Comparing Breastmilk Composition of Vegan, Vegetarian and Omnivorous Women: A Systematic Review

"the author not receiving breastmilk"

Hugo Koppens
h.j.koppens@amsterdamumc.nl

Mentor: Prof. dr. J.B. (Hans) van Goudoever
Department of Pediatrics, AMC, The Netherlands

PI: Prof. dr. J.B. (Hans) van Goudoever
Department of Pediatrics, AMC, The Netherlands

October 2019

Index

Abbreviations

Se: Selenium

Cbl: Cobalamin = Vitamin B12

DHA: Docosahexaenoic acid

Vit: Vitamin

Fe: Iron

Ca: Calcium

Mg: Magnesium

K: Potassium

Na: Sodium

Cu: Copper

Zn: Zinc

DDT: Dichlorodiphenyltrichloroethane

DDE: Dichlorodiphenyldichloroethylene

PCB: Polychlorinated biphenyl

a-HCH, b-HCH: a- and b-hexachlorocyclohexane

Px: Peroxidase

GSH-Px: Glutathione peroxidase

MMA: Methylmalonic acid

Mo: Month(s)

JBI: Joanna Briggs Institute

OCP: Organochlorine pesticide

Y: year(s)

ALA: Alpha-linolenic acid

LA: Linoleic acid

PCB: polychlorinated biphenyl

CVD: Cardiovascular disease

EPA: Eicosapentaenoic Acid

DHA: Docosahexaenoic Acid

Abstract

Background:

Changing view of the world by climate changes and subsequently adaptation to different diets with less meat may have an impact on the composition of breastmilk of lactating women. In just four years (2010-2014) the proportion of vegans and vegetarians in the Dutch population increased fourfold. The composition of breastmilk in itself is very diverse and depends on multiple factors. To know the impact of diet on breastmilk composition is of great importance to secure the health of our future offspring as early nutrition is associated with later health and disease. Furthermore, breastmilk may also be used for other children e.g. donor milk banks and therefore may impact the health of other children than mothers own as well. The objective of this study was to evaluate and summarize the effects of vegan and vegetarian diets on the composition of breastmilk.

Methods:

Pubmed and Embase were searched for relevant studies with no publication date limit. Inclusion criteria were: humans, breastmilk and Europe & North America. Exclusion criteria were: other diets than vegan and vegetarian, case series and articles written in other languages than English. Data were extracted by one author (HK) and all studies were critically appraised.

Results:

Out of 424 initially identified publication, 12 studies were included in this systematic review. A total of 478 participants following a vegan, vegetarian or omnivorous diet were studied. Five studies reported that unsaturated fats are higher in breastmilk of vegans and vegetarians and that saturated fats are higher in omnivores. Furthermore, total level of omega-3 fats was higher in vegans and vegetarians. Four studies reported differences in Vitamin B12 (Vit B12) concentrations (higher in omnivores) and one also reported on the impact of different Vit B12 supplements. Three studies reported on other nutrients and component. The main outcomes were a significant higher level of Selenium (Se) and lower levels of organochlorine pesticide (OCPs) and polychlorinated biphenyls (PCBs) in milk of vegans and vegetarians.

Conclusion:

Small sample sizes and wide variation in outcome measures do not allow a definitive statement. However, in general these data suggest, although preliminary, that it seems safe to allow all diets during lactation. But vegans and vegetarians should be advised to use specific Vit B12 supplementations during lactation.

Samenvatting

Achtergrond:

Een veranderende kijk op de wereld vanwege klimaatverandering en de hierop volgende aanpassing aan verschillende diëten met minder vlees hebben mogelijk invloed op de samenstelling van borstvoeding. In slechts vier jaar tijd (2010-2014) is de groep veganisten en vegetariërs in de Nederlandse bevolking verviervoudigd. De eigenlijke samenstelling van borstvoeding is erg divers en hangt af van verschillende factoren. Om de invloed van een dieet op de samenstelling van borstvoeding te weten is van groot belang voor de gezondheid van onze toekomstige kinderen omdat voeding in de vroege levensfase is geassocieerd met gezondheid en ziektes later in het leven. Bovendien mag borstvoeding ook gebruikt worden voor andere kinderen door middel van onder meer donormelk banken en daarom heeft het mogelijk invloed op de gezondheid van andere kinderen dan van de moeder zelf. Ons doel was om de effecten van veganistische en vegetarische diëten op de samenstelling van borstvoeding te evalueren en samen te vatten.

Methode:

Pubmed en Embase zijn doorzocht voor relevante studies met hieraan geen publicatie datum limiet verbonden. Inclusie criteria waren: mensen, borstvoeding en Europa & Noord Amerika. Exclusie criteria waren: andere diëten dan veganistische en vegetarische diëten, case reports en artikelen geschreven in een andere taal dan in het Engels. De data is onderzocht door één auteur (HK) en alle studies zijn tevens kritisch beoordeeld.

Resultaten:

Van de 424 initieel geïdentificeerde publicaties werden 12 studies geïncludeerd in deze systematische review. Een totaal van 478 deelnemers die een veganistisch, vegetarisch of omnivoor dieet volgden werden bestudeerd. Vijf studies gaven weer dat ongesatureerde vetten hogere waarden hebben bij veganisten en vegetariërs en dat gesatureerde vetten hogere waarden hebben in omnivoren. Verder was het totale level van omega-3 vetten hoger bij veganisten en vegetariërs. Vier studies rapporteerden over verschillen in vitamine B12 (Vit B12) concentraties (hoger bij omnivoren) en één van die studies rapporteerde ook over de invloed van verschillende Vit B12 supplementen. Drie studies gaven andere voedingsstoffen en samenstellingen weer. De belangrijkste uitkomsten waren dat er significant hogere waarden waren van Selenium (Se) en lagere waarden van organochlorine pesticiden (OCPs) en polychlorinated biphenyls (PCBs) in veganisten en vegetariërs.

Conclusie:

De kleine groepen deelnemers en de grote variatie in uitkomstmaten zorgen ervoor dat het niet mogelijk is om een definitieve uitspraak te doen. In het algemeen suggereert deze data echter, alhoewel niet sluitend, dat het veilig lijkt om alle diëten tijdens de borstvoeding toe te staan. Maar

veganisten en vegetariërs zouden wel geadviseerd moeten worden om specifieke Vit B12 supplementen te gebruiken tijdens de periode van het geven van borstvoeding.

Background information

People are changing, the world is changing. Nowadays climate changes gain more attention than before and consequently, so does the attention for food and food consumption in order to reduce our footprint. Our view of food consumption has changed dramatically over recent years and more and more people start adapting their daily food intake. The percentage no-meat eaters increased from 3.9% in 2010 to 4.5% in 2012 and the group with people eating specific vegan and vegetarian diets among other things increased from 1.1% to 4.4% in just four years (2010-2014) (1). This changing view and adaptation to another diets may affect the composition of breastmilk of lactating mothers who follow specific diets. Early nutrition is important as it may affect later health and disease and therefore, the composition of human milk is also important (2).

The composition of breastmilk in itself is very diverse. The composition changes over days/weeks, during the day and even during one single episode of lactation. Diet has an impact as well. For instance, fish oil supplementation has a strong positive correlation with breastmilk fish oil content (3). However, not exactly known is whether nowadays more common diets such as vegan or vegetarian may affect the composition. Many types of specific diets are now defined; Among others you have strict vegetarian, lacto-ovo vegetarian, semi-vegetarian and fish-eater. In this thesis we only use the terms of 'vegan', 'vegetarian' and 'omnivore'. To prevent misunderstandings and to facilitate data interpretation at a higher level, all different forms of diet are put together with their synonyms and their meanings in *Table 1*. To know the impact of these diets on the composition of breast milk is of high importance as the likelihood for a prolonged breast feeding period is higher with mothers who consume vegan or vegetarian diets (4).

There are numeral case reports available about the influence of vegan and vegetarian diets on the composition of breastmilk. These reports state that vegan and vegetarian diets cause, among other things, vitamin deficiency (5), megaloblastic anemia (6-8) and developmental delay (9, 10). Most frequently the role of vitamin B12 (Vit B12) was discussed in those case reports.

Stollhoff and Schulte described a 1 1/2-year-old male with megaloblastic anemia and a progressive neurological disorder clinically resembling leucodystrophy and the relationship between vitamin B12 and brain development (11). Graham et al. described the clinical findings in six infants with nutritional vitamin B12 deficiency and long-term neurologic consequences of nutritional vitamin B12 deficiency in infants (12). However, those are all case reports, which are considered low in the pyramid of evidence. On the other hand, case reports are an excellent tool to find out safety issues, and are thus of great value. So both case reports and also a systematic review are needed to

determine whether it is safe to have a vegetarian or vegan diet while breastfeeding.

Breastmilk is not only the preferred food for mother's own child, it may also be used for other children via e.g. donor milk banks. Milk from donormilk banks is intended for preterm infants primarily, usually with a gestational age below 32-34 weeks. Breastmilk is donated by mothers who have given birth in the past couple of months and have a natural surplus of milk. At present, the national Dutch Human Milk bank excludes mothers who use a vegan or vegetarian diet. The question is whether that is appropriate.

So our objective was to identify, appraise and summarize the effects of vegan and vegetarian diets on the composition of breastmilk and to provide an advice to the Dutch national Milk Bank whether it is appropriate to exclude mothers with the aforementioned diets from donating milk.

Methods

Criteria for considering studies for this review

Studies eligible for including in this review were systematic reviews, randomized controlled trials (RCTs), cohort studies, case-control studies, cross-sectional studies and case series including >4 cases (arbitrarily set). Studies were eligible if they reported on (a) humans, (b) breastmilk and (c) Europe & North America. Studies investigating the effects of other diets than vegan or vegetarian were excluded. In addition, animal studies and case reports were also excluded.

Outcomes

The outcome was the composition of breastmilk of vegans and vegetarians compared with the composition of breastmilk of omnivores. To be more specific a comparison was made between: milk fat; fatty acid; Cobalamin (Cbl = Vitamin B12); Iron (Fe); Calcium (Ca); Magnesium (Mg); Potassium (K); Sodium (Na); Copper (Cu); Zinc (Zn); Selenium (Se); lipid; protein; lactose; dichlorodiphenyltrichloroethane (DDT); dichlorodiphenyldichloroethylene (DDE); polychlorinated biphenyl (PCB); dieldrin, a-hexachlorocyclohexane (a-HCH); b-hexachlorocyclohexane (b-HCH); hexachlorobenzene; Peroxidase (Px); glutathione peroxidase (GSH-Px); erythrocyte lipid fatty acid; infant's urinary methylmalonic acid (MMA); maternal serum Vit B12; brain-derived neurotrophic factor (BDNF); and hemoglobin.

Search methods for identification of studies

The electronic search for relevant articles was accomplished on the 17th of September 2019 by one author (HK), using Pubmed and Embase. The search included terms for 'mother', and 'breastmilk', and synonyms. The full search strategies can be found in *Appendix 1 and 2.* There were no restrictions for date of publication. Only articles written in English and with full text available were included, articles written in other languages or only available in abstract form were excluded.

Selection of studies, data collection and analysis

Studies eligible for this review were selected with help of the inclusion criteria above. One author (HK) selected the articles and sorted them by class. Of the articles selected, an abstract review or a full-text review was done for further evaluation. EndNote X9 program, by A Clarivate Analytics company, was used for managing and handling extracted references that were searched from databases. Review Manager 5.3.5, by Cochrane Community, was used to create tables to show the methodological quality of the included observational studies.

Assessment of risk of bias

Methodological quality of the included cohort studies was determined by using the Joanna Briggs Institute (JBI Critical Appraisal Checklist for respectively Cohort Studies, Analytical Cross Sectional Studies and Case Series. (13) These included respectively checklists of 11, eight and 10 items for each to assess the methodological quality of a study and to determine the extent to which a study has addressed the possibility of bias in its design, conduct and analysis.

Quality assessment of included studies was done by one author (HK). To summarize the quality of these studies, risk of bias of all items was classified in three groups: 'low', 'high' and 'unclear' risk of bias. The author (HK) of this review was not blinded to the names of the authors of the included articles while assessing the risk of bias.

Results

Study characteristics

The search strategy resulted in 170 articles identified in PubMed and 254 identified by Embase. Of the 424 articles, 142 were excluded because they were a duplicate. 203 were excluded because they were classified as 'not relevant'. Of the 79 final articles, the full text was read. Based upon this full text, 67 articles were excluded. The reasons for exclusion are mentioned in *Figure 1*.

Year of publication of all included articles varied between 1978 and 2018. *Table 2* shows an overview of study and patient characteristics of each study, if reported.

Cohort

Study characteristics

Nine cohort studies were identified in this review (14-22). These nine cohort studies together included a total of 331 patients (excluding a group of 44 patients in the study of Sanders et al. (14) and a group of 55 patients in the study of Sanders and Reddy (15) because in both studies patients were nonlactating). Four studies described the difference in fatty acid composition of breastmilk (14-16, 21), were Finley et al. (16) and Specker et al. (21) also described the difference in milk fat composition. Honzik et al. (18) and Specker et al. (22) investigated the Vit B12 composition of breastmilk. Furthermore, Specker et al. (22) also investigated the relationship between Vit B12 concentrations and maternal serum Vit B12 concentrations and the relationship between Vit B12 concentrations and infant urinary MMA. Finley et al. (17) focused mainly on the difference of micro- and macronutrients in the composition of breastmilk. They investigated the difference of Fe, Ca, Mg, K, Na, Cu, Zn, lipid, protein and lactose in breastmilk composition. Norén (19) investigated the difference in organochlorine pesticides (OCPs) (DDE, DDT, a-HCH, b-HCH and dieldrin) and PCBs in the composition of breastmilk. Debski et al. (20) mainly investigated the difference in Se concentrations in the composition of breastmilk but also focused on the enzymatic activities in breastmilk of Px and GSH-Px.

At least 558 milk samples were used to compare the influence of different maternal diets. These samples were divided over four studies (16, 17, 19, 20). Four other studies did not mention how many samples were used to do the analyses (14, 15, 21, 22). Honzik et al. (18) didn't use sample analyses to compare but used serum Cbl levels to compare the effects of different diets. Furthermore, Honzik et al. (18) didn't use diet as main outcome measurement whereas the other eight cohort studies (14-17, 19-22) did use diet as main outcome measurement. Five studies described the difference in composition of breastmilk between vegetarian and omnivore diets (16-

20). Sanders et al. (15) also used vegan diet as comparison. Three studies (14, 21, 22) used only vegan and omnivore diets to describe the difference in composition of breastmilk.

Collection of breast milk samples

Two studies (16, 17) reported that "each woman was instructed to express all of the milk from one breast suckled previously that morning, at the time of the second nursing period of the day." Furthermore, "only samples expressed between 06.00 and 13.00 hour were included" and "only samples from women nursing at least four times per day were included" Finley et al. (16) also reported that the samples a wide time period reflected; 1st through 31st month of lactation. Two studies (21, 22) described that "milk samples were collected at the first daylight feeding and were expressed by hand or by pump from one breast." Sanders et al. (14) reported that "breast-milk samples, about 20 ml, were obtained at the start of a morning feed between the 2nd and 6th months postpartum; smaller samples, about 2 ml, were also obtained in the middle and end of the feed from the same breast" Sanders et al. (15) reported that "midstream human milk samples (10 ml) were obtained approximately 6 weeks postpartum." Three studies (18-20) didn't report about the time of expressing milk.

Used definition or descriptions of diets

Two studies (16, 17) described vegetarian diet as women who consumed no meat or poultry and occasionally fish, at most twice monthly. Omnivorous diet was described as: "consumed meat, poultry, fish, and other animal products." According to two studies (21, 22) vegans were described as "women who consumed a macrobiotic diet that excluded meat, eggs and dairy products. The diet consisted of approximately 50-60% whole cereal grains, 5% soups, 20-25% vegetables, and 5-10% beans and sea vegetables. Occasionally they ate supplementary foods which included seafood, nuts and fresh fruits." Specker et al. (21) also described the omnivorous diet as a diet that contained meat, eggs and dairy products. Honzik et al. (18) described vegetarian diet as "eating only dairy product but excluding meat and eggs from their diet" and used "excluded all animal products from her diet" to describe vegan diet. Nóren (19) used no specific description for the different diets. Debski et al. (20) used "consumed eggs and dairy products in addition to foods of vegetable origin" to describe vegetarian diet. Sanders et al. (14) described vegans as "their diets should not contain any foods of animal origin , including margarines made from animal and fish oils." Sanders et al (15) used "avoid all food of animal origin" to describe vegan diet and "exclude only meat and fish" to describe vegetarian diet.

Characteristics of participants and offspring

Three studies (16, 17, 20) described that the mean age of the women in their study was 29 years. Furthermore, Finley et al. (16) and Debski et al (20) mentioned that the women came from northern California. Finley et al. (16) also mentioned that "fifty-eight percent of the women were primiparous; 19% had 2 children, 19% had 3 children, and 4% had 4 children." Debski et al. (20) mentioned that the average number of children was 1,7. None of the three studies reported on the duration of the followed diet. Honzik et al. (18) described that the mean age of the women in the applicable group (group A) was 33 years. Mean parity was 1,9. Four mothers were on a long-term vegetarian diet (4-13 years) and one mother was on a long term vegan diet. Nóren (19) described that the mean age of the mothers was 28 years and that 20 mothers were nursing infant number one, 14 mothers infant number two and 11 mothers infant number three. No information was provided about other study demographics. Only that the reported fatty fish in the diet came from the Baltic. Furthermore, vegetarian mothers have been devoted to their diet for 5 to 35 years. Sanders et al. (14) described that "vegan mothers had been on the diet for an average of 7 years (range 3 to 12 years)." They didn't mention the mean age and the parity of the vegan mothers but they did point out that the mothers were residents from the United Kingdom. Sanders et al. (15) did mention that statistical analyses was used for maternal age and parity but they didn't mention the exact data. Also there was no information in regards to duration of the followed diet. "White vegetarian and vegan subjects were identified by placing advertisements in vegan and vegetarian magazines. Indian vegetarian and omnivore subjects were randomly selected from general practice lists and from patients attending the Northwick Park Hospital in North London." Specker et al. (21) reported that mean maternal age was 30 years (range 22-35 years) and the women on a vegetarian diet adhered the diet for 36-132 months. "The comparison group was recruited from the Boston area." No data were available for the vegetarian group. Also no data about parity were available. Specker et al. (22) reported that maternal age was 31 years (range 22-40 years) and that mothers were practicing a vegetarian diet for 14-137 mo (median 72 mo). No information was reported about the duration of the followed diet or other study demographics.

Outcomes

Four studies (14-16, 21) reported on fatty acid as outcome measurement. Two of these studies also mentioned milk fat (16, 21). Finley et al. (16) stated that of the 14 identified fatty acids in breastmilk four (C16:0, C16:1w9), C18:0 and C18:1w9) were significantly lower at vegetarians. However, one (C18:2w6) was significantly higher because of dietary vegetable fat. According to Finley et al. (16) children nursed milk similar in fat composition to the diet of the mother. Furthermore they stated that as parity increased, percentage milk fat becomes less but the fat contained a higher proportion

of fatty acids synthesized in the mammary gland. Specker et al. (21) stated that total milk fat did not differ between vegans and omnivores. Furthermore, they mentioned that dietary length may be of an influence of the percentage of some fatty acids (resp. palmitoleic = C16:1) in milk. Resulting in a lower percentage for women consuming vegan diet for a longer period. Their main observation was that the percentage of four (mono)saturated fatty acids (palmitic = C16:0, palmitoleic = C16:1, stearic =C18:0 and oleic = C18:1. n-9) were significantly lower in woman consuming a vegan diet. Three unsaturated fatty acids (linoleic = C18:2. n-6, eicosadienoic = C20:2. n-6 and linolenic = C18:3) were significantly higher. No relationships with length of diet were discovered. Sanders et al. (14) only used a small part of their investigation to establish the percentage of fatty acids in breastmilk. They stated that breastmilk of vegans contained lower percentages of four fatty acids (resp. 16:0 (= palmitic), 16:1, 18:0 and 20:4w3) and higher percentages of three fatty acids (resp. 18:2w6 (= Linoleic), 18:3w3 and 20:2w6). Also interesting to mention is that they discovered that erythrocytes of breast-fed infants of vegans contained significantly less 18:1, 20:5w3 and 22:6w6. Significantly higher values of 18:2w6, 20:2w6 and 22:4w6 were found. Sanders et al. (15) didn't devote their entire research on fatty acid composition of breastmilk but nevertheless they came up with the finding that percentages of five fatty acids (resp. 10:0, 12:0, 14:0, 18:2n-6 and 18:3n-3) in breastmilk of omnivores were significantly lower and that those of five other fatty acids (resp. 16:0, 16:1, 18:0, 18:1 and 22:6n-3) were significantly higher. They also reported findings about fatty acid composition in erythrocyte phospholipids in infants exclusively breastfed by omnivore or vegan mothers but compared these with cow milk formula.

Two studies (18, 22) reported on Vit B12 composition in breastmilk. Honzik et al. (18) analyzed Cbl levels in breastmilk only at the end of their study. They found out that six mothers with infants with low Cbl levels, from which five mothers were on a normal diet and one on a vegetarian, had lower levels of Cbl in breastmilk than seven healthy controls. Furthermore, they stated that among other things vegan and vegetarian diet of mother had an impact on Cbl levels of their infants. But they didn't relate this with breastmilk. They concluded that duration and severity of maternal Cbl deficiency were main factors in the degree of expression of Cbl deficiency in infants. They also stated that in vegetarian families using only a few Cbl-containing supplements or in children exclusively breastfed far beyond one year relatively mild deficiency cumulates over time leading towards severe clinical symptoms. Specker et al. (22) concluded that vegans had a significantly lower Vit B12 concentration in breastmilk. This was also inversely correlated with length of time on vegan diet. So increasing length of diet caused a significant decrease in Vit B12 concentrations in breastmilk. Furthermore, they concluded that breastmilk Vit B12 concentrations were correlated with maternal serum Vit B12 concentrations, breastmilk Vit B12 concentrations were inversely correlated with

maternal urinary MMA concentrations and that there was a significant relationship between infant urinary MMA and Vit B12 concentrations of breastmilk.

Two studies (17, 20) reported other micronutrient outcomes than Vit B12. Finley et al. (17) stated that besides some differences in protein levels (vegetarians had lower milk concentrations at 7-20 mo) and fatty acids (both protein levels and fatty acids aren't investigated any further) dietary intake, provided that participants are well-nourished, did not significantly affected milk composition between vegetarians and omnivores. They found out that during the first six months of lactation levels of Zn, Cu, Na, and K decreased significantly. Fe and protein concentrations only decreased very strongly during the first two months of lactation. Furthermore, between month 9-20 of lactation Na concentrations increased and between month 12-20 Zn levels also increased significantly. Breastmilk lipid concentrations remained constant during the first six months of lactation. After that lipid concentrations increased. Lactose remained constant for the entire period of 20 months. They also found out that during the first six months of lactation three pairs of inorganic constituents were positively correlated with each other. These pairs were Cu and Zn, K and Cu, and Ca and K. Finally they stated that there were four other significantly correlations. Fe was positively correlated with Na and Ca, Mg negatively with Zn and Na positively with Cu. Debski et al. (20) focused on Se concentrations in breastmilk. They found out that the quantity of Se in vegetarian breastmilk was 132% compared with milk of omnivores. Activity of GSH-Px in milk samples from vegetarians was significantly higher but total Px activity was not significantly different. Overall milk concentration of Se was linearly correlated with GSH-Px activity. Furthermore they found out that there was a significant relationship between milk GSH-Px activity and linoleic acid content. However, there was no difference found in dietary intake of Se so this implies that there are other factors playing a part. Debski et al. (20) mentioned that bioavailability may be one of these factors. Because bioavailability of Se has been shown lesser in animal sources. Geographic location of the mother may also play a role.

Finally, Norén (19) reported about OCPs and PCBs as outcome measurement. Norén (19) reported that PCBs and OCPs levels except a-HCH and b-HCH were found lowest in vegetarians. Furthermore, a positive correlation between DDT, DDE and PCBs and age for mothers nursing their first infants is discovered. However, Norén (19) stated that many factors besides diet may influence concentrations of OCPs and PCBs. Smoking habits, age and degree of polluted environment among other things may affect the levels of OCPs and PCBs.

Quality assessment

The methodological quality of Finley et al. (16) and Sanders et al. (15) was assessed as high (*Table 3*). All information was available or not applicable (*Appendix 3*). Four studies (17, 18, 20, 21) were overall assessed to be of moderate methodological quality (*Table 3*). Two studies (18, 21) gave no information about follow-up. Furthermore, Debsik et al. (20) reported no strategies to deal with confounding factors. Finley et al. (17) didn't report strategies to address incomplete follow-up. For detailed information see *Appendix 3*. Three studies (15, 19, 22) were overall assessed to be of low methodological quality (*Table 3*). Norén (19) reported no strategies to deal with confounding factors. Furthermore, Norén (19) provided no information about statistical analyses or about the similarity of exposure measurement. Sanders et al. (15) didn't report about strategies to deal with confounding factors, nor about follow-up or similarity of exposure measurement. Specker et al. (22) reported nothing about similarity of exposure measurement, follow-up or the identification of confounders. For detailed information see *Appendix 3*.

Cross-sectional

Study characteristics

Two cross-sectional studies were identified in this review (4, 23). The two studies had a total of 148 patients together. Pawlak et al. (4) described the difference in Vit B12 content of breastmilk. Perrin et al. (23) wanted to assess the difference in fatty acid and BDNF concentrations in breastmilk of mothers on a vegan, vegetarian and omnivorous diet.

148 milk samples were used to compare the influence of different maternal diets. Samples were equally divided over both studies (4, 23).

Collection of breast milk samples

Pawlak et al. (4) reported that "each participant was asked to provide a breast-milk sample, which was either collected in person or received via a shipment on dry ice to our laboratory. Participants were instructed to collect the sample in the morning, during the first or second feeding of the day and ≥2 h since the previous feeding. Milk was to be collected in a dimly lit room to help protect light-sensitive nutrients. Women were instructed to completely express the content of 1 breast using the expression method of their choice." Perrin et al. (23) reported exactly the same with note that "to ensure a representative milk sample was collected, participants received written collection instructions."

Used definition or descriptions of diets

Pawlak et al. (4) described vegans as "participants who did not ingest any meat but may have ingested other animal products <1 time/mo." Vegetarians were described as "participants who did not eat meat but regularly ingested other animal products, such as eggs or dairy." Furthermore, they stated that "any B-vitamin supplementation was defined as using any of the following: individual vitamin B-12 supplements, Bcomplex supplements, multivitamin supplements, or prenatal vitamin supplements." Perrin et al. (23) described vegans as participants that "never consumed meat and never or rarely consumed other animal products." Vegetarians were described as participants that "never or rarely consumed meat and sometimes or often consumed other animal products." Furthermore, omnivores were described as participants that "consumed meat sometimes or often."

Characteristics of participants and offspring

Pawlak et al. (4) described that maternal age was between 18-46 y and that participants were recruited from the United States. Furthermore, they mentioned that "vegan participants adhered to their diet for a mean ± SD of 6.2 ± 5.5 y (range: 0.5–23 y; median: 4.3 y), whereas vegetarians practiced their diet for a mean of 7.5 ± 5.5 y (range: 1 mo to 20 y; median: 6.0 y)." Finally they stated that "there were no significant differences between the mean age of participants (P = 0.438), gravida (P = 0.170), parity (P = 0.158), ethnicity (P = 0.900), or education (P = 0.279) by diet group. There was a significant difference in stage of lactation (P = 0.017) and maternal BMI between diet groups (P = 0.021), with vegans having the lowest and nonvegetarians having the highest BMI" Perrin et al. (23) stated that women came from the United States and had to be 18 years or older. Perrin et al. (23) also stated that "there was no significant difference in age, parity, ethnicity, or education ($p > 0.05$), but maternal BMI and lactation stage differed ($p < 0.05$) by diet type."

Outcomes

Pawlak et al. (4) reported that there was no significant difference in low Vit B12 between the three diet groups. But they also stated that 58 of the 74 participants used supplements containing Vit B12 and that there was a significant difference between diet groups (vegan = 44.8%, vegetarian = 26.3%, nonvegetarian = 3.9%; P = 0.001). Furthermore, individual Vit B12 supplements had a significant positive predictor of milk Vit B12 concententrations in contrast with other supplements who had no or even a negative relation with Vit B12 milk concentration. Perrin et al. (23) reported that there was no BDNF detectable in any of the samples, possibly due to excluding participants from the early postpartum period. Perrin et al. (23) also found no differences in docosahexaenoic acid (DHA) concentrations at different diet groups, but over 80% had "milk concentrations below the 0,30% target suggested by expert committees." Possibly due to widespread inadequate DHA levels in the

United States. Furthermore, there were differences found in numerous fatty acids. Levels of saturated fats and four of its fatty acids (resp. C15:0, C16:0, C17:0 and C18:0) were significantly higher in omnivores compared to vegans (lowest) and vegetarians. The levels of eight other fat compositions of breastmilk were also significantly higher (resp. ratio of linoleic acid (LA) : alpha-linolenic acid (ALA), C:18:3 *cis*, Omega-6: omega-3, C14:1 *cis*, C:16:1 *cis* 9, C:17:1 *cis*, total *trans* and C16:1 *trans*). Levels of unsaturated fats and two other fat compositions (resp. Total omega-3 and ALA (C18:3 *cis*)) were significantly higher in vegans than they were in vegetarians and omnivores (lowest). Furthermore, levels of C18:1 *cis* were higher in vegans.

Quality assessment

The studies of Pawlak et al. (4) and Perrin et al. (23) were overall assessed to be of high methodological quality (*Table 4*). All information was accounted for. For detailed information see *Appendix 4*.

Case series

Study characteristics

One case series was identified including a total of six patients (12). Graham et al. (12) described the long term consequences of nutritional Vit B12 deficiency in infants, but milk samples were not analyzed. Furthermore, no specific descriptions about diet were given except for two vegan and one vegetarian mother being part of the study.

Characteristics of participants and offspring

Graham et al. (12) described that two 14-mo old girls, two 14-mo old boys, one 8-mo old girl and a 15-mo old boy participated. The two 14-mo old girls had a normal intellectual outcome on later age, a 15-mo old boy and an 8-mo old boy had borderline on later age and one 15-mo old boy and the 8-mo old girl had uncertain intellectual outcomes on later age. All the children were diagnosed in Australia.

Outcomes

Graham et al. (12) stated that after treatment with Vit B12 all six children had a prompt recovery of their symptoms. The follow-up period differed between 6 mo and 8 y. After follow-up three of the children had a normal neurological recovery. Two did recover but not towards normal levels. One patient had no follow-up. Due to the fact that Vit B12 is not synthesized in humans, Graham et al. (12) states that diet is important to meet nutritional requirements. Therefore, Graham et al. (12) state that “vegans and vegetarians should be made aware of the importance of adequate Vit B12

intake during pregnancy and lactation."

Quality assessment

The study of Graham et al. (12) was assessed to be of moderate methodological quality (*Table 5*). No information was provided about inclusion criteria. Furthermore, Graham et al. (12) reported much too little about the presenting clinic's demographic information or even nothing about demographics of the participants. For detailed information see *Appendix 5*.

Discussion

Key results

This systematic review identified, appraised and summarized a total number of 12 studies, including nine cohort studies (14-22), two cross-sectional studies (4, 23) and one case series (12).

Five studies (14-16, 21, 23) reported on fatty acid concentrations of breastmilk. They all came to the conclusion that the concentration of saturated fats is lower in vegans and vegetarians compared to omnivores and the concentration of unsaturated fats is higher in vegans and omnivores compared to omnivores. Furthermore, Perrin et al. (23) stated that the total level of omega-3 fats was also higher in vegans and vegetarians compared to omnivores. This all makes perfect sense when you compare the diet of vegans, vegetarians and omnivores. Maternal plant-based diets are well known for their richness in unsaturated fats (23) where meat is richer in saturated fats. Sanders et al. (14) states that due to these differences in fatty acid composition of breastmilk you could suggest that vegan and vegetarian diets have a hypocholesterolemic effect compared to omnivorous diets although the most recent Cochrane stated that moderate- and high-quality evidence suggests that increasing eicosapentaenoic acid (EPA) and (DHA) has little or no effect on mortality or cardiovascular health. On the other hand, low-quality evidence suggests ALA may slightly reduce cardiovascular disease (CVD) event and arrhythmia risk (24). Taken together vegan and vegetarian certainly do not have a negative effect on the quality of lipid composition in breast milk.

Four studies (4, 12, 18, 22) reported about the Vit B12 concentrations of breastmilk. Three studies (12, 18, 22) indicated a significant lower level of Vit B12 concentrations in breastmilk of vegans and vegetarians. Only Pawlak et al. (4) reported no significant differences but most of the participants in this study used Vit B12 supplements. Interesting to mention is that individual Vit B12 supplementations were the only ones with a positive effect on Vit B12 levels in breastmilk. Interestingly, Honzik et al. (18) and Specker et al. (22) concluded that extended duration of maternal Vit B12 deficiency caused a significant decrease of Vit B12 concentrations in breastmilk. So vegans and vegetarians should take Vit B12 supplementation to avoid low concentrations in breast milk.

One study (17) reported about several micro- and macronutrients in breastmilk. Finley et al. (17) reported numerous differences in levels of micronutrients during different stages of lactation. However, provided that participants are well-nourished, dietary intake did not significantly affected milk composition between vegetarians and omnivores.

One study (20) reported about levels of Se in breastmilk. Debski et al. (20) found out that the mineral content of Se in breastmilk was significantly higher for vegetarians than with omnivores. Interesting to mention is that there was no difference found in dietary intake of Se. Debski et al. (20) stated that there must be other factors playing a part among which bioavailability may be one of them.

Finally, one study (19) reported about levels of organochlorine contaminants in breastmilk. Norén (19) reported that PCBs and OCPs levels (except a-HCH and b-HCH) were found on a lower level in breastmilk of vegetarians compared with omnivores. Norén (19) stated that levels of organochlorine pesticides and PCBs are also found most frequently in animal food. However, levels of organochlorine contaminants depend on numerous factors among which age, smoking habits and environmental pollution could be of importance.

Limitations

This review has several limitations. First, our search strategy for eligible articles did probably not reveal all relevant studies. The search strategy was executed by only one author (HK) and in just two databases (Pubmed and Embase).

Second, there was a wide heterogeneity between studies. Various types of outcome measurements were used so there was no primary outcome. Furthermore, some studies made use of the exact same population to test other outcome measurements.

Third, several other factors besides the diet of the mother might play an important role. Smoking habits, age of mother, parity and gravida, period of lactation and so on. The length of time in which a mother has devoted herself to a diet might also be of importance. Most studies did not mention this at all and a couple only mentioned it briefly.

Fourth, among other things protein levels were only mentioned briefly (resp. Finley et al. (17)) but not investigated thoroughly. To get a better picture of the impact of different diets on the composition of breastmilk we first have to decide what nutrients are essential for the development of the infant. Only then can we decide what is important for further investigation and research.

Fifth, only small groups of mothers participated in the investigated studies. It is far more difficult to draw accurate conclusions based on small populations. Furthermore, the difference between diet compositions makes it almost impossible to draw a final conclusion on the matter. Almost all studies had their own diet compositions and to make things even more difficult not all studies investigated the same diets.

In addition, the wide variation between the years of publication of studies (1978-2018) might have an impact on the overall results of this review. During this period of 40 years science has developed itself with great speed. Likewise, so has the composition of food and the environment. Results found in 1978 might not be valid in 2019.

Quality of evidence

All 12 included studies were assessed for methodological quality. There was a wide variation in methodological quality of the included studies. Overall, the quality of the cross-sectional studies was

assessed as high. Four of the cohort studies were overall assessed as moderate, five had a different methodological quality, two of which had a high methodological quality and three had a low methodological quality. The case series had an overall moderate methodological quality.

Overall completeness and applicability of evidence

The aim of this review was to summarize all available evidence of different breastmilk compositions of mothers who adhere to vegan, vegetarian or omnivorous diets.

The author (HK) included different study designs and formats with clear inclusion and exclusion criteria. Furthermore, the methodological quality of all included studies was assessed. Thereby using adequate tools for each study separately. All studies were assessed individually and the results are demonstrated in the attached tables.

The overall applicability of this review can be interpreted from two perspectives. Because of all the limitations mentioned above this review cannot be assumed to be of high applicability. However, because it is the only review found about the topic this review might be of greater value.

Implication for research

Further investigation of the effect of different maternal diets on the composition of breastmilk has to be done. Because of the small sample sizes, the wide variety of outcome measures and last but not least the growing variety of diets and people following these diets it is of great importance for our future offspring to investigate and research all possible implications. For now, we can build on this and move forward to a direction in which we need to make clear what the implications of different compositions of breastmilk are on the development of infants. Following that we can further investigate these different outcome measures in a larger cohort over time so there will be less gaps in the future evidence.

Author's conclusion

After carefully considering and reading all the available evidence the author (HK) came to the conclusion that if anybody wanted to make a statement about the effects of vegan and vegetarian diets on the composition of breastmilk the topic should be investigated more thoroughly. This systematic review showed that there lies an open field to exploit in front of us. For example, pescatarians eat only fish but no meat. Fish are well known to contain heavy metals so maybe this will also affect the quality of the breastmilk composition. But fish also contains unsaturated fats, that might affect health in a positive manner. So the question raises whether or not fish is beneficial in your diet regarding the composition of the composition of your breastmilk. Furthermore, meat contains unhealthy saturated fats but it is also a rich source of protein and Vit B12. So should we ban meat or advise people to often include meat in their diet. The solution is probably a bit of both worlds. We should not ban certain types of food entirely from our diet but we also should not consume it in excess.

Therefore, the author (HK) advises that it is best to follow an omnivorous diet which contains all ingredients of a healthy meal during lactation. But should anybody consider a different diet it is of great importance to at least add specific Vit B12 supplements during all stages of lactation to avoid Vit B12 deficiencies in (breastfed) children. Furthermore, more research is needed to determine whether there are no other significant and harming deviations in the breastmilk composition of different diets.

Acknowledgements

This work was supported by my thesis group containing seven participants, mentor E. Mank and PI and personal mentor J.B. van Goudoever. Furthermore, I would like to thank my two great friends A.D. van der Made and E.H.P.J. Radix. Special thanks to my PI for finding so much time in his busy schedule to check and appraise my work.

References

1. Nederlandse Vegetariërsbond. Factsheet Consumptiecijfers en aantallen vegetariërs 2019 [Available from: https://www.vegetariers.nl/bewust/veelgestelde-vragen/factsheet-consumptiecijfers-en-aantallen-vegetariers-.
2. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475-90.
3. Ribeiro P, Carvalho FD, Abreu Ade A, Sant'anna Mde T, Lima RJ, Carvalho Pde O. Effect of fish oil supplementation in pregnancy on the fatty acid composition of erythrocyte phospholipids and breast milk lipids. Int J Food Sci Nutr. 2012;63(1):36-40.
4. Pawlak R, Vos P, Shahab-Ferdows S, Hampel D, Allen LH, Perrin MT. Vitamin B-12 content in breast milk of vegan, vegetarian, and nonvegetarian lactating women in the United States. Am J Clin Nutr. 2018;108(3):525-31.
5. Baatenburg de Jong R, Bekhof J, Roorda R, Zwart P. Severe nutritional vitamin deficiency in a breast-fed infant of a vegan mother. Eur J Pediatr. 2005;164(4):259-60.
6. Cheron G, Girot R, Zittoun J, Mouy R, Schmitz J, Rey J. [Severe megaloblastic anemia in 6-month-old girl breast-fed by a vegetarian mother]. Arch Fr Pediatr. 1989;46(3):205-7.
7. Gambon RC, Lentze MJ, Rossi E. Megaloblastic anaemia in one of monozygous twins breast fed by their vegetarian mother. Eur J Pediatr. 1986;145(6):570-1.
8. Monfort-Gouraud M, Bongiorno A, Le Gall MA, Badoual J. [Severe megaloblastic anemia in child breast fed by a vegetarian mother]. Ann Pediatr (Paris). 1993;40(1):28-31.
9. Bousselamti A, El Hasbaoui B, Echahdi H, Krouile Y. Psychomotor regression due to vitamin B12 deficiency. Pan Afr Med J. 2018;30:152.
10. Baatenburg de Jong A, Bekhof J, Zwart P, Langenhorst VJ, Roorda RJ. [Developmental delay in breastfed children due to inadequate diet of the mother]. Ned Tijdschr Geneeskd. 2006;150(9):465-9.
11. Stollhoff K, Schulte FJ. Vitamin B12 and brain development. Eur J Pediatr. 1987;146(2):201-5.
12. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. J Pediatr. 1992;121(5 Pt 1):710-4.
13. Joanna Briggs. Critical Appraisal Tools [Available from: https://joannabriggs.org/research/critical-appraisal-tools.html.
14. Sanders TAB, Ellis FR, Dickerson JWT. Studies of vegans: The fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue, and breast milk, and some indicators of susceptibility to ischemic heart disease in vegans and omnivore controls. American Journal of Clinical Nutrition. 1978;31(5):805-13.
15. Sanders TAB, Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. Journal of Pediatrics. 1992;120(4 II SUPPL.):S-77.
16. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr. 1985;41(4):787-800.
17. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Inorganic constituents of breast milk from vegetarian and nonvegetarian women: relationships with each other and with organic constituents. J Nutr. 1985;115(6):772-81.

18. Honzik T, Adamovicova M, Smolka V, Magner M, Hruba E, Zeman J. Clinical presentation and metabolic consequences in 40 breastfed infants with nutritional vitamin B12 deficiency--what have we learned? Eur J Paediatr Neurol. 2010;14(6):488-95.
19. Noren K. Levels of organochlorine contaminants in human milk in relation to the dietary habits of the mothers. Acta Paediatr Scand. 1983;72(6):811-6.
20. Debski B, Finley DA, Picciano MF, Lonnerdal B, Milner J. Selenium content and glutathione peroxidase activity of milk from vegetarian and nonvegetarian women. J Nutr. 1989;119(2):215-20.
21. Specker BL, Wey HE, Miller D. Differences in fatty acid composition of human milk in vegetarian and nonvegetarian women: long-term effect of diet. J Pediatr Gastroenterol Nutr. 1987;6(5):764-8.
22. Specker BL, Black A, Allen L, Morrow F. Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. Am J Clin Nutr. 1990;52(6):1073-6.
23. Perrin MT, Pawlak R, Dean LL, Christis A, Friend L. A cross-sectional study of fatty acids and brain-derived neurotrophic factor (BDNF) in human milk from lactating women following vegan, vegetarian, and omnivore diets. Eur J Nutr. 2019;58(6):2401-10.
24. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database of Systematic Reviews. 2018(11).
25. Wikipedia. Lacto vegetarianism 2019, October 3 [Available from: https://en.wikipedia.org/wiki/Lacto_vegetarianism.
26. Wikipedia. Ovo-lacto vegetarianism 2019, September 23 [Available from: https://en.wikipedia.org/wiki/Ovo-lacto_vegetarianism.
27. Wikipedia. Semi-vegetarianism 2019, August 30 [Available from: https://en.wikipedia.org/wiki/Semi-vegetarianism.
28. Wikipedia. Carnivore 2019, June 23 [Available from: https://en.wikipedia.org/wiki/Carnivore.
29. Wikipedia. Macrobiotic diet 2019, October 20 [Available from: https://en.wikipedia.org/wiki/Macrobiotic_diet.

Tables and Figures

Figure 1. Flowdiagram search results 17-09-2019

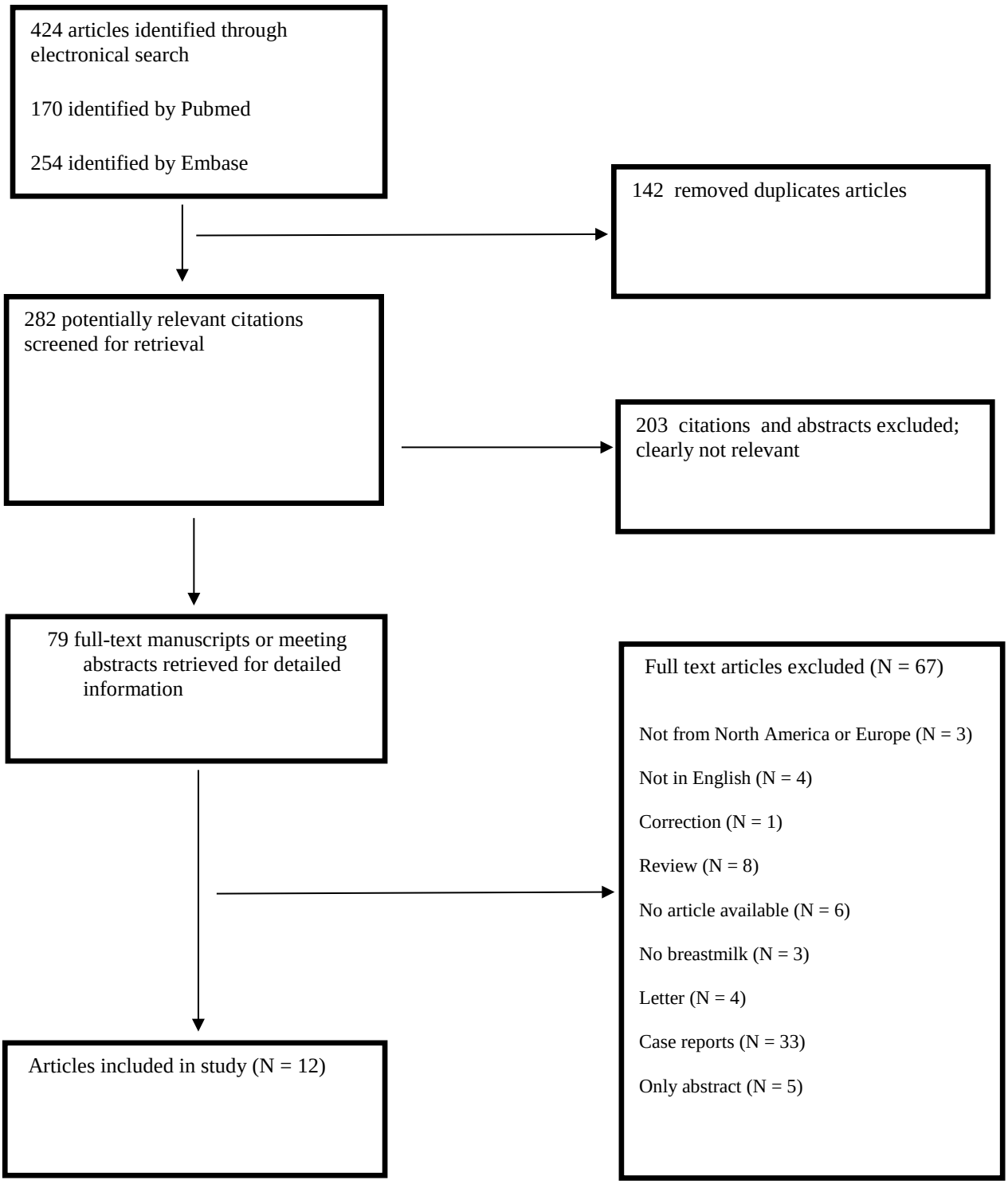


Table 1a. Diet characteristics of vegans, vegetarians and omnivores used by various authors.

Diet	Meaning
Vegan	Avoid all food of animal origin (15).
Vegetarian	Exclude only meat and fish from their diet (15).
Omnivore	Diet containing meat, eggs and dairy products (21).
Strict vegetarian	Avoid all food of animal origin (15).
Lacto-vegetarian/Lactarian	Consume dairy products, but not eggs or meat (25).
Lacto-ovo-/Ovo-lacto vegetarian	Diet containing some animal products like eggs and dairy. Consume no meat and fish (26).
Nonvegetarian	Diet containing meat, eggs and dairy products (21).
Semi-vegetarian/Flexitarian	Diet that is plant-based with the occasional inclusion of meat (27).
Fish-eater/Pescatarian	Consume no meat or poultry, but ate fish at least once weekly (16).
Carnivore/Meat eater	Diet consisting mainly or exclusively of animal tissue (28).
Macrobiotics	Reduce animal products, eat locally grown foods that are in season, and consume meals in moderation (29).

Table 1b. Diet terminology of vegans, vegetarians and omnivores used in this study and other words/description used in the studied reports that we used as synonyms.

DIET TYPES	SYNONYMS
VEGAN	Strict vegetarian
VEGETARIAN	Lacto-vegetarian/Lactarian, Lacto-ovo-/Ovo-lacto vegetarian
OMNIVORE	Nonvegetarian, Semi-vegetarian/Flexitarian, Fish-eater/Pescatarian, Carnivore/Meat eater, Macrobiotics

Table 2a. Study and patient characteristics of included studies.

	Year	N	Vegan	Vegetarian	Omnivore
Cohort					
Finley (16)	1985	59		30_1	29
Honzik (18)	2009	40	NA	NA	NA
Finley (17)	1985	52		26_1	26
Norén (19)	1983	49		18	20_2 / 11_3
Debski (20)	1988	38		26	12
Sanders (14)	1978	10	4_4		6
Sanders (15)	1992	45	19	5	21
Specker (21)	1987	21	12_5		7
Specker (22)	1990	19	13_5		6
Cross-sectional					
Perrin (23)	2018	74	26_6	22_7	26
Pawlak (4)	2018	74	26_8	22_9	26
Case Series					
Graham (12)	1992	6	1	3	2

N = number of participants; $_1$ = consumed no meat or poultry and only occasionally fish in very limited quantity, at most twice monthly; NA = not applicable; $_2$ = mothers eating a mixed diet; $_3$ = mothers regularly including fatty fish from the Baltic in their mixed diet; $_4$ = diets should not contain any foods of animal origin , including margarines made from animal and fish oils; $_5$ = women who consumed a macrobiotic diet that excluded meat, eggs and dairy products. The diet consisted of approximately 50-60% whole cereal grains, 5% soups, 20-25% vegetables, and 5-10% beans and sea vegetables. Occasionally they ate supplementary foods which included seafood, nuts and fresh fruits; $_6$ = Never consumed meat and never or rarely consumed other animal products; $_7$ = Never or rarely consumed meat and sometimes or often consumed animal products; $_8$ = participants who did not ingest any meat but may have ingested other animal products <1 time/mo; $_9$ = participants who did not eat meat but regularly ingested other animal products, such as eggs or dairy.

Table 2b. Study and patient characteristics of included studies.

	Year	N	Milk samples	Micronutrients	Macronutrients	Other
Cohort						
Finley (16)	1985	59	242		Milk fat, fatty acid	
Honzik (18)	2009	40		Cbl		
Finley (17)	1985	52	222	Fe, Ca, Mg, K, Na, Cu, Zn	Lipid, protein	Lactose
Norén (19)	1983	49	49			DDT, DDE, PCB, dieldrin, a-HCH, b-HCH, hexachlorobenzene
Debski (20)	1988	38	45	Se		Px, GSH-Px,
Sanders (14)	1978	10	Not clear		Fatty acid	Erythrocyte lipid fatty acid
Sanders (15)	1992	45	Not clear		Fatty acid	
Specker (21)	1987	21	Not clear		Milk fat, fatty acid	
Specker (22)	1990	19	Not clear	Vit B12		Infant's urinary MMA, maternal serum Vit B12
Cross-sectional						
Perrin (23)	2018	74	74		Fatty acid	BDNF
Pawlak (4)	2018	74	74	Vit B12		
Case Series						
Graham (12)	1992	6		Vit B12		Hemoglobin

N = number of participants; BDNF = brain-derived neurotrophic factor.

Table 3. Methodological quality of observational studies following JBI Critical Appraisal Checklist for Cohort Studies. Green = 'low', red = 'high' and white = 'unclear' risk of bias.

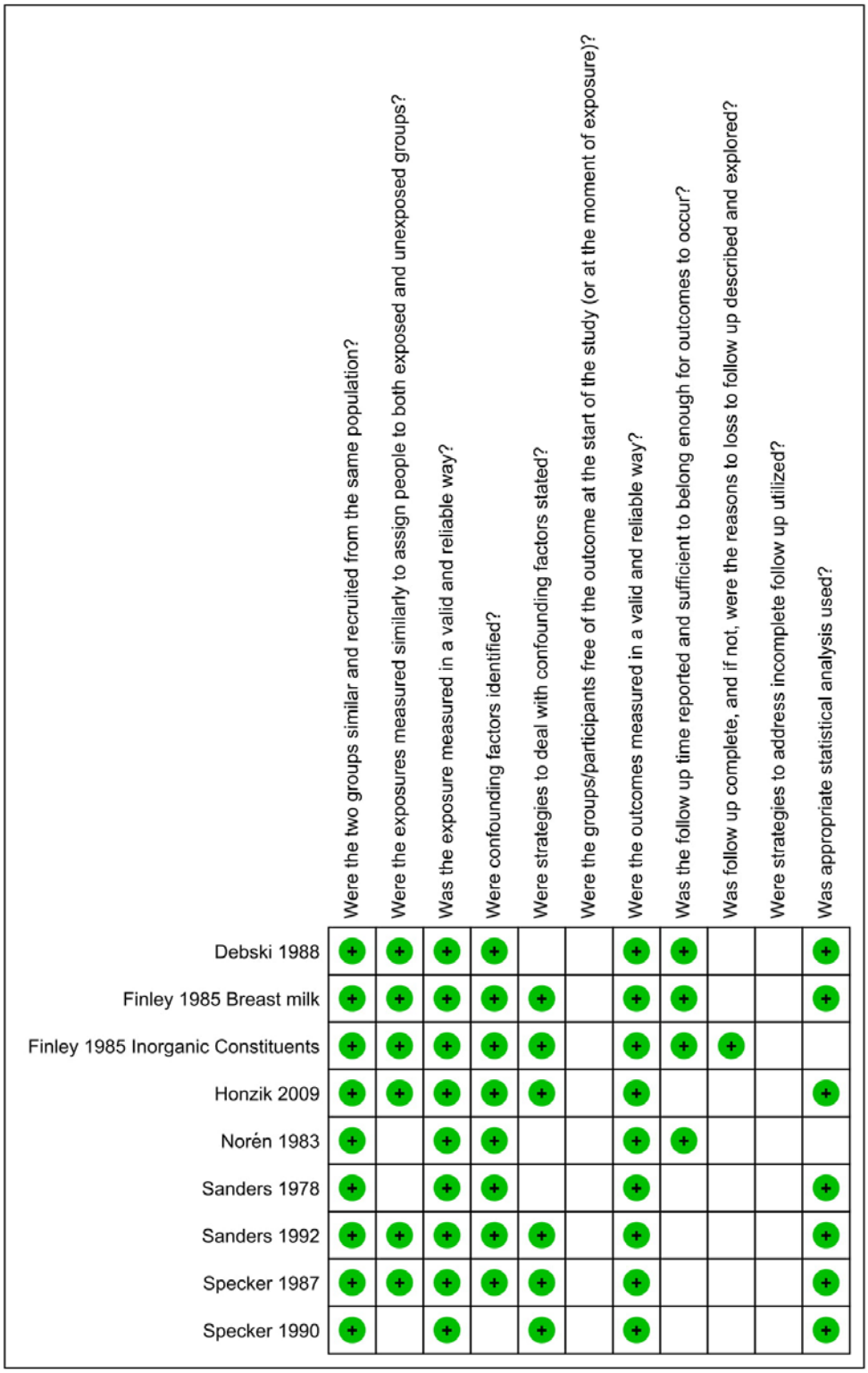


Table 4. Methodological quality of observational studies following JBI Critical Appraisal Checklist for Analytical Cross Sectional Studies. Green = 'low', red = 'high' and white = 'unclear' risk of bias.

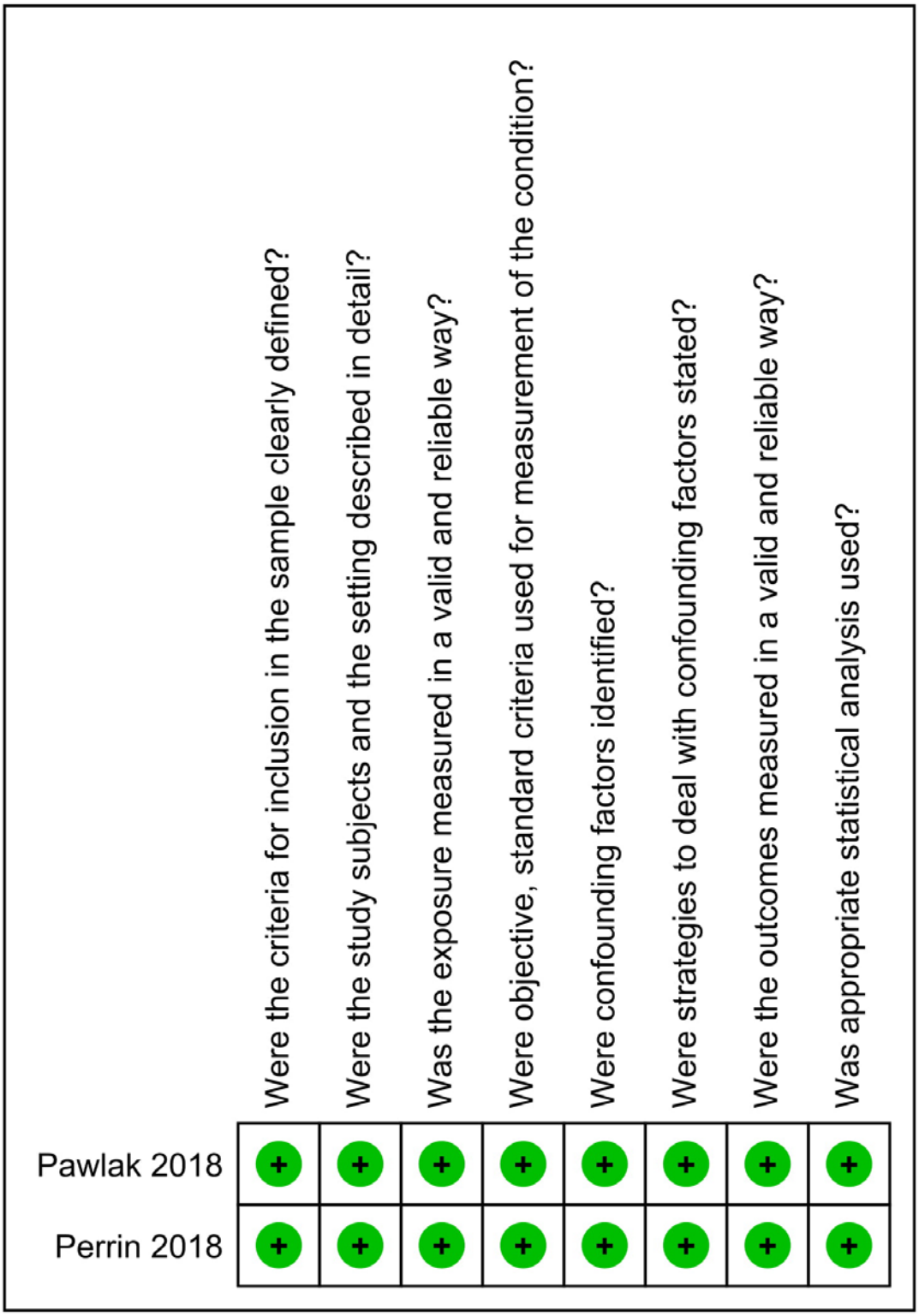
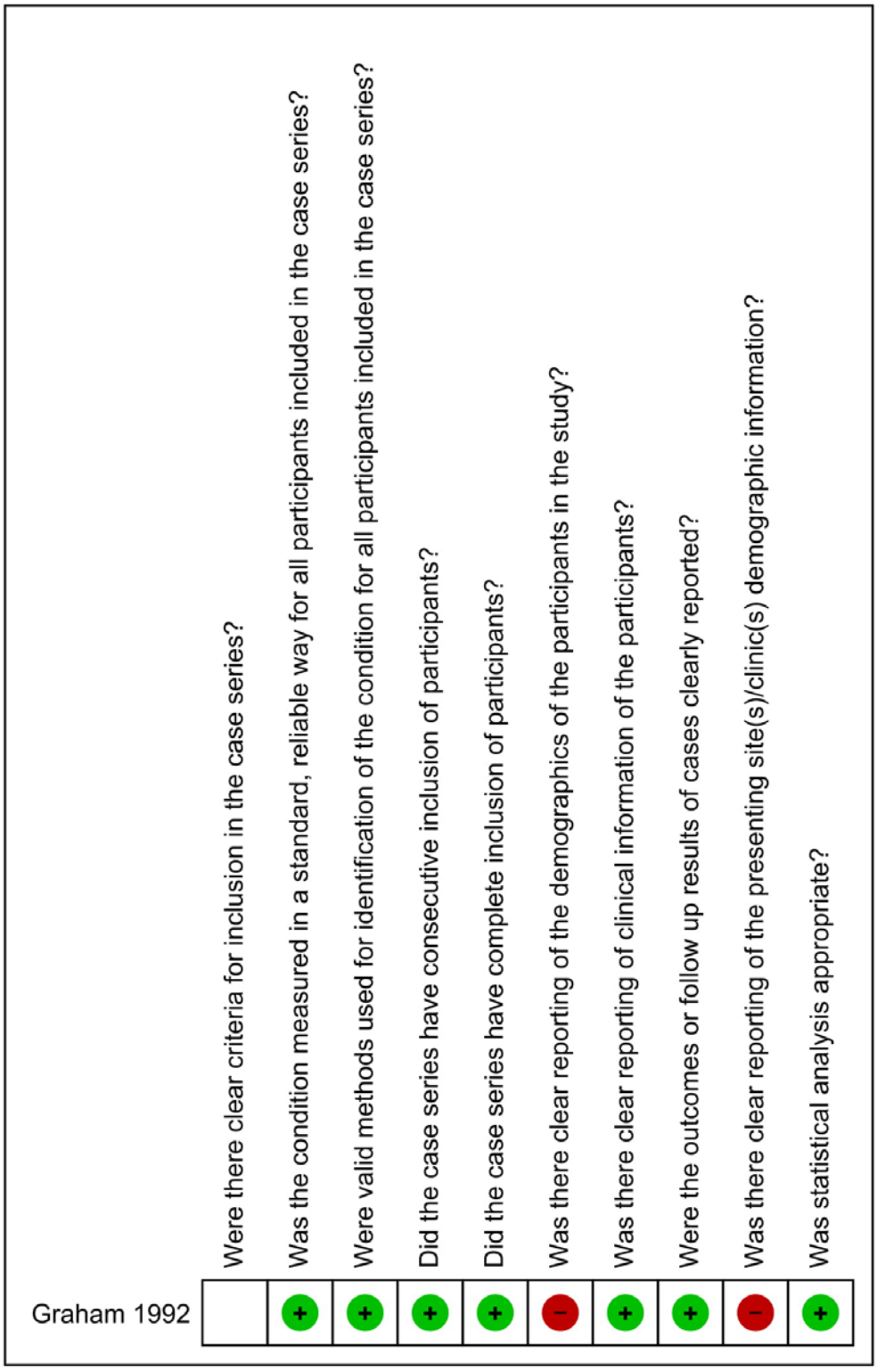


Table 5. Methodological quality of observational studies following JBI Critical Appraisal Checklist for Case Series. Green = 'low', red = 'high', and white = 'unclear' risk of bias.



Appendix 1. Electronic search strategy Pubmed.

(("vegetarians"[MeSH Terms] OR "Diet, Vegetarian"[MeSH] OR "Vegetarian*"[All Fields] OR "vegan*"[All Fields]))
AND
("Milk, Human"[Mesh] OR "Breast Feeding"[Mesh] OR "human milk"[All Fields] OR "breast milk"[All Fields] OR "breastmilk"[All Fields] OR "breastfeeding"[All Fields] OR "breast feeding"[All Fields])

Appendix 2. Electronic search strategy Embase.

1. exp vegetarian/ or exp vegetarian diet/ or vegetarian.mp.
2. exp vegan diet/ or exp vegan/ or vegan.mp.
3. 1 OR 2
4. breast milk.mp. or exp breast milk/
5. breast feeding.mp. or exp breast feeding/
6. human milk.mp. or exp breast milk/
7. 4 OR 5 OR 6
8. 3 AND 7

Appendix 3. Review authors' judgement for assessing risk of bias in included cohort studies.

Source of bias	**Finley 1985 (Breast)**
1. Were the two groups similar and recruited from the same population?	**Low** – 57 northern California women.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – The exposures were measured with help of questionnaires and survey forms. Dietary information was obtained from each of 57 women at monthly intervals. At the initial interview, each provided a 24-h dietary recall, including dietary supplements, for the previous day. Monthly, thereafter, each kept a 2-d diet record.
3. Was the exposure measured in a valid and reliable way?	**Low** – The exposures were measured with an initial interview and at monthly intervals. Vegetarians: consumed no meat or poultry and only occasionally fish in very limited quantity, at most twice monthly, *2)* Semivegetarians or fish-eaters: consumed no meat or poultry, but ate fish at least once weekly, and *3)* Nonvegetarians or omnivores: consumed meat, poultry, fish, and other animal products.
4. Were confounding factors identified?	**Low** – Percent fat in milk or percent of each of 14 fatty acids in milk fat with month of lactation, parity of mother, nursing interval length, time of day of milk expression, percent milk fat (fatty acids only), and intake level of eight dietary variables.
5. Were strategies to deal with confounding factors stated?	**Low** – Only samples with interval length (number of hours since last nursing) between I and 6 h were used since milk fat percent decreases with interval length. Mean interval length of samples included for analysis was 3.2 h with more than 50% having interval lengths of 3 or 4 h. Only samples expressed between 6 AM and 1 PM were included because of reported diurnal variation in percent milk fat. Only samples from women nursing at least four times per day were included since percent milk fat increases as number of nursings per day decreases. Stepwise multivariate regression analysis was used to identify changes.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Percent milk fat was determined gravimetrically using a modification of the method of Erickson and Dunkley as described by Emery and co-workers. Fat was extracted from 10 ml of milk using saturated sodium chloride, acidified ethanol (90% ethanol; 10% 1 N HC1**)** and hexane. This method has been validated against the Mojonnier method which is a modification of the Roese-Gottlieb method of fat analysis. Fatty acid composition of milk was determined by gas chromatography5 using 1.8 microliter of ester-containing solution.
8. Was the follow up time reported and sufficient to belong	**Low** – A total of 242 samples were provided by the 57 women. The samples reflect a wide time period; 1st through 31st month of lactation. Twenty-four women entered the study during their first 2 months of lactation; all but three

enough for outcomes to occur?	women joined the study before the 18th month of lactation. Women participated in the study an average of 5 months (range 1 to 9 months).
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**X** – Not applicable.
10. Were strategies to address incomplete follow up utilized?	**X** – Not applicable.
11. Was appropriate statistical analysis used?	**Low** – Student's *t* test was used to identify significant differences in nutrient intake between vegetarians and non-vegetarians. As most women in the study contributed more than one sample, specific statistical procedures were necessary. If a statistically significant regression coefficient (or difference based on a Student's *t* test) was identified for any milk or dietary variable, the standard error of the coefficient (or of the mean) for that variable was adjusted upward by using number of women providing samples, rather than number of samples, when calculating standard error and degrees of freedom.

Source of bias	**Honzik 2009**
1. Were the two groups similar and recruited from the same population?	**Low** – 40 breastfed infants and small toddlers (19 boys and 21 girls) from 40 families with nutritional Cbl deficiency were included in the study, recognized in participating clinics between 2002 and 2006. Over 5000 children were referred to our centres with a suspected metabolic disease during a five year period (January 2002 - December 2006). (Our centres = Prague, Czech)
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – The children were divided into two groups. Profound Cbl deficiency (69 ± 17 ng/l, reference range 200–900) was found in 17 infants (group A) and a milder Cbl deficiency in 23 infants (167 ± 40 ng/l) (group B). The cut-off value for the designation of patients to group A was chosen as Cbl level less than 100 ng/l.
3. Was the exposure measured in a valid and reliable way?	**Low** – Fasting blood samples were collected into tubes with and without EDTA. Laboratory investigations included the full blood count and a peripheral blood smear. Cbl levels in serum and breast milk were determined by a electrochemiluminescence immunoassay (Cobas, Roche Diagnostics), methylmalonic acid levels by the GC/MS technique, and total plasma homocysteine levels by HPLC with postcolumn fluorescence detection. Propionylcarnitine concentration was analysed in dried blood spots on API 2000 triple quadrupole MS/MS (Applied Biosystems/MDS SCIEX) with TurboIonSprayk interface. Cbl levels in serum and breast milk were measured by a kit (Cobas, Roche Diagnostics, USA).
4. Were confounding factors identified?	**Low** – Propionylcarnitine, detectible in expanded newborn screening, is considered to be an indirect marker of Cbl deficiency. Furthermore, even newborns of Cbl-deficient mothers can have some neonatal Cbl stores, and deficiency occurs only after these stores are depleted. Anthropometric data are presented in percentiles for the specific sex and age groups. They are not aware of any socio-economic problems in patients causing the Cbl deficiency.

5. Were strategies to deal with confounding factors stated?	**Low** – Blood samples in newborns and mothers.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Cbl deficiency in mothers was examined with help of gastroscopy, endoscopy, oral Cbl test or diet.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Unclear** – No information was provided.
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**Unclea**r – No information was provided.
10. Were strategies to address incomplete follow up utilized?	**Unclear** – No information was provided.
11. Was appropriate statistical analysis used?	**Low** – Statgraphics Plus version 6.0 was used for statistical analyses. Simple linear regression was used to test for the correlation between serum Cbl levels and P-HCY or U-MMA concentrations in infants and between Cbl levels in infants and their mothers.

Source of bias	**Finley 1985 (Inorganic)**
1. Were the two groups similar and recruited from the same population?	**Low** – well-nourished women living in an industrialized society (USA).
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – Dietary information was obtained from each woman at monthly intervals. Intake from dietary supplements was also determined.
3. Was the exposure measured in a valid and reliable way?	**Low** – Women who consumed no meat or poultry and only occasionally fish in very limited quantity (at most twice monthly during the study period) were identified as vegetarians. Women who consumed no meat or poultry, but ate fish at least once weekly, were identified as semivegetarians. Women who consumed meat, poultry, fish and other animal products during the study period were identified as nonvegetarians.
4. Were confounding factors identified?	**Low** – Milk nutrient with level of other milk nutrients, month of lactation, nursing interval length, and time of day of milk expression.

5. Were strategies to deal with confounding factors stated?	**Low** – Only samples with interval length (number of hours since last nursing) between 1 and 6 h were used since milk fat percent decreases with in terval length. Only samples expressed between 0600 and 1300 h were included because of diurnal variation in percent milk fat, protein and some minerals. Only samples from women nursing at least four times per day were included, since levels of certain nutrients change as number of nursings per day decreases. Regression analysis was used to identify significant correlations between levels of milk nutrients and month of lactation. Time periods in which levels changed and in which they did not change were identified.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Milk samples were analyzed for protein, lactose, lipid, minerals and trace elements. Protein was analyzed by a modification of the assay of Lowry et al. Lactose was assayed enzymatically by using lactase and glucose oxidase according to Somogyi and Dahlquist. Fat (total lipids) was analyzed by a colorimetrie method by using the sulfuric acid-vanillin reaction. For most of the samples analyzed for the present report, fatty acid composition was also determined by gas chromatography, and percent fat was determined gravimetrically by using a modification of the method of Erickson and Dunkley as described by Emery and co-workers. Milk samples were digested with nitric acid, as previously described. Calcium, magnesium, iron, zinc, copper, sodium and potassium were analyzed by a flame atomic absorption spectrophotometer (Perkin-Elmer 370, Norwalk, CT), by using the emission mode for sodium and potassium.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Low** – The follow up time was 20 months.
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**Low** – Women entered the study at various stages of lactation and provided between one and nine samples at monthly intervals. Month one was defined as the third and fourth weeks post partum, thereby excluding colostrum in which levels of many milk constituents change drastically.
10. Were strategies to address incomplete follow up utilized?	**Unclear** – No information was provided.
11. Was appropriate statistical analysis used?	**Low** – Data were analyzed by using the Statistical Package for the Social Sciences (SPSS). Regression analysis was used to identify significant correlations between levels of milk nutrients and month of lactation. A Student's *t*-test was used to identify significant differences between vegetarians and nonvegetarians.

Source of bias	Norén 1983
1. Were the two groups similar and recruited from the same population?	**Low** – Only Swedish mothers who had not lived abroad participated in the investigation.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Unclear** – No information was provided.
3. Was the exposure measured in a valid and reliable way?	**Low** – Lacto-vegetarian mothers, mothers eating a mixed diet and mothers regularly including fatty fish from the Baltic in their mixed diet.
4. Were confounding factors identified?	**Low** – The mothers were requested to give information about their age, number of infants nursed, duration of nursing, age of children, duration of vegetarian diet, estimated percentage of vegetable food grown without use of insecticides, fish consumption, smoking habits and domiciles.
5. Were strategies to deal with confounding factors stated?	**Unclear** – No information was provided.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Each mother provided about 100 ml of milk, 50.0 g of which were analyzed for organochlorine pesticides and PCBs by methods described in earlier studies from K. Norén.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Low** – The samples were collected in the course of one year (1978).
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**X** – Not applicable
10. Were strategies to address incomplete follow up utilized?	**X** – Not applicable.
11. Was appropriate statistical analysis used?	**Unclear** – No information was provided.

Source of bias	Debski 1988
1. Were the two groups similar and recruited from the same population?	**Low** – 38 apparently healthy lactating women living in northern California.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – Dietary data (2-d intake records) were obtained at monthly intervals.
3. Was the exposure measured in a valid and reliable way?	**Low** – Vegetarians consumed eggs and dairy products in addition to foods of vegetable origin. Nonvegetarians consumed an omnivorous diet.
4. Were confounding factors identified?	**Low** – Milk Se content is known to vary according to the geographic location of the donor. Bioavailability of Se has been shown to be greater from some vegetables than from animal sources. Kumpulainen et al. likewise reported that milk concentration was dependent upon the form consumed since maternal supplementation with selenium-yeast was more effective than sodium selenite in increasing milk Se concentration and correspondingly higher plasma values of the nursing infant.
5. Were strategies to deal with confounding factors stated?	**Unclear** – No information was provided.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Selenium intakes were calculated using Se values of foods tabulated by Schubert, Holden and Wolf. Milk Se was determined as previously described by McCarthy et al. Bovine non-fat milk powder (National Bureau of Standards reference material no. 1549, Gaithersburg, MD) served as the reference standard for assessing the accuracy and reproducibility of selenium analyses. Glutathione peroxidase (GSH-Px) activity was measured in both undialyzed skim milk and column fractions using a minor modification of the coupled assay of Paglia and Valentine.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Low** – 4-6 months during lactation.
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**X** – Not applicable.
10. Were strategies to address incomplete follow up utilized?	**X** – Not applicable.

11. Was appropriate statistical analysis used?	**Low** – Statistical evaluations of data was made by using the Student's *t*-test and regression analysis.

Source of bias	**Sanders 1978**
1. Were the two groups similar and recruited from the same population?	**Low** – Twenty-two vegans (12 males, 10 females) were selected for examination; the subjects were Caucasians and belonged predominantly to the professional and middle classes; they were not members of any particular religious sect. The mean age of these subjects was 38 years (range 21 to 66 years) and they had taken a vegan diet for a mean duration of 8 years (range 1 to 30 years). Controls were chosen from the general omnivorous population. The criteria for choosing these controls were that they should be healthy, and not taking any special diet, and they should match the vegan subjects with regard to age, sex, height, ethnic origin, and socioeconomic background. The first volunteers fulfilling these criteria were taken. In addition to the 22 vegans and 22 omnivore controls already described, 10 mothers, four vegans and six omnivores, and their infants were studied.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Unclear** – No information was provided.
3. Was the exposure measured in a valid and reliable way?	**Low** – The criteria used for the selection of vegans were that their diets should not contain any foods of animal origin, including margarines made from animal and fish oils, and that they had taken such a diet for at least 1 year; whether they took vitamin B12 supplements was noted. The criteria for controls were that they are not taking any special diet.
4. Were confounding factors identified?	**Low** – Medical histories were taken and attention paid to any symptoms that could be associated with nutritional deficiency. Blood samples were obtained by venipuncture with stasis in the morning from subjects who had been fasting overnight.
5. Were strategies to deal with confounding factors stated?	**Unclear** – No information was provided.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Lipids were extracted from breast-milk with chloroform:methanol (2:1 v/v) and non-lipid contaminants removed. Serum vitamin B12 was assayed microbiologically.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Unclear** – No information was provided.

9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**Unclear** – No information was provided.
10. Were strategies to address incomplete follow up utilized?	**Unclear** – No information was provided.
11. Was appropriate statistical analysis used?	**Low** – Wilcoxon's test for pair difference was used to test for between group differences. A two sample *t* test (two-tailed) was used to test between group differences for the fatty acid composition of breast-milk and for the fatty acid composition of erythrocyte lipids from the breast fed infants.

Source of bias	**Sanders 1992**
1. Were the two groups similar and recruited from the same population?	**Low** – White vegetarian and vegan subjects were identified by placing advertisements in vegan and vegetarian magazines. Indian vegetarian and omnivore subjects were randomly selected from general practice lists and from patients attending the Northwick Park Hospital in North London.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – Dietary intakes were assessed by a 7-day, weighed food intake inventory in nonlactating women and a 3-day, weighed food intake inventory in lactating women. Nutrient intakes were calculated from food tables (Foodtabs, Sanders T.A.B., unpublished data).
3. Was the exposure measured in a valid and reliable way?	**Low** – There are different degrees of vegetarianism: vegans, or strict vegetarians, avoid all food of animal origin, whereas most vegetarians exclude only meat and fish from their diets.
4. Were confounding factors identified?	**Low** – Birth weight, parity, maternal age, height, duration of gestation, and smoking habits.
5. Were strategies to deal with confounding factors stated?	**Low** – Multiple regression analysis was used to investigate the relationships between cord artery and fatty acid composition of plasma phospholipids and birth weight with the use of SPSS/PC (SPSS Inc., Chicago, Ill.). No relationship between birth weight, length, and head circumference and the proportions of DHA in plasma or cord artery phospholipids was evident from multivariate analyses.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Plasma, cord artery, and human milk lipids were extracted with mixtures of chloroform:methanol in the presence of butylated hydroxytoluene (50 mg/L). Fatty acid analyses of human milk lipids were carried out by gas-liquid chromatography on packed columns coated with Silar 10C and on a column coated with Silar 5C.
8. Was the follow up time reported and	**X** – Not applicable.

sufficient to belong enough for outcomes to occur?	
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**X** – Not applicable.
10. Were strategies to address incomplete follow up utilized?	**X** – Not applicable.
11. Was appropriate statistical analysis used?	**Low** – Two group comparison was made with a *t* test; comparisons between more than two groups were made by analysis of variance.

Source of bias	**Specker 1987**
1. Were the two groups similar and recruited from the same population?	**Low** – Women consuming two types of diet. The mean maternal ages were similar between the two diet groups, with a mean of 30 years (range 22-35 years). The comparison group was recruited from the Boston area.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Low** – Three-day diet diaries were obtained for 16 of the women. Nutrient analyses for fat and cholesterol intake and percent of calories from protein. Fat, and carbohydrates were determined using a nutrient analysis system (Ohio State University Nutrient Data Base Analysis System, Department of Dietics).
3. Was the exposure measured in a valid and reliable way?	**Low** – Vegetarian = no consumption of meat, eggs, or dairy products vs. nonvegetarian. A macrobiotic diet that excluded milk, eggs, and dairy products. The diet consisted of approximately 50-60% whole cereal grains, 5% soup, 20-25% vegetables, and 5-10% beans and sea vegetables. Occasionally they ate supplementary foods, which included seafood, nuts, and fresh fruit. The comparison group consumed an omnivorous diet containing meat, eggs, and dairy products.
4. Were confounding factors identified?	**Low** – Length of lactation or length of time on a vegetarian diet.
5. Were strategies to deal with confounding factors stated?	**Low** – Regression analyses were used to determine whether milk fatty acid content was associated with either length of lactation time or length of time on a vegetarian diet.
6. Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	**X** – Not applicable.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Total lipids were extracted within 24h using the method of Folch et al. Percentage of milk fat was determined gravimetrically. Fatty acid composition was determined after samples were saponified by 0.5 *N* KOH in methanol. Fatty acid methyl esters were analyzed by gas liquid chromatography using a capillary fused silica glass column (Supelcowax 10, 0,32 mm x 0,30 m, 190°C isothermal).

8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Unclear** – No information was provided.
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**Unclear** – No information was provided.
10. Were strategies to address incomplete follow up utilized?	**Unclear** – No information was provided.
11. Was appropriate statistical analysis used?	**Low** – Analysis of covariance techniques were used to determine differences in milk fatty acid content by diet, controlling for length of lactation. Wilcoxon rank sum statistics were used to determine whether the percent of calories in the form of protein, fat, and carbohydrate differed between the two diet groups.

Source of bias	**Specker 1990**
1. Were the two groups similar and recruited from the same population?	**Low** – Nineteen women were studied, 13 of whom were consuming a strict vegetarian diet and 6 of whom were consuming an omnivorous diet. These women represented 19 of 23 mothers who participated in the original study. Maternal ages did not differ between the two groups (mean 31 y, range 22-40 y). The mean ages of vegetarian and omnivorous infants were similar: 7.3 mo (range 2.0-1 3.9 mo) and 7.8 mo (range 5.0-1 1 .9 mo), respectively. The study was approved by Cincinnati Children's Hospital Medical Center Institutional Review Board and informed consent was obtained.
2. Were the exposures measured similarly to assign people to both exposed and unexposed groups?	**Unclear** – No information was provided.
3. Was the exposure measured in a valid and reliable way?	**Low** – The vegetarian women were practicing a macrobiotic diet, which generally consists of 50-60% whole cereal grains, 5% soups, 20-25% vegetables, and 5-10% beans and sea vegetables. There are occasional supplementary foods. In addition to avoidance ofanimal products, no dairy products or eggs were eaten and vitamin supplements generally were not taken. None of the vegetarian women and five of six omnivorous women took commercially prepared vitamins.
4. Were confounding factors identified?	**Unclear** – No information was provided.
5. Were strategies to deal with confounding factors stated?	**Low** – Regression-analysis techniques, Student's *t* test, and the Wilcoxon rank-sum test (indicated when used) were performed to determine the statistical significance of the relationships between variables.
6. Were the groups/participants free of the outcome at the start of the study (or at	**X** – Not applicable.

the moment of exposure)?	
7. Were the outcomes measured in a valid and reliable way?	**Low** – The vitamin B-12 content of human milk was determined by radio assay after digestion of the sample with papain (Sigma, St Louis). Blood samples were obtained from all mothers and serum vitamin B- 12 concentrations were determined with a radio assay technique that employs purified hog intrinsic factor as binder (boil method, Diagnostic Products Corp. Los Angeles). Urine samples were collected from all infants and vegetarian mothers, and MMA concentrations per millimole creatinine were determined.
8. Was the follow up time reported and sufficient to belong enough for outcomes to occur?	**Unclear** – No information was provided.
9. Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	**Unclear** – No information was provided.
10. Were strategies to address incomplete follow up utilized?	**Unclear** – No information was provided.
11. Was appropriate statistical analysis used?	**Low** – Regression-analysis techniques, Student's *t* test, and the Wilcoxon rank-sum test (indicated when used) were performed to determine the statistical significance of the relationships between variables.

Appendix 4. Review authors' judgement for assessing risk of bias in included cross-sectional studies.

Source of bias	**Perrin 2018**
1. Were the criteria for inclusion in the sample clearly defined?	**Low** – Inclusion criteria were: maternal age of 18 or older; stage of lactation 2 weeks or more postpartum; healthy, term birth; willingness to provide one breast milk sample for the study and complete a short diet survey. Exclusion criteria included any of the following conditions: pregnant; taking metformin; Crohn's disease; Celiac disease; MTHFR gene mutation; liver disease; myeloproliferative disorders; hyperthyroid or hypothyroid. Interested individuals were asked to complete an online basic screening questionnaire (BSQ) to determine eligibility and self-identify their diet pattern (vegan, vegetarian, omnivore).
2. Were the study subjects and the setting described in detail?	**Low** – Lactating women in the United States who were recruited between November 2016 and April 2017 through online communities focused on breastfeeding or vegetarianism, and through local religious communities with a vegetarian population. There was no significant difference in age, parity, ethnicity, or education ($p > 0.05$), but maternal BMI and lactation stage differed ($p < 0.05$) by diet type.
3. Was the exposure measured in a valid and reliable way?	**Low** – Participants were asked to complete a digital questionnaire which included demographic, diet, and supplement information. Consumption of meat (beef, lamb, pork, and poultry), dairy (milk, cheeses, and yogurts), egg, fish, and omega-3 supplemented margarine were categorized as follows: Never; Rarely (less than 1 times per month); Sometimes (1–4 times per month), Often (more than 1 time per week). A diet category was assigned to each participant based on detailed responses to the diet survey as follows: vegans never consumed meat and never or rarely consumed other animal products; vegetarians never or rarely consumed meat and sometimes or often consumed other animal products; omnivores consumed meat sometimes or often.
4. Were objective, standard criteria used for measurement of the condition?	**Low** – 74 lactating women in the United States following a vegan ($n = 26$), vegetarian ($n = 22$), or omnivore ($n = 26$) diet pattern. Vegans never consumed meat and never or rarely consumed other animal products; vegetarians never or rarely consumed meat and sometimes or often consumed other animal products; omnivores consumed meat sometimes or often.
5. Were confounding factors identified?	**Low** – Maternal age, parity, BMI, and stage of lactation.
6. Were strategies to deal with confounding factors stated?	**Low** – Linear regression was used to identify significant predictor variables.
7. Were the outcomes measured in a valid and reliable way?	**Low** – Total fat was measured by creamatocrit, using methods for human milk that were validated against reference methods. The fatty acids present in the samples were measured as fatty acid methyl esters according to the method of Bannon. Total BDNF was measured in duplicate using a Quantikine Total BDNF ELISA kit (part number DBNT00, R&D Systems, Minneapolis, MN) which has a standard curve range of 15.6–1000 pg/mL. This method was selected because it was used in two of the three published studies on BDNF in human milk, and was the only published methods available at the time that had been validated by the manufacturer for use with human milk.

8. Was appropriate statistical analysis used?	**Low** – Descriptive statistics were computed for all outcome variables. Distribution of data for normalcy was evaluated using a Shapiro–Wilk test. Normally distributed outcome variables were evaluated using a one-way ANOVA. Distributions of non-parametric variables were evaluated using a Kruskal–Wallis test. Due to the large number of statistical tests performed on numerical outcome variables, a Bonferroni correction to the *p* value was computed as 0.05/41 which resulted in a significance threshold of 0.001. Differences in categorical variables were evaluated using a Fisher's exact test with a significance threshold of 0.05. Analysis was conducted using SAS Enterprise Software 9.4 (SAS Corporation; Cary, NC).

Source of bias	**Pawlak 2018**
1. Were the criteria for inclusion in the sample clearly defined?	**Low** – Inclusion criteria included living in the United States, maternal age 18–46 y, giving birth to a healthy term infant currently older than 2 wk, and willingness to collect 1 milk sample per the study collection protocol and to complete a diet survey. Exclusion criteria included being diagnosed with methylene tetrahydrofolate reductase (*MTHFR*) gene mutation; health conditions that affect vitamin B-12 status (e.g., patients with known intrinsic factor deficiency, history of bariatric surgery, inflammatory bowel disease, celiac disease); hypo- or hyperthyroidism; advanced liver disease (cirrhosis, hepatitis); myeloproliferative disorders; or being pregnant.
2. Were the study subjects and the setting described in detail?	**Low** – Participants (*n* = 74) of this cross-sectional study were recruited through vegetarian and vegan organizations, faith-based institutions, and online parenting and breastfeeding support groups. Participants were asked to complete a basic online screening questionnaire (BSQ) to determine eligibility for participation. The study took place in the United States of America.
3. Was the exposure measured in a valid and reliable way?	**Low** – Individuals were selectively invited to participate in the study on the basis of their self-reported diet type, with a goal of recruiting a similar number of participants (*n* = 25) per diet group. Vegans were defined as participants who did not ingest any meat but may have ingested other animal products <1 time/mo. Vegetarians consisted of participants who did not eat meat but regularly ingested other animal products, such as eggs or dairy. Any B-vitamin supplementation was defined as using any of the following: individual vitamin B-12 supplements, Bcomplex supplements, multivitamin supplements, or prenatal vitamin supplements.
4. Were objective, standard criteria used for measurement of the condition?	**Low** – Vegans were defined as participants who did not ingest any meat but may have ingested other animal products <1 time/mo. Vegetarians consisted of participants who did not eat meat but regularly ingested other animal products, such as eggs or dairy.
5. Were confounding factors identified?	**Low** – Only gravida (β ± SE: 56.6 ± 21.0; standardized β = 0.305; *P* = 0.009, *R*2 = 0.093) and parity (β ± SE: 79.6±25.2; standardized β =0.351; *P*=0.002, *R*2 0.123), but not maternal age, BMI, or stage of lactation, were predictive of milk vitamin B-12 concentrations across the study population.
6. Were strategies to deal with confounding factors stated?	**Low** – Linear regression was performed to probe for significant predictors of vitamin B-12 milk concentration.

7. Were the outcomes measured in a valid and reliable way?	**Low** – A low milk vitamin B-12 concentration was defined as <310 pmol/L. A high milk vitamin B-12 concentration was defined as above the assay detection threshold of 1122 pmol/L. The analysis was performed by using a competitive chemiluminescent enzyme immunoassay that accounts for interference effects caused by high concentrations of haptocorrin in breast milk. This assay has the ability to detect vitamin B-12 in the range of 24–1122 pmol/L. Milk samples with vitamin B-12 concentrations above the upper detection limit were assigned a maximum vitamin B-12 value of 1122 pmol/L and were flagged as being above assay detection range.
8. Was appropriate statistical analysis used?	**Low** – The distributions of vitamin B-12 among the 3 diet groups were compared by using side-by-side boxplots and summarized with the use of means and medians. The null hypothesis that all 3 distributions were the same was tested by using the F-statistic from a 1-factorANOVAand by using the Kruskal-Wallis test. The groups were compared on dichotomous variables with the use of Fisher's exact test. The significance level of $P < 0.05$ was used in all analyses. Data analysis was performed with the statistical software R, version 3.4.2, and SAS 9.4 (SAS Institute).

Appendix 5. Review authors' judgement for assessing risk of bias in included case series.

Source of bias	**Graham 1992 (9)**
1. Were there clear criteria for inclusion in the case series?	**Unclear** – No information was provided.
2. Was the condition measured in a standard, reliable way for all participants included in the case series?	**Low** – Clinical and hematological.
3. Were valid methods used for identification of the condition for all participants included in the case series?	**Low** – Serum levels of vitamin B12.
4. Did the case series have consecutive inclusion of participants?	**Low** – Six infants with this diagnosis have been identified at our institution, and we have been able to obtain long-term follow-up of four of them.
5. Did the case series have complete inclusion of participants?	**Low** – Six infants with this diagnosis have been identified at our institution, and we have been able to obtain long-term follow-up of four of them.
6. Was there clear reporting of the demographics of the participants in the study?	**High** – No geographic region and ethnicity mentioned.
7. Was there clear reporting of clinical information of the participants?	**Low** – Disease status, comorbidities, stage of disease, previous interventions/treatment and results of diagnostic tests were described.
8. Were the outcomes or follow up results of cases clearly reported?	**Low** – Results of follow up were clearly reported.
9. Was there clear reporting of the presenting site(s)/clinic(s) demographic information?	**High** – No, only that the patients came from Prince of Wales Children's Hospital, Randwick, Australia, and Wollongong Hospital, Woliongong, Australia.
10. Was statistical analysis appropriate?	**X** – Not applicable.