Safety of Cow's Milk-Derived Fortifiers Used with an All-Human Milk Base Diet in Very Low Birthweight Preterm Infants

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Abstract

Background: Very low birthweight (VLBW) preterm infants fed mothers own milk (MOM) need nutritional supplementation, traditionally achieved with cow's milk (CM) derived fortifier CMDF) and preterm formula (PTF) if MOM is insufficient. CM products have been associated with diverse major morbidities. The current recommendation is to preferentially replace PTF with donor milk (DM) to produce a 100% human milk (HM) base diet, usually fortified with CMDF. Objective: To identify whether CMDF, even when fed with a 100% HM base diet, is related to an increased risk of major morbidities.

Methods: We identified a randomized trial with an all-HM base diet, comparing CMDF with a fortifier derived from human milk (HMDF), and two additional studies of this design were generated from raw data as subgroup analyses of a randomized controlled trial and a quasi-experimental study. Using these studies, we calculated the impact of CMDF on major morbidities of death, necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), sepsis, bronchopulmonary dysplasia (BPD) and patent ductus arteriosus (PDA).

Results: Each study individually provided support for an increase in major morbidities with CMDF. Meta-analyses of pooled data showed that compared to HMDF, the CMDF group had large increases in NEC (RR=3.3; P=0.001), ROP (RR=2.2; P=0.007), PDA (RR=1.6; P=0.009), interruption of feeding (RR=3.4; P=0.001) and a positive mortality/morbidity index based on one or more of death, NEC, sepsis, ROP and BPD (RR=1.4; P=0.006).

Conclusions: Despite the increased use of HM in modern neonatal care as a base diet, we found a greater risk of critical morbidities with CMDF compared with HMDF. This burden of morbidity provides evidence that the benefits of an HM base diet, might be, in part, counteracted by multiple adverse outcomes relating to the use of CMDF.

Key Words: preterm infant feeding, cow's milk-derived fortifiers, human milk-derived fortifiers, neonatal morbidity, donor milk

Abbreviations: MOM: Mothers own milk, CM: Cow's milk, CMDF: Cow's milk-derived fortifier, PTF: Preterm fortifier, HMDF: human milk-derived fortifier, NEC: Necrotizing enterocolitis, ROP: Retinopathy of prematurity, PDA: Patent ductus arteriosis, BPD: bronchopulmonary dysplasia, NICU: Neonatal intensive care unit

Introduction

MOM is strongly recommended for very low birth weight (VLBW) preterm infants, but does not fully meet their nutritional needs, traditionally met by adding a CMDF to MOM, and when MOM is insufficient, by using a PTF. CM-based products have an important role in current neonatal practice in promoting growth, but evidence indicates that VLBW infants fed partly or wholly on CM products may have a greater risk of adverse outcomes relating to NEC1-4, late-onset sepsis (LOS) (5-8), mortality (7,8), ROP (7,9-11), BPD,(10,12) , brain development (13,14), cardiovascular risk (15-17), bone health (18), atopic disease (19) and structural development of the heart, lungs and great vessels (20). It is unknown if these adverse outcomes relate to CM exposure, displacement of HM exposure, or both.

With increasing focus on using human milk in the NICU, official bodies (21,22) recommend using DM rather than PTF when MOM is insufficient, thus increasing HM exposure. Most units would then use a CMDF as the sole source of CM. Given the international emergence of this practice, testing the safety of CMDF, as used in this common practice, is critical. The ideal safety study is one where the base diet is 100% HM, and where it is possible to compare a CMDF versus an HMDF for a range of morbidities. However, remarkably few such studies have been undertaken. We identified only three studies of this design; the OptiMoM trial (9) together with two subgroup analyses of existing studies (1,10).

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Our hypothesis, based on historical evidence of adverse outcomes seen with CM products (cited above), is that even when the base diet is 100% HM, CMDF is associated with major morbidity. A large, well-powered, hypothesis-testing trial has not been done to test this comprehensively. However, since feeding an all HM diet with a CMDF is so prevalent, we considered that the combined analysis of morbid outcomes and mortality from the three studies identified, providing 453 subjects, should be evaluated as this is the largest dataset of its type and may help to guide practice and research.

Methods

Screening of PUBMED, MEDLINE, Google Scholar, and recent reviews, revealed only one study, the OptiMoM trial (2018) (9),

that met our criterion of 100% human milk base diet with the experimental comparison of CMDF with a modern HMDF. However, we reanalyzed part of this study, and the authors later corrected the findings (see results). The other two studies – the Sullivan trial 2010 (1) and the Assad study 2014 (10) – were ones where we had access to raw data and could generate a subgroup with the desired design (100% HM base diet and experimental comparison of CMDF versus HMDF). We rejected a group of very small studies, notably those of Polberger et al (23) and Hagelberg et al (24) from before the modern HMDF era where investigative groups made non-standardized, clinically unavailable fortifier preparations from skimmed DM for physiological studies that were experimental precursors of current standardized HMDFs.

The three studies presented here are considered separately; and then combined in meta-analyses if two or all three studies included major individual morbidities, previously linked to CM exposure, including NEC, death, ROP, BPD, LOS, and PDA. A secondary outcome was feeding interruption (enteral feeding withheld 24 hours; FW24) since the increased need for parenteral nutrition (PN) with feed intolerance may increase morbidity.

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The original studies included here for further reanalyses and meta-analyses all received ethical approval. The OptiMoM trial was approved by the Human Research Ethics Board of each participating hospital; for the trial by Sullivan et al (2) Institutional Review Board (IRB) approval was obtained at each center; and for the Assad, study (3) approval was obtained from the IRB of the Herman and Walter Samuelson Children's Hospital.

OptiMoM trial (9)

This was a blinded, multicenter RCT from Canada comprising 125 infants <1250g birthweight, who received a base diet of MOM, plus DM as required, and were randomly assigned to CMDF or HMDF. The trial was powered for feeding interruption, but a secondary outcome was a dichotomous mortality/morbidity index, affirmative for any one or more of death, LOS, BPD, ROP or NEC (Bells stage II or greater) – each reported separately as well as part of the morbidity/mortality index. Partial BM fortification commenced at a feeding volume of 100ml/kg per day and full fortification at 140ml/kg/day.

Sullivan trial; subgroup analysis

The original trial (1), reported elsewhere, was a 3-limb, 12 center RCT (11 centers from the USA: one from Austria) including infants 500-1250g at birth. All infants received MOM. Groups HM100 and HM40 received HMDF (Prolacta Bioscience, USA), at feeding volumes of 100 mL/kg/day and 40 mL/kg/day, and both groups received standardized DM (Prolacta Bioscience) when MOM was insufficient. The CM group received CMDF (Enfamil, Mead Johnson, USA or Similac; Abbott Laboratories, USA) and a PTF if MOM insufficient.

We have recently reported25 an analysis of a subgroup from that trial in which the base milk was 100% mother's milk. Thus, the only difference between groups was whether HMDF or CMDF was added. Those randomized separately into the HM40 and HM100 were merged for analysis (no differences were seen based on the timing of initiation of feeds for any outcome), explaining why there were 82 subjects fed HMDF and 32 fed CMDF.

NEC was the principal outcome in our subgroup reanalysis, justified by the fact that it was a predetermined key outcome in the main trial (Clinicaltrials.gov reg. #NCT00506584.). Our further key outcome was a severe morbidity index: NEC surgery or death. Because mortality is high in those requiring surgery, death is logically included because of its censoring effect on the incidence of NEC surgery. Secondary outcomes included: BPD (7,10-12), ROP7,9-11, and sepsis (5-8).

Assad subgroup analysis

Assad et al. (2014) (10) conducted a single-center study in 293 preterm infants (mean gestation 28 weeks). The study is reported elsewhere (10). Three groups of infants were fed CM-based products and a 4th, EHM feeding. Two of the groups allowed a pre-post (quasi-experimental) comparison of an all HM base diet with CMDF pre-2012 versus an all HM base diet with HMDF post-2012. Since some original data analyses were from combined groups, our new 2-subgroup study required a reanalysis of primary data, allowing us to examine any association between fortifier type and multiple outcomes.

Publication history

The data in the Assad subgroup reanalysis, have not been published previously as an original manuscript. This analysis now also includes new data from the medical records for death and LOS that were not included in the analysis of the original Assad study (10). Both the Sullivan reanalysis and OptiMoM studies have been published (9,25) but have been further analyzed in this current study. None of the meta-analyses of the three studies combined have been previously published.

Type of human milk

The exclusive human milk group in the three studies all included both MOM and donor milk-derived fortifier. We note that in two of the three studies (Sullivan and Assad), the base diet in the CM group was only MOM, whereas in the OptiMoM trial, the base diet was MOM plus DM as required.

Diagnosis of NEC

In the OptiMoM and Sullivan trials, the diagnosis of NEC was made by radiologists, blind to the dietary assignment to improve diagnostic reliability, as described previously (2).

ROP

Two studies examined severe ROP (Sullivan(1) and OptiMoM(9)), and one examined all ROP (Assad10). For our meta-analyses, we elected to combine the three studies despite the heterogeneity. Previous studies have done this, and prior work shows both severe and all ROP are related to the use of CM(11) – as seen here when the studies were analyzed separately.

Statistical analysis

The baseline comparisons of categorical data used the chi-square test for homogeneity or Fisher's exact test for small cell sizes. Comparisons of baseline quantitative variables used the two-sample t-test. In considering whether fixed or random-effects models

would be most appropriate for our meta-analyses, we note the view of Borenstein et al (26). that if the number of studies is very small, as in this case, it may be impossible to estimate adequately between-study variance (tau-squared), rendering the fixed effect model the most viable option. In effect, we have treated included studies as the only ones of interest. Nevertheless, for the more major analyses, we also checked whether similar findings emerged with a random-effects model. We performed meta-analyses using REVMAN 5.3 software.

Results

Table 1 shows a lack of statistical evidence for differences in baseline characteristics between CMDF and HMDF groups within any of the three studies (P>0.05 in all cases).

Table 2 shows the relationship between fortifier type and outcomes for the three individual studies.

OptiMoM trial (9)

For all outcomes shown for OptiMoM in table 2, RR was greater in the CMDF group ranging from negligible for NEC stage II or greater, to 6.4 for severe ROP, a significant effect (P=0.04). There was also evidence for higher risk of sepsis with CMDF (RR=1.84; that is, an 84% increase in risk: P=0.07).

Additionally, the authors reported a dichotomous combined overall morbidity index, which was positive if the subject exhibited one or more of the following: death, NEC (stage II or higher), BPD, ROP or LOS. This aspect of the trial results is material, yet historically complex and clarified here,

The initial publication of the trial findings showed this morbidity index was not related to fortifier type: the incidence of a positive index in the CMDF and HMDF groups was almost identical at 49% and 48%. (9)

"However, we were concerned that this analysis was not an accurate representation of the data. Our own analysis utilized the authors' data on individual morbidities to calculate the average number of adverse events per subject. There were 31 adverse events in 64 subjects fed HMDF (0.48 events per subject); yet, for CMDF there were 45 adverse events among 61 subjects (0.74 events per subject)."

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The authors of the OptiMoM trial re-explored their findings and published a corrigendum (27), noting that there had been an overcounting of BPD cases resulting in their removal of 8 cases with a positive index selectively from the HMDF group. Thus, a revised version of the OptiMoM trial results now shows 23/64 subjects with a positive mortality/morbidity index in the HMDF group and 30/61 subjects in the CMDF group, reflecting, in our own analysis, an increased RR of having a positive index in the CMDF group of approaching 40% (RR 1.37; 95% CI 0.90-2.07) with an authors' adjusted P=0.07. The revised version is the version of the record at the journal website (direct link to AJCN).

Sullivan trial subgroup analysis (25)

Table 2 shows that use of CMDF was related to an increase in NEC compared to use of HMDF (RR=4.2; P=0.04); and also to a severe morbidity index of NEC surgery or death (RR=5.1; P=0.01) For BPD, sepsis, and ROP the relationship with fortifier type was not found.

Assad study reanalysis

The quasi-experimental pre-post subgroup analysis of Assad's study showed a major impact of fortifier type in those otherwise fed 100% human milk base diet.

Table 2 shows 7 outcomes in relation to fortifier type: death, late-onset sepsis, BPD, NEC, ROP, PDA, and FW24, all of which showed at an increase in risk in the CMDF group, and significantly so for NEC (RR=7.5:P=0.02); ROP (RR=2.5; P=0.001); PDA (RR=2.7; P=0.007); FW24 (RR=5.9; P=0.001).

In the original 4-limb Assad study, BPD incidence was significantly higher in those exposed to CM, but this subgroup analysis that allowed us to compare fortifiers was underpowered to study BPD; nevertheless, there was a 60% higher risk of BPD in the CMDF group (RR=1.6).

Meta-analyses

All three studies, Assad, Sullivan, and OptiMoM, contributed data for our meta-analyses of NEC, ROP. Death, BPD, and sepsis (all shown in Fig 1). Using fixed effect models, as planned, CMDF was associated with a higher risk of NEC (RR=3.3; P=0.008) and ROP (RR=2.4; P=0.001); with significance also shown in random-effects models (not depicted). In the CMDF group, there was also a more than doubling of the risk of death (RR=2.1; P=0.1); and a 32% higher risk of BPD (RR= 1.32; P=0.1). Both effects trended towards statistical significance. No overall effect of fortifier type was found for late-onset sepsis: the 80% and 30% increases in risk with CMDI in the OptiMoM and Assad studies were counterbalanced by a decreased risk in the Sullivan reanalysis

Two studies provided data for the impact of fortifier type on PDA, and our secondary outcome feeds withheld for >24 hours (FW24). Figure 2 shows the CMDF group had a higher risk of PDA (RR=1.6; P=0.009) and FW24 (RR=3.4; P=0.0001).

A meta-analysis of mortality/morbidity indices

In OptiMoM, those fed CMDF had a large, near 40% increase in the risk of a positive mortality/morbidity index compared to the HMDF group. (P=0.07) - see table 2. Because of the poten-

Figure 1: meta-analyses for NEC, ROP, BPD, Death, LOS; all 3 studies contributed

Figure 1a NEC

Study or Subgroup	CM Events	CM Total	HM Events	HM Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Assad 2014	11	127	1	87	20.5%	7.54 [0.99, 57.31]
OptiMoM 2018	3	61	3	64	50.5%	1.05 [0.22, 5.00]
Sullivan 2019	5	32	3	82	29.0%	4.27 [1.08, 16.84]
Total (95% CI)		**220**		**233**	**100.0%**	**3.31 [1.36, 8.07]**
Total events	19		7			

Heterogeneity: Chi² = 2.84, df = 2 (P = 0.24); I² = 30%
Test for overall effect: Z = 2.63 (P = 0.008)

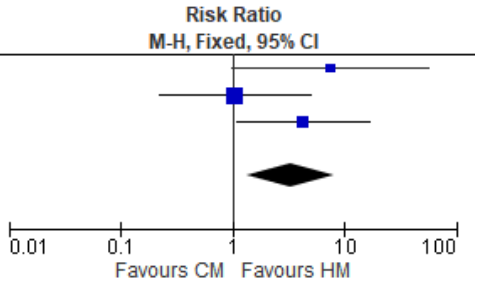


Figure 1b ROP

Study or Subgroup	CM Events	CM Total	HM Events	HM Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Assad 2014	40	127	11	87	75.1%	2.49 [1.35, 4.58]
OptiMoM 2018	6	58	1	62	5.6%	6.41 [0.80, 51.67]
Sullivan 2019	2	32	6	82	19.4%	0.85 [0.18, 4.01]
Total (95% CI)		**217**		**231**	**100.0%**	**2.39 [1.40, 4.08]**
Total events	48		18			

Heterogeneity: Chi² = 2.58, df = 2 (P = 0.28); I² = 22%
Test for overall effect: Z = 3.20 (P = 0.001)

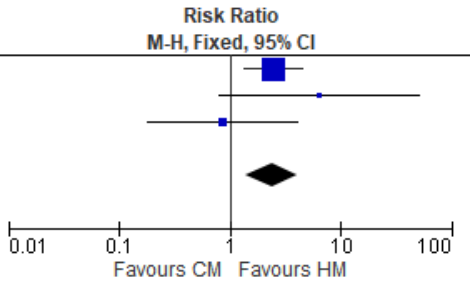


Figure 1c BPD

Study or Subgroup	CM Events	CM Total	HM Events	HM Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Assad 2014	30	127	13	87	34.7%	1.58 [0.88, 2.85]
OptiMoM 2018	18	61	16	64	35.1%	1.18 [0.66, 2.10]
Sullivan 2019	11	32	24	82	30.3%	1.17 [0.65, 2.11]
Total (95% CI)		**220**		**233**	**100.0%**	**1.32 [0.94, 1.85]**
Total events	59		53			

Heterogeneity: Chi² = 0.65, df = 2 (P = 0.72); I² = 0%
Test for overall effect: Z = 1.60 (P = 0.11)

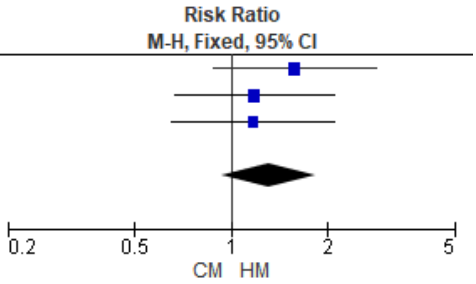


Figure 1d Death

Study or Subgroup	Cows Milk Events	Cows Milk Total	Human Milk Events	Human Milk Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Assad 2014	3	127	1	87	20.5%	2.06 [0.22, 19.43]
OptiMoM 2018	4	61	3	64	50.5%	1.40 [0.33, 6.00]
Sullivan 2019	4	32	3	82	29.0%	3.42 [0.81, 14.42]
Total (95% CI)		**220**		**233**	**100.0%**	**2.12 [0.85, 5.31]**
Total events	11		7			

Heterogeneity: Chi² = 0.74, df = 2 (P = 0.69); I² = 0%
Test for overall effect: Z = 1.60 (P = 0.11)

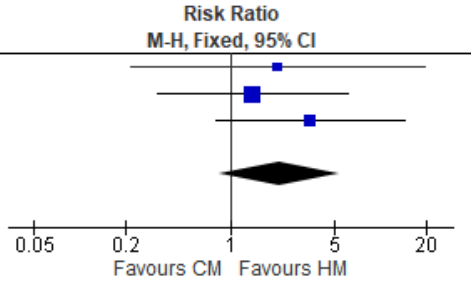


Figure 1e Late onset sepsis (LOS)

Study or Subgroup	Cows Milk Events	Cows Milk Total	Human Milk Events	Human Milk Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Assad 2014	20	127	11	87	40.7%	1.25 [0.63, 2.47]
OptiMoM 2018	14	61	8	64	24.3%	1.84 [0.83, 4.06]
Sullivan 2019	5	32	20	82	35.0%	0.64 [0.26, 1.56]
Total (95% CI)		**220**		**233**	**100.0%**	**1.18 [0.76, 1.83]**
Total events	39		39			

Heterogeneity: Chi² = 3.02, df = 2 (P = 0.22); I² = 34%
Test for overall effect: Z = 0.73 (P = 0.47)

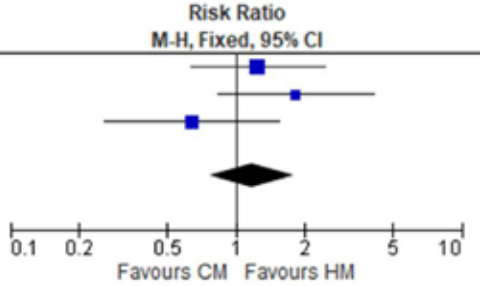


Figure 2: meta-analyses where data on 2 out of the 3 studies were available. Outcomes were PDA, and the secondary outcome: feeds withheld >24 hours

Figure 2a PDA

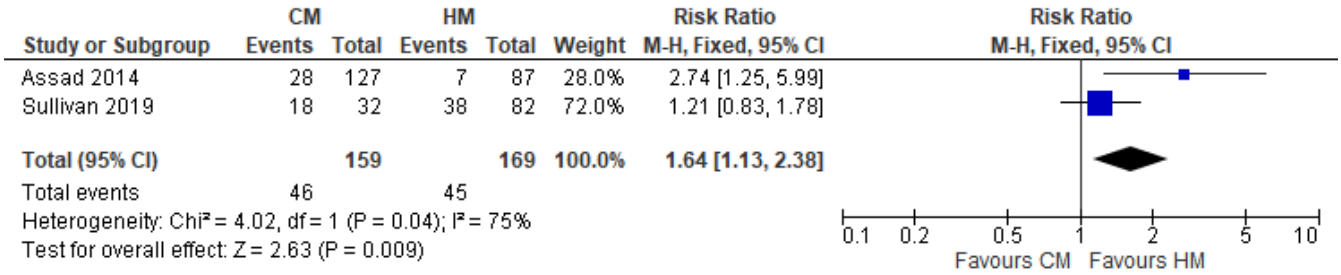


Figure 2c Feed withheld >24 hours

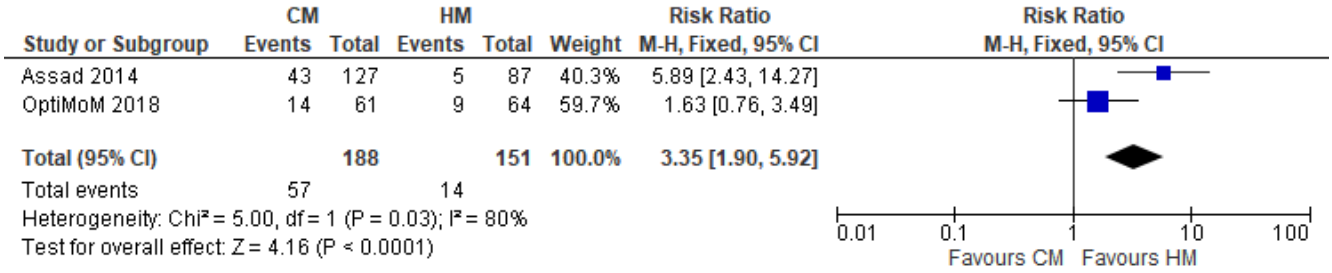
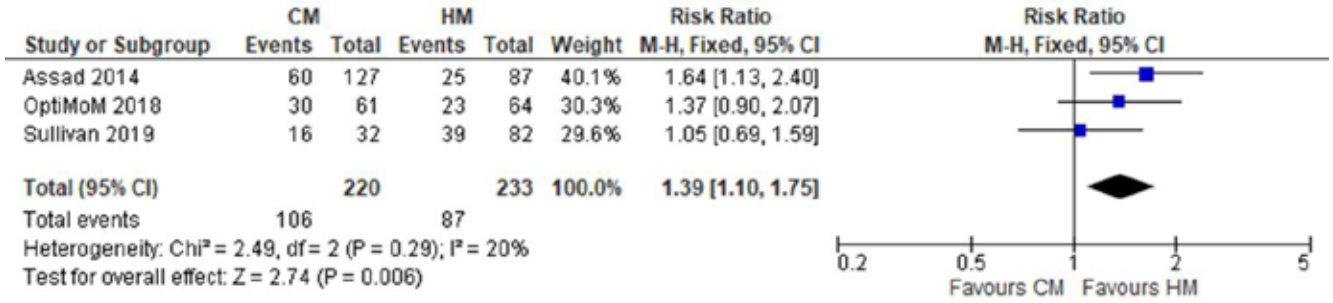


Figure 3: Meta-analysis of morbidity indices in the OptiMoM, Assad, and Sullivan studies, A positive index is defined as one or more of death, sepsis, NEC, ROP and BPD. The index was based on the one published in the OptiMoM corrigendum (27), and equivalent indices (based on the same 5 outcomes) were derived from raw data in the Sullivan and Assad reanalysis.



tial clinical importance of this, we derived the same index for the Assad and Sullivan studies based on the raw data (one or more of NEC, sepsis, BPD, ROP, or death). Fig. 3, shows a significant 40% higher risk of a positive mortality/morbidity index in the CMDF group (RR=1.4; P=0.006) when the three studies were combined in a meta-analysis.

Discussion

We found strong evidence that using CMDF, even with a 100% HM base diet, was associated with an increased risk of major neonatal morbidities. This finding is consistent with previous studies, including RCTs, cohort studies, systematic reviews, and meta-analyses, which indicated that feeding CM based products (PTF and CMDF) was associated with increased risk of multiple major neonatal morbidities (1-12).

In each of the three studies, analyzed separately, individual adverse outcomes were increased in the CMDF groups. When the studies were combined in meta-analyses, a clearer pattern of increased risk emerged for CMDF, most strongly expressed for NEC, ROP, PDA and FW24, and a dichotomous mortality/morbidity index based on death, late-onset sepsis, NEC, ROP or BPD.

One factor that may have an important bearing on these findings is that even with a 100% HM base diet, CM protein consumption of VLBW infants is still unexpectedly high. The current guideline that trades use of PTF for the use of DM is focussed on maximizing HM intake to improve outcomes. Yet, this practice also increases the use of fortifier – most commonly CMDF. We estimate a VLBW baby targeted to receive 4g protein/kg/day would obtain only 50% of this from 160 ml/kg per day of mother's preterm milk

Table 1: Baseline characteristics comparing infants fed a human milk (HMDF) vs cow's milk (CMDF) derived fortifier

Parameter	HMDF	CMDF
OptiMoM trial		
Number of subjects	64	63
Sex(female)	39/64 (61%)	34/63 (54%)
Race (non-European)	37/64 (59%)	44/63 (71%)
Antenatal steroids	56/64 (88%)	56/63 (89%)
Apgar at 5 min	7.4 (SD 2.1)	7.3 (SD 2.3)
Gestation (weeks)	27.9 (SD 2.7)	27.5 (SD 2.3)
Birthweight (grams)	887 (SD 208)	889 (SD 196)
SGA at birth	13/64 (20%)	16/63 (25%)
Sullivan trial re-analysis		
Number of subjects	82	32
Sex (female)	47/82 (57.3%)	15/32 (46.9%)
Race (black)	16/82 (19.5%)	3/32 (9.4%)
Antenatal steroids	15/82 (18.3%)	6/32 (18.8%)
APGAR<7	8/82 (9.8%)	6/32 (18.8%)
Gestation (weeks)	27.3 ± 2.2	27.1 ± 1.8
Birthweight (grams)	937 ± 199	938 ± 190
SGA at birth	10/82 (12.2%)	3/32 (9.4%)
Assad study re-analysis		
Number of subjects	87	127
Sex (female)	34/87 (39%)	64/127 (50%)
Race (black)	53 (61%)	85/127 (67%)
Gestation (weeks)	27.7 (SD 2.7)	28.3 (SD 2.8)

Statistical tests used: t-test for quantitative variables and chi-square/Fisher's exact test for categorical variables; all comparisons between groups were non-significant (p>0.05)

Table 2: Outcomes comparing infants fed a human milk (HMDF) vs cow's milk (CMDF) derived fortifier

Parameter	HMDF	CMDF	RR[1]	P-value[2]
Total number subjects (453)	233	220		
OptiMOM trial				
Number of subjects	64	61		
ROP (severe)	1 /62 (1.6%)	6 /59; 10.%)	6.4	0.04
Late onset sepsis	8 /64 (13%)	14/61 (23%)	1.8	0.07[3]
Death	3 /64 (4.7%)	4 (6.6%)	1.4	0.65
BPD	16/64 (25%)	18/61 (30%)	1.2	0.73[3]
NEC (Bells stage II or greater)	3/64 (4.7%)	3/61 (4.9%)	1.0	0.95
NEC all stages	3/64 (4.7%)	6/61 (9.8%)	2.1	0.27
Feeds withheld 12h (FW12h)	17/64 (27%)	20/61 (33%)	1.2	0.34[3]
Feeds withheld 24h (FW24h)	9/64 (14%)	14/61 (23%)	1.6	0.19[3]
Positive morbidity index[4]	23/64 (36%)	30/61 (49%)	1.4	0.07[3]
Sullivan RCT – subgroup reanalysis				
Number of subjects	82	32		
NEC (Bells Stage II or greater)	3/82 (3.7%)	5 (15.6%)	4.2	0.04
NEC surgery or death	3 /82 (3.7%)	6/32 (18.8%)	5.1	0.01
Death only	3/82 (3.7%)	4/32 (12.5%)	3.4	0.10
Proven sepsis	20 /84 (24.4%)	5/32 (15.6%)	0.6	0.45
BPD	24/84 (29.3%)	11/32 (34.4%)	1.2	0.60
ROP (grade 3 or 4)	6 /84 (7.3%)	2/32 (6.3%)	0.9	1.0
Assad study – subgroup group reanalysis				
Number of subjects	87	127		
NEC (Bells stage II or greater)	1/87 (1.1%)	11/127 (8.7%)	7.5	0.02
ROP	11/87 (14%)	40/127 (32%)	2.5	0.001
BPD	13/87 (15%)	30/127 (24%)	1.6	0.20
PDA[5]	7/87 (8%)	28/127 (22%)	2.7	0.007
Feeds withheld 24h (FW24)	5/87 (6%)	43/127 (34%)	5.9	0.001
Late onset sepsis	11/87 (13%)	20/127 (16%)	1.3	0.66

[1]RR = relative risk of adverse outcome in the CMDF group
[2]chi-square/Fisher's exact test
[3]adjusted P value based on multivariate logistic regression model
[4]Positive mortality/morbidity indxex = one or more of: ROP, sepsis, death, BPD, NEC stage II or greater
[5]Data on PDA collected in original database but not published in the original Assad Study

Death	1/87 (1.1%)	3/127 (2.4%)	2.2	0.89

of average composition (28) and only around 40% of this intake from 160 ml/kg of typical DM. The rest of this protein requirement must be from CMDF. Thus, babies fed HM as recommended may derive more protein from CMDF than HM, perhaps accounting for the apparently large adverse impact of CMDF.

"We found strong evidence that using CMDF, even with a 100% HM base diet, was associated with an increased risk of major neonatal morbidities."

An aspect of note was the variation between studies in the diversity of outcomes related to the use of CMDF. The OptiMoM trial showed that compared to the HMDF group, those fed CMDF had a strong trend to a near 40% increase in the proportion of subjects with a positive morbidity index, based on diverse morbidities. The Assad reanalysis illustrated this diversity of adverse outcomes with CMDF even more strikingly. Nevertheless, conversely, in the Sullivan reanalysis, the dominant effect of CMDF was in relation to NEC, NEC surgery, and death: whereas sepsis, ROP and BPD appeared unaffected. A likely possibility is that this simply reflects random variation in small individual studies, and hence justifies our approach in using meta-analyses to combine the data,

One finding in our trial that deserves attention is the hitherto unexplained higher risk of patent ductus in those fed CMDF. This was observed here in a meta-analysis of two of our three studies but has been previously reported by Hair and co-workers7, who found a highly significant increase in the risk of PDA in those exposed to CM products versus an exclusive human milk base diet. This requires further investigation.

Many studies lack the power to detect significant effects on the outcomes studied. To increase power, dichotomous morbidity indices are often used where having one or more of a group of adverse outcomes is treated as a positive result. In the OptiMoM trial, a mortality/morbidity index was used (one or more of death, NEC, sepsis, ROP, or BPD); and the CMDF group had a 40% increased risk of a positive index, based on the most serious common outcomes in neonatal care. Yet because P=0.07, the authors rejected this as "not significant" (27). However, Amrhein et al. in a seminal 2019 Nature paper (29), with 800 signatories, argue that P values have been misused, that P should not be a dichotomous variable with an arbitrary cut off value such as 0.05 and cannot be used to determine that findings are 'not significant,' particularly with large RRs, since this may result in incorrect rejection of key findings. Because of the potential clinical importance here, we conducted a meta-analysis of this same mortality/morbidity index for all three of our studies (n=453 versus 125 in OptiMoM alone: fig 3). The increased risk of having a positive index in the CMDF group remained at 40%, but now, in this more powered analysis, P=0.006.

Our secondary outcome and a primary one in OptiMoM (9) was the interruption of feeds – a major problem that increases the need for PN and its associated morbidity. In OptiMoM, feed interruption was measured as feeds withheld for 12 hours (FW12) but also as FW24. The increased risk for feed interruption for 12 and 24 hours in OptiMoM in the CMDF group was sizeable at 22% and 63%, respectively, but rejected as not significant. In OptiMoM, CM was introduced late (see below), which could have reduced the power of the study. Since FW24 was measured in both OptiMoM and Assad, a more robust meta-analysis was possible, showing a 3.4-fold increase in FW24 (P=0.0001) with the use of CMDF (see figure 2); an effect size potentially of considerable clinical significance.

Limitations

Our study is not a conventional systematic review or meta-analysis of published evidence and could not be conducted according to conventional guidelines. For 2 of our three studies (Sullivan and Assad), these could not be searched since they did not pre-exist but were created from subgroup analyses of the raw data from the original studies; and we accept there might be further, suitable raw datasets that could be correspondingly analyzed.

Two of our studies were subgroup analyses, theoretically more prone to chance imbalances between groups; greater morbidity in the CMDF group might simply have reflected chance generation a higher risk population. The evidence is strongly against that. Our studies, conducted in different years and centers, consistently showed greater morbidity in those fed CDMF. Baseline risk factors were well balanced between groups in each study. Also, much evidence links CM exposure to the same adverse outcomes shown here.

The NEC incidence amongst our three studies deserves some comment. The most modern of the three studies (OptiMoM) showed a particularly low incidence of NEC with no difference between randomized groups. Whilst we agree that future studies will resolve if this is an exceptional finding or not, we would note that the adverse effects of CM may be ameliorated by delaying its introduction into the diet (though with corresponding downsides for growth and requirement for PN). In the OptiMoM trial, the mean age of introduction of CM was late at day 17 (9), compared to the possibility of receiving CM in the early part of the first week in the Sullivan trial (1). The incidence of NEC in the Sullivan reanalysis was higher than commonly seen today, even in very small infants. However, the key point is that despite the heterogeneity in feeding practice, age of study, etc., our meta-analysis indicates a major increase in NEC in those assigned CMDF vs. HMDF: 8.6% incidence vs. 3.0% (derived from the data in figure 1).

"Our secondary outcome and a primary one in OptiMoM (9) was the interruption of feeds – a major problem that increases the need for PN and its associated morbidity."

Our study does not address the use of liquid CMDF used now in the US, though powder-based fortifiers are used in many countries and the three studies here. Whether liquid fortifiers by displacing about 1/6th of the MOM or DM volume with a fortifier could further increase any adverse impact of CMDF has received little attention. It would be hard to explore if HMDF, which is also a liquid, commercially derived from pasteurized DM, could have any measurable impact on the outcome by displacing a significant volume of MOM when the available volume is high. However, overall, compared to VLBW infants exposed to CM products, those fed on an EHM diet including HMDF, studied by us here and by other groups (1-3,7,10) have in general significantly lower morbidities

Finally, our findings apply to intact protein fortifiers in widespread international use. We do not consider here partially or extensively hydrolyzed fortifiers, now often used in the US (30-32). Such fortifiers have been compared with each other with some differences but not with HMDF and not studied for the broad range of morbidities reported to be differentially affected by CM vs. HM exposure.

Moreover, the hypothesis that using hydrolyzed CM protein in feeds for preterm infants would overcome the adverse effects of intact CM products is unproven, and not supported conceptually by a recent Cochrane review (33), albeit focussed on hydrolyzed formulas rather than fortifiers.

Conclusion

Current recommendations to maximize human milk intake in preterm infants have been enthusiastically implemented internationally, but without adequate research on the impact of the CM component of the diet with this new regime. This now needs scientific attention. We have identified three studies all with some form of experimental design that show individually, and collectively in meta-analyses, that VLBW preterm infants fed CMDF with an otherwise 100% human milk base diet had a significant increase in major morbidities some of which may reduce survival or have significant adverse post-neonatal effects. This burden of morbidity, indicated by the findings, provides evidence that the benefits of an HM base diet, might be in part counteracted by multiple adverse outcomes relating to the use of CMDF– and this needs further research attention. Our study demonstrated a significantly lower burden of morbidity with HMDF. Finally, the use of CMDF is designed to meet nutrient needs, yet our study emphasizes further the increasingly recognized potential importance of non-nutritional impacts of diet on clinical course and health outcomes in this high-risk population.

"We have identified three studies all with some form of experimental design that show individually, and collectively in meta-analyses, that VLBW preterm infants fed CMDF with an otherwise 100% human milk base diet had a significant increase in major morbidities some of which may reduce survival or have significant adverse post-neonatal effects."

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Author Contributions
***Professor Alan Lucas** conceived and initiated the study; analyzed raw data; and wrote the paper*
***Dr Maushumi Assad** provided her raw data (Assad study) and assisted us with the understanding of her database, She read and commented constructively on the manuscript*
***Professor Jan Sherman** was our advisor on evidence-based medicine. She performed all the meta-analyses and provided the data for figures. She read and advised on the manuscript.*
***Dr. John Boscardin** was our statistician who advised on: the statistical analysis, interpretation of data and data presentation*
***Professor Steven Abrams** key collaborator involved in every aspect of the study and made major intellectual input.*

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