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Vegan or vegetarian diet and breast milk composition – a systematic review

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ABSTRACT

It is known that nutritional composition of breast milk is, to a certain extent, related to maternal diet. The question of nutritional adequacy of mothers' milk is often raised whenever a vegetarian or vegan diet during the lactation process is concerned. For this reason, in some countries, the recruitment of vegan lactating women as milk donors is excluded by milk banks. The purpose of this systematic review is to summarize existing knowledge on variability of specific nutrients in breastmilk of mothers adhering to a plant-based diet. The databases, including MEDLINE (Pubmed) and Scopus, were used to identify relevant publications. Data extraction and analysis were conducted following a PRISMA protocol. Thirteen publications concerning the impact of dietary pattern and levels of animal-origin food intake on breast milk composition were included. The systematic review has shown that all non-vegetarian, vegetarian and vegan mothers produce breast milk of comparable nutritional value. Several differences are primarily attributed to fatty acids and some micro-components, primarily vitamin B12. Regardless of dietary choices, nourishment and adequate nutrition have a significant impact on human milk composition – on the basis of the current evidence, vegetarian and vegan mothers are capable of producing nutritionally valuable milk for their infants, as far as the appropriate supplementation compensating for breast-feeding mother's nutritional requirements is provided. Dietary choices should not be a permanent exclusion criterion for donor candidates in human milk banks.

KEYWORDS

Lactation; human milk; nutrients; plant-based diet; milk banks

Introduction

Breast milk is the basis of infants nutrition. According to current knowledge, human milk consists not only of nutrients, but immunologically active compounds, which provides both short- and long-term health benefits for infants (Agostoni et al. 2009). Therefore, the World Health Organization (WHO) recommends exclusive breastfeeding for 6 months of life along with its continuation during the introduction of complementary foods, up to 2 years or longer (World Health Organization 2017). The American Academy of Pediatrics (AAP) adopted a similar approach, recommending exclusive breastfeeding for 6 months of life and its continuation until 1 year along with the weaning period, in accordance with the needs of the mother and the child (American Academy of Pediatrics 2012).

As far as the nutritional adequacy of mother's milk is concerned, breast milk is usually sufficient to support infant growth and its composition is believed to be remarkably unaltered in case of limited energy and nutrient intake in mother's diet. However, persistent dietary restrictions during the lactation period can result in depletion of maternal body reserves and negatively affect both breast milk volume and its content of specific nutrients (Institute of Medicine 1991). In addition, some of these critical nutrient concentrations are potentially of significant importance for child's growth and development in their first months of life – proteins

providing amino acids, polyunsaturated fatty acids (with impact on omega-3 fatty acids), vitamin D, vitamins of group B (especially B12), iron and iodine (Kleinman 2009). Thus, nutritional counseling for breastfeeding mothers should include careful examination of maternal dietary habits.

The issue of adequate nutrient intake is often raised in the aspect of following vegetarian, especially vegan, diet during the lactation period. The vegetarians' eating patterns may vary considerably, depending on the extent to which animal products are excluded. First of all, vegetarians do not consume any kind of meat – both red and white, and fish as well. The most common types of vegetarianism include: lacto-ovo-vegetarianism – eliminates meat and fish but allows consumption of eggs and dairy products; lacto-vegetarianism – allows dairy products, but eliminates eggs; ovo-vegetarianism – allows eggs but eliminates dairy products; veganism – exclude consumption of any animal products, vegans do not use honey or beeswax, gelatin and other animal-derived ingredients (Melina, Craig, and Levin 2016; Craig and Mangels 2009). The more restrictive the vegetarian diet, the risk of dietary inadequacy is greater. Nutrition specialists state that vegetarian, including vegan, diets can provide sustainable development and cover all dietary needs; however, they warn about the risk of nutritional deficiencies and their consequences. Generally, it is assumed that a vegan diet should meet specific, higher than average,

nutritional requirements while breastfeeding, with a particular emphasis on bioavailability and intake of micronutrients – for instance: vitamin B12, calcium, selenium, zinc, iodine and omega-3 fatty acids including docosahexaenoic acid (DHA) (Federal Commission for Nutrition (FCN) 2018). Vegan diets can be adequate for people at all ages, including pregnant or lactating women, provided that meal plans are well-balanced or sufficient supplementation is provided (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Federal Commission for Nutrition (FCN) 2018; Agnoli et al. 2017).

It is already known that not only the nutritional status of the mother appears to influence the composition of breast milk, but also her dietary habits – the issue of maternal diet and content of particular nutrients in human milk have been already analyzed (Bravi et al. 2016; Andreas, Kampmann, and Mehring Le-Doare 2015; Emmett and Rogers 1997; Ballard and Morrow 2013; Lönnerdal 1986; Innis 2014). According to the American Academy of Nutrition and Dietetics, breast milk of vegan mothers is similar in its composition to that of non-vegetarians, except for fatty acids concentration, and an appropriately planned plant-based diet can contribute to sustainable growth of the infants (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Mangels and Messina 2001). On the contrary, the Swiss Federal Commission for Nutrition (FCN) (Federal Commission for Nutrition 2018) and the German Nutrition Society (DGE) (Richter et al. 2016) state that a vegan diet cannot be recommended for general population, particularly not for pregnant and lactating women, infants, children and elderly people. Both organizations emphasize the importance of supplementation and nutrition counseling in aforementioned groups. Comparably, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Fewtrell et al. 2017) draws attention to the risk of nutrient deficiencies in infants of vegan mothers, as the intense process of growth and development, especially in the first year of life when milk, optimally breast milk, is a basis of nutrition, requires adequate supply of nutrients (Fewtrell et al. 2017; Van Winckel et al. 2011). To ensure a proper maintenance of nervous system, the regular supplementation of vitamin B12 is strongly recommended in both vegan mothers and their infants, even if the mother do not present any symptoms of deficiency. It might be also advised in lacto-vegetarians, as well as other groups, whenever the dietary intake of reliable sources of cobalamin is low (Fewtrell et al. 2017; Van Winckel et al. 2011; Van Winckel 2017).

On the basis of the aforementioned stance, most of worldwide milk banks recruit vegan mothers as donors, on condition that they regularly supplement vitamin B12 as well as other micronutrients if necessary. According to European Milk Bank Association (EMBA) Guidelines, mothers who follow a vegan diet without supplementation with Vitamin B12 should not donate their milk, but there is no recommendation to disqualify all vegan mothers without any other premise apart from the way of nutrition (Weaver et al. 2019). The similar position was supported by PATH

organization (PATH 2019), as well as adopted by Human Milk Banking Association of South Africa (HMBASA) (HMBASA Milk Banks Guidelines 2014). Vegan donors might be recruited conditionally – provided the mother is in good health condition, the diet is well-composed according to the medical and dietary advice, and the supplementation of an additional dose of vitamins and other micronutrients is regular. Nevertheless, national guidelines might vary in particular countries, ex. human milk banks in Switzerland, Germany and Austria do not accept donations from vegans (The European Foundation for the Care of Newborn Infants (EFCNI) 2018; Frischknecht et al. 2010). Similarly, in Poland, vegan diet is an absolute and constant exclusion criterion for breast milk donation – on the basis of official recommendations for human milk banks, breast milk of vegan mothers is not considered as wholesome due to the restrictive diet (Wesołowska et al. 2018).

In this paper, we aimed to analyze existing research on the effects of vegetarian, including vegan, diet on maternal breast milk composition.

Methods

Literature search strategy

The full search for published literature was performed twice: on 12th December 2018 and on 20th May 2019. The databases including MEDLINE (PubMed) and Scopus were used to find relevant papers. There was no language restrictions, neither date limits. As the process of writing the article had prolonged, an additional search was conducted on 20th January 2020, concentrating on studies published from May to the current date. The searches were conducted by using various word combinations, such as: “vegetarian,” “vegan,” “vegetarianism,” “human milk,” “breast milk,” “lactation,” “breastfeeding,” “breastfed,” “breast fed,” “diet,” “nutrition,” “intake,” “status,” “composition,” “fat,” “fatty acids,” “protein,” “amino acids,” “carbohydrates,” “glucose,” “fructose,” “galactose,” “micronutrients,” “vitamins,” “iron,” “choline,” “calcium,” “zinc,” “selenium,” “iodine,” “infant,” “newborn,” “neonate,” “pregnancy outcome,” “delivery,” “deficiency.” Each term search involved a wide range of synonyms and keyword connections to ensure maximum access to relevant papers. Studies were based on the assumption that search keywords were present in either the title, or abstract, or keywords, provided by authors of the manuscript.

Studies selection and data extraction

The papers found in initial search were removed of duplicates and they were searched for titles and abstracts in accordance with the eligibility criteria. The inclusion criteria were stated as follows:

1. primary studies;
2. studies on humans;
3. papers written in Polish, English or German;

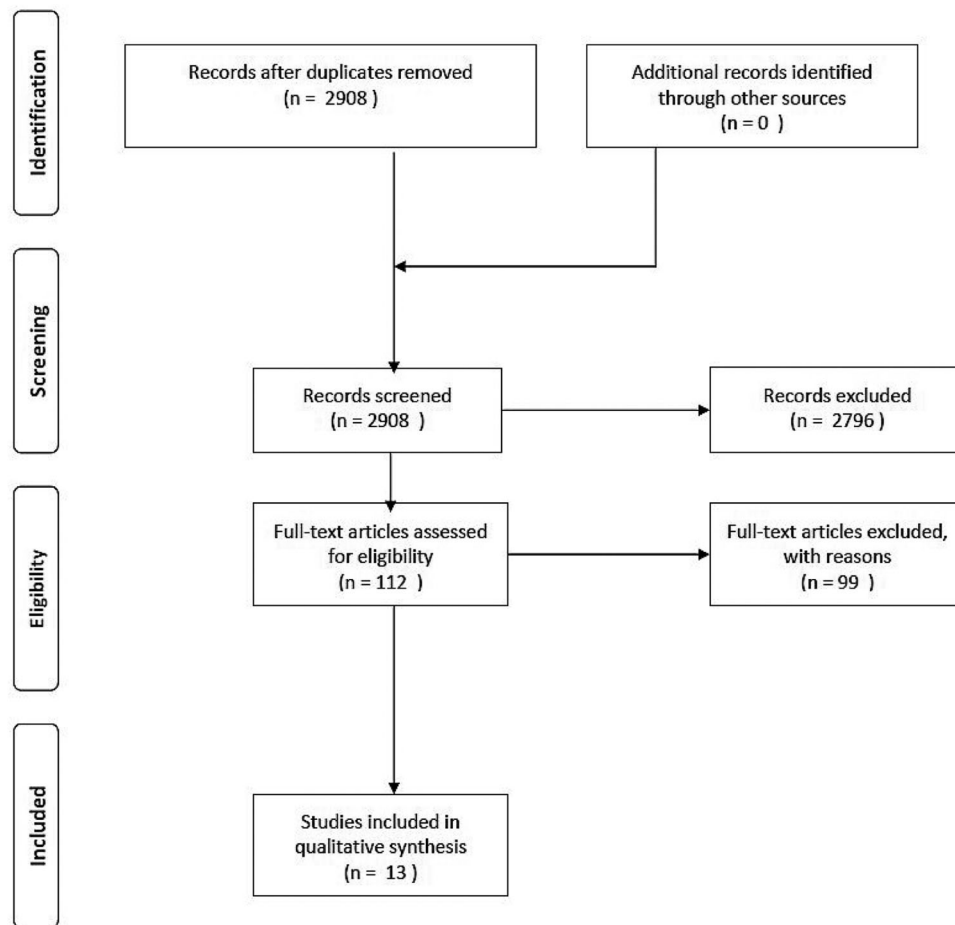


Figure 1. Procedure of publication selection – The PRISMA flow chart.

4. references concerning the influence of dietary pattern on breast milk composition
5. categorization due to dietary habits along with consideration given to the levels of animal-origin food intake;
6. inclusion of vegetarians or vegans in a study group;
7. inclusion of breastfeeding mothers in the study group;
8. references concerning the exposure to various nutrients or a type of diet and its impact on maternal or neonatal status and breast milk composition

The following exclusion criteria were applied

1. secondary studies;
2. case reports;
3. studies on animals;
4. full text access unavailable;
5. insufficient data on mothers' diet, lactation, nutritional status;
6. no dietary assessment/categorization;
7. lack of control group;
8. non-assessed impact of dietary patterns on breast milk composition;
9. no animal-derived food exclusion in any participating group or non-detailed data on eating pattern.

Overall, the selection process aimed at identifying any original studies determining the associations between breast

milk composition and mother's adherence to plant-based diet. Extracted data included details on methodology, study group characteristics, and main findings of the original study. If the abstract did not provide sufficient data, the paper was qualified for further full-text analysis. Each time a literature review was found, we checked its reference list to ensure our search results include all relevant publications. No additional articles were found. Then, if available, full texts of selected papers were retrieved. The articles were checked for inclusion twice at an interval of 6 months – the corresponding lists of selected studies were consistent. After reviewing the full texts, the rejected articles ($n = 99$) were divided into groups, on the basis of such categories as: (1) no animal source foods excluded or no detailed information on dietary pattern provided ($n = 60$); (2) insufficient information on the impact of diet on maternal status, including mother's breast milk composition ($n = 17$); (3) no dietary assessment ($n = 7$); (4) focus on poor condition of a breastfed child, without any assessment of breast milk composition ($n = 3$); (5) insufficient data on lactation or breast milk quality ($n = 12$).

The literature search and the reviewing process were conducted according to the PRISMA guidelines (Figure 1) (Moher et al. 2009).

All eligible studies were formally evaluated using the Quality Assessment Tool, provided by the National Heart, Lung and Blood Institute (National Heart, Lung and Blood

Institute 2018). This tool refers to 13 elements of quality assessment. Each component was rated using descriptive score: “yes”, “no”, “not applicable”/“not reported”/“cannot determine”. For summary purposes, we derived a numeric score for the final grade assigned to each study by counting the number of ‘yes’ answers obtained. For overall assessment, the following criteria were applied: 0–3 points “very poor”, 4–5 points “poor”, 6–8 points “fair”, 9–10 points “good”, 11–13 points “very good”.

The first author (KK) designed the methodology, established the criteria of data extraction, performed the process of records screening and identification of the literature, under the constant supervision of the second author (BK-O). Both two authors (KK and BK-O) reviewed all of the articles consecutively and performed the study selection independently, according to the inclusion and exclusion criteria. Any disagreements at each stage of the verification process were resolved by consensus.

Results

Literature search

The selection process using the PRISMA flow diagram is depicted in Figure 1. After removing duplicates, the search yielded 2908 articles, including all search results. Having read title and abstract, 112 papers, with an access to full text, were identified. After considering the full-text, further 99 publications were excluded. A total of 13 independent studies met the inclusion criteria and were included in this systematic review. On search conducted in November 2019, 8 additional publications were found and all of them (4 systematic reviews, 2 papers in Spanish or Chinese, 2 irrelevant observational studies) were rejected.

Study characteristics – summary

The characteristics of formally evaluated studies are presented in a chronological order in Table 1.

None of 13 included papers received a full score in quality assessment. Not every study provided a clear description of study population, nor the detailed criteria of the recruitment process (Bijur and Desai 1985; Finley et al. 1985a, 1985b; Sanders and Reddy 1992). In addition, the sample size justification and power calculations were missing. In some papers, the exposure measures were not always clearly defined, including the details on the exposure time frame (Bijur and Desai 1985; Finley et al. 1985a, 1985b; Debski et al. 1989; Sanders and Reddy 1992; Liao et al. 2011), the level of exposure (Sanders, Ellis, and Dickerson 1978; Bijur and Desai 1985; Specker et al. 1990; Liao et al. 2011), multiple evaluation of exposures (Sanders, Ellis, and Dickerson 1978; Bijur and Desai 1985; Rana and Sanders 1986; Specker et al. 1990; Patel and Lovelady 1998; Perrin et al. 2019, as far as the impact of mother’s nutrition on breast milk composition was concerned.

Mostly, the level of exposure was not blinded to the assessors. In some studies, all missing details were said to be

provided in another papers (Rana and Sanders 1986; Debski et al. 1989); however, the full texts of those publications were unavailable. For these reasons, in evaluation studies they obtained from 4 to 9 points – most of the analyzed studies (76.9%) got a score of 7 points or more, which was assessed as “fair” or “good” due to the adopted scale (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a, 1985b; Rana and Sanders 1986; Debski et al. 1989; Sanders and Reddy, 1992; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019).

The studies included were published between 1978 and 2018. Only 3 articles were published after 2000 (Liao et al. 2011; Pawlak et al. 2018; Perrin et al. 2019). The remaining papers were published in 1970s ($n=1$) (Sanders, Ellis, and Dickerson 1978), 1980s ($n=5$) (Bijur and Desai 1985; Finley et al. 1985a, 1985b; Rana and Sanders 1986; Debski et al. 1989) and 1990s ($n=4$) (Specker et al. 1990; Sanders and Reddy 1992; Kim et al. 1996; Patel and Lovelady 1998). The studies were conducted mainly in the USA ($n=7$) (Finley et al. 1985a, 1985b; Debski et al. 1989; Specker et al. 1990; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019). The other ones were carried out in the UK ($n=3$) (Sanders, Ellis, and Dickerson 1978; Rana and Sanders 1986; Sanders and Reddy 1992) and in Asian countries: India ($n=1$) (Bijur and Desai 1985), Taiwan ($n=1$) (Liao et al. 2011), Korea ($n=1$) (Kim et al. 1996). Seven publications considered data on ethnicity of the participants and 3 of them included Hindu (Bijur and Desai 1985; Patel and Lovelady 1998) and Indian (Sanders and Reddy 1992) women in a study group (Table 2). All the studies included women in reproductive age, mostly aged from 18 to 40 years, however, the authors of 6 papers did not refer to the age of breastfeeding mothers (Sanders, Ellis, and Dickerson 1978; Rana and Sanders 1986; Sanders and Reddy 1992; Liao et al. 2011).

As far as the inclusion criteria are considered, detailed eligibility criteria for participants are provided in 5 studies (Sanders, Ellis, and Dickerson 1978; Rana and Sanders 1986; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019), whereas the remaining articles include only characteristics essential for recruitment to study and control groups (Bijur and Desai 1985; Finley et al. 1985a, 1985b; Debski et al. 1989; Specker et al. 1990; Sanders and Reddy 1992; Kim et al. 1996; Liao et al. 2011).

Study and control group characteristics

The size of study and control groups varied among publications. In general, the study samples were small with fewer than 100 participants. The number of participants ranged from 19 to 74. However, it should be also mentioned that in one of the studies, specimens of breast milk were involved in the analysis from previous research (Rana and Sanders 1986), whereas in another study, breastfeeding mothers constituted a minor part of all participants, thus the breast milk analysis was not the major concern of that research (Sanders, Ellis, and Dickerson 1978).

Table 1. Characteristics of evaluated studies.

First author, year (ref)	Location	Quality score	Study participants	Maternal dietary assessment	Breast milk samples	Components measured in breast milk	Results
Sanders 1978 (Sanders, Ellis, and Dickerson 1978)	UK	8	22 vegans and 22 omnivore controls, of whom 4 vegans and 4 omnivore controls were breastfeeding mothers.	Not specified; Interview regarding inclusion criteria for vegans: diet does not include any products of animal origin; Supplementation of vitamin B12 was noted.	20 ml of breast milk were obtained from each mother at the start of morning feed; 2ml of breast milk were obtained from the same breast in the middle and the final part of the feed.	Fatty acids.	Wilcoxon's test: Significantly lower amount of C16:0, C16:1, C18:0 and C20:4:03 and higher amount of C18:2:06, C18:3:03, C20:2:06 in vegans' breast milk ($p < 0.05$). Tendency to lower proportions of C20:5:03 and C22:6:03 in vegans' breast milk.
Bijur, 1985 (Bijur and Desai 1985)	India	5	50 women from Bombay: 18 non-vegetarian frequent meat eaters, 16 non-vegetarian occasional meat eaters, 16 lacto-vegetarians.	Not described.	Morning samples of manually expressed breast milk were collected before feeding the baby.	Total protein, free amino acids (FAA), vitamin B12.	Low levels of vitamin B12 in breast milk were found among mothers who had low serum B12 level – lower level were found, both in serum and breast milk ($p < 0.01$), in lacto-vegetarian mothers in comparison with non-vegetarian mothers. Contents of total protein and FAA were similar in all groups.
Finley 1985 (Finley et al. 1985a)	USA	7	57 women: 30 vegetarians, 8 semi-vegetarians (fish-eaters), 29 omnivores.	24-h dietary recall including supplements at the initial interview; 2-day diet record every month.	Morning samples of breast milk were collected on the day after dietary record. Women expressed all of the milk in the breast.	Total fat, fatty acids.	Student-t test: No significant difference in total breast milk fat content between vegetarians and non-vegetarians was found. Breast milk from vegetarians contained less C16:0, C16:1:09, C18:0, C18:1:09 and more C18:2:06 fatty acids. Higher level of C10:0 and C12:0 in milk of vegetarians correlated with lower fat intake.
Finley 1985 (Finley et al. 1985b)	USA	7	68 women: vegetarians, semi-vegetarians, omnivores.	24-h dietary recall and 2-day diet record including supplements obtained at monthly intervals	Portion of all of the milk from one breast expressed at the time of second breastfeeding of the day	Protein, lactose, lipid, minerals, trace elements	Student-t test: No significant influence of the diet on breast milk composition was found – all women were well-nourished. Milk from vegetarians and non-vegetarians differed in concentration of some fatty acids, due to maternal diet and daily intake. Correlation analysis: level of milk lipid remained constant during 6 months of lactation and increased during next 14 months ($r = +0.29$); level of milk lactose remained constant during 20 months of lactation; level of milk protein decreased during 6 months of lactation ($r = -0.43$) and remained constant during next 14 months (vegetarians at 7–20 months of lactation had lower levels of milk protein than non-vegetarians). Student-t test ($p < 0.01$): mean taurine concentration in the vegan breast milk (35 mg/dl) was significantly lower than in the breast milk of omnivores (53 mg/dl).
Rana 1985 (Rana and Sanders 1986)	UK	9	For milk analysis 14 vegan and 14 omnivore mothers included - analysis included samples from previous study.	Food intake record of seven consecutive days (including weight); collection of duplicate meals from 3 days.	Frozen mid-stream milk samples collected 4–6 weeks post-partum.	Taurine.	

(continued)

Table 1. Continued.

First author, year (ref)	Location	Quality score	Study participants	Maternal dietary assessment	Breast milk samples	Components measured in breast milk	Results
Debski 1989 (Debski et al. 1989)	USA	7	45 milk samples from 38 lactating women; 26 vegetarians and 12 nonvegetarians.	2 - day diet record at monthly intervals for 4-6th month of lactation.	Milk samples collected by hand expression or with breast pump.	Selenium (Se); Glutathione peroxidase (GSH-Px) activity.	The quantity of Se in milk from vegetarian women was 132 % of that from non-vegetarian women ($22.2 \pm 0.8 \text{ ng/ml}$ vs $16.8 \pm 1.3 \text{ ng/ml}$). Protein content in milk was similar in both groups. Milk Se concentration was linearly correlated with GSH-Px activity ($r = 0.76$, $p < 0.0001$). Significant relationship ($r = 0.68$, $p = 0.0001$) between milk GSH-Px activity and linoleic acid content was found. Milk Se and linoleic acid content were not correlated ($r = 0.30$, $p = 0.09$). Vitamin B12 concentration in vegetarian mothers' milk was lower than in omnivores' milk ($p = 0.007$). In strict vegetarians, an inverse correlation between milk vitamin B12 concentrations and length of time on a vegetarian diet was found ($r = -0.605$, $p = 0.03$). Milk vitamin B12 concentrations were inversely related to maternal urinary MMA concentrations ($r = -0.830$, $p < 0.001$) and associated with maternal serum vitamin B12 content ($r = 0.787$, $p < 0.001$). In comparison to omnivores' breast milk, vegans' breast milk contained higher proportions of short chain FA (C10-C14) and lower proportions of medium chain FA (C16-C18) ($p < 0.01$). The proportions of dihomogammalinolenic acid (20:3 ω 6) and arachidonic acid (20:4 ω 6) were comparable in all groups. The proportion of breast milk DHA (22:6 ω 3) was lower in vegans than in omnivores and vegetarians ($p < 0.01$). The ω 6 / ω 3 FA ratio was higher in the vegans than in the other groups.
Specker 1990 (Specker et al. 1990)	USA	5	19 women: 13 vegetarians, 6 omnivores.	Interview - not specified; exclusion of dairy products and eggs from vegetarian diet; none of vegetarians and 5 omnivores took commercial supplements.	Sample of breast milk from the first morning feeding - all milk of the breast was expressed.	Vitamin B12.	
Sanders 1992 (Sanders and Reddy 1992)	UK	7	45 women: 19 vegans, 5 vegetarians, 21 omnivores.	3-day weighed food intake inventory in lactating women; 7-day weighed food intake inventory in non-lactating women; analysis of 3-day duplicate meals.	Midstream breast milk samples obtained 6 weeks postpartum (10 ml).	Fatty acids (FA).	
Kim 1996 (Kim et al. 1996)	Korea	7	22 non-vegetarian women from Seoul, 23 lacto-ovo-vegetarian women from Seoul and its suburban areas.	Mothers' food intake measured by the weighing method - not specified. Infants' taurine intake was calculated by multiplying taurine concentrations by human milk intakes.	Sample of 30-50 ml of initial milk expressed manually from 10-12 a.m. at 3, 5, 15, 30, 60, 90, 120, 150 days postpartum.	Taurine	Taurine concentrations of human milk decreased significantly during the course of lactation among all participants ($p < 0.05$). After 90 days postpartum, the decline in taurine concentrations in breast milk was more remarkable in lacto-ovo-vegetarians than in non-vegetarians ($p < 0.05$). Non - vegetarians had higher energy intakes than lacto-ovo-vegetarians at 30, 120 and 150 days postpartum, however the overall protein intake was higher among lacto-ovo-vegetarians.

Patel 1998 (Patel and Lovelady 1998)	USA	8	8 East Indian women: 5 lacto-vegetarians, 3 lacto-ovo-vegetarians. 11 omnivores: 5 East Indian women, 6 Caucasians.	Food frequency questionnaire; supplements intake was recorded.	Samples of all the breast milk from one breast expressed at the first feeding, collected at least 4 weeks postpartum.	Vitamin B12	No significant correlation between the number of servings of dairy products consumed daily and milk vitamin B12 concentrations among all participants was found. Vitamin B12 concentrations were significantly lower in the vegetarian group than in the omnivore group ($p < 0.05$). The mean breast milk vitamin B12 concentrations decreased significantly with the duration of lactation ($p = 0.04$), and were lower in vegetarians than in omnivores ($p = 0.006$). Total free nucleotide concentration in breast milk was significantly higher in vegetarians than in non-vegetarians ($p = 0.037$), but there was no significant difference in nucleoside concentration. Cytidine diphosphate was found to be the predominant nucleotide in human milk, indifferent to the stage of lactation, diet and place of residence. No difference in vitamin B12 concentrations in breast milk was found between diet groups. Use of individual vitamin B12 supplements had a significantly positive impact on milk B12 concentrations ($p = 0.024$). No relation with vitamins B-complex nor prenatal vitamins was found.
Liao 2010 (Liao et al. 2011)	Taiwan	4	24 women from Taiwan; 8 vegetarians among participants.	Not described.	Sample of 30 ml of breast milk categorized due to lactation stages: first week postpartum; first month postpartum; second month postpartum; third to ninth month postpartum.	Free nucleotides and nucleosides	
Pawlak 2018 (Pawlak et al. 2018)	USA	9	74 lactating women: 26 vegans, 22 vegetarians, 26 non-vegetarians.	Diet type was self-reported by participants (survey); vitamins supplementation was recorded.	Samples of milk collected during the first or second feeding and over 2 h since the previous feeding; complete expression of 1 breast content.	Vitamin B12.	
Perrin 2019 (Perrin et al. 2019)	USA	9	74 lactating women: 26 vegans, 22 vegetarians, 26 omnivores.	Diet survey; vitamins supplementation was recorded.	Samples of milk collected during the first or second feeding and over 2 h since the previous feeding; complete expression of 1 breast content.	Total fat, fatty acids (FA), brain-derived neurotrophic factor (BDNF).	The breast milk composition of saturated fat, unsaturated fat, and trans fat differed significantly by diet ($p \leq 0.001$). A significant difference in total omega-3 fatty acids was observed between diet groups, with higher percentages of ALA ($p \leq 0.001$). No difference in the prevalence of low milk DHA between diet groups was found. BDNF was not detectable in collected samples.

Table 2. Material and methods – characteristics of the study groups.

First author, year (ref)	Age of breastfeeding participants	Socioeconomic status	Ethnicity	Duration of meatless diet	Duration of lactation	Anthropometrics	Inclusion criteria	Additional exclusion criteria
Sanders 1978 (Sanders, Ellis, and Dickerson 1978)	Not specified.	Predominantly professional and middle classes.	Caucasians.	Range 3–12 years, average 7 years (vegan mothers).	Minimum of 3 months.	Not specified regarding mothers, however vegans proved to have lower body weight and lower sum of skinfold measurements.	Study group: Diet should not contain any food of animal origin. Diet should last at least 1 year. Control group: Healthy, not taking any special diet volunteers. Omnivores matching the vegans regarding the sex, age, height, ethnic origin and socioeconomic background.	–
Bijur, 1985 (Bijur and Desai 1985)	Range 18–38 years.	Low class.	80 % Hindus, 20 % not specified.	Not specified.	Not specified.	Mean weight of the mothers 45.42 kg (range of 36.4–57.6 kg). Mean weight of the infant 2.57 kg (range of 1.1–4.1 kg).	Not specified. 50 mothers admitted to the hospitals in Bombay - they were representative for the majority of population.	–
Finley 1985 (Finley et al., 1985a)	Range 22–37 years, average 29 years.	Not specified.	Not specified; Women living in northern Canada.	Not specified.	Range 2–31 months.	Maternal pre-pregnancy body weight (% ideal weight for height): mean 103 %, range 82–137 %.	Not specified.	–
Finley, 1985 (Finley et al. 1985b)	Mean 20 years.	Low median household income.	Not specified.	Not specified.	Minimum 3–4 weeks postpartum.	Maternal pre-pregnancy body weight (% ideal weight for height): mean 103 %.	Not specified; study included well-nourished women living in industrialized society.	Breastfeeding women before the stabilisation of lactation - exclusion of colostrum.
Rana, 1985 (Rana and Sanders 1986)	Range 18–40 years; age of breastfeeding women not specified.	Not specified.	Not specified; both study and control group were recruited in England.	Vegans: Average of 6 years (range 1–15 years); previously followed vegetarian diet average of 4 years (0–14 years).	Not specified; the study included breast milk samples from another research.	Maternal pre-pregnancy body weight (% ideal weight for height): mean 103 %.	Study group: Following vegan diet for at least 1 year; Taking no medications other than oral contraceptives. Control group: volunteers matching the vegan subjects for age, sex, similar height, similar body built.	–
Debski 1989 (Debski et al. 1989)	Mean 29 years.	Not specified, but reported elsewhere -full text unavailable.	Not specified, but reported elsewhere -full text unavailable.	Not specified, but reported elsewhere -full text unavailable.	Not specified, but reported elsewhere -full text unavailable.	Not specified, but reported elsewhere -full text unavailable.	Healthy lactating women - vegetarians and omnivores.	–
Specker 1990 (Specker et al. 1990)	Mean 31 years, range 22–40 years.	Not specified.	Not specified.	Range 14–137 months (median 72 months).	Vegetarian infants: mean 7.3 months (range 2–13.9 months); Omnivorous infants: mean 7.8 months (5.0–11.9 months).	Not specified.	Lactating women and their infants – omnivores and mothers following macrobiotic diet.	Inadequate volume of breast milk or infant's urine or maternal serum samples obtained.

Sanders 1992 (Sanders and Reddy 1992)	Not specified.	Not specified.	White vegetarians and vegans; Indian vegetarians; white omnivores.	Not specified.	14 weeks.	Mean birth weight of infants born: to Hindu vegetarian women 3.18 kg (range 3.04–3.33 kg), to white omnivores 3.48 kg (3.35–3.62 kg), white vegan 3.31 kg (3.08 –3.35 kg).	Not specified; vegan, vegetarian and omnivore subject of white and Hindu ethnicity, matched for gestational age, parity, maternal age, sex of the infant.	-
Kim 1996 (Kim et al. 1996)	Not specified.	Not specified.	Mothers living in Seoul; Seventh Day Adventist Church members.	Not specified.	3, 5, 15, 30, 60, 90, 120, 150 days postpartum.	Not specified.	Study group: lacto-ovo-vegetarians Control: non-vegetarians.	-
Patel, 1998 (Patel and Lovelady 1998)	Not specified.	Not specified.	Study group: East Indian women (mostly Hindu); Control: East Indian and Caucasian women.	Not specified.	Minimum 4 weeks.	Not specified.	Lactating women, free of any illness, not smoking, who gave birth to healthy full-term infants. Infants at least 4 weeks old, exclusively breastfed.	-
Liao 2010 (Liao et al. 2011)	Not specified.	Not specified.	Asian, Taiwanese.	Not specified.	Range 1 week–9 months postpartum.	Not specified.	Taiwanese women, vegetarians and omnivores.	-
Pawlak 2018 (Pawlak et al. 2018)	Mean age (± SD): vegans 32.7 (± 5.2); vegetarians 32.2 (± 4.6); non-vegetarians 31.0 (± 4.7) years.	Not specified, participants categorized by education.	Black; Asian; Hispanic; White; Mixed/Other.	Mean length (± SD): vegans 6.2 (± 5.5) years - range 0.5–23 years; median 4.3 years; vegetarians 7.5 (± 5.5) years - range 1 month - 20 years; median 6 years; non-vegetarians 25.8 (± 11.5) years.	Mean length (± SD): vegans 36.6 (± 27.7) months; vegetarians 54.6 (± 46.0) months; non-vegetarians 27.5 (± 19.8) months.	Mean BMI (± SD) of mothers: vegans 22.8 kg/m ² (± 5.1), vegetarians 23.9 kg/m ² (± 3.8), non-vegetarians 25.8 kg/m ² (± 4.5).	Diagnosis of MTHFR gene mutation; health conditions affecting B12 status (intrinsic factor deficiency, bariatric surgery in the past; celiac disease; inflammatory bowel disease); hypothyroidism; hyperthyroidism; myeloproliferative disorders; advanced liver disease; pregnancy.	-
Perrin, 2019 (Perrin et al. 2019)	Mean age (± SD): vegans 32.7 (± 5.2); vegetarians 32.2 (± 4.6); non-vegetarians 31.0 (± 4.7) years.	Not specified, participants categorized by education.	Asian; Hispanic; White; Mixed / Other.	Mean length (± SD): vegans 6.2 (± 5.5); vegetarians 7.5 (± 5.5) years; non-vegetarians 25.8 (± 11.5) years.	Mean length (± SD): vegans 36.6 (± 27.7) months; vegetarians 54.6 (± 46.0) months; non-vegetarians 27.5 (± 19.8) months.	Mean BMI (± SD) of mothers: vegans 22.8 kg/m ² (± 5.1), vegetarians 23.9 kg/m ² (± 3.8), non-vegetarians 25.8 kg/m ² (± 4.5).	Living in the United States; mothers at age of 18–46 years; giving birth to a healthy term infant; currently, infant at age of at least 2 weeks; mothers willing to complete the diet survey and donate their milk.	Diagnosis of MTHFR gene mutation; health conditions affecting B12 status (intrinsic factor deficiency, bariatric surgery in the past; celiac disease; inflammatory bowel disease); hypothyroidism; hyperthyroidism; myeloproliferative disorders; advanced liver disease; pregnancy.

The authors of only three papers referred to the socio-economic status of the participants – one study included representatives of middle class (Sanders, Ellis, and Dickerson 1978), two studies were carried out among lower class (Bijur and Desai 1985; Finley et al. 1985b). In 2 papers, the study groups were categorized by level of education (Pawlak et al. 2018; Perrin et al. 2019). The remaining papers do not provide any data on the socio-economic status of lactating mothers (Rana and Sanders 1986; Debski et al. 1989; Specker et al. 1990; Sanders and Reddy 1992; Kim et al. 1996; Patel and Lovelady 1998; Liao et al. 2011).

The characteristics of participants, who took part in evaluated studies, are presented in a chronological order in Tables 1 and 2.

Maternal nutrition

The study groups of all studies included women following meatless diet, with various extent in exclusion of products of animal-origin, and omnivore control groups. In 3 studies, participants were classified as vegans, vegetarians and non-vegetarians (Sanders and Reddy 1992; Pawlak et al. 2018; Perrin et al. 2019), in 2 studies: vegans and omnivores (Sanders, Ellis, and Dickerson 1978; Rana and Sanders 1986), while in 8 studies: vegetarians and omnivores (Bijur and Desai 1985; Finley et al. 1985a, 1985b; Debski et al. 1989; Specker et al. 1990; Kim et al. 1996; Liao et al. 2011) (2 of which additionally distinguished a semi-vegetarian group) (Finley et al. 1985a, 1985b).

The type of maternal diet was defined by mothers themselves. Furthermore, for more precise definition of dietary habits, the authors used such methods as: dietary recall ($n=2$) (Finley et al. 1985a, 1985b), food record ($n=4$) (Finley et al. 1985a, 1985b; Rana and Sanders 1986; Debski et al. 1989), dietary survey or food frequency questionnaire ($n=3$) (Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019), interview ($n=3$) (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a; Specker et al. 1990), duplicate diet method ($n=1$) (Rana and Sanders 1986), weighing method ($n=2$) (Sanders and Reddy 1992; Kim et al. 1996) and non-specified one ($n=2$) (Bijur and Desai 1985; Liao et al. 2011). The supplement intake was reported in 7 papers (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a, 1985b; Rana and Sanders 1986; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019).

Since time might be a key predictor of how dietary habits affect breast milk composition and maternal body reserves, the duration of meatless diet was reported in 5 studies (Sanders, Ellis, and Dickerson 1978; Rana and Sanders 1986; Specker et al. 1990; Pawlak et al. 2018; Perrin et al. 2019). In most cases, the mean duration of vegan or vegetarian diet was 6–7 years.

Another factor affecting human milk composition is undernutrition. As far as the authors provided information on mothers' anthropometry, including weight and Body Mass Index (BMI), the women who participated in the study group were within normal range (accepted in authors'

country). Physical measures were the only reported indicators of nutritional status.

All the factors described above are summarized in Table 2.

Breast milk samples collection

The detailed information on breast milk samples collection is presented in Table 1.

It is known that the composition of breast milk varies during the lactation period. Of the all studies included in this systematic review, 8 papers concerned mature breast milk (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a, 1985b; Specker et al. 1990; Sanders and Reddy 1992; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019), 2 – colostrum or transitional milk (Kim et al. 1996; Liao et al. 2011). Authors of 3 papers did not provide any information on the stage of lactation while collecting samples (Bijur and Desai 1985; Rana and Sanders 1986; Debski et al. 1989). Furthermore, in one study the breast milk specimens were collected from the same mother several times in ongoing lactation period (Kim et al. 1996). In another study, the human milk specimens were classified into to 4 stages of lactation (Liao et al. 2011) (Tables 1 and Tables 2).

The way of collecting mother's milk varied in those studies as well. Hand expression was a predominant method, but in 3 studies milk was allowed to be collected using a breast pump (Finley et al. 1985a, 1985b; Pawlak et al. 2018). In 9 papers, the analyzed breast milk was collected in the morning: before feeding the baby ($n=1$) (Bijur and Desai 1985), from the 1st feeding ($n=2$) (Specker et al. 1990; Patel and Lovelady 1998), from the 1st or 2nd feeding ($n=2$) (Pawlak et al. 2018; Perrin et al. 2019), from the 2nd feeding ($n=2$) (Finley et al. 1985a, 1985b), at 10–12 am ($n=1$) (Kim et al. 1996), at non-specified time ($n=1$) (Sanders, Ellis, and Dickerson 1978) (Table 1).

In 4 papers, authors did not provide the details regarding the part of a day for sample collection (Rana and Sanders 1986; Debski et al. 1989; Sanders and Reddy 1992; Liao et al. 2011).

In 6 studies, mothers were asked to express whole content of one breast (Finley et al. 1985a, 1985b; Specker et al. 1990; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019). Another 2 studies carried out an analysis of a midstream sample of breast milk (Rana and Sanders 1986; Sanders and Reddy 1992). In another paper, the milk specimens contained initial part of milk (Kim et al. 1996). One study, however, included smaller samples – taken in the middle and at the end of the same feeding (Sanders, Ellis, and Dickerson 1978) (Table 1).

The majority of articles (76.9%) specified that milk was frozen after collecting the samples and stored in cold. In one study, mothers froze breast milk just after expressing it, at a temperature about -4°C (Liao et al. 2011). The milk was later kept frozen in laboratories. The storage temperature was -20°C (Finley et al. 1985a, 1985b; Rana and Sanders 1986; Sanders and Reddy 1992; Patel and Lovelady 1998; Pawlak et al. 2018; Perrin et al. 2019) or -70°C

(Debski et al. 1989; Liao et al. 2011). In one of the studies there was no information given regarding the freezing temperature (Specker et al. 1990).

Breast milk composition – assessment

The researchers' interest concentrated on various human milk components. The authors of analyzed publications examined the content of: vitamin B12 ($n=4$) (Bijur and Desai 1985; Specker et al. 1990; Patel and Lovelady 1998; Pawlak et al. 2018), folic acid activity ($n=1$) (Bijur and Desai 1985), fatty acid ($n=4$) (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a; Sanders and Reddy 1992; Perrin et al. 2019), total fat ($n=3$) (Finley et al. 1985a, 1985b; Perrin et al. 2019), amino acids and total protein ($n=3$) (Bijur and Desai 1985; Finley et al. 1985b; Debski et al. 1989), taurine ($n=2$) (Rana and Sanders 1986; Kim et al. 1996), selenium ($n=1$) (Debski et al. 1989), minerals ($n=1$) (Finley et al. 1985b), free nucleotides and nucleosides ($n=1$) (Liao et al. 2011), lactose ($n=1$) (Finley et al. 1985b). In addition, in one study breast milk was analyzed for brain-derived neurotrophic factor (BDNF) in correlation with omega-3 fatty acids content (Perrin et al. 2019); in another paper, because of the examination of selenium content, the glutathione peroxidase (GSH-Px) activity was determined (Debski et al. 1989).

Breast milk composition – main results

Total fat

The total breast milk fat was measured in 3 papers (Finley et al. 1985a, 1985b; Perrin et al. 2019). Perrin et al. observed a significant difference in dietary groups ($p=0.041$) – mean concentrations of total milk fat were 3.0 (± 1.7) g/dl for vegans, 4.0 (± 2.9) g/dl for vegetarians and 4.0 (± 2.9) g/dl for omnivores. The total fat content in breast milk seemed to be affected by maternal age ($\beta=0.1$, $R=0.047$; $p=0.036$), with no significant relationship to the stage of lactation and maternal BMI, although the maternal BMI in mothers following a meatless diet was significantly lower ($p=0.021$) than in omnivores. When it comes to trans-fat, the mean breast milk trans-fat concentrations were relatively low – below 1.1% in all study groups, with the lowest levels in vegans (Perrin et al. 2019).

Finley et al. found that the milk fat maintained at a constant level in the first 6 months of lactation and increased in subsequent 14 months ($r=+0.29$). As all the study participants were well-nourished, the dietary fat intake did not significantly affect the breast milk composition (correlation coefficient: 0.03), even though the vegetarians consumed less fat than non-vegetarians (Finley et al. 1985b).

Finley et al. continued the analysis of breast milk fat content in another study. Since the participants were well-nourished, both vegetarians and non-vegetarians had similar dietary fat intake, which constituted 36% of energy intake – with no significant impact on fat content in breast milk samples. The mean percentage of fat in breast milk samples was $3.21 \pm 1.78\%$ for vegetarians and $3.23 \pm 2.13\%$ for non-

vegetarians. In further analysis, maternal parity and nursing intervals were negatively correlated with a milk fat percentage ($r=-0.38$ and $r=-0.28$, respectively), with no significant correlation between a part of a day and expressing milk (Finley et al. 1985a).

Regardless of meat consumption patterns, the participants were divided into groups of low animal-fat intake (LAF) – less than 35 g daily, and high animal-fat intake (HAF) – more than 35 g daily (the fat intake was estimated on the basis of two-day records made prior to the milk samples collection). For LAF group the milk fat percentage was positively correlated with the amount of animal-fat consumption ($r=+0.35$, $p \leq 0.001$). However, there was no significant correlation in HAF group ($r=0.06$, $p \leq 0.10$). Neither the total fat intake nor vegetable fat intake affected significantly the milk fat percentage in any of dietary groups (Finley et al. 1985a).

Free fatty acids

A detailed analysis of particular fatty acids was conducted in 4 studies. When it comes to polyunsaturated fatty acids, the main $\omega 3$ acids include: α -linolenic acid (ALA; 18:3 $\omega 3$), DHA, EPA, and docosapentaenoic acid (DPA; 22:5 $\omega 3$) and $\omega 6$ acids: linoleic acid (LA; 18:2 $\omega 6$) and arachidonic acid (AA; 20:4 $\omega 6$).

In both studies, Finley et al. found some differences in fatty acid composition of breast milk, based on the maternal dietary habits. In general, fourteen fatty acids were identified in human milk. In comparison to non-vegetarians, the vegetarians' breast milk contained less C16:0 ($23.96 \pm 2.92\%$ vs $22.50 \pm 3.63\%$), C16:1 $\omega 9$ ($3.23 \pm 0.79\%$ vs $2.81 \pm 0.68\%$), C18:0 ($8.70 \pm 1.31\%$ vs $7.44 \pm 1.53\%$) and C18:1 $\omega 9$ ($32.98 \pm 3.51\%$ vs $30.74 \pm 4.13\%$) fatty acids and was more rich in 18:2 $\omega 6$ fatty acids ($14.65 \pm 4.24\%$ vs $18.38 \pm 4.67\%$). The results seemed to reflect the difference in dietary intake of fatty acids – vegetarians consumed less oleic acid (C18:1 $\omega 9$) than non-vegetarians (mean 30 ± 13 g vs 34 ± 12 g), and more linoleic acid (C18:2 $\omega 6$) (mean 14 ± 9 g vs 12 ± 8 g). As vegetarians consumed less fat, their milk proved to have a higher level of C10:0, C12:0, C14:0 fatty acids. All in all, vegetarians' breast milk contained a reduced level of saturated long-chain fatty acids and a higher level of polyunsaturated fatty acids (Finley et al. 1985a, 1985b).

The analysis of fatty acids in relation to the animal-fat intake (low animal-fat intake – LAF, high animal-fat intake – HAF) has revealed no correlation between fatty acid pattern and total fat in milk in LAF group, whereas in HAF group a significant relationship was found: C10:0 ($r=+0.38$), C12:0 ($r=+0.31$), C18:3 ($r=+0.26$), C20:2 $\omega 6$ ($r=+0.31$), C16:0 ($r=-0.29$), C18:0 ($r=-0.28$). Considering de novo synthesis of fatty acids in mammary glands, no significant difference in breast milk composition, including fat content, was found between vegetarians and non-vegetarians (Finley et al. 1985a).

Perrin et al. discovered that breast milk samples were predominantly rich in long-chain fatty acids, counting more than 15 carbons. No significant difference in distribution of total long-chain ($p=0.906$) or total medium-chain ($p=0.879$) fatty acids by the dietary group was observed. Of

the predominant fatty acids, the dietary patterns proved to affect the content of C16:0, C16:1cis, C18:0, C18:1cis, C18:3cis ω 3 fatty acids ($p \leq 0001$) in milk significantly. Furthermore, in particular dietary groups ($p \leq 0.001$), a dietary pattern had an impact on the content of saturated, unsaturated and trans fat in breast milk (Perrin et al. 2019).

The authors conducted further analysis of polyunsaturated fatty acids in milk samples: omega-3 and omega-6 fatty acids. As regards omega-3 fatty acids, a statistically significant difference was found in dietary groups due to a higher percentage of ALA in vegan milk ($p < 0.001$) – the mean percentage of ALA in breast milk samples was 2.09% for vegans, 1.55% for vegetarians and 1.19% for omnivores. There was no statistically significant difference in DHA concentration in any dietary group ($p = 0.543$). Moreover, the majority of participants had breast milk DHA concentration below 0.3% and no significant correlation was found among them ($p = 0.555$). In the analysis of total omega-6 fatty acids concentrations, there was no statistically significant difference in any dietary group ($p = 0.492$). However, omnivores had more gamma linolenic acid (0.08%; $p < 0.001$). 13.5% of participants supplemented DHA/EPA – taking supplements proved to be a positive predictor of ALA ($\beta = 0.62$, $p = 0.002$), DHA ($\beta = 0.10$, $p = 0.017$), total omega-3 fatty acids ($\beta = 0.71$, $p < 0.001$) and a decreased concentration ratio of omega-6, omega-3 fatty acids in breast milk ($\beta = -2.84$, $p = 0.005$) (Perrin et al. 2019).

Sanders et al. assessed human milk samples with regard to fatty acid composition. As a result, the difference in the fatty acid profile was significant ($p < 0.001$) due to dietary groups, apart from arachidonic acid (AA 20:4 ω 6) and dihomogamma-linolenic acid (20:3 ω 6). The amount of short-chain fatty acids, i.e. with 10–14 carbon atoms, transpired to be higher in vegans than in vegetarians and omnivores; similar results were found for linoleic acid and α -linolenic acid. As far as fatty acids with 16–18 carbon atoms were concerned, their ratio was higher in omnivores than in vegetarians and vegans. In comparison to other dietary groups, the proportion of DHA in milk of vegans tended to be lower. In addition, vegans had lower ratio of LA/ALA in breast milk, however the omega-6 and omega-3 ratio was higher than in other groups. The results of milk analysis seemed to be parallel to the analysis of dietary habits – the intake of linoleic acid (18:2 ω 6) was greater in vegans than in other groups and the intake of α -linolenic acid (18:3 ω 3) turned out to be higher in vegans and white vegetarians than in Hindu vegetarians and omnivores (Sanders, Ellis, and Dickerson 1978).

In another study by Sanders et al. analyzing fatty acid composition, breast milk of vegan mothers tended to have the lower proportion of C16:0, C16:1, C18:0, C20:4 ω 3 ($p < 0.05$) fatty acids and the higher proportion of C18:2 ω 6, C18:3 ω 3 and 20:2 ω 6 ($p < 0.05$) fatty acids than omnivores (Sanders and Reddy 1992).

Proteins

The total protein content was estimated in 3 studies (Bijur and Desai 1985; Finley et al. 1985b; Debski et al. 1989).

Both Bijur et al. and Debski et al. observed no significant differences in particular dietary groups with regard to total protein concentrations in breast milk (Bijur and Desai 1985; Debski et al. 1989). According to Bijur et al. mean values constituted 1.122 (± 0.072) gm/dl for lacto-vegetarians, 1.221 (± 0.064) gm/dl for occasional meat eaters and 1.216 (± 0.049) gm/dl for frequent meat eaters. On the basis of the data available from authors, the estimated protein intake, including animal protein intake in non-vegetarians, transpired to be lower than in western countries (Bijur and Desai 1985). According to Debski et al. mean protein concentration in vegetarians' milk was 10.2 (± 1.4) g/100 ml and 9.9 (± 1.1) g/100 ml in non-vegetarians' milk (Debski et al. 1989).

Finley et al. discovered that the protein levels in milk from mothers in particular dietary groups were different because of the course of lactation. After 7–20 months of lactation, milk samples from vegetarian mothers had lower content of proteins ($0.44 \pm 0.46\%$, $n = 52$ samples from 11 women) than those from non-vegetarian ones ($1.83 \pm 0.48\%$, $n = 21$ samples from 16 women). As far as the dietary protein intake is concerned, even though vegetarians consumed less total proteins than non-vegetarians, both groups covered 100% of the Recommended Dietary Allowance. However, in both groups the levels of milk proteins were unrelated to dietary protein intake (correlation coefficient = 0.06). Similarly, neither a part of a day of milk expression, nor month of lactation, nor an interval between milk expressions (partial correlation coefficient = 0.02) are related. In both groups, the level of milk proteins decreased in the first 6 months of lactation ($r = -0.43$) and did not change significantly in subsequent 14 months of lactation (Finley et al. 1985b).

Taurine

The analysis of taurine concentration in breast milk samples with regard to the dietary preferences was conducted in 2 studies (Rana and Sanders 1986; Kim et al. 1996). Rana et al. compared the group of omnivores with the group of vegans, both living in Seoul, in terms of taurine dietary intake, plasma concentration, urinary excretion and breast milk concentration. The breast milk samples analyzed in the study included also samples taken from another research. The authors found significantly lower (than in omnivore samples) mean taurine concentrations in vegan milk samples ($p < 0.01$). Further analysis revealed that, when compared with omnivores, vegans consumed significantly less protein and their meals contained no preformed dietary taurine. Furthermore, the urinary excretion of taurine was significantly higher in omnivores, whereas the plasma mean concentrations were similar in both groups (Rana and Sanders 1986).

Kim et al. analyzed the taurine concentrations in human milk in several stages of lactation, comparing samples from lacto-ovo-vegetarians and omnivores. In both groups, the longer the course of lactation was, the less taurine the breast milk contained ($p < 0.05$). With regard to the dietary group, lacto-ovo-vegetarians had significantly lower milk taurine

concentrations after 90 days postpartum than omnivores. The taurine intake in breastfed infants was estimated by calculations – multiplying mean taurine concentrations in several stages of lactation by an average breast milk intake, measured by weighing method. Until 120 days postpartum, taurine intake was similar among infants of non-vegetarian mothers. After 30 days postpartum, a gradually decreasing taurine intake was observed in infants of vegetarian mothers. After 60 days postpartum, there was a significant difference in comparison to the control group – $p < 0.05$ (Kim et al. 1996).

Vitamin B12

Vitamin B12 was analyzed in 4 articles (Bijur and Desai 1985; Specker et al. 1990; Patel and Lovelady 1998; Pawlak et al. 2018). Two studies incorporated Indian vegetarian women to the study groups (Bijur and Desai 1985; Patel and Lovelady 1998). The results of both studies revealed that mean serum and vitamin B12 levels were lower in milk from vegetarian women than from the non-vegetarian control groups (Bijur and Desai 1985; Patel and Lovelady 1998). Bijur et al. found those differences between dietary groups as statistically insignificant ($p > 0.05$) (Bijur and Desai 1985), whereas Patel et al. – statistically significant ($p < 0.05$) (Patel and Lovelady 1998). As far as the vitamin B12 concentrations in milk are concerned, in both studies vegetarian mothers proved to have lower levels, with a statistically significant difference according to Patel et al. ($p = 0.006$) (Patel and Lovelady 1998). However, according to Patel et al. the number of daily servings of dietary cobalamin sources consumed did not significantly correlate with vitamin B12 concentrations in milk, although the mean dietary intake was significantly lower in the vegetarian group. In addition, among the control groups, Indian women proved to have lower vitamin B12 concentrations in breast milk and comparable levels in serum ($p = 0.06$) (Patel and Lovelady 1998).

Pawlak et al. and Specker et al. also found lower vitamin B12 concentrations in milk from mothers following meatless diet, than in omnivore control groups (Specker et al. 1990; Pawlak et al. 2018). Nevertheless, according to Pawlak et al. the measured values of breast milk vitamin B12 did not differ significantly by dietary pattern. It should be mentioned, that 78.4% of all participants used multivitamins containing vitamin B12, whereas 25.7% of them used individual vitamin B12 supplements (Pawlak et al. 2018).

In all 3 studies, where serum and milk vitamin B12 concentrations were assessed concomitantly, the positive correlation between measured values was found (Bijur and Desai 1985; Specker et al. 1990; Patel and Lovelady 1998). Moreover, Patel et al. has proved that the duration of lactation negatively affects the content of vitamin B12 in breast milk ($p = 0.04$) (Patel and Lovelady 1998), whereas neither Pawlak et al. nor Specker et al. did not consider the duration of lactation as a factor affecting vitamin B12 levels in milk ($p > 0.05$) (Specker et al. 1990; Pawlak et al. 2018).

In 3 papers, the supplementation was taken into consideration during analyses. According to Pawlak et al. taking vitamin B12 supplements proved to be a positive predictor

of breast milk vitamin B12 concentration ($p = 0.028$), whereas taking multivitamins did not affect milk vitamin B12 content significantly (Pawlak et al. 2018). On the contrary – Patel et al. did not find any significant correlation with regard to that issue (Patel and Lovelady 1998). None of the participants of Specker et al.'s study was administered commercial supplements.

According to Specker et al. the length of any type of vegetarian diet is a factor inversely correlated with breast milk vitamin B12 concentration ($p = 0.03$) (Specker et al. 1990).

Minerals

Finley et al. analyzed milk samples for iron, copper, zinc, calcium, magnesium, potassium and sodium concentrations. As both vegetarian and non-vegetarian women were well-nourished, there was no statistically significant difference in breast milk's inorganic constituent composition. Moreover, some differences in dietary mineral intake did not affect milk composition significantly. Longitudinal changes in concentration of inorganic constituents in milk have revealed to be similar in both groups. During the first 6 months of lactation, there was a decreasing trend in the concentrations of zinc ($r = -0.7$) copper ($r = -0.69$), sodium ($r = -0.56$), potassium ($r = -0.56$), iron ($r = -0.43$), whereas the level of magnesium increased ($r = +0.31$). Within subsequent 14 months the level of calcium decreased ($r = -0.41$), whereas the level of zinc increased ($r = +0.39$). Between the 9th and 20th month of lactation, there was an increase in sodium level ($r = +0.39$). Between the 7th and 20th month of lactation, the concentrations of sodium and potassium remained stable, without any significant fluctuations (Finley et al. 1985b).

Selenium

In undialysed milk samples, the selenium concentration was significantly higher in the case of vegetarian mothers in comparison to non-vegetarian ones (22.2 ± 0.8 ng/ml vs 16.8 ± 1.3 ng/ml), whereas there was less significant difference between both study groups in dialyzed milk samples (10.9 ± 0.7 ng/ml vs 10.6 ± 0.6 ng/ml). Both groups of mothers had comparable dietary intake of selenium (vegetarian women: 101 ± 6 ng; non-vegetarian women: 106 ± 5 ng) during the 4-6 months of lactation. According to Debski et al. a variance in concentrations of undialysed milk samples was unlikely to result from the dietary habits (Debski et al. 1989).

Selenium was also found as a fraction associated with proteins, mainly of high molecular weights. However, the increase in vegetarian milk selenium content was not associated with a proportional increase in selenoprotein concentrations (Debski et al. 1989).

Lactose

The lactose concentration in breast milk samples from vegetarian mothers was found to be similar to the lactose levels

in samples from non-vegetarian mothers. Furthermore, the lactose concentration remained stable during the course of lactation among all participants. The dietary intake of carbohydrates did not affect the lactose concentration in breast milk significantly (Pearson correlation coefficient: 0.04) (Finley et al. 1985b).

Folic acid activity

The folic acid content in human milk was assessed using microbiological method of folic acid activity evaluation by Bijur et al. No statistically significant difference between vegetarians and omnivores was found (Bijur and Desai 1985).

Non-nutritive components

Brain derived neurotrophic factor (BDNF)

BDNF was not detected in any of breast milk samples. The analysis was proceeded on two groups of samples - prior to the analysis, one group was prepared according to the instruction of commercial ELISA kit, another group was centrifuged with higher speed prior to improve BDNF capacity (Perrin et al. 2019).

Free nucleotides and nucleosides

Liao, et al. provided data on mean concentrations of nucleosides and nucleotides among all mothers. The mean total nucleotides and nucleosides concentrations constituted 213.15 (SD \pm 73.26) μ mol/l and 16.38 μ mol/l. The concentrations of both nucleotides and nucleosides decreased during the course of lactation. Cytidine diphosphate was found to be the prevalent nucleotide in breast milk, regardless of the stage of lactation and dietary habits of the mothers. In comparison to the omnivore group (*U* Mann Whitney test, $\alpha=0.05$), vegetarian mothers had high free nucleotide concentration in breast milk ($p=0.037$), with no statistical difference in free nucleoside concentration ($p=0.076$) (Liao et al. 2011).

Glutathione peroxidase activity

The analysis of breast milk samples has revealed a high activity of GSH-Px in milk of vegetarian women when compared to non-vegetarian women. A linear correlation between Se concentration and GSH-Px activity was found ($r=0.76$, $p<0.001$) along with a correlation coefficient equal to 0.72 in vegetarian women's milk and 0.60 in omnivore women's milk. In addition, a relationship between milk GSH-Px activity and linoleic acid content was revealed ($r=0.68$, $p=0.0001$). However, on the basis of other studies, the authors suggested that GSH-Px activity partially resulted from the presence of GSH-Px isozymes in human milk - their biological function is to protect lipids from oxidative stress. Overall, the GSH-Px activity in vegetarian and non-vegetarian women's milk accounted, respectively, for 37% and 23% of total peroxidase activity. The total peroxidase activity in milk samples from both groups remained

without any statistically significant difference (Debski et al. 1989).

Discussion

Over the past few decades the attempts to investigate the relationship between such maternal factors as the nutritional status and dietary habits, including the intake of particular nutrients, and the composition of human milk have been made (Bravi et al. 2016; Innis 2014; Butte et al. 1984; Brown et al. 1986). The impact on particular milk components is related to not only current mother's dietary intake, nutrients reserves and nutrients utilization, but depends on genetic determinants, effectiveness of metabolic pathways, comorbidities, environmental conditions, infant's gender, gestational age at birth and postnatal age, as well nutrients digestibility and bioavailability - all of the aforementioned factors might result in positive, neutral or negative change in the concentrations of the breast milk compounds (Institute of Medicine 1991; Bravi et al. 2016). In general, milk production and milk quality is not significantly affected by mild or moderate variations in maternal nutrition (Dewey 1998; Lovelady 2004; Keikha et al. 2017). Longer periods of dietary restrictions, both quantitative and qualitative, do not have any pronounced effect on human milk, provided that the mobilization of maternal body reserves is sufficient for milk synthesis (Institute of Medicine 1991; Butte et al. 1984; Dewey 1998; Lovelady 2004). As the energy demands to exceed normal metabolic needs during the lactation process, the nutrient needs of breastfeeding mothers increase to compensate for the daily nutrient output into breast milk (Institute of Medicine 1991; Butte et al. 1984). Studies conducted on overweight lactating women revealed that neither physical activity, nor dietary restrictions affect the quantity and composition of breast milk, as well as the infant's growth - but the lactation should be stabilized (Lovelady 2011). On the contrary, the composition of some nutrients in human milk can be significantly affected by prolonged nutritional deprivation and insufficient mother's tissue stores (Institute of Medicine 1991; Brown et al. 1986). Due to e.g. low socioeconomic status, temporary periods of fasting preceded by insufficient daily provision of nutrients, might affect both mother's nutritional status and micronutrients concentrations of breast milk (Rakicioglu et al. 2006).

As the mother's nutritional status is considered to have impact on the quality and quantity of breast milk, several studies investigated the association of maternal anthropometric measurements with breast milk composition. In the recent studies, the milk protein concentrations were positively correlated with bioelectrically estimated maternal body composition: muscle, fat and fat-free mass. The milk fat concentrations and milk caloric value depended on body weight and body mass index (BMI) (Bzikowska-Jura et al. 2018; Bzikowska et al. 2018).

The studies included in this systematic review were conducted on women whose anthropometric measures were within normal range, what was adopted for indicator of

nutritional status (data from 46.15% studies) – regardless of their socio-economic status and educational background. Even though a tendency to lower body weight was found among vegans, their anthropometry remained within normal range. Nevertheless, the physical measures (e.g. weight, BMI, skinfold thickness) do not constitute a reliable tool for the assessment of nutritional status. Apart from the risk of errors arising out of inadequate training of personnel, improper technique of measurements, difficulties in measurement of certain anthropometric characteristics such as skinfolds, the reference data should be representative of the investigated community. Moreover, anthropometry is insensitive to detect changes in nutritional status following inadequacy of food over short periods of time, nor unable to distinguish specific nutrients deficiencies as a cause of undernutrition.

The results of the reviewed studies did not present any significant impact of body weight on breast milk components. However, the detailed data concerning participants' nutritional status and anthropometric measures was limited, since further investigation is needed to verify whether body composition differs due to the diet and intake of animal-origin products, and whether it affects breast milk components.

In addition, regardless of the methods of analyses as well as the preparation and storage of the collected human milk samples, the results of investigations were comparable.

As far as the impact of mother's diet on human milk nutritional components is concerned, the macronutrient content seems to be less affected by dietary habits and changes during the course of lactation as a result of the infant's needs. According to current knowledge, maternal dietary intake do not affect the breast milk proteins and lactose composition (Bravi et al. 2016; Schanler 2011). Similar conclusions have been reported in the analyzed studies – regardless of other factors, the breast milk composition was not significantly different between mothers following a meatless diet and omnivores. In fact, a properly planned and well-balanced vegan diet can fully cover nutritional needs for proteins. However, as the digestibility of plant-derived proteins can be limited, the daily intake of protein should be increased by 10% during lactation (Agnoli et al. 2017; Baroni et al. 2019). Considering amino acids profile, the difference in taurine concentrations was found, as omnivores' milk was significantly more rich in this amino acid, what might have been attributable to dietary intake (Kim et al. 1996). Taurine is considered as a factor regulating the development of fetal and neonatal nervous system. This amino acid is provided by mother via placenta or with breast milk. According to existing knowledge, both maternal obesity and maternal malnutrition can adversely influence the transfer of taurine from mothers to fetuses or infants and probably impair the neural development (Tochitani 2017). Further research with consideration given to other factors, e.g. maternal nutritional status, comorbidities, stage of lactation are needed to assess whether dietary habits have a significant impact on taurine concentration in breast milk.

On the contrary, maternal dietary fat intake, regardless of the type of the diet, has an impact on the quality of fat in

breast milk, with insignificant effect on its quantity. Mothers on a low-fat diet produce breast milk with a slightly higher fraction of medium-chain fatty acids when compared with mothers following a high-fat diet (Lönnerdal 1986; Innis 2014; Kelishadi et al. 2012).. As plant-based diet is generally rich in linoleic acid and omega-6 polyunsaturated fatty acids and deficient in good sources of omega-3 fatty acids and monounsaturated oils, the tissue fatty acids profile in vegetarians and vegans is thought to have higher proportions of omega-6 fatty acids and lower proportions of omega-3 fatty acids, compared to omnivores (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Agnoli et al. 2017; Sanders 1999). Similar findings were reported in the reviewed studies (Sanders, Ellis, and Dickerson 1978; Finley et al. 1985a; Sanders and Reddy 1992). Nevertheless, fatty acids occur in milk not only as a result of dietary intake, but also as mobilization from body fat reserves and endogenous synthesis by the mammary glands. For this reason, even if maternal diet is deficient in some fatty acids, they are still present in human milk (Institute of Medicine 1991; Neville and Picciano 1997).

The impact of maternal dietary habits on the fatty acid composition of milk concerns mainly fluctuations in the content of essential polyunsaturated fatty acids, with a special interest on DHA and EPA (Sanders 1999). Vegans consume quite big amounts of omega-6 but marginal amounts of omega-3 fatty acids, which is mainly ALA (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Van Winckel et al. 2011). In the periods of pregnancy and lactation, the metabolites of ALA, DHA and EPA, are indispensable for the development of the retina and central nervous system (Agostoni et al. 2009; Van Winckel et al. 2011; Sanders 1999). The daily intake of DHA and EPA is very low among vegans, as these omega-3 fatty acids might be found only in algae or fortified products, mainly plant-based beverages (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Baroni et al. 2019). It is assumed, that DHA and EPA can be synthesized from ALA, which requires sufficient amounts of ALA, optimal omega-6/omega-3 ratio of fatty acids daily intake, but also proteins, pyridoxine, biotin, calcium, copper, magnesium, and zinc involved in DHA production. However, the process of endogenous conversion to DHA is highly inefficient and do not cover daily requirements. Serum levels of DHA and EPA were found to be lower in vegan and vegetarians compared to non-vegetarians. Similar results were obtained in studies investigating omega-3 fatty acids content in breast milk, cord blood, and erythrocytes lipid membrane (Van Winckel et al. 2011; Baroni et al. 2019). All these conclusions have been reflected in the results of analyzed studies – breast milk of vegan mothers is deficient in DHA, since the supplementation of preformed forms of DHA is essential. As an alternative for vegan mothers, algal-derived DHA, accepted by FDA, is available (Baroni et al. 2019).

The longitudinal changes in human milk during the course of lactation appears to be identical in all mothers, regardless of their dietary habits. If lactating women are exposed to the micronutrient deficiency or daily

requirements to cover both mother's and infant's needs exceed the dietary intake, the depletion of maternal reserves affects the breast milk composition. During lactation, mothers should provide proper amounts of calcium, vitamin D, iron, iodine, as well as zinc, magnesium, vitamin B6, folate (Institute of Medicine 1991; Bravi et al. 2016). Furthermore, vegetarians, and especially vegans, should pay attention to adequate vitamin B12 intake (Melina, Craig, and Levin 2016; Agnoli et al. 2017; Baroni et al. 2019) The biggest risk of developing vitamin B12 deficiency is attributed to the vegan diet. Since cobalamin is essential for DNA creation and maintenance of nervous system, deprivation or insufficiency of its body reserves can lead to irreversible cognitive impairment and other neurological disorders, hematologic complications and even death due to qualitative malnutrition. For this reason, supplementation of vitamin B12 is strongly recommended for all vegans and advisable for vegetarians, especially for breastfeeding mothers and children – an emphasis is put on reliable additional source of cobalamin for breastfed infants whose mothers do not supplement this vitamin (Van Winckel 2017; Van Winckel et al. 2011; Sebastiani et al. 2019). In this systematic review, the time of following a plant-based diet, the duration of lactation, supplementation of vitamin B12 by mothers as well as serum level of vitamin B12 in mothers have been found as main determinants of vitamin B12 concentrations in breast milk. Proper preparation of meals, with consideration given to the digestibility and assimilation of nutrients, should not be omitted (Melina, Craig, and Levin 2016; Craig and Mangels 2009; Federal Commission for Nutrition (FCN) 2018; Agnoli et al. 2017; Baroni et al. 2019).

The purpose of this study was to collect evidence on the relationships between maternal plant-based diet and breast-milk composition, including the comparison with healthy mothers following a typical omnivore diet. The subject of breast milk composition, concentrated on particular compounds, has been already well studied. According to the current knowledge, the nutritional profile of breast milk depends not only on dietary habits, but also nutritional status, comorbidities, genetics, stage of lactation, gestational age at labor, and other environmental factors (Institute of Medicine 1991; Kleinman 2009; Keikha et al. 2017; Sanders 1999; Tochitani 2017; Miliku et al. 2019). Due to the aforementioned factors, nutritional requirements vary among individuals. As far as the nutritional adequacy of the diet is concerned, a dietary pattern that provides sufficient intake of essential nutrients and prevent depletion of the nutrients from the body ensuring its proper function can be called “well-balanced”. If the nutritional requirements exceed the daily dietary intake, especially for micronutrients, the proper supplementation should be considered.

As it was already mentioned, the official statements of professional dietetic societies and organizations regarding the impact of vegetarianism on both mothers and infants' health condition are inconsistent. Although, AND (Melina, Craig, and Levin 2016; Craig and Mangels 2009) supported the thesis that well-balanced meatless diet is safe and support sustainable growth and development in all age groups,

the FCN (Federal Commission for Nutrition (FCN) 2018), the DGE (Richter et al. 2016) and ESPGHAN (Fewtrell et al. 2017) indicates the risk of nutritional deficiencies and their irreversible consequences, thus do not recommend a vegan diet as a way of nutrition during pregnancy, lactation, infancy and childhood. Moreover, the issue of restricting animal-derived nutrients during pregnancy and lactation still arises doubt of healthcare providers managing donations of human milk.

Our study included papers published in vast period of time. The results have shown that the breast milk composition in women following plant-based and traditional diet is similar, with few differences in fatty acids profile, including strong emphasis on low DHA and EPA concentrations in milk of vegans. Moreover, among differences in micronutrient content, a significant consideration should be given to the low levels of vitamin B12 and the need of proper supplementation, optimally under the medical supervision. Regardless of a laboratory method used and the time of publication, the conclusions were consistent. However, the anthropometric measurements were the only indicators of participants' nutritional status. Future studies on the current topic are therefore recommended.

Conclusions

The question whether vegan diet disqualifies mother from breast milk donation to the milk banks is debatable. Due to the solid evidence, breast milk provides basic alimentation for preterm infants. Mother's milk is the first choice, but when its amount is insufficient, the donor's milk is an alternative (Agostoni et al. 2009; Wesolowska et al. 2018; Arslanoglu et al. 2019). With regard to human milk banking, the Neonatal Intensive Care Units require recruiting donors and donor profiles, including the data on nutritional status, dietary habits and health history, because these all factors have an impact on individual composition of breast milk (PATH, 2019; HMBASA Milk Banks Guidelines 2014; The European Foundation for the Care of Newborn Infants (EFCNI) 2018; Frischknecht et al. 2010; Wesolowska et al. 2018; Moher et al. 2009; National Heart, Lung and Blood Institute 2018; Sanders, Ellis, and Dickerson 1978; Bijur and Desai 1985; Finley et al. 1985a, 1985b; Rana and Sanders 1986; Debski et al. 1989; Specker et al. 1990; Sanders and Reddy 1992; Kim et al. 1996). The individual high needs of premature infants for nutrients – mainly proteins, calcium and phosphorus, require improving breast milk quality with fortified breast milk (Wesolowska et al. 2018; Arslanoglu et al. 2019). Provided that nourishment is satisfactory, the diet covers nutritional requirements, and the obligatory supplementation of at least DHA and vitamin B12 is provided, vegans are likely to produce as nutritionally valuable milk as omnivores. For this reason, dietary choices, if rational, should not permanently forbid breast milk donation. Each donor candidate should be assessed individually, with evaluation of health condition, nutritional assistance, medical supervision and lactational support.

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