Enhancing the endogenous nutrient levels of DHM to help support the needs of extremely and very preterm infants

Mother's own milk (MOM) is the first choice for feeding preterm infants; however, if MOM is limited or unavailable, donor human milk (DHM) is the recommended alternative. Exclusively MOM or DHM diets do not provide sufficient levels of key nutrients required by extremely or very preterm infants. This article explores how modifications to donor nutrient intake and milk banking practices can potentially be used to enhance the endogenous nutrient levels of DHM.

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Key points

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1. Current milk banking practices (eg storage, freeze-thawing, processing) negatively affect the nutritional quality of DHM. We provide recommendations to mitigate some of these effects.
2. Milk banks do not currently provide specific dietary advice for donors. We highlight how donor diet influences the nutritional content of DHM and provide recommendations for donor nutrient intake.
3. Through a combination of these approaches, it may be possible to enhance the endogenous nutrient levels of DHM to better support the needs of extremely and very preterm infants.

Appropriate nutrition is fundamental for newborns; especially preterm infants, where their materno-fetal nutrient supply has been prematurely terminated.[1] Breast milk provides nutrients, hormones, enzymes and immunological factors that are essential for infant development. The nutritional composition of human milk varies widely, not only over the course of lactation, but also between individuals and populations.[2-4] Factors that have been shown to affect nutritional composition include, maternal lifestyle and dietary habits.[5]

Maternal breast milk is the first choice for feeding neonates;[6] however, an inadequate milk supply is nearly three times more likely in preterm mothers than in term mothers.[7] Underlying reasons can be physiological, such as incomplete development of the mammary glands, or poor hormonal response, as well as psychological.[8] Furthermore, in some cases, maternal breast milk might not be appropriate, due to illness or medication. In these instances, DHM from a human milk bank (HMB) is the recommended alternative.[9] In the UK, the British Association of Perinatal Medicine (BAPM) has set out a framework for practice, *The Use of Donor Human Milk in Neonates*,[7] whereby, 'DHM may be considered in babies born at <32 weeks' gestation and/or <1,500g to establish enteral feeding when MOM is unavailable or insufficient to meet the baby's requirements.' However, it's noted, 'There is insufficient evidence to make specific recommendations about duration of DHM use, fortification of DHM and use of DHM in moderate/late preterm and term babies.'

Nutritional needs of extremely and very preterm infants

The nutritional needs of extremely and very preterm infants are not currently met by exclusive diets of maternal or donor milk. For example, a recent study identified that extremely preterm infants fed an entirely human milk diet receive significantly less than the recommended daily vitamin K intake and are consequently at increased risk of vitamin K-dependent bleeding.[10] Similarly, we have shown that extremely preterm infants receiving standard care with maternal and DHM receive very low levels of the long-chain polyunsaturated fatty acids (PUFAs), arachidonic acid (ARA, 20:4n-6), and docosahexaenoic acid (DHA, 22:6n-3).[1] DHA and ARA are essential for optimal brain, visual and immune system function,[11] and during the last trimester, the brain weight increases approximately five-times and at the same time around 80% of the brain DHA and ARA are accumulated.[1] Furthermore, we have shown that both donor milk and milk expressed from mothers of preterm infants provide protein levels significantly below the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommendations, and an exclusive DHM diet would provide levels of linoleic acid (LA; 18:2n-6), α-linolenic acid (ALA; 18:3n-3), ARA, and DHA significantly below ESPGHAN recommendations.[12]

Nutrients: PUFA/SFA	Relevance	Quality of evidence*	Observations
DHA	Essential for optimal brain and visual system development	High	DHA levels in BM proportional to maternal dietary DHA intake Maternal diet quality affects the fatty acid composition of breast milk. Lower DHA and long chain fatty acids proportion in vegan BM For RCTs, supplementation ranged from 200mg to 1,200mg/day EFSA adequate DHA intake: 100-200mg/day
EPA	Important for cardio-vascular function and precursor of bioactive mediators with anti-inflammatory and pro-resolving properties	High	EPA levels in BM less proportional to maternal dietary EPA intake Effect of maternal EPA supplementation on BM concentration more modest than DHA For RCTs, supplementation ranged from 70mg to 300mg/day EFSA adequate EPA intake: 100-200 mg/day
ALA	Precursor of longer chain omega-3 PUFAs and may have independent roles	Low	BM ALA content correlated with intake of canola and soybean oils; seeds and nuts (eg flaxseed, chia, walnuts) and some green leafy vegetables (kale, spinach) For RCTs, supplementation was 10.1g/day EFSA adequate intake: 0.5% energy intake of ALA
ARA	Essential for brain development and immune system function	Low	No effect observed in studies but very low doses provided Same proportion of ARA in omnivores and vegans' BM For RCTs, supplementation was 54mg/day EFSA adequate intake: not available
SFA/TFA		High	TFA in the milk appeared to follow dietary trans changes after a 12-36 hour lag period. No association between TFA consumption and BM TFA level Overweight women's BM compared to normal weight women's BM contained higher amount of SFA, lower ratio of unsaturated to saturated FAs than those of normal weight EFSA adequate intake: not applicable

TABLE 1 PUFA/SFA nutrients in breast milk responsive to maternal diet, based on 29 articles (15 experimental studies and 14 observational studies). Key: PUFA= polyunsaturated fatty acids; BM=breast milk; SFA=saturated fatty acids; TFA=trans fatty acids; EFSA=European Food Safety Authority; ALA=α-linolenic acid; ARA=arachidonic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; RCT=randomised controlled trial. *Based on GRADE rating system.

The recent position paper of the ESPGHAN Committee on Nutrition and Invited Experts, sets out revised guidelines for the enteral nutrition of preterm infants,[13] where it's noted: 'In general, there is a lack of strong evidence for most nutrients and topics' and, 'We strongly support the use of human milk and recognise that the variation in nutrient density and absorption make precise recommendations for supplements or fortifiers challenging.' The question therefore arises as to how best enhance and/or preserve the endogenous levels of these nutrients in DHM to support the needs of extremely or very premature infants.

Modification of endogenous nutrient levels of DHM

Breast milk is a highly dynamic fluid and the nutritional content appears to be affected by a wide range of factors, including maternal nutritional intake.[4] We therefore suggest, that there are two areas where endogenous nutrient levels of DHM may be modified:

1. At the level of maternal nutrient intake, and/or
2. After expression when the milk is stored and processed by the HMB.

DHM typically undergoes prolonged cold storage, freeze-thaw cycles and processing before it is given to infants.[14] We have reviewed the evidence for the negative effects of these practices on the levels of long-chain PUFAs, lipid mediators and antioxidants in DHM, and have proposed a series of recommendations to mitigate some of these negative effects.[14] We suggest that DHM should be protected from light exposure and storage at 4°C minimised, to prevent decreases in vitamin C and endocannabinoids and increases in free fatty acids and lipid peroxidation products. Storage at -20°C prior to pasteurisation should also be minimised, to prevent free fatty acid increase and total fat and endocannabinoid decrease. Storage ≤-70°C is preferable wherever possible, although post-pasteurisation storage at -20°C for three months appears safe for free fatty acids, lipid peroxidation products and total fat content.

A mixed-methods survey

In terms of modifying maternal diet, we reviewed the dietary guidance and advice currently available to donors by the HMBs. Previously (November 2016 to February 2018), we undertook a mixed-methods survey to investigate not-for-profit HMB practices in the UK related to donor selection, nutritional information, and donor milk transport and storage.[15] There was an overall response rate of 93% (14 of 15). With regards to nutritional aspects:

- five HMBs reported asking potential donors about their dietary habits (besides alcohol consumption) before the first milk donation; one asked both before and during the time of milk donation; five did not ask at all
- two HMBs excluded mothers from donating if they followed a restricted diet, whereas 11 did not exclude
- only three HMBs had general leaflets or information available to donors about a

Nutrients: Vitamins	Relevance	Quality of evidence	Observations
A	Optimum visual, growth, immune system and cognitive development	High	Level in BM is responsive to maternal intake Higher deficiency risks in developing countries For RCTs, supplementation was either 30mg/day, 54mg/day or 60mg single dose EFSA average requirement: 1,020µg RE/day
B1, B2, B5 and B9	B1 deficiency symptoms: gastrointestinal, cardiac failure, lactic acidosis B2 deficiency: anaemia and cataracts B5 deficiency: fatigue, insomnia, depression, irritability, vomiting B9: essential role in fetal brain and spinal cord development	Very low	B1 supplementation did not significantly affect B1 concentration in BM B2 supplementation seems to have positive impact on BM concentration No evidence found on folic acid (B9) and pantothenic acid (B5) For RCTs, B1 supplementation was 1.7mg/day For RCTs, B2 supplementation was 2mg/day EFSA average requirement B1: 0.072mg/MJ EFSA adequate requirement B2: 1.7mg/day EFSA adequate intake B5: 7mg/day EFSA adequate requirement B9: 380µg DFE/day
B6	Essential for brain development	Moderate	B6 supplementation seems to have positive impact on BM concentration For RCTs, B6 supplementation ranged from 2.5 to 20mg/day EFSA average requirement: 1.4mg/day
B12	Essential for brain development	Moderate	Maternal B12 supplementation may prevent declines in BM content For RCTs, B12 supplementation was 8µg/day EFSA adequate intake: 5µg/day
C	Essential for collagen formation and role in immune and nervous system functions	Moderate	BM levels responsive to maternal supplementation only when lactating mothers have low intake Overall, level in BM is responsive to maternal intake but levels are regulated, (response to highest dose modest in European women vs 3-fold increase in African women) For RCTs, C supplementation ranged from 90 to 1,000mg/day EFSA average requirement: 140mg/day
D	Essential for calcium absorption and skeletal growth. Deficiency can cause nutritional rickets	Moderate	BM levels responsive to maternal supplementation. Greater effect when high unique dose supplementation For RCTs, supplementation ranged from 50µg/day to 3,750µg single dose EFSA adequate intake: 15µg/day
E	Deficiency can compromise immune system and affect lung development Premature infants are more susceptible to deficiency	High	Dose response relationship, and supplementation effects are confirmed for alpha-tocopherol Milk total tocopherol found to be associated only with mother's total fat and saturated fat dietary intake An increase in PUFA content may increase peroxidation and increase vitamin E requirements For RCTs, supplementation ranged from 40mg to 536mg single dose EFSA adequate intake: 11mg/day
K1	Deficiency can lead to classical haemorrhagic disease of the newborn	Low	Dose response relationship and supplementation effects are confirmed For RCTs, supplementation ranged from 0.5 to 5mg/day EFSA adequate intake: 70µg/day

TABLE 2 Vitamins in breast milk responsive to maternal diet (vitamin A based on 8 experimental studies; B vitamins based on 5 experimental and 1 observational study; vitamin C based on 3 experimental studies; vitamin D based on 5 experimental studies; vitamin E based on 5 experimental and 1 observational study; vitamin K1 based on 3 experimental studies). Key: RE=retinol equivalents; DFE=dietary folate equivalent.

healthy diet for breastfeeding mothers
- one had a registered dietitian at the HMB, five had registered dietitians at the hospital who could be consulted, and four had no one available
- following donation, nutritional assessment of the DHM for energy, PUFAs, minerals, or peroxidation products was not performed by any of the HMBs, four assessed macronutrients (fat, protein and carbohydrates), one assessed other nutritional factors. Nine did not do any nutritional assessments
- only one HMB recorded fortifying donated milk at the milk bank, and this was on very rare occasions.

A structured semi-quantitative survey

Between July 2021 and November 2022, we conducted a structured semi-quantitative survey with milk bank managers in the UK through phone interviews. The aim of this survey was to investigate in further detail

Nutrients: minerals and other	Relevance	Quality of evidence	Observations
Iodine	Deficiency can damage brain and increase mortality	High (1 experimental and 3 observational studies)	Dose response relationship and supplementation effects are confirmed Supplementation effects are immediate (peak six hours after supplementation) For RCTs, supplementation ranged from 75 to 150µg/day. One study administrated 450µg as a single dose EFSA adequate intake: 200µg/day
Selenium	Deficiency associated with increased respiratory morbidity	Low (4 experimental and 2 observational studies)	BM selenium correlated to fresh fish consumption; no correlation between multivitamin supplements during pregnancy Maternal supplementation increases BM levels and prevents decline with advancing lactation; appears to increase the BM concentration of PUFAs and decrease BM SFA For RCTs, supplementation ranged from 20 to 200µg/day EFSA adequate intake: 85µg/day
Iron	Iron deficiency anaemia associated with neurofunctional effects and can affect sleep pattern	Low (1 experimental and 3 observational studies)	Supplementation to non-anaemic women did not change BM content or prevent decline For RCTs, supplementation was from 80mg/day EFSA average requirement: 7mg/day
Zinc and copper	Zinc deficiency can cause cutaneous signs, diarrhoea, alopecia Copper deficiency can cause bone lesions	Low (3 observational studies)	Dietary intake of zinc/copper did not affect BM concentrations EFSA adequate intake copper: 1.5mg/day EFSA average requirement zinc: 2.4mg/day
Ovalbumin	Exposure to ovalbumin may reduce egg allergy development	Moderate (2 experimental studies)	Direct dose-response between amount of cooked egg ingested and BM ovalbumin For RCTs, egg intake ranged from no egg to >4 eggs/week EFSA adequate intake: not available
Protein and amino acids	Maternal protein consumption may impact BM nitrogen Taurine role: intestinal fat absorption, hepatic function, auditory and visual development	Low (1 experimental and 1 observational study)	Output of lactoferrin, α-lactalbumin and serum albumin higher with high protein diet, however differences not significant Mean taurine concentration in vegan BM 35mg/dL significantly lower than in BM of omnivores EFSA average requirement: 15mg/day from 0-6 months and 10g/day after 6 months postpartum
Tyrosine	Tyrosine deficiency may increase postpartum depression risk	Low (1 experimental study)	Rise in free, but not total tyrosine; supplementation effects immediate (peak six hours after supplementation) For RCTs, supplementation ranged from 2g to 10g single dose EFSA adequate intake/average requirement: not available
Choline	Needed for growth and development, and role in membrane and signalling functions	Low (1 observational study)	No significant effects by maternal diet pattern EFSA adequate intake: 520mg/day

TABLE 3 Minerals (and other) in breast milk responsive to maternal diet.

how HMBs were managing the nutritional aspects of donors' milk. We first contacted the 15 HMBs present in the UK by email; eight agreed to answer the survey.

With regards to nutritional restriction or specific requirements:

- two HMBs confirmed they applied the NICE guidelines, three mentioned they had developed their own questionnaire
- all the HMBs confirmed selecting exclusively smoke and recreational drug-free donors; three requested caffeine-free donors; two requested total alcohol-free donors; four requested donors not to take any medication or were postponing milk collection over the treatment
- two HMBs requested donors to take supplements in case of restricted diets, either vitamin B12 or postnatal multivitamin and mineral supplements
- most HMBs confirmed not classifying the donors in any sort; two did classify the donors depending on whether they had had preterm or full-term babies
- out of the eight HMBs, six said they did not provide any nutritional information to donors; two did ask donors to take supplements, eat healthy fats or asked the donors to compress their breast to get some more fat content in milk
- regarding where they believe there is a need for further research, five HMBs mentioned they wanted more guidance around vegan and vegetarian donors to better understand the implications. One HMB mentioned they had issues with low protein content milk and could not advise donors how to improve. One wanted better understanding of maternal

Nutrients: contaminants	Relevance	Quality of evidence	Observations
Arsenic, cadmium, lead, mercury	Heavy metals disrupt cellular events including growth; can induce cancer	Moderate (4 observational studies)	Factors significantly related to metal levels in BM were area of residence (Hg, Pb), prematurity (Hg), fish (Pb) and cereal (Hg) consumption, vitamin supplementation (Hg) and smoking (Pb, Cd) No significant difference found in BM between fish and non-fish eaters for THg concentration; negative association found between THg in BM and freshwater fish consumption PTWI (JECFA): no appropriate safety guidelines for As and Pb; Cd PTWI = 2.5μg/kg/week; IHg = 4μg/kg/week; MeHg = 1.6μg/kg/week
PCBs	PCBs can induce skin and liver damage	Moderate (1 observational study)	Levels were three times higher for very high seafood consumers (average 300g/day) than for low seafood consumers (average 12g/day) EFSA tolerable intake: 2pg/kg

TABLE 4 Contaminants in breast milk responsive to maternal diet. Key: PCBs=polychlorinated biphenyls; As=arsenic; Cd=cadmium; Pb=lead; Hg=mercury; THg=total mercury; IHg=inorganic mercury; MeHg=methylmercury; PTWI=provisional tolerable monthly intake; JECFA=joint FAO/WHO (Food and Agriculture Organization/World Health Organization) Expert Committee on Food Additives.

diet on milk organoleptic properties and infant preferences.

Regarding how to make nutritional resources easier to adopt by HMBs:
- two mentioned that having standardised nutritional advice across the country would be helpful
- two mentioned that using easy language, translatable into practice, relevant to donors and cost-free would be critical
- from a donor perspective, four HMBs said that any nutritional information given to donors would have to be carefully communicated so they do not see it as an additional constraint or think their milk is not good enough.

A systematic review

These findings are consistent with a recent online European Milk Bank Association survey to European HMBs exploring practices relating to donors who were following vegan or vegetarian diets.[16] The survey identified extremely variable approaches between HMBs, which could result in exclusion of potential donors and nutritional deficiencies. There is therefore a need to develop nutritional resources and guidance for donors, which can be provided through the HMBs. To support the development of these resources we recently undertook a systematic review and narrative synthesis to identify the responsivity of milk nutrient content to changes in maternal nutrient intake.[17] In total, 88 articles were included in this review, 54 were experimental studies and 34 were observational, comprising a total of 6,577 participants:
- 29 articles examined fatty acids
- 31 examined vitamins
- 23 examined minerals, amino acids and proteins
- five examined contaminants (heavy metals: arsenic, boron, lead and mercury) and polychlorinated biphenyls (PCBs).

TABLES 1-4 provide a summary of the results along with ratings of the overall quality of the evidence using the GRADE system for each nutrient and contaminant. The tables also summarise the doses of supplementation provided in the experimental studies. Where relevant, European Food Safety Authority (EFSA) recommended intake levels are provided for comparison, as well as toxicity information on contaminant levels.

The main findings are:
- there is strong evidence of response to maternal intakes of DHA, eicosapentaenoic acid (EPA), vitamins A, E and K, iodine and selenium in breast milk composition
- there is some evidence of response for ALA, B vitamins, vitamins C and D, ovalbumin, tyrosine and some contaminants
- there is insufficient evidence to determine the effects of ARA, copper, iron, zinc and choline.

It should be noted that only a high dose of vitamin C was shown to produce an increase in breast milk vitamin C content, and although ARA intake was not found to affect breast milk ARA content, the supplemental dose used was too low to allow definitive conclusions.

Although maternal intake of iron, copper, zinc and total choline levels was not shown to affect their levels in breast milk, these findings are based on a limited number of studies and so there remains uncertainty for these nutrients, consistent with previous systematic reviews.[5,18-20]

The most researched nutrients were DHA and EPA, where supplementation was consistently shown to increase their respective milk levels and this was in a dose-dependent manner. ALA maternal intake was similarly shown to increase breast milk ALA levels. A significant positive correlation between maternal consumption of fatty fish intake and breast milk DHA, EPA and ALA was also reported. DHA levels were either significantly lower in vegans than omnivores or vegetarians, or low across all groups. For ARA, only one study was identified and in this experimental study participants were supplemented with 54mg ARA per day for two weeks and no relationship was identified between maternal intake and breast milk levels. Trans fatty acids, saturated fatty acids and hydrogenated fats consumption and their content in breast milk were investigated in four studies.

It is important to highlight that in most of the reviewed experimental publications, the nutrient doses were higher than the EFSA adequate intake or average requirements.[21] For instance, in all nine reviewed experimental studies, DHA and EPA supplementation was shown to increase breast milk DHA and EPA levels in a dose-dependent manner. The highest dose of DHA provided was 1.2g for 14 days. The levels of supplemen-tation provided were also higher than EFSA recommendations for vitamins A, B6, B12, E and K.[21] It may therefore be that dietary supplementation is needed to produce the greatest levels of nutrients.

Vegan and vegetarian diets

When considering specific restrictive diets such a vegan or vegetarian, breast milk from vegan mothers was consistently shown to contain low DHA levels. The choline content was also shown to be lower in breast milk from mothers following a vegan diet. In terms of protein, taurine was lower in vegan mothers' breast milk. Our review did not identify any studies that specifically compared the vitamin B12 content of breast milk from mothers following vegan, vegetarian and non-vegetarian diets, although vitamin B12 content was shown to be responsive to maternal intake. Therefore, our results support the previous recommendations that mothers following a vegan diet should take supplementation with preformed DHA and vitamin B12, as maternal levels of intake of these nutrients may be low.[18] Furthermore, as vegan and vegetarian diets become more popular, there is an urgent need to conduct further high-quality studies in this area, so lactating mothers and milk bank donors can be provided with specific nutritional recommendations. HMBs should identify if donors are following these types of restricted diets.

There is often a concern around the possible negative effects of increasing dietary fish intake, which may theoretically increase the milk levels of contaminants such as heavy metals and PCBs. Our review identified that the number of studies published in this area is limited, with very few publications focusing on dietary sources. However, the overall presence of contaminants in breast milk was below toxicity levels, and based on these observations, fish intake was not identified as a potential source of elevated mercury in breast milk, or as an area of concern with other contaminants.

Conclusion

In conclusion, we have proposed two areas where it may be possible to enhance and/or preserve the nutrient content of DHM. The first area concerns the practices employed by HMBs, looking at storage, transport and processing techniques used, where we have proposed recommendations for practice (protect donor milk from light, minimise storage times, store at the lowest possible temperature).[14]

The second involves developing nutritional resources for healthcare professionals (such as dietitians and lactation consultants), and for the donors and HMBs. Our systematic review provides the foundation for the development of these nutritional resources. Maternal intake, particularly DHA, EPA, ALA, and vitamins A, D, E, B6, B12 and K, ovalbumin and tyrosine were found to be responsive to maternal diet, whereas there is insufficient evidence to ascertain the effects of intake on breast milk ARA, vitamin C, iron, zinc, copper and choline.[17]

These results support the recommendation that donors should adopt a healthy and diversified diet, such as the Mediterranean diet, and consider dietary supplementation with nutrients such as DHA, vitamins B12, K and D when their diets are restricted or limited by external factors. Future work will explore how to implement these changes and whether they produce the expected effects on the nutritional content of DHM.

Author contributions

SCD: Conceptualisation, methodology, resources, visualisation, writing - original draft, review and editing, project administration. CF: Data curation, methodology, formal analysis, investigation, resources, writing – original draft, review and editing. YJ: Resources, writing – review and editing.

All data are available upon request from the corresponding author.

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