6{\cdot}25$)	24-h recall for two consecutive days	No significant association was observed between maternal protein and energy consumption with BM protein content
Rakicioglu *et al.*, 2006[38]	8	21 Turkish mothers with mean age 27·3 (5·4) years at postpartum age of 2–5 months; measurements were done during second week of Ramadan and 2 weeks after Ramadan	Mature post-feed hind milk was collected in the morning between 9.00 and 11.00 h by hand expression; samples were frozen before analysing for BM protein content by Kjeldahl method ($n \times 6{\cdot}25$), BM fat content by Rose–Gottlieb method and BM lactose content by enzyme-specific YIS method	3-d food record was obtained during and after Ramadan; difference between average maternal intake during *v.* after Ramadan was significant only for carbohydrate intake (P-value = 0·016); intake of energy (P-value = 0·06), protein (P-value = 0·12) and total fat (P-value = 0·87) was not significantly different	No significant association was observed for maternal dietary intake and BM macronutrient composition (P-value for BM protein, lactose and fat content was 0·398, 0·765 and 0·853, respectively)
Nikniaz *et al.*, 2009[35]	7	182 Iranian mothers with average age 26·5 (6) years at postpartum age of 90–120 d were included from rural (*n* 91) and urban (*n*-91) area	15 ml of mature foremilk was expressed once; the samples were frozen and thawed at 38°C before analysing for fat content by Gerber method	24-h recall for 3 d including one weekend	Maternal carbohydrate intake was positively significantly correlated ($\beta = 0{\cdot}39$, P-value < 0·01) with BM fat content; no significant correlation between maternal intake of protein and fat and energy with BM fat content
Antonakou *et al.*, 2013[42]	9	46 Greek mothers with average age of 32·5 (3·1) years were enrolled at postpartum age of 1 month and followed (and measured at) 3 (*n* 39) and 6 (*n* 24) months postpartum	30 ml mature foremilk was collected using an electric breast pump at any point of the day; samples were frozen at −80°C and thawed before analysing for fat content by modified Folch method	3-d food record was used to collect current dietary intake of the participants once at 1-month, 3-month and 6-month postpartum	No significant correlation was observed between BM fat content (g/100 ml) and maternal intake of protein ($r = -0{\cdot}02$), fat ($r = 0{\cdot}12$), carbohydrate ($r = -0{\cdot}10$) and energy ($r = -0{\cdot}04$)
Ogechi *et al.*, 2013[48]	7	27 Nigerian mothers with average age of 26·0 (0·4) years at postpartum age 2–4 weeks	5 ml of mature milk was collected using a breast pump; analysis for BM protein content by Kjeldahl, BM fat content by Soxhlet, BM carbohydrate content by difference method and BM energy content was calculated using modified Atwater factor	3-d weighed inventory (food record of weighed food item) and 24-h recall method was used to collect information on dietary intake of participants	No correlation between BM protein content and maternal energy ($r = 0{\cdot}015$, P-value = 0·91), fat ($r = 0{\cdot}088$, P-value = 0·54), protein ($r = -0{\cdot}093$, P-value = 0·517) and carbohydrate ($r = -0{\cdot}006$, P-value = 0·97) intake was observed; BM fat content not correlated with maternal protein ($r = 0{\cdot}027$, P-value = 0·85), fat ($r = 0{\cdot}082$, P-value = 0·57), energy ($r = -0{\cdot}020$, P-value $r = 0{\cdot}89$) and carbohydrate ($r = -0{\cdot}066$, P-value = 0·66) intake; **BM lactose was significantly correlated with maternal intake of energy ($r = 0{\cdot}454$, P-value < 0·01), protein ($r = 0{\cdot}310$, P-value = 0·03),**

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Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
					fat ($r = 0{\cdot}40$, *P*-value $< 0{\cdot}01$) and carbohydrate ($r = 0{\cdot}385$, *P*-value $< 0{\cdot}01$); BM energy content was significantly correlated with maternal protein ($r = 0{\cdot}345$, *P*-value $= 0{\cdot}01$) and fat ($r = 0{\cdot}341$, *P*-value $< 0{\cdot}01$) intake but not correlated with energy ($r = 0{\cdot}251$, *P*-value $= 0{\cdot}08$) and carbohydrate ($r = 0{\cdot}134$, *P*-value $= 0{\cdot}35$) intake
Yang *et al.*, 2014[17]	8	436 Chinese mothers aged between 20 and 41 years at postpartum age of 5–240 d from MING study group were included	Transition and mature milk was collected between 09.00 and 11.00 h by whole breast emptying using either hand or breast pump; 40 ml aliquot was used for further analysis; sample was stored at −80˚C and thawed before analysing for BM protein, fat, lactose and energy content using a MIRIS human milk analyser	24-h recall was used to obtain current dietary intake of participants by trained interviewer, measuring cups and food pictures were used to conduct the recall	No correlation between maternal dietary intake and BM composition at different postpartum age was observed except for **BM fat content and maternal energy intake ($r = 0{\cdot}216$, *P*-value $< 0{\cdot}05$) at 61–120 d postpartum**; **significant association was observed between maternal fat intake and BM lactose ($\beta = 0{\cdot}001$, *P*-value $= 0{\cdot}03$) and protein ($\beta = 0{\cdot}0025$, *P*-value $= 0{\cdot}02$) content,** whereas no significant association was observed with BM fat content
Kim *et al.*, 2017[25]	8	238 Korean mothers with average age 31·6 (3·2) years at postpartum age of 140 (44) d (range: 30–360 d) were included; 51·2 % of the participants took dietary supplements; data collection was done between April 2013 and May 2015	150 ml of mature milk was collected by whole breast emptying at any point of the day; sample was frozen at −18˚C and thawed at 37˚C before analysing for protein, fat and lactose content using an Infrared Spectrometry (MilkoScan FT2) device	3 d (2 weekdays and 1 weekend) dietary records were collected from mothers 1 week before milk collection and 1 week after milk collection	BM protein was negatively not correlated with energy, carbohydrate, protein and fat intake; **BM fat was significantly correlated with fat intake($r = 0{\cdot}14$; *P*-value $< 0{\cdot}05$)** and not associated with maternal carbohydrate, protein and energy intake; BM lactose was not associated with maternal energy, carbohydrate, protein and fat intake; **BM energy was significantly correlated with maternal fat intake ($r = 0{\cdot}132$ *P*-value $< 0{\cdot}05$)** and not correlated with maternal carbohydrate protein and energy intake
Bzikowska-Jura *et al.*, 2018[29]	8	40 Polish mothers with average age 31·1 ± 4·4 years at postpartum age 1 month; participants were followed till 6 months postpartum, and measurements were taken at 3 (*n* 22) and 6 (*n* 15) months postpartum	10–20 ml mature fore (5–10 ml) and hind milk (5–10 ml) were collected for a whole day from 06.00 till 06.00 of following day using a breast pump or hand expression; sample was frozen at −20˚C and thawed at 40˚C before analysing for BM protein, fat and lactose content using MIRIS human milk analyser; BM energy content by Atwater factor; protein conversion factor of × 0·825	3-d dietary record (self-reported); maternal intake of protein, fat carbohydrate and energy reported	No significant correlation was observed between maternal dietary intake and macronutrient composition of BM; **BM energy was positively correlated with maternal intake of energy, fat and carbohydrate at 1 and 3 months and negatively correlated at 6 months, while BM energy was positively correlated with protein intake at all 3 postpartum ages**

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Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Aumeistere *et al.*, 2019[30]	6	61 Latvian mothers with median age of 31 years (range: 23–39 years) at median postpartum age of 4 months (range: 1·5–21 months)	Mature hind milk was collected using hand expression or breast pump in the morning, midday and evening; pooled sample of 60 ml was used for analysis; samples were frozen and thawed before analysing for BM protein content using Kjeldahl method ($n \times 6{\cdot}25$), BM fat content using Gerber method and BM lactose using high-performance liquid chromatography method	Self-reported 72 h-food dairy; maternal intake of protein, fat carbohydrate and energy reported	No significant correlation was observed when maternal intake of a macronutrient and BM composition of corresponding nutrient was investigated: protein ($r = 0{\cdot}009$); fat ($r = 0{\cdot}052$) and lactose ($r = 0{\cdot}213$)
Minato *et al.*, 2019[31]	8	91 Japanese mothers with average age of 34 years at postpartum age of 1 month (*n* 91) were followed till 3 months postpartum (*n* 57)	30 ml mature milk was collected from the participants; milk samples were frozen at −80°C and thawed at 40°C before analysing for BM protein, fat and lactose content using MIRIS human milk analyser	FFQ for previous month of BM collection; maternal intake of energy, protein, phosphorous and Ca was reported	At 1 month postpartum: negative correlation was observed between the maternal nutrient intake and BM protein content; however, these correlations were not statistically significant; **only energy ($r = 0{\cdot}180$, P-value $< 0{\cdot}1$) and phosphorous ($r = 0{\cdot}187$, P-value $< 0{\cdot}1$) intake of mother were significantly correlated with BM fat content**; **only maternal protein ($r = 0{\cdot}175$, P-value $< 0{\cdot}1$) intake was positively significantly correlated with BM carbohydrate content**; **only phosphorous ($r = 0{\cdot}176$, P-value $< 0{\cdot}1$) intake was positively significantly correlated with BM energy at 1 month postpartum**; At 3 months postpartum, no significant correlation between maternal intake and BM composition was observed
RCT/Intervention studies					
Karmarkar *et al.*, 1963[39]	5	60 Indian mothers at postpartum age of 1–3 months were enrolled and followed for 5 months; participants were from low socio-economical household and **marginally undernourished**	Mature foremilk was collected before feeding the baby; no pre-treatment of sample; analysis of BM protein content by MicroKjeldahl and BM fat content according to Chiba *et al.*	Duplicate portion method for 3 d to collect dietary information; intake of protein before intervention was between 18 and 23 g/d; participants were categorised into three groups (fat supplement group, protein supplement group and control group), fat (further classified into four groups provided with no supplement, 15 g protein, vitamins or 15 g protein + vitamins) and protein group (further classified into four groups provided with no supplements, vitamins, 20 g fat or 20 g fat + vitamins were; fat was supplied in the form of butter and protein in the form of skimmed milk powder; in the fat and protein group the supplement of fat (5, 15, 25, 35, 45 g/d) and protein (10, 20, 30, 35, 40 g/d) was increased every month, respectively	**BM protein content was significantly higher in the protein supplement group but not significant in other treatment groups**; **BM fat was significantly different in fat supplement group and not significant in other treatment group**

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Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Edozien *et al.*, 1976[49]	6	12 Nigerian mothers at postpartum age 1–3 months were enrolled for intervention with diet containing 100 g protein/d; all mothers were sub clinically malnourished	10 ml mature mid- and hind milk samples were collected for the whole day; no pre-treatment of the sample; analysis of BM protein content by Kjeldahl method	Food was provided for both intervention groups: Group I: initially 25 g protein/d for 2 weeks followed by 2-week intervention with 100 g protein/d and Group II: initially 50 g protein/d for 2 weeks followed by 2-week intervention with 100 g protein/d	No statistically significant differences were observed between the two groups
Mellies *et al.*, 1978[57]	8	14 mothers residing in the USA at postpartum age of 1 month were enrolled in a crossover designed RCT	Mature milk was collected pre- or post-feeding at the second feeding session of the day; samples were frozen before analysing for BM fat content using Folch method	Participants were either assigned to Diet I (cholesterol poor and phytosterol and PUFA-rich (PUFA/SFA: 1·8) or Diet II (cholesterol-rich and phytosterol and PUFA poor (PUFA/SFA: 0·12)); participants were followed for 4 weeks after each *ad libitum* diet and alternate diet	No statistical significance was observed between BM fat content during *ad libitum* intake *v.* diet I *v.* diet II (3·58 *v.* 2·69 *v.* 2·66 g/100 ml)
Forsum *et al.*, 1980[44]	5	3 Swedish mothers with average age 29 years at an average postpartum age of 17 weeks were enrolled in a crossover designed RCT	5–10 ml mature milk was collected before and after feeding, pooled sample of fore and hind milk was used for further analysis; samples were frozen before analysing for BM protein content using Kjeldahl method	Duplicate portion method was used to measure the dietary intake of participants at the end of each intervention; High- *v.* Low-Protein intake in a crossover design was used as intervention, each diet was consumed for 4 d, with washout of 1 d	**Positive significant ($P < 0·01$) difference was observed for true protein content between low-protein *v.* high-protein group (0·8 (0·02) *v.* 0·9 (0·03) g/100 ml)**
Cant *et al.*, 1991[45]	8	36 mothers residing in the UK were enrolled for a double-blinded RCT in two equal groups (placebo *v.* control); postpartum age during enrolment was 2–6 months; intervention lasted for 8 months	Mature hind milk at the end of feeding was collected in the morning; no pre-treatment was done before analysing for BM fat content using Folch method	Control (normal diet with additional 2800 mg linoleic acid + 320 mg Gamma linoleic acid) *v.* Placebo (normal diet); comparison was made between baseline measurement and end measurement at the termination of study	No statistical significance was observed (P-value = 0·092); however, total BM fat content of control group increased by 15 % from baseline measurement, whereas for placebo group, fat content reduced by 23 % compared with baseline value
Dusdieker *et al.*, 1994[58]	6	22 mothers residing in the USA with postpartum age of 30–100 d; average age of the mothers was 32·1 (4·1) years; intervention lasted for 10 weeks	30 ml mature milk was collected from the whole BM expressed using either hand expression or by hand pump; no pre-treatment of sample; analysis of BM fat was done by creamatocrit method (then converted to % of fat in milk using equation by Lucas *et al.*)	Self-reported 2-d dietary record was collected once every week for the study period; participants were provided with low-fat, energy-controlled diet as intervention; energy intake baseline and during intervention were reported as 9636 (2519) kJ/d *v.* 7385 (1753) kJ/d, respectively	No statistical significant difference in BM %fat composition between baseline (4·06 (2·15)) and end measurement (4·00 (2·56)) after the intervention was observed; total nitrogen of BM was analysed but not translated to protein content hence not included in this study
Park *et al.*, 1999[53]	10	16 mothers residing in the USA with average age of 32 (2) years (21–38 years) at postpartum age of 9·1 (2) months (1–26 months) were enrolled in a crossover designed RCT; participants were categorised into two equal groups for intervention with either low-fat dairy product or with high-fat dairy product (milk, cheese, ice-cream and yogurt)	Mature milk was collected by whole breast emptying using an electric breast pump at 15.00–22.00 hours for one breast simultaneously while feeding the baby; sample was frozen before analysing for BM lipid content using solvent extraction method	Self-reported 3-d food record was collected every week for 3 weeks during the study period; intervention groups were defined as high dairy product *v.* low dairy product: high dairy group had significantly high maternal consumption of energy, protein and fat but not for carbohydrate intake	**BM lipid concentration was significantly higher in the high dairy product group than in low dairy product group (38·3 (1·6) *v.* 45·6 (5·0) mg/g milk, respectively (P-value < 0·05)**

Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Cisse *et al.*, 2002[50]	7	133 pregnant Senegalese mothers with average age of 27 (6) years were enrolled in an intervention study (intervention with supplement based on millet (*n* 41), maize (*n* 35), and control group (*n* 57)); participants were followed till 4 month lactation; however, for BM composition analysis, samples were collected at 14-d postpartum; participants were residents of poor urban area of Senegal	Mature milk was collected by manual expression twice a day (midmorning and midafternoon); pooled sample was used for further analysis; no pre-treatment of sample; analysis for BM protein content by Kjeldahl method ($n \times 6{\cdot}38$); BM lactose and TAG content by enzymatic method	Additional supplemental product based on millet that provided +1766 kJ/d, based on maize that provided +1674 kJ/d, and control group with no additional supplementation was provided; supplementation was initiated during pregnancy and lasted for more than 60 d	**Positive significant difference ($P < 0{\cdot}01$) observed between supplemented and control group (millet *v.* maize *v.* control) for BM protein content (1·5 (0·3) g/100 ml *v.* 1·5 (0·2) g/100 ml *v.* 1·2 (0·3) g/100 ml) and BM lactose content (6·2 (0·8) g/100 ml *v.* 6·0 (0·8) g/100 ml *v.* 5·5 (0·8) g/100 ml)** but not for TAG (2·9 (0·7) g/100 ml *v.* 3·3 (1) g/100 ml *v.* 3·1 (1) g/100 ml) composition
Masters *et al.*, 2002[59]	11	91 mothers residing in the USA with average age of 31·5 (0·8) years and at average postpartum age of 5·3 (0·9) months (range: 1–12 months) were enrolled in a crossover double-blinded randomised trial	Mature milk was collected by emptying whole breast using an electric breast pump between 13.00 and 15.00 h; milk was collected on the last day of intervention period; sample was frozen at −70°C and thawed before analysing for fat content using Folch method	3-d dietary record was collected from participants; Intervention (5 d) with 1500 mg/d conjugated linoleic acid *v.* 1500 mg/d placebo (olive oil) followed by wash-out of 7 d and crossover for alternate intervention (5 d)	**% BM milk fat was significantly lower ($P < 0{\cdot}05$) during the conjugated linoleic acid treatment when compared with the placebo treatment (2·3 (0·2) *v.* 3·0 (0·2) %, respectively)**
Chien *et al.*, 2009[66]	8	23 Taiwanese mothers with mean age of 24·5 (3·4) years; mothers were categorised into two intervention group (each group was feed with either soup containing alcohol or soup without alcohol)	Mature milk was collected by emptying whole breast using an electric breast pump in the morning; milk from both breast was collected for 15 min and pooled; milk was collected before intervention and after 120 min of the intervention; no pretreatment of sample; analysis of protein was done by commercial assay kits TP 1630, while lactose and fat content were determined by ELISA method modified from Arthur *et al.* and Cox *et al.*, respectively	No dietary information on habitual/ current intake was reported; intervention was soup with alcohol. Only energy and TAG content of the soup were significantly different between soup groups (*P*-value < 0·05) and not in carbohydrate and protein content	**Mean values of BM protein and TAG were significantly higher (*P*-value < 0·05) from baseline in both soup group; BM lactose content was significantly lower from baseline in alcoholic soup group** but not lower in non-alcoholic soup group
Mohammad *et al.*, 2009[14]	7	7 healthy mothers normal BMI (African-American *n* 2, Hispanic *n* 3 and white *n* 2) with average age of 29·3 (1·0) years at postpartum age of 6–14 weeks were included in a crossover design RCT; the participants were studied for 8 d for each intervention (high-carbohydrate diet: 60 % carbohydrate and 25 % fat *v.* high-fat diet: 30 % carbohydrate and 55 % fat) with washout period of 1–2 weeks	15 ml mature (fore + middle + hind) milk was collected from both breasts using an electric breast pump 8 times a day (i.e. every 3 h); milk was collected on days 5–8 of the study period of each intervention; no pretreatment of sample was reported before analysing for BM protein content by bicinchoninic acid protein assay kit (Novagen, Madison, WI), BM fat content by gravimetric method, and BM lactose content by enzyme-specific method (YSI Glucose Analyser)	Fixed energy calculated diet was provided, and left-over food was collected, addition food consumed was reported by the participants	BM protein and lactose concentrations were unaffected after both interventions; **BM fat (4·3 (0·3) g/100 ml *v.* 4·8 (0·3) g/100 ml)* and energy (2590** (96) **kJ/d *v.* 654** (24) **kJ/d)* content were significantly (*P*-value < 0·05) higher after the high-fat diet intervention than after high-carbohydrate diet**

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Table 1. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutrition intake	Findings (including analysis method)
Yahvah *et al.*, 2015[32]	10	15 mothers residing in the USA with average age of 27 (1.0) years at postpartum age of 26 (2) weeks were enrolled in a crossover double-blinded randomised trial; participants were included in high dairy product intervention group or low dairy product intervention group for 14 d followed by a washout period of 14 d before switching groups; only mothers feeding formula to their infants were included for ethical purposes	7–160 ml of mature whole BM was collected using an electric pump in the morning between 06.00 and 10.00 hours; milk samples were collected on the day before intervention and on the 14th d of each intervention; samples were not pre-treated before analysing for fat content using Folch method	Dietary intake was measured using weighted inventory method; milk, cheese, and yogurt (skimmed for low-fat group and full fat for high-fat group) were provided to the participants for consumption and requested not to consume any other form of dairy product throughout the intervention period; high dairy product group had significantly higher consumption of energy, and fat; however, protein and carbohydrate intake was not significantly different	**%BM fat was significantly (P-value $< 0{\cdot}05$) lower when women consumed the low-fat dairy intervention (2·41 (0·31) %) compared with the full-fat dairy intervention (3·35 ± 0·28 %) diet**

FFM, fat-free mass; IBW, ideal body weight; MIRIS, human milk analyser; MUAC, mid upper arm circumference; TBW, total body water; TCSF, triceps skin fold; #, same study looking at between mother nutritional status and within mother change in weight.
Significant differences are bold.
* Data inside parenthesis are presented as mean (SEM).

duplicate portion method[39,44], FFQ[31,59], weighed food record[32,48] and controlled dietary intervention studies[14,49,50,66].

Milk analysis

Collected samples were frozen and thawed before analysis in 32 (61·5 %) of the studies; the majority (87·5 %) of these studies analysed fat content. Nine (17·3 %) studies used the MIRIS human milk analyser, whereas 4 (7·7 %) used MilkoScan human milk analyser; other methods were total nitrogen estimation and colorimetry method for protein, solvent extraction method and creamatocrit for lipid/fat estimation, and Infrared Spectrometry and colorimetry method for carbohydrates. Nine different methods were used to determine BM protein content, ten different methods were used to identify BM carbohydrate content and fifteen different methods were used to quantify BM fat content. Besides, these differences in analytical methods also laboratory procedures varied across studies. Only one study provided information on validation with standard technique.

Breast milk energy content

A total of sixteen studies investigated and reported BM energy composition and its association with either maternal dietary intake or maternal nutritional status. Five studies calculated BM energy content using Atwater general factor[27–29,34,48], and three studies used Atwater specific factor (i.e. conversion factor for protein, carbohydrate and fat of 5·65, 3·95 and 9·25 kJ/g, respectively)[24,37,51]. BM energy was measured using the bomb calorimetry method by only one cross-sectional study[61]. Five studies used infrared-based devices such as MIRIS and Milkoscan to measure BM composition[18,21,22,25,29]. These studies/devices calculate BM energy composition based on Atwater factor for individual macronutrient composition. Only one study used specific conversion factors of 38·7, 16·7 and 18·4 kJ/g for fat, carbohydrate and protein, respectively, to calculate BM energy composition[22].

Range of mean BM energy based on the studies included is 259 (29) kJ/100 ml with a range of 21-301 kJ/100 ml. Out of six studies that investigated association of BM energy with maternal dietary intake, three showed significant associations for maternal intake of protein[29,48,51], fat[25,29,48], carbohydrate[29] and total energy[29], while 2 (33 %) did not observe any association with the macronutrient intakes studied[31,61]. All six studies investigated BM composition of three macronutrients. Five studies investigated maternal intake of protein, fat, carbohydrate and total energy and their association with BM energy content. One study only investigated association of maternal intake of protein and total energy with BM energy content[31].

Out of eleven studies that investigated associations between maternal nutrition status (either pre-pregnancy BMI or maternal status at the time of lactation) and BM energy content, 7 (64 %) showed significant associations[18,21,24,25,28,34,61], whereas 4 (36 %) did not[22,27,29,36]. An overview of the available result is presented in Fig. 2. Current maternal nutritional status and BM energy density content were reported to have a significant association in four studies, that is, n 30, $r = 0{\cdot}35$, P-value $= 0{\cdot}07$[61]; n 60, $\beta = 65$ (kJ/d per kg maternal weight), P-value $< 0{\cdot}01$[34]; n 2632, $r = 0{\cdot}10$, P-values $< 0{\cdot}01$[21];

Table 2. Studies investigating maternal nutritional status and breast milk (BM) composition of macronutrients

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Observational studies					
Khin Maung Naing *et al.*, 1980[33]	9	90 (*n* 83 used for analysis of interest) Burmese mothers, aged 18–35 years with postpartum age 1–12 months belonging to a low-socio-economic group; mothers classified as stage 1 = 1–4 month, stage 2 = 4–7 month and stage 3 = 7–12 month postpartum	10 ml mature foremilk, collected once in the morning before nursing the child; milk from three different stages were pooled for BM energy (modified Atwater method), fat (by Rose and Gottlieb method) and lactose (by Benedict's solution method) content estimation; pooled sample from stage 1 and 2 or stage 2 and 3 was computed separately for protein composition by Kjeldahl ($n \times 6{\cdot}38$); no pre-treatment reported	Maternal height and weight were measured and classified into 4 categories based on weight-for-height (WH) percentile: > 95·85 % (*n* 16) *v.* 95·85–94·75 % (*n* 20) *v.* 94·74–84 % (*n* 34) *v.* < 75 % (*n* 13)	No statistically significant differences were observed between the different WH groups in terms of macronutrient and energy content of BM
Steenbergen *et al.*, 1983[46]	8	98 Kenyan mothers, aged 28 (8) years (WH-plus group) and 27 (7) years (WH-minus group) with postpartum age 92 (51) d (WH-plus group, *n* 52) and 90 (47) d (WH-minus group, *n* 46)	25–100 ml mature, fore and hind milk collected once in the morning (11.00 hours); milk samples were frozen and thawed; BM analysed for Protein by Kjeldahl, Fat by Rose and Gottlieb, and Lactose by Modified Folin method	Weight and height during last trimester were measured to calculate weight-for-height (WH); two groups: WH-plus (90–115 % of reference population) and WH-minus (70–80 % of reference population)	No significant association was identified for any of the BM macronutrient at *P*-value < 0·05; BM fat content was higher but not significant in the WH-minus group (WH-plus: 2·6 (1·3) g/100 ml) *v.* WH-minus: 3·2 (1·3) g/100 ml)
Marin Spring *et al.*, 1985[60]	8	81 Brazilian mothers exclusively breast-feeding; low (*n* 71) and high (*n* 10) socio-economic group	2·5 ml aliquot from mature, whole BM expressed by either nipple shield or manual expression at any point of the day; no pre-treatment; analysis for BM fat content by Creamatocrit method; average of triplicate measurements	Weight, height, triceps skin-fold thickness and mid upper arm circumference were measured to calculate weight-for-height ratio; mothers were classified as severely undernourished, undernourished and well nourished; further classified for comparison purpose as malnourished low socio-economic status (*n* 10); well-nourished low socio-economic status(*n* 61) and well-nourished high socio-economic status (*n* 10)	No significant association was recognised for any of the BM macronutrient at *P*-value < 0·05; a higher BM fat concentration was observed in malnourished mothers 5·25 (2·50) g/100 ml compared with well-nourished (low socio-economic) 3·84 (2·04) g/100 ml and well-nourished (high socio-economic) 4·1 (2·65) g/100 ml mothers
Brown *et al.*, 1986[34]	9	60 Bangladeshi mothers with median age of 19 years (range 14–39 years) at 7–188 d postpartum; mothers were classified into 2 groups based on postpartum age: <90 d and ≥90 d	Whole breast emptying by breast pump from both breasts was done for 24 h to collect mature milk sample; aliquot samples were frozen and thawed; analysis for BM fat by Gravimetric and BM lactose by Colorimetry method; BM nitrogen measured by Kjeldahl (no conversion to protein), BM energy by Atwater factor	Weight, height, TCSF thickness (left hand) and MUAC (left hand) were measured; mothers were classified as undernourished, normal and over-nourished within the group classified based on postpartum age; mothers were further classified based on weight gain group I: < 0·2 kg weight gain and group II: ≥0·2 kg weight gain	**Significant association was observed for fat ($\beta = 0{\cdot}94$, *P*-value < 0·05), lactose ($\beta = 2{\cdot}20$, *P*-value < 0·01), nitrogen content ($\beta = 0{\cdot}069$, *P*-value < 0·01) and energy ($\beta = 15{\cdot}44$, *P*-value < 0·01) content of milk between the groups classified based on maternal weight;** however, no significance was observed between groups based on MUAC and TCSF classification; **in a sub-sample, BM nitrogen content between mother gaining < 0·2 kg was significantly lower than mother gaining ≥ 0·2 kg weight regardless of their initial weight**; no comparison was reported for other macronutrient in the sub-sample

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Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Michaelsen, *et al.*, 1990[40]	7	2554 milk samples were collected from 244 Danish mothers donating milk at milk bank during June 1984–December 1986; median age of the mothers was 27 years range (17–43 years)	Milk samples included irrespective of the type and time of milk collection; samples were frozen at −20°C and heated at 40°C; analysis for BM protein, fat and lactose by infrared analysis (device: MilkoScan)	Self-reported weight and height during enrolment at the bank was used to calculate the BMI; mothers were classified into seven BMI groups: BMI < 21 kg/m^2; BMI = 21 kg/m^2; BMI = 22 kg/m^2; BMI = 23 kg/m^2; BMI = 24 kg/m^2; BMI = 25–26 kg/m^2 and BMI ≥ 27 kg/m^2	BM protein, fat and carbohydrate content had no significant association with maternal BMI
Nommsen *et al.*, 1991[51]	8	73 mothers from DARLING cohort study USA with age 30·4 (4·6) years were followed for 12 months; milk samples were collected at 3, 6, 9 and 12 months postpartum	Whole breast emptying by hand expression from alternate breast was done for 24-h to collect mature milk sample; no pre-treatment of samples; analysis for BM protein content by Lowry, fat content by Folch, lactose content by colorimetry and BM energy by bomb calorimetry method	Weight was measured once every 3 months to calculate %IBW, weight loss (kg/month), and TCSF on the left arm; mothers were not classified based on nutritional status	**BM fat was positively and significantly correlated with %IBW at 6 ($\beta = 0{\cdot}28$; $R^2 = 0{\cdot}19$; P-value < 0·01), 9 ($\beta = 0{\cdot}32$; $R^2 = 0{\cdot}35$; P-value < 0·01) and 12 ($\beta = 0{\cdot}30$; $R^2 = 0{\cdot}37$; P-value < 0·05) months postpartum**; **BM protein concentration was positively and significantly correlated with %IBW at 9($\beta = 0{\cdot}06$; $R^2 = 0{\cdot}38$; P-value < 0·01) months postpartum only**; Lactose did not show any relationship with %IBW; other associations reported were non-significant
Villalpando *et al.*, 1992[61]	8	30 Mexican mothers of average age 26 (6·1) years (range 18–35 years) with postpartum age of 4 months (*n* 15) or 6 months (*n* 15); measurements were taken on day of enrolment (day 1) and at day 15	Mature milk; whole breast emptying using a breast pump from one breast at 10.00, 14.00 and 18.00 hours; no pre-treatment; individual sample used for BM fat content by Jeejebhoy and BM, and lactose content by YSI automated enzyme	Maternal weight and height were measured to calculate BMI and TBW, FFM and FM (Doubly labelled water) were measured on day 1 and 14 after enrolment and average of these two measurement was used for further analysis; mothers were not categorised into groups based on nutritional status	BM protein and lactose content did not show any significant association with maternal nutrition status; **BM fat content was positively significantly associated with BMI ($P = 0{\cdot}05$), FM ($P = 0{\cdot}001$), maternal weight ($P = 0{\cdot}002$) and % weight ($P = 0{\cdot}002$)**; **BM energy content was positively associated with FM ($P = 0{\cdot}001$), maternal weight ($P = 0{\cdot}002$) and %weight ($P = 0{\cdot}004$)**
Michaelsen *et al.*, 1994[41]	8	249 BM samples from 28 Danish mothers from Copenhagen cohort study; participants followed from birth to 5 months postpartum; samples were collected on 4th day, 14th day, every 2weeks till 3rd month, once on 4th month, and once on 5th month postpartum	16 ml of transition (4 d postpartum) and mature milk were collected once in the morning; both fore (8 ml) and hind (8 ml) milk were collected and analysed separately; collected samples were frozen and thawed; analysis for protein, fat and lactose content by infrared analysis method	Weight gained during pregnancy was derived from hospital record; mothers were categorised into three groups based on pregnancy weight gain: low (< 11·2 kg) *n* 7; medium (11·2–16·9 kg) *n* 14 and high (> 16·9 kg) *n* 7	**BM fat content was significantly correlated with weight gained during pregnancy at 4 month postpartum ($r = 0{\cdot}43$; P-value < 0·01)**; no other correlation between BM protein or lactose with weight gain during pregnancy was observed; no correlation was observed between BM protein, fat or lactose content with maternal BMI and height individually
Barbosa *et al.*, 1997[62]	7	40 Mexican mothers were included at 3 months postpartum and followed till 6 months postpartum; mean age of mothers in group low BMI was 21·3 (4·3) years and for high BMI was 22·4 (3·0) years	Mature milk was collected by whole breast emptying using an electric breast pump at 10.00, 14.00 and 18.00 hours; pooled sample was used to analyse protein content and individual sample was used to analyse fat content; no pre-treatment was done before analysing for protein content by Kjeldahl, fat content by Jeejebhoy and Lactose by YSI automated enzyme method	Weight and height were measured to calculate BMI; mothers were classified as low BMI (BMI < 23 kg/m^2: *n* 21) and high BMI (BMI > 23 kg/m^2: *n* 19); maternal body circumference (measuring tape); triceps, biceps, subscapular and supercilia skin fold thickness (Lange Callipers); TBW, FFM and body fat% (deuterium dilution) were also measured	**BM fat concentration was significantly higher in high BMI group (P-value = 0·04) at 3 and 6 months postpartum and positively correlated with body fat at 3 and 6 months ($r = 0{\cdot}32$ and 0·40; $P = 0{\cdot}04$ and 0·01, respectively)**; BM protein and lactose did not show any significant association with maternal nutritional status indicators assessed in the study

Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Ruel *et al.*, 1997[63]	8	52 Guatemalan mothers with average age 25 (6) years at 1–4 months postpartum	Mature milk was collected by whole breast emptying using an electric breast pump simultaneously while feeding from one breast every 2 h (i.e. 13 times); 10 % aliquot of pooled sample was frozen and thawed; analysis for BM fat content by TAG method	Weight and height were measured to calculate BMI; mothers were not classified into categories	**Maternal weight ($\beta = 0{\cdot}04$; $P = 0{\cdot}05$) and BMI ($\beta = 0{\cdot}36$; $P < 0{\cdot}05$) were significantly associated with the fat concentration of** BM; maternal height was not significant with BM fat variation between mother
Rocquelin *et al.*, 1998[47]	8	102 Congolese mothers with average age 27·0 (6·8) years at 5 months postpartum	2–10 ml mature milk from both breast (1–5 ml from each breast) was collected between mid-morning and mid-afternoon; no pre-treatment of samples; analysis for BM fat by Folch method (quantified by gravimetric method)	Weight and height were measured to calculate BMI; participants were categorised as underweight (*n* 14); normal (*n* 66); overweight (*n* 22) based on international classification	**BM fat content was significantly different between the BMI groups (underweight 3·55 (1·56) g/100 ml *v.* normal 2·89 (1·05) g/100 ml *v.* overweight 2·17 (0·63) g/100 ml); a statistically negative correlation ($r = -0{\cdot}28$) was observed**
Marin *et al.*, 2005[64]	6	46 Argentinean mothers within age range of 16–39 years and 1–3 months postpartum	Mature foremilk was collected by hand expression after 3 min of initiating to feed the baby; samples were frozen and thawed; analysis for protein by Lowry and fat by Folch method	Maternal BMI was used to categorise participants as normal (*n* 21), overweight (*n* 16) and obese (*n* 9) based on the international classification	No association between BM protein content and BMI; **significant positive difference in BM fat content between the group normal *v.* obese (6·92 (0·43) g/100 ml *v.* 9·81 (0·94) g/100 ml *P*-value < 0·01) and overweight *v.* obese (7·15 (0·77) g/100 ml *v.* 9·81 (0·94) g/100 ml: *P*-value < 0·05)**
Nikniaz *et al.*, 2009[35]	7	182 Iranian mothers with average age 26·5 (6) years at postpartum age of 90–120 d were included from rural (*n* 91) and urban (*n*-91) area	15 ml of mature foremilk was expressed once; the sample was frozen and thawed at 38˚C; analysis of BM fat content by Gerber method	Weight and height were measured to calculate BMI; no classification of the participant was done	**Maternal BMI was positively significantly correlated with BM fat content ($\beta = 0{\cdot}28$ *P*-value = 0·02)**
Bachour *et al.*, 2012[36]	6	65 Lebanese mothers with average age 28·5 (5·9) and 29 (6) years (for smoker and non-smoker, respectively) at postpartum age of 5 d and 30 d were included	5–10 ml transition and mature foremilk was collected by hand expression once a day at day 5 and 30 postpartum; sample was frozen and thawed; analysis for BM protein content by Bradford and fat content by gravimetric method	Self-reported height and height was used to calculate BMI; mothers were categorised into normal (*n* 23), overweight (*n* 23), obese (*n* 9) based on the international classification	**BM protein content was significantly negatively associated with BMI (normal *v.* overweight; 1·52 (0·07) g/100 ml *v.* 1·29 (0·06) g/100 ml *P*-value < 0·05)** but not for (normal *v.* obese; 1·52 (0·07) g/100 ml *v.* 1·59 (0·02) g/100 ml); BM fat content was not significantly associated with maternal BMI
Quinn *et al.*, 2012[37]	6	126 Pilipino mothers from Cebu cohort aged 24–25 years; breast-feeding mothers < 18 months postpartum	10 ml mature foremilk was collected by hand expression after initiating feeding the baby; samples were frozen at –35˚C and thawed; analysis of BM protein content by combustion ($n \times 6{\cdot}38$), fat content by Rose–Gottlieb, sugar by phenol sulphuric acid method, and energy using Atwater specific factor	Weight and height were measured to calculate BMI; triceps, biceps, subscapular and supercilia skin fold thickness were measured to calculate % body fat; MUAC was measured; participants were not classified based on nutritional status	BM protein has no association with maternal BMI and body fat %; **BM fat showed significant negative relationship with maternal BMI ($\beta = -0{\cdot}073$; *P*-value < 0·1),** while no association was seen with body fat% ($\beta = -0{\cdot}012$); **BM sugar showed significant negative relationship for both BMI ($\beta = -0{\cdot}044$; *P*-value < 0·05) and body fat% ($\beta = -0{\cdot}032$; *P*-value < 0·01);** BM energy showed not association with BMI ($\beta = -0{\cdot}009$) and body fat% ($\beta = -0{\cdot}002$)

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Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Antonakou *et al.*, 2013[42]	9	46 Greek mothers with average age of 32·5 (3·1) years at postpartum age of 1 month	30 ml mature foremilk was collected using an electric breast pump at any point of the day; samples were frozen at −80˚C and thawed; analysis of BM lipid content by modified Folch method	Weight gained during pregnancy was recorded from hospital record; weight and height were measured; self-reported pre-pregnancy weight was used to calculate pre-pregnancy BMI; mothers were categorised as underweight (*n* 4), normal (*n* 50), overweight (*n* 7) and obese (*n* 3) based on international classification	No significant correlation between BM fat and maternal pre-pregnancy body weight ($r = 0·141$), maternal body weight at 1 month postpartum ($r = 0·07$) and weight gain during pregnancy ($r = 0·01$) was observed
Schueler *et al.*, 2013[52]	7	12 mothers residing in the USA with average age of 25·6 (4·5) years at postpartum age of 29–38 d; primiparous non-smoking mother from a longitudinal study measured once between fall 2009 and spring 2011	60 ml mature (fore = 30 ml + hind = 30 ml) milk was collected between 07.00 and 10.00 hours using a breast pump was collected once; analysis for BM fat content by Creamatocrit method; hind and foremilk were analysed separately	Weight and height were measured to calculate BMI; body composition was measured using dual-energy x-ray absorptiometry; self-reported pre-pregnancy weight and weight gain during pregnancy was recorded; no classification was made based on nutritional status	**Fat content of both hind and fore milk was positively correlated *r* (*P*-value) with all maternal anthropometrics: fore milk *v.* maternal fat mass 0·65 (0·022); BMI 0·65 (0·024); body weight 0·63 (0·028); and body fat% 0·55 (0·064) and hind milk *v.* maternal fat mass 0·67 (0·018); BMI 0·75 (0·005); body weight 0·59 (0·044); and body fat % 0·63 (0·029); the correlations observed still hold the significance after correcting for pre-pregnancy weight or weight gain during pregnancy**
Yang *et al.*, 2014[17]	9	436 Chinese mothers aged between 20 and 40 years at postpartum age of 30–240 d from MING study group	Mature milk was collected between 9.00–11.00 hours by whole breast emptying using either hand or breast pump; 40 ml aliquot was stored at −80˚C and thawed; analysis of BM protein, fat and lactose content was done using MIRIS human milk analyser	Weight and height were measured to calculate BMI; no classification was made based on nutritional status	**BMI was correlated positively to BM protein ($\beta = 0·007$; *P*-value = 0·037) and BM fat ($\beta = 0·054$; *P*-value = 0·019) concentration and negatively with BM lactose content ($\beta = -0·0012$; *P*-value = 0·047)**
Chang *et al.*, 2015[21]	9	2632 Korean mothers with average age 32 ± 3·3 years at postpartum age 0–8 months; data collection was done between March 2013 and July 2014; participants selected from 7 metropolitan cities	Mature milk was collected at any point of the day by whole breast emptying; 45 ml aliquot sample was stored at −20˚C and thawed at 37˚C; analysis for BM protein, fat and lactose content was done using Infrared Spectrometry (MilkoScan FT2) device	Self-reported weight and height were used to calculate BMI; no classification was made based on nutritional status; statistical analysis was adjusted for maternal age	**BM protein was positively correlated with BMI ($r = 0·247$, $\beta = 0·007$: $P < 0·001$); BM fat was positively correlated with BMI ($r = 0·06$, $\beta = 0·029$: $P < 0·01$); BM lactose was negatively correlated with BMI ($r = -0·046$, $\beta = -0·005$: $P < 0·05$); BM energy was positively correlated with BMI ($r = 0·097$, $\beta = 0·272$: *P*-value < 0·001)**
De Luca *et al.*, 2016[22]	8	100 mother residing in the USA with average age of 30·6 years for normal BMI and 30·8 years for obese at postpartum age of 1 month were included; groups were matched for pregnancy status (parity, i.e. first child *v.* second or more), ethnic origin (European descent, African descent, Asian descent) and maternal educational level; data collection was done between 22 February 2010 and 10 September 2012	Mature milk was collected between 09.00 and 11.00 hours by whole breast emptying using an electric breast pump; 2 ml aliquot sample was frozen at −80˚C and thawed; analysis of BM protein, fat and carbohydrate content was done using MIRIS human milk analyser; BM energy was calculated based on conversion factors 38·7, 16·7 and 18·4 kJ/g for fat, carbohydrate and protein, respectively	Weight and height were measured to calculate BMI; pre-pregnancy weight was derived from hospital record to calculate pre-pregnancy BMI; Participants were categorised as normal (*n* 50) and obese (*n* 50); participants in both groups were significantly different in terms of pre-pregnancy weight and BMI, and BMI at delivery and BMI at 1 month postpartum	No significant difference was observed in BM macronutrient composition between normal and obese group

Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Dias *et al.*, 2016[23]	7	63 Indian mothers with average age of 28 years at in the postnatal ward of the hospital	5 ml of foremilk was collected between 09.00 and 11.00 hours; sample was analysed for carbohydrate content by Benedict's test, protein by Biuret test, and TAG using glycerol-3-phosphate oxidase method	Pre-pregnancy weight was used to calculate BMI; participants were divided into three groups based on BMI as Group I (BMI < 18·5 kg/m^2; *n* 21), Group II (BMI 18·5–24·9 kg/m^2; *n* 21) and Group III (BMI > 25 kg/m^2; *n* 21)	**BM protein content was significantly lower in milk of mothers from group I (1·51 (0·48) g/100 ml) compared with group II (3·29 (1·27) g/100 ml; *P*-value = 0·015) and group III (2·58 (1·15) g/100 ml; *P*-value = 0·001);** BM lactose and TAG content was not significantly different between the three groups
Quinn *et al.*, 2016[24]	9	82 Tibetan mothers residing in Nepal with average age of 29·4 (7·2) years at postpartum age of 11·14 (8·11) months; participants were living in high altitude (2090–3830 m; *n* 41) rural area and low altitude (1400 m; *n* 41) urban area	8–10 ml mature foremilk 2 min after initiating to feed the baby was collected between 06.00 and 10.00 hours; 1 ml aliquot was frozen at −80°C and thawed; analysis of BM protein by combustion, fat by Rose–Gottlieb, sugar by phenol sulphuric acid method, and energy using Atwater specific factor	Maternal weight, height, MUAC and triceps, biceps, subscapular and supercilia skin fold thickness (Lange Calipers) were measured; BMI was calculated, and maternal triceps skink fold thickness was used as maternal adiposity indicator for final analysis; no classification was made based on nutritional status	BM protein was not associated with maternal triceps thickness ($\beta = 0{\cdot}002$, *P*-value > 0·05) or BMI ($\beta = 0{\cdot}013$, *P*-value > 0·05); **BM fat was significantly positively associated with maternal triceps thickness ($\beta = 0{\cdot}095$; *P*-value < 0·02)** but was not significantly associated with BMI ($\beta = 0{\cdot}113$; *P*-value < 0·089); **BM sugar content was negatively associated with maternal triceps thickness ($\beta = -0{\cdot}083$; *P*-value < 0·01)** but not associated with BMI ($\beta = -0{\cdot}022$; *P*-value = 0·051); **BM energy was significantly positively associated with maternal triceps thickness ($\beta = 0{\cdot}812$; *P*-value < 0·01)** but not with BMI ($\beta = 1{\cdot}022$; *P*-value > 0·05)
Kim *et al.*, 2017[25]	8	238 Korean mothers with average age 31·6 (3·2) years at postpartum age of 140 (44) d (range: 30–360 d); 51·2 % of the participants took dietary supplements; data collection was done between April 2013 and May 2015	150 ml of mature milk was collected by whole breast emptying at any point of the day; sample was frozen at −18°C and thawed at 37°C; analysis of BM protein, fat and lactose content was done by Infrared Spectrometry (MilkoScan FT2) device	Self-reported pre-pregnancy weight and height were used to calculate pre-pregnancy BMI; participants were not categorised based on BMI; range of BMI was 16–33 kg/m^2	BM protein (1·2 (0·2) g/100 ml) was not correlated with BMI ($r = 0{\cdot}095$ $P > 0{\cdot}05$); **BM fat (3·3 (1·3) g/100 ml) and energy (261 (50) kJ/100 ml) was positively correlated with BMI ($r = 0{\cdot}213$ and $r = 0{\cdot}232$: $P < 0{\cdot}01$)**; BM lactose (7·0 (0·4) g/100 ml) was not correlated with BMI ($r = -0{\cdot}026$ $P > 0{\cdot}05$)
Kugananthan *et al.*, 2017[26]	9	59 Australian (mainly Caucasian) mothers with average age of 33·4 (4·2) years at postpartum age 2, 5, 9 and 12 months postpartum; 21 mothers contributed milk samples at multiple times; pooled data were used for this study	10 ml of mature pre-feed foremilk (5 ml) and post-feed hind milk (5 ml) was collected between 09.30 and 11.30 hours using a hand pump; sample was frozen at −20°C and thawed; analysis of BM protein and lactose content was done by Bradford essay and by enzymatic spectrophotometric method, respectively	Weight (measured) and height (self-reported/measured) were used to calculate BMI (24·6 (5·7) kg/m^2); bioelectric impedance analysis device (ImpediMed SFB7 tetra-polar) was used to calculate %fat mass (32·3 (7·0) %); participants were not categorised based on nutritional status	No association was observed between BMI and BM protein; however, **with every 1 % increase in %fat mass, a significant (*P*-value = 0·028) increase of protein concentration by 0·016 g/100 ml is predicted, this significance hold true when adjusting for month of lactation (*P*-value = 0·036);** no significant association was observed between BM lactose content and %fat mass (*P*-value = 0·48) BM lactose and maternal BMI (*P*-value = 0·66**)**

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Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
Young *et al.*, 2017[27]	8	56 Korean mothers with average age of 31·7 (4·5) years at postpartum age 2 weeks and follow-up at 4 months; data collection was carried out between 2002 and 2005	**Transition and mature milk** were collected between 10.00 and 13.00 hours by whole breast emptying using a breast pump; sample was frozen at −80˚C and thawed; analysis of BM protein content by Bradford essay, fat by creamatocrit and lactose by enzymatic spectrophotometric method and energy by modified Atwater factor	Maternal weight and height were measured to calculate BMI; participants were categorised as normal (18·5 < BMI < 24·8 kg/m^2, *n* 33) and overweight or obese (BMI > 25 kg/m^2, *n* 23)	**For BMI and BM protein content, a significant difference observed at 2 weeks (*P*-value = 0·05)** but no significance was observed at 4 months (*P*-value = 0·28); for BMI and BM fat content no significant difference observed at 2 weeks and 4 months; **for BMI and BM lactose content, a significant negative difference observed at 4 months (*P*-value = 0·02)** but no significant difference observed at 2 weeks; for BMI and BM energy content, a no significant difference observed at 2 weeks and 4 months
Bzikowska *et al.*, 2018[28]	8	40 Polish mothers with median age of 29·5 years at postpartum age of 3–4 weeks	10–20 ml mature fore (5–10 ml) and hind milk (5–10 ml) were collected for 24 h using a breast pump or hand expression; sample was heated till 40˚C before analysing for BM protein, fat and lactose content using MIRIS human milk analyser; BM content was estimated using Atwater factor	Maternal weight and height were measured to calculate BMI; participants were categorised as normal (18·5 < BMI < 24·8 kg/m^2, *n* 30) and overweight or obese (BMI > 25 kg/m^2, *n* 10); self-reported pre-pregnancy weight was recorded to calculate pre-pregnancy BMI	For BM protein content, no significant difference was observed for both current (r = 0·27; *P*-value = 0·09) and pre-pregnancy (r = 0·24; *P*-value = 0·16) BMI; **for BM fat content, positive significant difference was observed for both current (r = 0·37; *P*-value = 0·02) and pre-pregnancy (r = 0·30; *P*-value = 0·01) BMI;** for BM carbohydrate content, no significant difference was observed for both current (r = 0·02; *P*-value = 0·91) and pre-pregnancy (r = 0·03; *P*-value = 0·87) BMI; **For BM energy, positive significant difference was observed for both current (r = 0·39; *P*-value = 0·01) and pre-pregnancy (r = 0·33; *P*-value = 0·04) BMI**
Bzikowska-Jura et al., 2018[29]	8	40 Polish mothers with average age 31·1 (4·4) years at postpartum age 1 month; participants were followed, and measurements were taken at 3 (*n* 22) and 6 (*n* 15) months postpartum	10–20 ml mature fore (5–10 ml) and hind milk (5–10 ml) were collected for 24 h using a breast pump or hand expression; sample was frozen at −20˚C and thawed at 40˚C; analysis of BM protein, fat and lactose content using MIRIS human milk analyser; BM energy content was estimated using Atwater factor	Weight and height were measured to calculate BMI; total body water, fat mass, fat-free mass and muscle (kg) were measured using BIA device (Maltron BioScan 920-II multifrequency bioelectrical impedance analyser); participants were not categorised based on nutritional status	**For women in the third month postpartum, moderate to strong significant correlations (*r* ranging from 0·47 to 0·64) between total BM protein content and the majority of body composition measures as follows were observed: positive correlations: % fat mass (r = 0·60; *P* = 0·003), fat-free mass expressed in kg (r = 0·63; *P* = 0·001), and muscle mass (r = 0·47; *P* = 0·027); and negative correlation: % total body water (r = −0·60; *P* = 0·003)**; total protein content was not significantly correlated with any of the maternal nutritional status variables at 1 and 6 month postpartum; **the variance in milk fat content was**

Table 2. (*Continued*)

Author year	QAS	Study and participant characteristics	Milk characteristic (including analysis method)	Maternal nutritional status	Findings (including analysis method)
					related to the BMI, with a significant positive correlation in the first month postpartum ($r = 0{\cdot}33$; $P = 0{\cdot}048$) and with maternal weight at 6 month postpartum ($r = 0{\cdot}49$; $P < 0{\cdot}05$); no other significant association was observed; carbohydrate content of BM was not significantly correlated with any of the maternal nutritional status variables at 1, 3 and 6 month postpartum; **energy content of BM was significantly positively correlated with maternal weight and BMI at postpartum 1 and 3 months; with fat mass% at postpartum 1 month; significantly negatively with total body water% at 3 month postpartum**; BM energy content was not significantly correlated with other maternal nutritional status variables
Hahn *et al.*, 2018[18]	8	80 Korean mothers with age range of 20–39 years at 4 weeks postpartum	Mature fore and hind milk were collected between 14.00 and 15.00 hours; sample was frozen at −20˚C and thawed at 45˚C; analysis for BM protein, fat, lactose and energy was done using MIRIS human milk analyser	BMI was collected, and mother was classified into four groups (20 participants in each) based on BMI and age as follows: Normal BMI 20 s; Overweight BMI 20 s; Normal BMI 30 s; Overweight BMI 30 s	**For BM protein, a positive significant difference was observed between BMI groups (P-value = 0·03)** but not with interaction of BMI and age (P-value = 0·51); **for BM fat, lactose and energy positive significant difference was observed with interaction of age and BMI (P-value = 0·001, 0·045 and 0·001)** but no significant difference was observed between BMI groups (P-value = 0·620, 0·32 and 0·657)
RCTS					
Park *et al.*, 1999[53]	10	16 American mothers were enrolled in a crossover deign randomised control trial for 3 weeks (week 1 baseline, week 2 and week 3 intervention); mean age 32 (2) years and 1–26 months postpartum (blocked for <12 months and >12 months postpartum for analysis purpose)	Mature milk was collected by whole breast emptying using an electric breast pump simultaneously while feeding from one breast between 15.00 and 22.00 hours once a day on days 3 and 7 of each intervention period; samples were frozen at −80˚C and thawed; analysis for fat content using solvent extraction method	Weight and self-reported height were recorded at baseline and measured every week during the intervention (low and high dairy product) in 11 participants (self-reported height and weight were used for 5 participants); BMI was calculated	**BMI was related positively to milk lipid concentration during all periods; reported regression coefficient of BM fat content in high dairy intervention $\beta = 5{\cdot}55$ (2·30) *v.* low dairy intervention $\beta = 5{\cdot}31$ (2·02)**

BIA, bioelectrical impedance analysis; FFM, fat-free mass; IBW, ideal body weight; MIRIS, human milk analyser; MUAC, mid upper arm circumference; TBW, total body water; TCSF, triceps skin fold.
Significant differences are in bold.

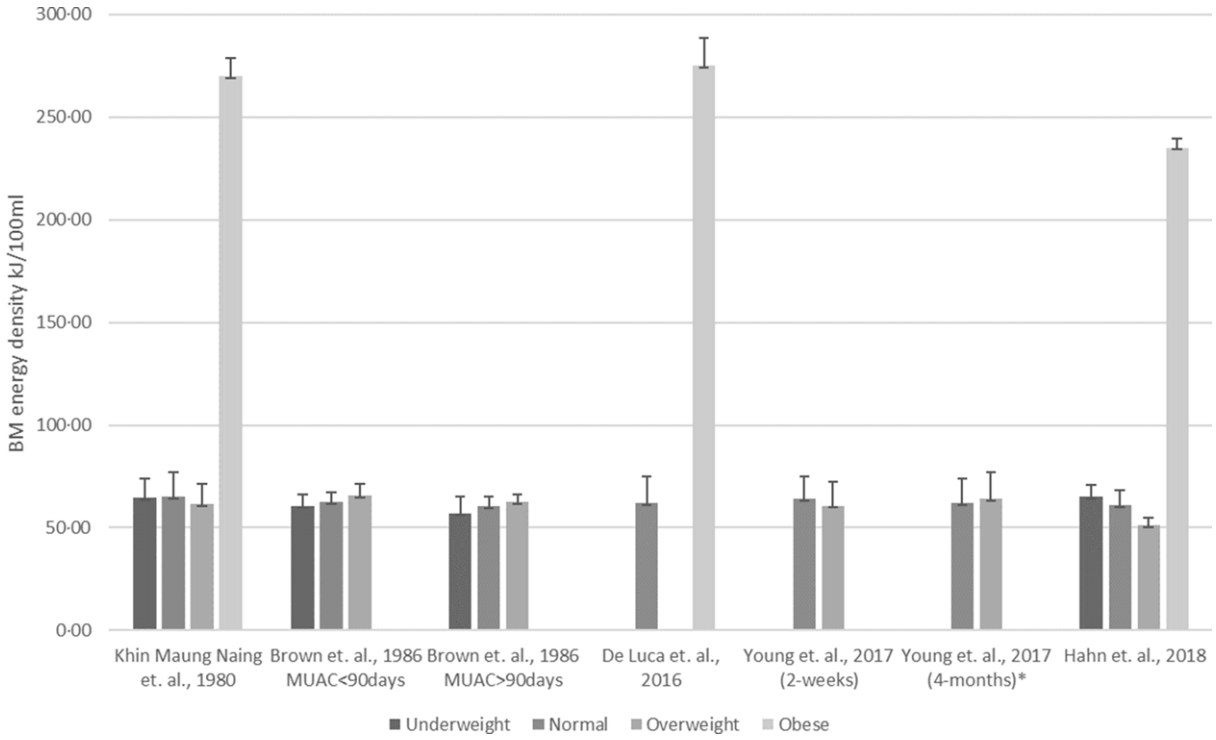

Fig. 2. Association between maternal nutrition status with breast milk energy content. ■, Underweight; ■, Normal; ■, Overweight; ■, Obese. Studies followed with an asterisk (*) reported a significant association

and *n* 40, $r = 0{\cdot}33$, *P*-value < 0·04[28]. On the other hand, no significant associations between current maternal nutritional status and BM energy content were reported in observational studies by Quinn *et al.*, *n* 216, $\beta = -0.04$ (kJ/g per BMI unit), *P*-value > 0·05[37], and by De Luca *et al.*, *n* 100, Δ (normal-obese BMI) = 16 kJ/100 ml milk, *P*-value = 0·33[22]. Mixed results were reported within one publication where fat mass was positively significantly associated with BM energy density ($\beta = 0{\cdot}81$, *P*-value < 0·01), while no significance was observed for maternal BMI and BM energy density ($\beta = 1{\cdot}02$, *P*-value > 0·05)[24].

Breast milk protein content

Methods used to determine protein content of the collected human BM samples by the included studies are: Kjeldahl (*n* 14), Colorimetry (*n* 1), Lowry (*n* 3), Bradford (*n* 3), Milkoscan (*n* 4), MIRIS (*n* 7), combustion (*n* 1), assays (*n* 2) and Infrared (*n* 1).

Range of mean BM protein concentration based on the studies included is 1·3 (0·3) g/100 ml with a range of 0·8 g/100 ml to 3·3 g/100 ml. Out of seventeen studies exploring the association of maternal dietary intake with BM protein composition, 6 (35 %) showed significant association with at least one macronutrient consumed by the mother[17,39,44,50,54,66], while 11 (65 %) did not show significant findings[13,14,25,29–31,38,48,49,51,65]. Significant association of maternal protein intake with the BM composition of protein was reported by Wurtman *et al.* (*n* 20, *P*-value < 0·01) and Forsum *et al.* (*n* 3, Δ(high protein-low protein diet) = 0·1 g/100 ml *P*-value < 0·01)[44,54]. Interestingly, there was an immediate postprandial effect of maternal protein consumption among Taiwanese mothers (*n* 23) showing a significant increase in BM protein content one and a half hour after consumption of both alcoholic (+1·4 mg/100 ml) and non-alcoholic (+1·2 mg/100 ml) soup[66]. An intervention study (*n* 60) conducted among marginally undernourished mother concluded that BM protein content was significantly higher in group supplemented with protein compared with group supplemented with fat and the control group[39].

Studies investigating relation between maternal nutritional status and BM protein content delivered mixed results as well: eight studies (42 %) showed significant associations[17,18,21,23,26,27,29,35,50,65], while eleven studies (58 %) did not show any significant findings[22,24,25,28,36,40,45,60,61,63,66]. An overview of the available result is presented in Fig. 3. A study conducted in Poland (*n* 40) reported significant positive association of maternal nutritional status with BM protein content at 3 months postpartum, but not at 1 or 6 months postpartum[29]. Pre-pregnancy nutritional status (BMI) was also significantly associated with BM protein composition in urban Chinese mothers (*n* 436)[17], but no such associations were observed in South Korean mothers (*n* 238)[25]. Mixed results were also reported for association between BM protein composition and maternal nutritional status (measured in terms of % fat mass and BMI, respectively) with significant positive associations reported by Hahn *et al.* (*n* 80) and Kugananthan *et al.* (*n* 59)[18,26], while Barbosa *et al.* (*n* 40) reported no such associations[62]. Other studies reported that BM protein composition was similar in mothers with normal BMI and obese mothers[22,36], while it was significantly lower in overweight mothers[36]. Similarly, a positive significant association was reported between

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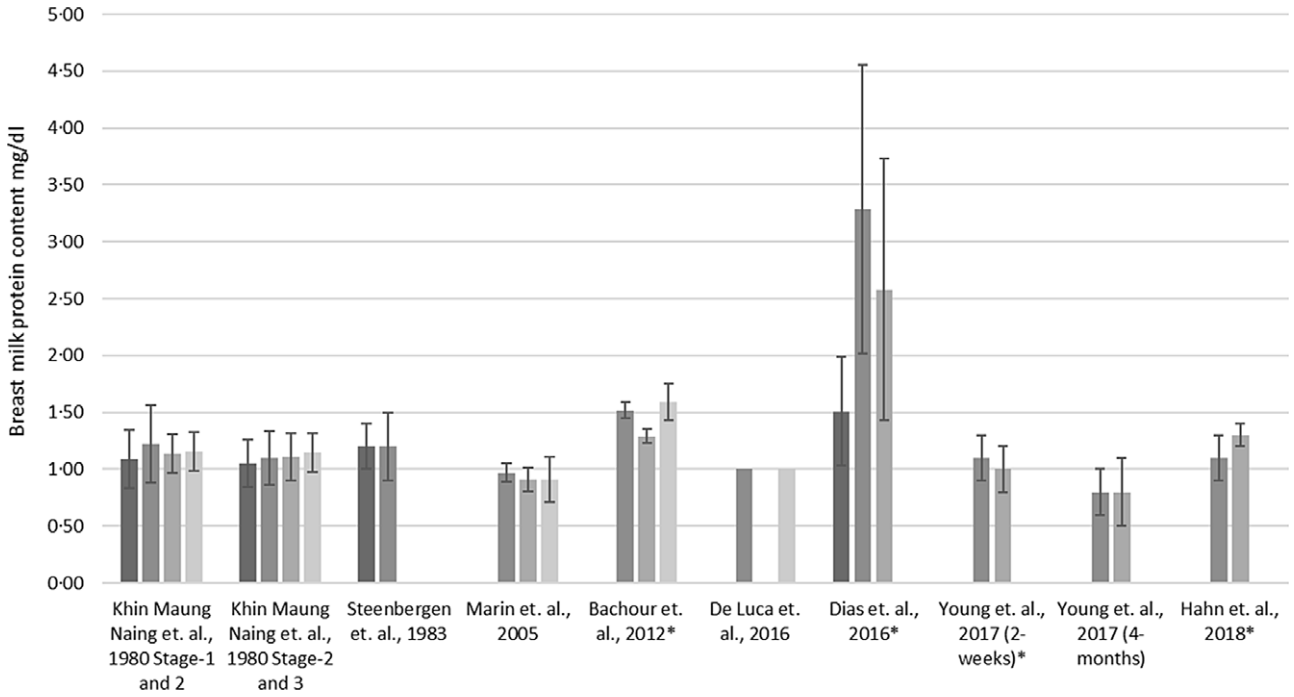

Fig. 3. Association of maternal nutritional status with breast milk (BM) protein content. ■, Underweight; ■, Normal; ■, Overweight; ■, Obese. Studies followed with an asterisk (*) reported a significant association

maternal fat mass and BM composition of protein[26,29] at 3 months postpartum, while no significant associations were observed at 1 and 6 months postpartum[29]. Although not all associations were statistically significant, protein composition of BM of undernourished mothers was reported to be lower than normally nourished mothers by all the studies included in this review[36,61,65]. In an intervention-based study (n 133) by Cisse *et al.*, diets of undernourished mothers were supplemented with millet or maize and compared with a placebo group with no supplement. After 60 d, authors reported significant positive change in maternal fat-free mass, while no change was observed in maternal fat mass. This increase in fat-free mass translated into a significantly higher BM protein and lactose content at 3 months postpartum age among the supplementation group compared with placebo group[50]. An observational study conducted by Brown *et al.* (n 35) on marginally nourished mothers reported a significant association between maternal weight gain at postpartum age of 3 months with BM nitrogen content. Mothers gaining <0·2 kg weight had significantly ($t = 2{\cdot}64$, P-value < 0·02) lower BM nitrogen (0·85 g/100 ml for mothers with initial weight < 39 kg and 1·02 g/100 ml for mothers with initial weight ≥ 39 kg) compared with mothers gaining more than 0·2 kg weight (1·09 g/100 ml for mothers with initial weight < 39 kg and 1·12 g/100 ml for mothers with initial weight ≥ 39 kg)[34].

Breast milk fat content

Methods used to determine human BM fat content of sample collected in the included studies were Rose and Gottieb (n 5), Crematocrit (n 4), Gravimetric (n 4), Colorimetry (n 1), Soxhlet (n 1), Milkoscan (n 4), MIRIS (n 7), Folch (n 9), Jeejebhoy (n 2), Infrared (n 1), Assays (n 1), TAG (n 1), solvent extraction (n 3), Gerber (n 2) and Chiba (n 1) method.

BM fat content is the macronutrient most frequently studied and most frequently reported to be influenced by maternal factors. Range of mean BM fat concentration based on the studies included in this study is 3·6 (0·9) g/100 ml with a range of 2·1–9·8 g/100 ml. Out of twenty-five studies that explored the association of maternal dietary intake with BM fat composition, 8 (32 %) showed significant association with at least one of the maternal macronutrient intakes[14,25,31,35,39,51,53,66], while 17 (68 %) did not show significant findings[17,29,30,32,38,42,43,45,48,50,54–59,61]. Two studies observed significant association between maternal intake of carbohydrate (n 182, $\beta = 0{\cdot}39$, P-value < 0·01)[35] and protein (n 73, $r = 0{\cdot}49$, P-value < 0·01)[51] with BM fat content, while no associations were observed for maternal fat intake with BM fat content[35,51]. Conversely, an observational study conducted in Korea (n 238) did not report a significant correlation ($r = 0{\cdot}21$, P-value < 0·01) between maternal fat intake and BM fat content[25]. Moreover, both intervention studies investigating the impact of a high-fat *v.* low-fat maternal diet with BM fat content showed a higher concentration of BM fat content in the high-fat diet group with the following significant differences (n 7, $\Delta = 0{\cdot}5$ g/100 ml, $P < 0{\cdot}05$)[14] and (n 16, $\Delta = 7{\cdot}3$ mg/g, $P < 0{\cdot}05$)[53] between the comparison groups.

Twenty-six studies included in this study explored the potential association between nutritional status and BM fat content of which 19 (73 %) showed significant associations[17,18,21,24,25,28,29,34,35,37,41,47,51–53,61–64], while 7 (27 %) did not[22,27,35,41,45,59,66]. An overview of the available result is

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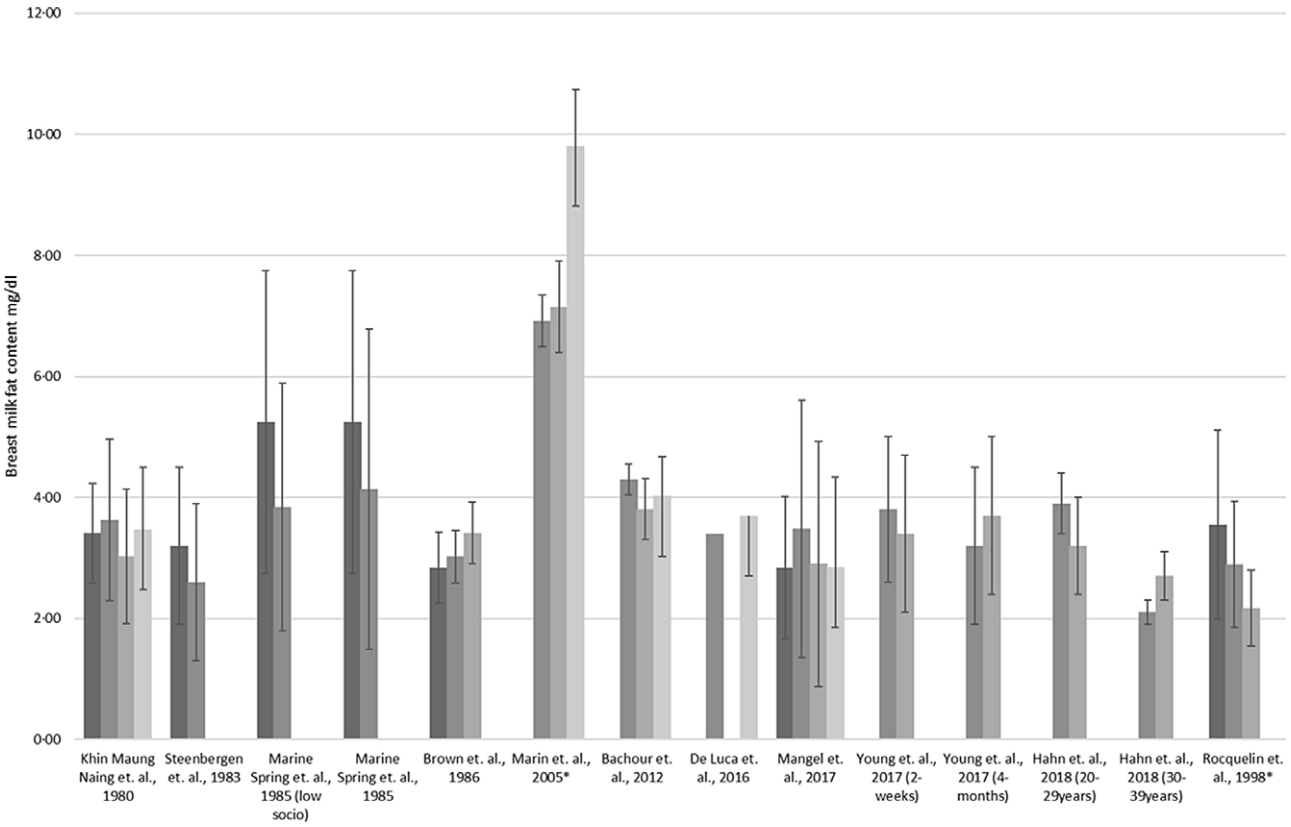

Fig. 4. Association between maternal nutrition status with breast milk composition of fat. ■, Underweight; ■, Normal; ■, Overweight; ■, Obese. Studies followed with an asterisk (*) reported a significant association

presented in Fig. 4. Pre-pregnancy maternal nutrition status was shown to be significantly associated with BM fat composition by two observational studies[25,28], while an observational study reported no such association[41]. Majority of studies (observational) reported positive significant[17,21,28,52] associations between current maternal nutritional status and BM fat content, whereas two observational studies did not show associations[22,42]. A longitudinal study conducted in the USA (n 12) reported that both fore (BMI: $r = 0{\cdot}65$, P-value = 0·024, % body fat: $r = 0{\cdot}55$, P-value = 0·064, weight: $r = 0{\cdot}63$, P-value = 0·028 and fat mass: $r = 0{,}67$, P-value = 0·018), and hind milk (BMI: $r = 0{\cdot}75$, P-value = 0·005, % body fat: $r = 0{\cdot}63$, P-value = 0·029, weight: $r = 0{\cdot}59$, P-value = 0·044, and fat mass: $r = 0{\cdot}67$, P-value = 0·018) were significantly positively correlated with maternal nutritional factors, which persisted after correction for maternal pre-pregnancy BMI[52]. This association is also supported by Iranian and Filipino studies[35,37], but not by various other studies included in this review[35,41].

Breast milk carbohydrate content

Methods used to determine BM carbohydrate content of the collected samples of the studies included are: Modified Folin (n 2), Colorimetry (n 2), Phenol-sulphuric acid (n 1), Milkoscan (n 4), MIRIS (n 7), Assays (n 1), Infrared (n 1), HPLC (n 1), Difference (n 1) and YIS enzymatic (n 7) method.

Range of mean BM carbohydrate concentration based on the studies included in this review is 6·7 (0·7) g/100 ml. Out of twelve studies that investigated the association of maternal dietary intake with BM carbohydrate composition, 5 (42 %) showed significant associations with the intake of at least one maternal macronutrient[17,31,48,50,66], while 7 (58 %) did not show significant findings[14,25,29,30,38,51,61]. Maternal dietary intake was reported to be positively associated with BM carbohydrate (some measured in terms of lactose) content for maternal protein intake[31] all nutrient intake[48] and only fat intake[17]. Other studies reported no significant associations[25,29,30,38,61]. An intervention study (n 7) comparing a normal carbohydrate (60 % of total energy) $v.$ a low-carbohydrate (30 % of total energy) diet among healthy American mothers showed no significant difference when comparing BM carbohydrate content of the two intervention groups[14]. Supplementing 113 undernourished Senegalese mothers with additional 1766 kJ (n 41) or 1674 kJ (n 35) $v.$ no supplementation (n 57) did show significant different BM lactose composition post-treatment among supplemented mothers[50]. In a study conducted among Taiwanese mothers (n 23), consumption of an alcoholic soup providing 120 kJ/100 ml energy had a significant negative effect on BM lactose composition. No such effect was observed when the mothers consumed a non-alcoholic soup providing 95 kJ/100 ml energy[66].

Nineteen studies investigated the potential association between nutritional status and BM carbohydrate content of which 7 (37 %) showed significant associations[17,18,21,24,27,34,37], while 12 (63 %) did not show significant findings[22,23,25,26,28,29,40,45,50,60,61,66]. An overview of the

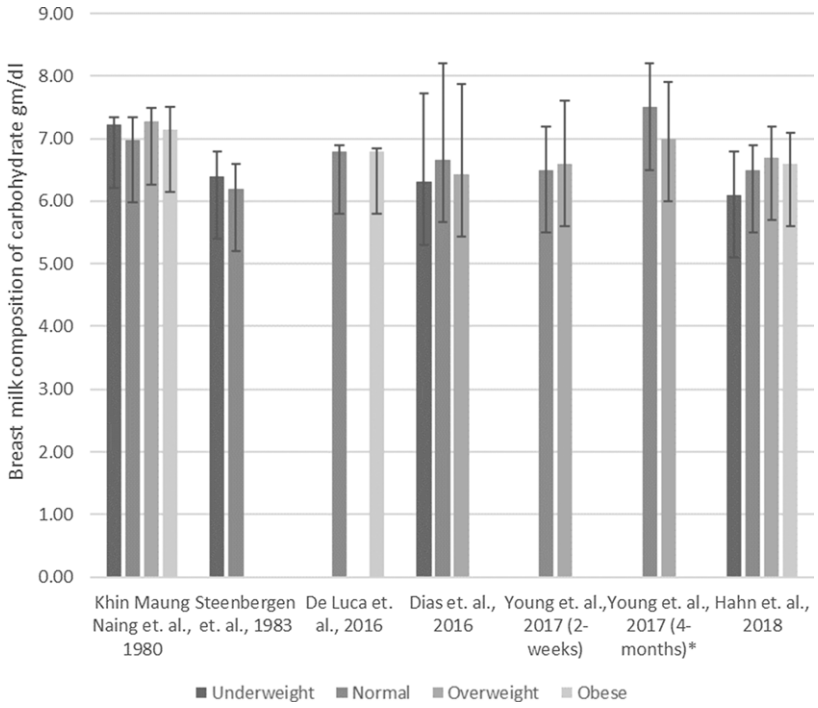

Fig. 5. Association between maternal nutrition status with breast milk composition of carbohydrate. ■, Underweight; ■, Normal; ■, Overweight; ■, Obese. Studies followed with an asterisk (*) reported a significant association

available result is presented in Fig. 5. Majority of studies reported that pre-pregnancy nutritional status[23,25,28] and current maternal nutrition status[28] were not significantly associated with BM composition of carbohydrate. This diversity in results can be illustrated by two of the identified studies. One of the observational studies (n 436) showed a significant negative association (n 436, $\beta = -0{\cdot}0012$, P-value $= 0{\cdot}047$) between pre-pregnancy maternal nutrition status and BM carbohydrate[17], while the other observational study (n 60) showed a positive significant association (n 60, $\beta = 2{\cdot}20$, P-value $< 0{\cdot}01$) of nutritional status during lactation with BM carbohydrate composition[34]. The association of maternal nutritional status and BM carbohydrate content was inconsistent along with the longitudinal variation of BM composition[17,34]. Another study (n 56) comparing Korean mothers with normal BMI with mothers with BMI higher than 25 kg/m^2 reported a non-significant positive difference observed at 2 weeks (Δ(BMI > 25 kg/m^2–Normal BMI) $= 0{\cdot}1$ g/100 ml, P-value $= 0{\cdot}46$), while a significant negative difference (Δ(BMI > 25 kg/m^2–Normal BMI) $= -0{\cdot}5$ g/100 ml, P-value $= 0{\cdot}02$) was observed at 4 months[27].

Discussion

Various reviews summarised available data on maternal dietary intake and BM composition[5,9,67]. However, an overview linking maternal nutritional status and BM composition is still lacking. We summarised current evidence on the potential relation between maternal nutrition status, nutrient intake and human BM composition. Evaluation of data from fifty human observational studies and trials among 'disease-free' mothers and their term-delivered children showed that literature is diversified, both in terms of methodology and results. The diversified results may relate to variability in sample size, participant characteristics, sample collection methods, statistical approach, markers for nutritional status used as well as various sources of bias related to dietary intake assessment such as response bias and reactivity bias. Future well-designed studies considering these aspects are needed to strengthen the evidence on the link between maternal nutritional status, dietary intake and BM composition. The potential role of above listed co-factors will be discussed in more detail below.

In terms of study design, sample sizes for the studies included in this review ranged from three participants to 2632 participants; twenty-three of fifty studies were conducted among < 50 participants and eight studies included < 20 participants. Small sample sizes may limit statistical power to detect associations if actually present, and as such lead to accepting a null hypothesis that is actually not true. However, so far twenty-one studies were conducted with less than fifty participants of which nine did not show any association, whereas of the twenty-nine studies with more than fifty participants, seven did not show any association. Furthermore, while designing the study, many studies did not account for potential relevant covariates such as maternal age[5,68] or postpartum age[69,70] of the participants. More specifically, only one study adjusted for maternal age[21], one stratified data according to age group[18] and eight studies included both mothers with postpartum age of ≥6 months and <6 months[21,24,25,30,37,53,54,59]. Data indicate that protein composition of BM produced at 10/12 weeks postpartum is 0·3 g/100 ml higher than BM produced 3/4 weeks

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postpartum[70]. As such, insufficiently accounting for postpartum age increases variation in BM macronutrients concentrations independent of maternal dietary intake, which may attenuate the associations under study. Moreover, a 24-h BM collection is considered the most representative technique to collect BM samples in order to account for circadian variation[75]. However, only eight studies conducted a 24-h BM collection[76]. Accordingly, whereas compositional differences have also been shown for fore and hind milk[8], fifteen studies used either foremilk or hind milk. Besides, definitions used for foremilk and hind milk differed across studies.

In terms of methodology, although short-term and long-term freezing of BM milk alters BM composition[77,78], milk samples were frozen before analysis in more than half of the included studies. Additionally, a diverse set of tools to assess maternal dietary intake were used, ranging from either weighed or estimated food records for 1 d or more days, recalls for 1 d or more days, to FFQ. Whereas 1- or 2-d food records and recalls usually represent actual intake, food records and recalls assessed at more days as well as FFQ are usually administered to assess habitual intake. In addition, besides the more technical aspects, maternal weight status is a more physiological aspect that deserves some discussion. BM composition of normal-weight mothers – based on BMI – has been shown to significantly differ compared with BM composition of overweight mothers but not with obese mothers[36]. Most of the studies did not classify mothers into overweight and obese. Barbosa *et al.* showed difference in BM composition of fat in mothers classified as lower BMI group (BMI < 23 kg/m^2) and higher BMI group (BMI ≥ 23 kg/m^2)[62]. In addition, this review was conducted without date restriction, which resulted in the inclusion of studies published from 1963 till 2018. Consequently, technological innovations may also have contributed to heterogeneity across studies. To illustrate, in terms of maternal nutritional status classification, the applied methods ranged from weight-for-height, skin fold thickness, BMI to using bioelectric impedance analysis to measure body fat content[71,72]. Although BMI and MUAC were the most frequently used methods, more recent methods such as bioelectric impedance analysis have been suggested to be more accurate to predict fat mass and fat-free mass[73]. It is also important to consider potential differences depending on the weight status of the mothers. This should be accounted for while studying BM composition with maternal nutritional status. Likewise, methods used to measure BM macronutrients composition varied across studies as well, whereas the human milk analyser was more frequently used in recent studies. Although the human milk analyser tends to underestimate fat content by 12 % as compared with the Rose–Gottlieb method, no significant differences in protein and lactose estimates have been observed as compared with the Kjeldahl and high-performance anion exchange chromatography-pulsed amperometric detection method[74]. However, it should be considered that human milk analysers are considerably rapid tools that can measure with smaller BM sample and have fairly reliable association with the standard methods of macronutrient measurement. Therefore, analytical methods, techniques used to assess nutritional status, circadian variation, use of fore or hind milk, sample handling, applied dietary assessment methodology as well as maternal weight status can be considered additional factors adding to the heterogeneity across studies. It may be clear, that the results of this review need to be evaluated in light of the above discussed methodological issues.

When focusing on the results of this review in more detail, 67 % of the studies on maternal dietary intake and 54 % studies on nutritional status showed significant associations with BM energy content, which again highlights the variability of current evidence. All studies, except one[29], that showed significant associations with BM energy content also showed associations with at least one BM macronutrient, particularly BM fat content[14,32,53]. Interestingly, some of the studies that did not show associations between maternal dietary intake and BM fat content were conducted using frozen milk samples[29,38,42,43,54,55]. Various studies, though definitely not all, also observed associations between maternal dietary intake and nutritional status, and BM protein intake. In addition to the more generic methodological factors as listed above, variability in BM protein concentration may be attributed to the type of measurement, for example, true protein measurement against calculated protein content using nitrogen estimates[70]. The inaccuracies in protein quantification can be attributed to protein and non-protein nitrogen determination, indirect conversion to protein content or interference from other chemical substances. While amino acid analysis, due to lack of interfering substances, can quantify protein content more accurately[79]. BM carbohydrate composition seemed to be most stable across studies, which is in line with current literature even during the course of lactation[80]. Though, variability in BM carbohydrate composition has been reported based on the quantity of milk produced, that is, showing higher BM lactose concentrations among mothers producing higher milk quantities[51]. Another factor worth mentioning is that in some cases, BM carbohydrate content is measured and referred to as lactose content. Although total BM carbohydrate mainly consists of lactose, it also consists of other oligosaccharides and as such total BM carbohydrate and lactose concentrations cannot be considered completely comparable measures[12].

Given the diversified methodology and results of current literature, future well-powered studies using the most optimal standardised approaches to further elucidate the nature and strength of the association between maternal dietary intake and nutritional status, and BM composition are clearly needed. In terms of maternal dietary intake, it is key to clearly define the parameter(s) of interest, identify whether the (SE) parameter(s) warrant assessment of actual or habitual diet or both and subsequently select the most suitable assessment tool, which may be a self-report tool – retrospective or real-time assessment[81] – but could also be an objective nutritional biomarker, or a combination[82]. A bioelectric impedance analysis uses population-specific equation to calculate body compositions and comes with limitations such as high vulnerability to hydration status of the participant and measurement errors. A dual-energy x-ray absorptiometry scan provides a more objective and accurate measure of lean body mass and fat mass within a relatively short time, but it should also be emphasised that dual-energy x-ray absorptiometry is financially and logistically more challenging as well as more burdensome for the participant[83,84].

In terms of nutritional status assessment by anthropometric assessments, a combination of measures would be ideal as related to potential measurement error. In terms of quantifying BM composition, collection protocols should ideally strive for a 24 h sample collection, that is, sample collection during each feeding, to account for circadian variation. Moreover, the sample should contain a balanced mixture of fore and hind milk, which can be done by either collecting equal amount of fore and hind milk or by collecting an aliquot from a whole breast emptying. In terms of study design, research should aim for minimal longitudinal variations related to postpartum age by targeting on a narrower inclusion window with respect to postpartum age (e.g. 1–3 months *v.* 3–6 months *v.* ≥ 6 months). Moreover, although logistically challenging, freezing of BM should ideally be avoided[77,78]. If deviations from the proposed approach are unavoidable, they should be properly accounted for, for example, by means of statistical approaches.

A limitation of this review is that the large variation in assessment methods for maternal dietary intake, maternal nutritional status and BM composition; limited sample sizes; as well as the often insufficient consideration of potentially confounding factors resulted in diversified findings across studies, which inhibited us to draw strong conclusions about the associations under study. Another aspect that needs to be noted is that we summarised available evidence on the associations between maternal diet and nutritional status, and BM macronutrient composition. As such, this review does not account potential influences of the maternal exposures on BM micronutrient composition. Strengths of this review include the systematic approach, duplicate screening of references, broad inclusion range in terms of publication year and the included quality assessment.

Conclusion

To conclude, it is widely acknowledged that BM is the best food for infants[1]. Still, given the fact that the global prevalence of child malnutrition (stunting) in the year 2019 is 21·3 %[85], research on maternal determinants of BM composition is crucial and may eventually help to improve nutritional status of infant[86]. This review provides an overview of the studies that reported on associations between maternal dietary intake, maternal nutritional status and BM composition of macronutrients and highlights the diversified methodological approaches as well as associated results. Future studies investigating association of maternal nutrition and BM composition should carefully define (postpartum) age ranges and apply standardised milk collection procedures, storage and analysis techniques. Moreover, attention should be given to ensure the most optimal statistical approach, including adequate adjustment for covariates.

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Supplementary material

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