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## Individualized versus standard diet fortification for growth and development in preterm infants receiving human milk (Review)

Fabrizio V, Trzaski JM, Brownell EA, Esposito P, Lainwala S, Lussier MM, Hagadorn JI

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[Intervention Review]

# Individualized versus standard diet fortification for growth and development in preterm infants receiving human milk

Veronica Fabrizio<sup>1,2</sup>, Jennifer M Trzaski<sup>1,2</sup>, Elizabeth A Brownell<sup>3,4</sup>, Patricia Esposito<sup>1</sup>, Shabnam Lainwala<sup>1,2</sup>, Mary M Lussier<sup>1</sup>, James I Hagadorn<sup>1,2</sup>

<sup>1</sup>Division of Neonatology, Connecticut Children's, Hartford, CT, USA. <sup>2</sup>Department of Pediatrics, University of Connecticut School of Medicine, Farmington, CT, USA. <sup>3</sup>School of Nursing, University of Texas Health Science Center, San Antonio, TX, USA. <sup>4</sup>Department of Pediatrics, University of Texas Health Science Center, San Antonio, Texas, USA

**Contact address:** Jennifer M Trzaski, [jtrzask@connecticutchildrens.org](mailto:jtrzask@connecticutchildrens.org).

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## ABSTRACT

### Background

Human milk as compared to formula reduces morbidity in preterm infants but requires fortification to meet their nutritional needs and to reduce the risk of extrauterine growth failure. Standard fortification methods are not individualized to the infant and assume that breast milk is uniform in nutritional content. Strategies for individualizing fortification are available; however it is not known whether these are safe, or if they improve outcomes in preterm infants.

### Objectives

To determine whether individualizing fortification of breast milk feeds in response to infant blood urea nitrogen (adjustable fortification) or to breast milk macronutrient content as measured with a milk analyzer (targeted fortification) reduces mortality and morbidity and promotes growth and development compared to standard, non-individualized fortification for preterm infants receiving human milk at < 37 weeks' gestation or at birth weight < 2500 grams.

### Search methods

We used the standard search strategy of Cochrane Neonatal to search the Cochrane Central Register of Controlled Trials (CENTRAL; 2019, Issue 9), in the Cochrane Library; Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R); and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), on September 20, 2019. We also searched clinical trials databases and the reference lists of retrieved articles for pertinent randomized controlled trials (RCTs) and quasi-randomized trials.

### Selection criteria

We considered randomized, quasi-randomized, and cluster-randomized controlled trials of preterm infants fed exclusively breast milk that compared a standard non-individualized fortification strategy to individualized fortification using a targeted or adjustable strategy. We considered studies that examined any use of fortification in eligible infants for a minimum duration of two weeks, initiated at any time during enteral feeding, and providing any regimen of human milk feeding.

### Data collection and analysis

Data were collected using the standard methods of Cochrane Neonatal. Two review authors evaluated the quality of the studies and extracted data. We reported analyses of continuous data using mean differences (MDs), and dichotomous data using risk ratios (RRs). We used the GRADE approach to assess the certainty of evidence.

**Individualized versus standard diet fortification for growth and development in preterm infants receiving human milk (Review)**

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## Main results

Data were extracted from seven RCTs, resulting in eight publications (521 total participants were enrolled among these studies), with duration of study interventions ranging from two to seven weeks. As compared to standard non-individualized fortification, individualized (targeted or adjustable) fortification of enteral feeds probably increased weight gain during the intervention (typical mean difference [MD] 1.88 g/kg/d, 95% confidence interval [CI] 1.26 to 2.50; 6 studies, 345 participants), may have increased length gain during the intervention (typical MD 0.43 mm/d, 95% CI 0.32 to 0.53; 5 studies, 242 participants), and may have increased head circumference gain during the intervention (typical MD 0.14 mm/d, 95% CI 0.06 to 0.23; 5 studies, 242 participants). Compared to standard non-individualized fortification, targeted fortification probably increased weight gain during the intervention (typical MD 1.87 g/kg/d, 95% CI 1.15 to 2.58; 4 studies, 269 participants) and may have increased length gain during the intervention (typical MD 0.45 mm/d, 95% CI 0.32 to 0.57; 3 studies, 166 participants). Adjustable fortification probably increased weight gain during the intervention (typical MD 2.86 g/kg/d, 95% CI 1.69 to 4.03; 3 studies, 96 participants), probably increased gain in length during the intervention (typical MD 0.54 mm/d, 95% CI 0.38 to 0.7; 3 studies, 96 participants), and increased gain in head circumference during the intervention (typical MD 0.36 mm/d, 95% CI 0.21 to 0.5; 3 studies, 96 participants). We are uncertain whether there are differences between individualized versus standard fortification strategies in the incidence of in-hospital mortality, bronchopulmonary dysplasia, necrotizing enterocolitis, culture-proven late-onset bacterial sepsis, retinopathy of prematurity, osteopenia, length of hospital stay, or post-hospital discharge growth. No study reported severe neurodevelopmental disability as an outcome. One study that was published after our literature search was completed is awaiting classification.

## Authors' conclusions

We found moderate- to low-certainty evidence suggesting that individualized (either targeted or adjustable) fortification of enteral feeds in very low birth weight infants increases growth velocity of weight, length, and head circumference during the intervention compared with standard non-individualized fortification. Evidence showing important in-hospital and post-discharge clinical outcomes was sparse and of very low certainty, precluding inferences regarding safety or clinical benefits beyond short-term growth.

## PLAIN LANGUAGE SUMMARY

### Individualized versus standard diet fortification for growth and development in very low birth weight infants receiving human milk

**Review question:** does individualized rather than standard, non-individualized addition of nutrients and calories to breast milk feeds safely improve growth and other outcomes in preterm infants?

**Background:** preterm infants are at risk for poor growth following birth. Breast milk reduces their risk of illness but does not meet their nutritional needs. Therefore, breast milk fed to preterm infants must be fortified with extra nutrients. Usual methods of fortifying breast milk treat all breast milk and all preterm infants the same. However, two methods are available for individualizing fortification for each preterm infant. Targeted fortification adds nutrients to breast milk based on the results of breast milk analysis. Adjustable fortification adds nutrients based on the results of preterm infant laboratory results. Individualized fortification may improve preterm infant growth or other outcomes. However, it is not known whether targeted or adjustable fortification is safe or improves outcomes for preterm infants compared with the standard method.

**Study characteristics:** through literature searches updated to September 2019, we found seven studies that tested the effects of targeted or adjustable fortification of breast milk feeds compared to standard fortification in preterm infants, yielding eight publications (521 total participants were enrolled in these studies). One study that was published after our literature search was completed is awaiting classification.

**Key results:** targeted or adjustable fortification improves short-term growth compared to standard fortification in preterm infants. Determining the best way to customize breast milk feeds is necessary, as is clarifying its safety and effects on other clinical outcomes.

**Certainty of evidence:** very low to moderate. Moderate certainty means that the true effect of individualized fortification on growth in preterm infants is likely to be close to the result of this review but there is a possibility that it is substantially different. Low certainty means that the true effect may be substantially different from the results of this review. Very low certainty means that the true effect of individualized fortification on growth in preterm infants is likely to be substantially different from the results of this review. Certainty of evidence was downgraded most often in this review due to small numbers of participants in included studies and significant differences in study design and outcome measures among included studies.

## SUMMARY OF FINDINGS

### Summary of findings 1. Targeted or adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk

#### Targeted or adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk

**Patient or population:** promoting growth and development in very low birth weight infants receiving human milk

**Setting:** neonatal ICU

**Intervention:** targeted or adjustable individualized fortification

**Comparison:** standard non-individualized fortification

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with standard non-individualized fortification	Risk with targeted or adjustable individualized fortification				
Growth velocity, weight, g/kg/d, end of intervention	Mean growth velocity, weight, g/kg/d, end of intervention was 17.1 g/kg/d	MD 1.88 g/kg/d more (1.26 more to 2.5 more)	-	345 (6 RCTs)	⊕⊕⊕⊖ MODERATE <sup>a</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, length, mm/d, end of intervention (length velocity)	Mean growth velocity, length, mm/d, end of intervention was 1.17 mm/d	MD 0.43 mm/d more (0.32 more to 0.53 more)	-	262 (5 RCTs)	⊕⊕⊖⊖ LOW <sup>a,b</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, head circumference, mm/d, end of intervention	Mean growth velocity, head circumference, mm/d, end of intervention was 1.18 mm/d	MD 0.14 mm/d higher (0.06 higher to 0.23 higher)	-	242 (5 RCTs)	⊕⊕⊖⊖ LOW <sup>a,b</sup>	No single study appeared to be an outlier compared to other studies
Necrotizing enterocolitis	No data available					

Culture-proven late-onset sepsis	No data available				
Mortality	No data available				
Bronchopulmonary dysplasia	Study population		RR 0.89 (0.71 to 1.12)	391 (4 RCTs)	⊕⊕⊕⊕ VERY LOW <sup>b,c,d</sup>
	443 per 1000	394 per 1000 (315 to 496)			
Retinopathy of prematurity, any	Study population		RR 0.79 (0.36 to 1.72)	60 (1 RCT)	⊕⊕⊕⊕ VERY LOW <sup>b,d</sup>
	350 per 1000	276 per 1000 (126 to 602)			
Osteopenia	Study population		RR 0.86 (0.40 to 1.84)	60 (1 RCT)	⊕⊕⊕⊕ VERY LOW <sup>b,d,e</sup>
	350 per 1000	301 per 1000 (140 to 644)			

\***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; ICU: intensive care unit; MD: mean difference; RCT: randomized controlled trial; RR: risk ratio.

#### GRADE Working Group grades of evidence.

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

<sup>a</sup>Downgraded by one for inconsistency due to high heterogeneity ( $\geq 75\%$ ) in estimate of effect.

<sup>b</sup>Downgraded by one for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects.

<sup>c</sup>Downgraded by one for inconsistency due to variation among studies in case definitions of outcome.

<sup>d</sup>Downgraded by two for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control.

<sup>e</sup>Downgraded by one for indirectness due to use of surrogate outcome (osteopenia) rather than patient-important outcome (fractures).

### Summary of findings 2. Targeted individualized compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk

**Targeted individualized compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk**

**Patient or population:** promoting growth and development in very low birth weight infants receiving human milk  
**Setting:** neonatal ICU  
**Intervention:** targeted individualized  
**Comparison:** standard non-individualized fortification

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with standard non-individualized fortification	Risk with targeted individualized				
Growth velocity, weight, g/kg/d, end of intervention	Mean growth velocity, weight, g/kg/d, end of intervention was 19.2 g/kg/d	MD 1.87 g/kg/d higher (1.15 higher to 2.58 higher)	-	269 (4 RCTs)	⊕⊕⊕⊕ MODERATE <sup>a</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, length, mm/d, end of intervention	Mean growth velocity, length, mm/d, end of intervention was 1.64 mm/d	MD 0.45 mm/d higher (0.32 higher to 0.57 higher)	-	166 (3 RCTs)	⊕⊕⊕⊕ LOW <sup>a,b</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, head circumference, mm/d, end of intervention	Mean growth velocity, head circumference, mm/d, end of intervention was 1.29 mm/d	MD 0.08 mm/d higher (0.01 lower to 0.18 higher)	-	166 (3 RCTs)	⊕⊕⊕⊕ LOW <sup>a,b</sup>	No single study appeared to be an outlier compared to other studies
Length of hospital stay, days	Mean length of hospital stay, days, was 86 days	MD 12 days lower (26.38 lower to 2.38 higher)	-	75 (1 RCT)	⊕⊕⊕⊕ VERY LOW <sup>c,d</sup>	
Retinopathy of prematurity	No data available					
Bronchopulmonary dysplasia	No data available					
Mortality	No data available					

In-hospital mortality	Study population	RR 0.14 (0.02 to 1.14)	334 (3 RCTs)	⊕○○○ VERY LOW <sup>b,d</sup>
	36 per 1000 5 per 1000 (1 to 41)			
Necrotizing enterocolitis	Study population	RR 0.40 (0.08 to 1.99)	257 (2 RCTs)	⊕○○○ VERY LOW <sup>b,d</sup>
	39 per 1000 16 per 1000 (3 to 78)			
Culture-proven late-onset bacterial sepsis	Study population	RR 1.29 (0.76 to 2.17)	257 (2 RCTs)	⊕○○○ VERY LOW <sup>b,d</sup>
	156 per 1000 202 per 1000 (119 to 339)			

\***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; MD: mean difference; RCT: randomized controlled trial; RR: risk ratio.

#### GRADE Working Group grades of evidence.

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

<sup>a</sup>Downgraded by one for inconsistency due to high heterogeneity in estimate of effect ( $I^2 \geq 75\%$ ).

<sup>b</sup>Downgraded by one for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects.

<sup>c</sup>Downgraded by one for risk of bias. Prolacta Bioscience provided the product for the study and assisted in data analysis. Two study authors received financial support and speaker honoraria from Prolacta Bioscience. Two other study authors were employees of Prolacta Bioscience. Allocation concealment, blinding of outcome assessment not described. Masking of study groups was not possible at one site.

<sup>d</sup>Downgraded by two for imprecision due to total enrolment insufficient for 50% power to detect 20% change compared to control.

### Summary of findings 3. Adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk

#### Adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk

**Patient or population:** promoting growth and development in very low birth weight infants receiving human milk

**Setting:** neonatal ICU

**Intervention:** adjustable individualized fortification

**Comparison:** standard non-individualized fortification

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	N° of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with standard non-individualized fortification	Risk with adjustable individualized fortification				
Growth velocity, weight, g/kg/d, end of intervention	Mean growth velocity, weight, g/kg/d, end of intervention was 15.2 g/kg/d	MD 2.86 g/kg/d higher (1.69 higher to 4.03 higher)	-	96 (3 RCTs)	⊕⊕⊕⊖ MODERATE <sup>a</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, length, mm/d, end of intervention	Mean growth velocity, length, mm/d, end of intervention was 1.06 mm/d	MD 0.54 mm/d higher (0.38 higher to 0.7 higher)	-	96 (3 RCTs)	⊕⊕⊕⊖ MODERATE <sup>a</sup>	Although <a href="#">Kadioglu Simsek 2019</a> appeared prominent compared to other studies in its effects favoring individualized fortification, this was explored further in the sensitivity analysis; thus evidence was not downgraded further
Growth velocity, head circumference, mm/d, end of intervention	Mean growth velocity, head circumference, mm/d, end of intervention was 0.98 mm/d	MD 0.36 mm/d higher (0.21 higher to 0.5 higher)	-	96 (3 RCTs)	⊕⊕⊕⊕ HIGH	
Mortality	No data available					
NEC stage ≥ 2	No data available					
Culture-proven late-onset sepsis	No data available					
Retinopathy of prematurity	No data available					
Bronchopulmonary dysplasia	No data available					

\***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; ICU: intensive care unit; MD: mean difference; NEC, necrotizing enterocolitis; RCT: randomized controlled trial.

**GRADE Working Group grades of evidence.**

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

<sup>a</sup>Inconsistency due to high heterogeneity in estimate of effect ( $I^2 \geq 75\%$ ).

**Summary of findings 4. Targeted individualized fortification compared to adjustable individualized fortification for promoting growth and development in very low birth weight infants receiving human milk**

**Targeted individualized fortification compared to adjustable individualized fortification for promoting growth and development in very low birth weight infants receiving human milk**

**Patient or population:** promoting growth and development in very low birth weight infants receiving human milk

**Setting:** neonatal ICU

**Intervention:** targeted individualized fortification

**Comparison:** adjustable individualized fortification

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	N° of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with adjustable individualized fortification	Risk with targeted individualized fortification				
Growth velocity, weight, g/kg/d - end of intervention	Mean growth velocity, weight, g/kg/d - end of intervention was 21.5 g/kg/d	MD 2.49 g/kg/d higher (0.44 higher to 4.54 higher)	-	72 (2 RCTs)	⊕⊕⊕⊖ MODERATE <sup>a</sup>	
Growth velocity, length, mm/d - end of intervention	Mean growth velocity, length, mm/d - end of intervention was 1.5 mm/d	MD 0.07 mm/d higher (0.06 lower to 0.2 higher)	-	72 (2 RCTs)	⊕⊕⊖⊖ LOW <sup>b</sup>	
Mortality	No data available					
NEC ≥ stage 2	No data available					
Culture-proven late-onset sepsis	No data available					

Retinopathy of prematurity	No data available
Bronchopulmonary dysplasia	No data available

<sup>a</sup>Downgraded by one for imprecision due to wide confidence intervals that include both clinically significant benefit and clinically insignificant effects.

<sup>b</sup>Downgraded by two for imprecision due to total enrolment insufficient for 50% power to detect 20% change compared to control.

## BACKGROUND

### Description of the condition

#### Growth failure in preterm infants

Preterm birth is a major cause of mortality and morbidity worldwide. A major morbidity faced by preterm infants is extrauterine growth restriction (EUGR), defined as weight at discharge less than the tenth percentile of expected intrauterine growth at the corresponding gestational age (Clark 2003; Ehrenkranz 2014; Hu 2019). Although rates of EUGR are decreasing, it remains a significant problem among very low birth weight (VLBW) infants in reports from large multi-center cohorts in North America and Israel (Griffin 2016; Horbar 2015; Ofek Shlomain 2014). Growth failure in VLBW infants results from the complex interaction of many factors, of which inadequate nutrition, especially during the first weeks of life, appears critically important (Embleton 2001). Growth failure during neonatal intensive care unit (NICU) hospitalization is associated with adverse neurodevelopmental outcomes including occurrence of cerebral palsy, scores less than 70 on the Bayley II Mental Development and Psychomotor Development Indices, and abnormal neurological examinations at 18 to 22 months (Ehrenkranz 1999), as well as abnormal performance in IQ and verbal flexibility, visual memory, and visual flexibility composite scores at a mean age of 25 years (Sammallahti 2014).

#### Fortification of human milk for preterm infants

The American Academy of Pediatrics recommends human milk for neonates due to its associated improved maternal and infant health outcomes (AAP 2012). These include decreased infections in the first year of life; reduced risk of sudden infant death syndrome; protective effects against asthma, atopic dermatitis, and eczema; reduction in certain gastrointestinal diseases, obesity, childhood leukemia, and lymphoma; and improved neurodevelopmental outcomes. Specifically, human milk protects against sepsis and necrotizing enterocolitis (NEC) in preterm infants, and is associated with fewer hospitalizations in the year after NICU discharge, lower rates of severe retinopathy of prematurity (ROP), and lower rates of metabolic syndrome and lower blood pressure in adolescence (AAP 2012). Human milk in preterm infants is also associated with improved neurodevelopmental outcomes, including mental, motor, and behavior skills (AAP 2012).

Although human milk has been established as the preferred enteral feeding option for preterm infants, its nutritional content is not sufficient to maintain the pace of intrauterine nutrient accretion. Intake of both protein and energy is crucial for the growth of preterm infants, and human milk does not adequately provide the recommended amounts at typical feeding volumes of between 135 and 200 mL/kg/d (Arslanoglu 2019). Thus, fortification of human milk can be used in the NICU setting to optimize nutritional intake and improve growth outcomes for preterm infants (Agostoni 2010; Ehrenkranz 2006). Bovine or human milk-derived multi-nutrient fortifier is typically introduced once the infant has demonstrated tolerance of enteral feeds advanced beyond minimal volumes. These fortifiers attempt to increase the protein and energy levels of enteral feeds to goals of 3.5 g/kg/d to 4.5 g/kg/d and 105 kcal/kg/d to 135 kcal/kg/d, respectively (Arslanoglu 2019). Fortifier amount is typically titrated clinically in response to infant growth and is usually continued until the infant approaches discharge.

Standard methods for fortifying human milk do not account proactively for variation in human milk nutrient content. However, when measured both within and among mothers, the macronutrient composition of human milk varies considerably (Wu 2018). In addition, the majority of banked donor milk is pooled from mothers of term infants and, when compared to preterm maternal milk, differs in macronutrient composition (Lawrence 2011; Radmacher 2013; Saarela 2005).

### Description of the intervention

This review compared three approaches to human milk fortification for preterm infants: standard, adjustable, and targeted (Adamkin 2014; Alan 2013; Radmacher 2017). Standard fortification, the most commonly used approach, assumes that all breast milk has an average caloric content and macronutrient composition, and then fortifies with a predetermined amount of fortifier. With adjustable fortification, addition of fortifying nutrients is individualized using the infant's metabolic response to enteral protein intake, as measured by blood urea nitrogen (BUN) (Alan 2013). Adjustable fortification typically increases protein content as tolerated using cutoff BUN levels typically around 9 mg/dL to 16 mg/dL, adding extra protein if BUN levels remain low (Arslanoglu 2019). Targeted fortification individualizes fortification using the results of human milk analysis, specifically by adding extra protein, fat, or carbohydrate based on the macronutrient concentration measured (Arslanoglu 2019). Milk analyzers assess breast milk content of carbohydrates, fat, protein, total solids, and energy, and may help healthcare providers meet the needs of infants requiring additional nutrients because of preterm birth or other health conditions. In 2018, the US Food and Drug Administration (FDA) approved a human milk analyzer for clinical use (US Food and Drug Administration 2018). NICUs are incorporating the use of analyzers into clinical care (Wake Forest/Baptist Medical Center 2018).

### How the intervention might work

The primary goal of fortifying human milk for preterm infants is to support postnatal growth at a velocity similar to in utero growth (AAP 1977). Standard fortification practice fails to account for variation in the composition of mother's milk and donor's milk, and is associated with postnatal growth failure. By individualizing nutritional support, adjustable or targeted fortification strategies may improve growth failure and, secondarily, the neurodevelopmental outcomes associated with growth.

### Why it is important to do this review

Given the known variation in human milk macronutrient composition before fortification, a systematic assessment of standard versus adjustable versus targeted diet fortification of VLBW infant feedings is warranted. This review is clearly distinct from existing reviews on topics involving human milk and preterm infants (donor milk versus formula, banked preterm milk versus banked term milk, maternal breast milk versus formula) and has incorporated sophisticated advances in human milk feeding techniques, for which important literature is just emerging. In addition, this review makes available summary results of randomized controlled trials on different fortification strategies as they emerge, supporting management and promotion of optimal VLBW outcomes.

Infrared human milk analyzers efficiently provide accurate macronutrient profiles for individual specimens of human milk. They have reached the market, they are cost-effective, and they are used in descriptive research studies to examine the composition of mother's own milk and donor human milk (Radmacher 2013; Rochow 2013; Sauer 2011). In 2018, the FDA approved a human milk analyzer for clinical use. Analyzers therefore allow for targeted human milk fortification (i.e. tailored to individual infants and milk specimens) in clinical care. The impact of routine use of analyzers upon nutritional support, clinical outcomes, or long-term neurodevelopment for VLBW infants receiving human milk is yet to be seen in the NICU setting. Similarly, the comparative merits of these fundamentally different approaches to fortification have not been well defined.

## OBJECTIVES

To determine whether individualizing fortification of breast milk feeds in response to infant blood urea nitrogen (adjustable fortification) or to breast milk macronutrient content as measured with a milk analyzer (targeted fortification) reduces mortality and morbidity and promotes growth and development compared to standard, non-individualized fortification for preterm infants receiving human milk at < 37 weeks' gestation or at birth weight < 2500 grams.

## METHODS

### Criteria for considering studies for this review

#### Types of studies

We considered randomized controlled trials (RCTs), quasi-RCTs, and cluster-RCTs for inclusion. We excluded cross-over trials.

#### Types of participants

Preterm infants at < 37 weeks' gestation or at birth weight < 2500 grams fed human milk exclusively - either mother's own milk or donor human milk - or a combination of mother's milk and donor milk.

#### Types of interventions

Interventions were human milk fortification methods. We compared each of the three fortification approaches: targeted and adjustable fortification; adjustable and standard fortification; targeted and standard fortification. We considered studies examining any use of fortification in eligible infants for a minimum duration of two weeks, initiated at any time during enteral feeding, and with any regimen of human milk feeding.

#### Types of outcome measures

##### Primary outcomes

1. End of intervention growth velocity
  - a. Weight (g/kg/d)
  - b. Length (mm/d)
  - c. Head circumference (mm/d)

Growth velocity may be expressed in various ways. For example, weight growth velocity may be expressed as g/d or as g/kg/d, and may be calculated, for example, as  $\text{growth velocity} = 1000 \times \ln(\text{Wt2}/\text{Wt1})/(\text{D2} - \text{D1})$ , where Wt1 and Wt2 are the weights measured on days D1 (birth) and D2 (discharge), respectively (Patel 2005).

##### Secondary outcomes

1. In-hospital growth outcomes (at 36 weeks' postmenstrual age; at hospital discharge)
  - a. Weight (g or Z score)
  - b. Length (cm or Z score)
  - c. Head circumference (cm or Z score)
  - d. Growth velocity in weight (g/kg/d), length (cm/week), and head circumference (cm/week)
  - e. Body mass index
  - f. Ponderal Index
  - g. Incidence of growth < 10th percentile for postmenstrual age
2. Post-discharge growth outcomes (up to six months' corrected age; at six months' or greater corrected age)
  - a. Weight (g or Z score)
  - b. Length (cm or Z score)
  - c. Head circumference (cm or Z score)
  - d. Growth velocity in weight (g/kg/d), length (cm/week), and head circumference (cm/week)
  - e. Body mass index
  - f. Ponderal Index
  - g. Incidence of growth < 10th percentile for corrected age
3. Other growth outcomes
  - a. Time to regain birth weight (days)
4. Clinical feeding/nutritional outcomes
  - a. Time to establishment of full enteral feedings (days)
  - b. Duration of parenteral nutrition (days)
  - c. Feeding intolerance defined as the number of days when feeds were stopped or reduced and parenteral nutrition was either commenced or increased during hospital stay secondary to the inability to digest enteral feeds as indicated by gastric residual volume > 50%, abdominal distention or emesis, or both, or as defined by study authors (Moore 2011)
  - d. Osteopenia
5. In-hospital clinical outcomes
  - a. In-hospital mortality
  - b. NEC stage  $\geq 2$  (Bell 1978)
  - c. Culture-proven sepsis
  - d. Any retinopathy of prematurity
  - e. Retinopathy of prematurity treated with retinal ablation or vascular endothelial growth factor (VEGF) inhibitor
  - f. Culture-proven late-onset sepsis
  - g. Bronchopulmonary dysplasia at 28 days of life and at 36 weeks' postmenstrual age (Jobe 2001)
  - h. Length of hospitalization (days)
6. Severe neurodevelopmental disability defined after 12 months' corrected age as the presence of one or more of the following: non-ambulatory cerebral palsy; developmental delay (Bayley Scales of Infant Development) (Bayley 1993; Bayley 2005); auditory impairment (any impairment requiring or unimproved by amplification); and visual impairment (visual acuity < 6/60)

##### Search methods for identification of studies

We used the criteria and standard methods of Cochrane and Cochrane Neonatal.

## Electronic searches

We conducted a comprehensive search including the Cochrane Central Register of Controlled Trials (CENTRAL; 2019, Issue 9), in the Cochrane Library; OVID MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) (1946 to September 20, 2019); MEDLINE via PubMed (September 1, 2018 to September 20, 2019) for the previous year; and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1981 to September 21, 2019). We have presented the search strategies used for each database in [Appendix 1](#). We did not apply language restrictions.

We searched clinical trial registries for ongoing or recently completed trials. We searched the World Health Organization's International Clinical Trials Registry Platform (ICTRP) ([www.who.int/ictrp/search/en](http://www.who.int/ictrp/search/en)), as well as the US National Library of Medicine's [ClinicalTrials.gov](http://ClinicalTrials.gov), via Cochrane CENTRAL. Additionally, we searched the [ISRCTN Registry](#) for any unique trials not found through the Cochrane CENTRAL search.

## Searching other resources

We handsearched the reference lists of identified clinical trials.

## Data collection and analysis

We used the standard methods of Cochrane Neonatal.

## Selection of studies

Two review authors reviewed abstracts and studies for inclusion in this review. We resolved disagreements in opinion through discussion.

## Data extraction and management

All review authors extracted data using an extraction form created for this study. Two review authors, assigned randomly, extracted data from each included study.

## Assessment of risk of bias in included studies

Two review authors (JH, FS) independently assessed risk of bias (low, high, or unclear) of all included trials using Cochrane's 'Risk of bias' tool ([Higgins 2011a](#)).

1. Sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)
5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)
7. Any other bias

We resolved any disagreements by discussion or by consultation with a third review author. See [Appendix 2](#) for a more detailed description of criteria used to assess each domain.

## Measures of treatment effect

We used the standard methods of Cochrane Neonatal. We performed analyses using the most recent version of the statistical package Review Manager 5 ([Review Manager 2014](#)). We assessed dichotomous data using risk ratio (RR) and risk difference (RD) with corresponding 95% confidence intervals (CIs). If we detected

a statistically significant difference, we calculated the number needed to treat for an additional beneficial outcome (NNTB) and the number needed to treat for an additional harmful outcome (NNTH). We presented means, standard deviations (SDs), and corresponding 95% CIs for continuous outcomes. We assumed a fixed-effect model.

## Unit of analysis issues

For each study, we reported whether the unit of randomization, and hence the unit of analysis, occurred at the individual level or at the cluster level. We did not identify any pertinent cluster-randomized trials. [Kadioglu Simsek 2019](#) tested all three fortification strategies in separate study arms. When targeted or adjustable fortification was compared with standard fortification (Comparison 1), the two individualized fortification arms (targeted, adjustable) were combined using the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2019](#)). For categorical outcomes, the targeted and adjustable fortification arms were combined into a single Individualized group and were used in a single comparison with the standard fortification arm. For continuous outcomes, targeted versus standard and adjustable versus standard were included as separate comparisons; however the total number of participants in the standard arm was divided in half for each comparison, and the means and standard deviations were left unchanged.

## Dealing with missing data

We obtained data from primary investigators when published data were incomplete.

## Assessment of heterogeneity

We evaluated heterogeneity of studies via the  $I^2$  statistic, using the following cutoffs and labels for heterogeneity.

1. Less than 25% indicates no heterogeneity.
2. 25% to 49% indicates low heterogeneity.
3. 50% to 74% indicates moderate heterogeneity.
4. 75% and above indicates high heterogeneity.

## Assessment of reporting biases

When appropriate, we identified potential reporting bias using funnel plots.

## Data synthesis

We assessed dichotomous data using risk ratio (RR) and risk difference (RD) with corresponding 95% confidence intervals (CIs). If we detected a statistically significant difference, we calculated the number needed to treat for an additional beneficial outcome (NNTB) and the number needed to treat for an additional harmful outcome (NNTH). We presented means, standard deviations, and corresponding 95% CIs for continuous outcomes.

## Certainty of evidence

We used the GRADE approach, as outlined in the GRADE Handbook ([Schünemann 2013](#)), to assess the certainty of evidence for the following (clinically relevant) outcomes: growth velocity during intervention; mortality; NEC  $\geq$  stage 2; culture-proven late-onset sepsis; retinopathy of prematurity; and bronchopulmonary dysplasia.

The GRADE approach yields an assessment of the certainty of a body of evidence as assigned to one of four grades.

1. High certainty: further research is very unlikely to change our confidence in the estimate of effect.
2. Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
3. Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
4. Very low certainty: we are very uncertain about the estimate.

Two review authors (JH, JMT) independently assessed the certainty of evidence for each outcome. We used the [GRADEpro GDT](#) Guideline Development Tool to create four 'Summary of findings' tables to report the certainty of evidence. We downgraded the certainty of evidence for imprecision due to insufficient power based on sample size calculations performed with a web-based calculator ([Kohn 2020](#)).

#### Subgroup analysis and investigation of heterogeneity

Planned subgroup analysis consisted of comparisons of standard versus adjustable versus targeted human milk fortification by birth weight (< 1000 grams; ≥ 1000 grams and < 1500 grams) and donor breast milk versus mother's own milk.

#### Sensitivity analysis

If we included a sufficient number of trials in this review, we planned to perform sensitivity analyses by excluding unblinded trials and those without adequate treatment allocation concealment.

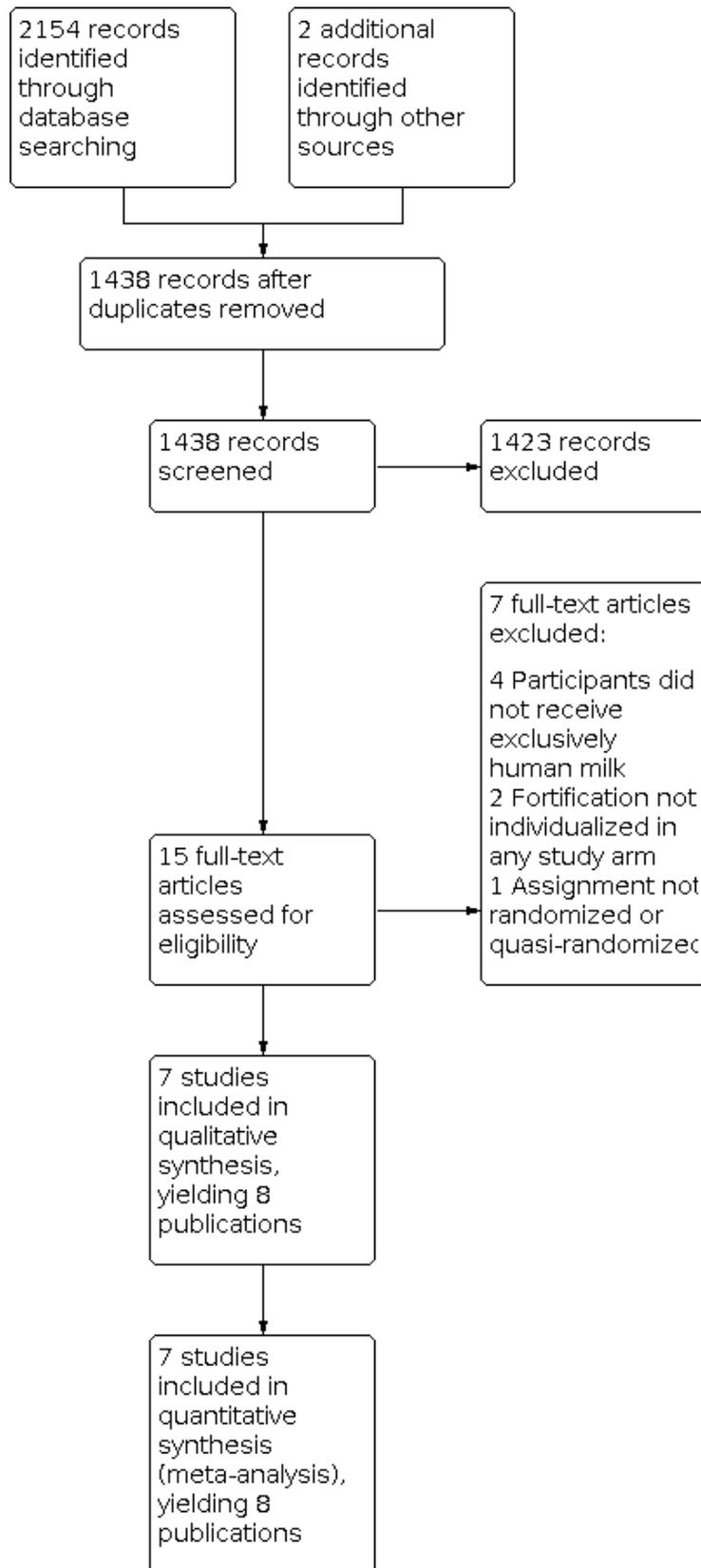
## RESULTS

### Description of studies

#### Results of the search

Our search generated seven randomized controlled trials that resulted in eight publications and met inclusion criteria ([Figure 1](#)). Four published reports described the effects of targeted versus standard fortification on preterm neonates ([Agakidou 2019](#); [Hair 2014](#); [Hair 2016](#) [secondary analysis of [Hair 2014](#)]; [Rochow 2020](#)). Two reports detailed the effects of adjustable versus standard fortification on preterm neonates ([Arslanoglu 2006](#); [Moro 1995](#)). The remaining two reports described the effects of targeted versus adjustable fortification ([Bulut 2019](#)), as well as targeted or adjustable versus standard fortification, on preterm neonates ([Kadioglu Simsek 2019](#)). One multi-center study was performed in the USA ([Hair 2014](#)). Six single-center studies were performed in Canada ([Rochow 2020](#)), Greece ([Agakidou 2019](#)), Italy ([Arslanoglu 2006](#); [Moro 1995](#)), and Turkey ([Bulut 2019](#); [Kadioglu Simsek 2019](#)).

**Figure 1. Study flow diagram.**



## Included studies

### Agakidou 2019

This randomized double-blinded study with parallel design took place in Greece between March 2013 and March 2016. Appropriate-for-gestational age preterm infants at between 25 and 32 weeks' gestation with birth weight < 1500 grams admitted to the study NICU within the first 24 hours of life were eligible for inclusion in the study. Infants with evidence of maternal health problems precluding breast-feeding, congenital infection, metabolic/genetic syndrome, early death, grade III to IV intraperiventricular hemorrhage, or necrotizing enterocolitis were excluded from the study. Infants were randomized in clusters through a computer-generated randomization list based on birth weight  $\geq$  or < 1200 grams. All infants were initially fed exclusively with own mother's milk fortified with a cow's milk-based, multi-nutrient human milk fortifier (HMF) (PreNAN FM-85; Nestlé, Vevey, Switzerland). Infants randomized to the standard fortification group received a fixed amount of fortification, 5 grams HMF/100 mL. Infants randomized to the targeted fortification group had fortification consisting of a daily protein content of 4 to 4.5 g/kg for infants with birth weight < 1200 grams and 3.5 to 4 g/kg for infants  $\geq$  1200 grams. The primary aims of this study were to compare the effects of protein-targeted fortification on:

1. insulin-like growth factor-1 (IGF-1) and ghrelin plasma levels up to the 35th week postmenstrual age; and
2. growth up to 12 months' corrected age.

The secondary outcome was to examine the effectiveness of two own mother's milk fortification protocols in attaining the recommended range of macronutrient intake.

### Arslanoglu 2006

Arslanoglu and colleagues performed a single-center RCT comparing adjustable fortification to standard fortification of feedings for very low birth weight infants. Infants with birth weight 600 to 1750 grams at gestational age 24 to 34 weeks who reached a feeding volume of 90 mL/kg/d of enteral feedings before 21 days of life were eligible for inclusion in this study. Infants with major congenital abnormalities, chromosomal aberrations, systemic disease, sepsis, necrotizing enterocolitis, or intraventricular hemorrhage, or who were ventilator-dependent on day of life 21, were excluded from the study. Randomization used stratification by birth weight (< 1250, 1251 to 1500, and 1501 to 1750 grams). All infants received standard fortification practices until an enteral feeding volume of 150 mL/kg/d was achieved. Standard fortification practice was to fortify human milk with 5 g/100 mL HMF. Once an enteral feeding volume of 150 mL/kg/d was achieved, infants randomized to the adjustable fortification arm had adjustments to fortification based on blood urea nitrogen (BUN) levels. The primary outcome of this study was weight gain (g/kg/d, g/d) determined from study day 1 to a weight of 2000 grams. Secondary outcome measures were BUN and serum creatinine, albumin, calcium, phosphorus, and alkaline phosphatase levels.

### Bulut 2019

This randomized controlled single-center trial, performed in Turkey between September 2013 and February 2014, compared effects of targeted and adjustable protein fortification on early growth of

VLBW infants receiving human milk. VLBW infants at  $\leq$  32 weeks' gestation who were receiving a diet exclusively of human milk were eligible for inclusion in this study. Infants were excluded if they had any congenital abnormality, metabolic disease, necrotizing enterocolitis, or moderate to severe bronchopulmonary dysplasia, or had received any formula feedings. Predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes. When enteral feeding volume reached 80 mL/kg/d, human milk was fortified in a stepwise manner up to 4 units fortifier/100 mL (Eoprotin; Aptamil, Milupa, Germany) per standard nursery practice. Randomization and study commencement occurred when the enteral feeding volume reached 150 mL/kg/d of fortified human milk. Infants randomized to the targeted fortification group received added protein (Protifar; Nutricia, Erlangen, Germany) following milk analysis with a mid-infrared spectrophotometer (Miris AB, Uppsala, Sweden) to maintain a target protein intake of 4.5 g/kg/d. Infants randomized to the adjustable fortification group received added protein based on BUN levels to reach a maximum estimated amount of protein of 4.5 g/kg/d. The goal of this study was to compare the effects of targeted and adjustable fortification on early growth of breast-fed VLBW infants.

**Hair 2014:** this study from the USA consisted of two separate reports published by the same investigators in 2014 and 2016.

### Hair 2014

Hair and colleagues randomized infants between 750 and 1250 grams birth weight to one of two groups - a standard fortification group and a targeted fortification group that received a human milk-derived cream supplement (Prolact CR; Prolacta Bioscience, City of Industry, CA, USA) if the human milk (HM) that infants were receiving was found to be < 20 kcal/oz based on milk analysis with a near-infrared milk analyzer (SpectraStar 2400RTQ; Unity Scientific, Brookfield, CT, USA). Infants were randomized via blocks of four. Exclusion criteria included infants with major congenital anomalies, clinically significant congenital heart disease, low expectation for survival, high potential for early transfer to a non-study institution, enrollment in another clinical study affecting nutritional management, failure to start minimum enteral feeds before 21 days of life, or intestinal perforation or stage 2 necrotizing enterocolitis before tolerating fortified feeds, or at the discretion of the study investigator. All study infants received standard fortification by the time they were tolerating 100 mL/kg/d of enteral feeds. Once feeds were established and tolerated, milk fed to infants randomized to the targeted fortification group was analyzed and fortified to a target level of 20 kcal/oz if analysis indicated caloric content < 20 kcal/oz. The primary outcomes of this study were growth velocity (weight, length, head circumference) and the amount of donor HM-derived fortifier used.

Hair 2016 (secondary analysis of [Hair 2014](#))

This report presented secondary analysis of outcomes from [Hair 2014](#), analyzing the effect of targeted fortification using a human milk-derived cream supplement on the growth velocity of preterm infants. Analysis of data in this publication pertained to clinical outcomes and length of stay. Inclusion and exclusion criteria and targeted fortification protocols were as described in [Hair 2014](#). Primary outcomes of this secondary analysis included comorbidities collected in the original study: medically or surgically managed patent ductus arteriosus, culture-proven late-onset

sepsis,  $\geq$  stage 2 necrotizing enterocolitis, and bronchopulmonary dysplasia (BPD), as well as length of stay and postmenstrual age at discharge. Study investigators also performed a subgroup analysis of infants with BPD comparing clinical outcomes, mortality, length of stay, and postmenstrual age at discharge for study infants who received standard fortification versus infants who received targeted fortification.

#### Kadioglu Simsek 2019

Study authors performed a single-center RCT in Turkey comparing the effects of adjustable, targeted, and standard fortification on early growth of very low birth weight infants. This study took place between January 2015 and December 2015. Infants were included if birth weight was  $<$  1500 grams and gestational age  $<$  32 weeks, and if they were fed only human milk. Infants with significant congenital anomalies, respiratory support requirements, sepsis, or a history of cardiac or intestinal surgery, or who received any formula feedings, were excluded. All study infants received fortification according to standard practice when milk intake reached 100 mL/kg/d and were randomized using computer-generated sequential numbers when full enteral feeds reached a volume of 160 mL/kg/d. Infants in the adjustable fortification group had protein supplement (Aptamil, Milupa) added or reduced based on twice-weekly BUN levels. In the targeted fortification group, milk analysis was performed with mid-infrared spectrophotometry (Miris), and protein supplement was added to achieve a target protein intake of 3.5 to 4.5 g/kg. The primary outcome of this study was the change in percentile of body weight, head circumference, and height before and four weeks after initiation of fortification.

#### Moro 1995

Moro and colleagues performed a single-center RCT in Italy. Infants were included from the study if their birth weight was between 900 and 1500 grams, and if they were no longer receiving intravenous fluids. Exclusion criteria included major congenital abnormalities and systemic illness. In the standard fortification arm of the study, infants were fed breast milk fortified with bovine milk protein-based fortifier in a fixed amount, 3.5 g/100 mL. Infants in the adjustable arm received fortified milk with the same bovine milk protein-based fortifier, but the amount of fortifier was based on corrected serum urea nitrogen levels. The primary objective of this study was to test a novel fortification in comparison with standard fortification practices, hypothesizing that adjustable fortification would lead to higher protein intake, which would result in more rapid growth. A secondary objective of this study was to evaluate a new bovine milk-protein-based fortifier in comparison with standard human milk protein concentrate.

#### Rochow 2020

This single-center RCT was performed in Canada. Infants were included from the study if they were  $<$  30 weeks' gestational age with an anticipated length of stay  $>$  21 days and were receiving fortified breast milk. Infants were excluded if they had gastrointestinal perforation, major congenital anomalies, stage 2 necrotizing enterocolitis, abdominal surgery, or gram-negative sepsis. Randomization was stratified by gestational age  $>$  or  $<$  28 weeks, with variable block sizes of 2, 4, and 6. Standard fortification (Enfamil HMF; Mead Johnson, Cleveland, OH, USA) was introduced at an enteral feeding volume of 120 mL/kg/d for all study infants. Infants randomized to the standard fortification arm received

1 package of HMF/25 mL, and those receiving donor human milk received an additional 0.4 grams of whey protein powder (Beneprotein; Nestlé, Vevey, Switzerland)/100 mL. In the targeted fortification arm, macronutrients were measured using a near-infrared milk analyzer (SpectraStar; Unity Scientific, Brookfield, CT, USA). Fortification aimed to achieve milk contents according to European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommended intakes. The primary outcome of this study was weight gain velocity (g/kg/d) during the first 21 days of intervention. Secondary outcomes were macronutrient intake, nutritive efficiency, weight, head circumference, length, body composition, major morbidities of prematurity, and weekly clinical chemistries.

#### Excluded studies

We excluded six studies (reported in seven publications).

#### Boehm 1993

Boehm and colleagues investigated the effects of different types of protein - a human milk protein, bovine whey protein hydrolysate, and a mixture of bovine proteins, peptides, and amino acids - on the growth and plasma amino acid profiles of low birth weight infants. Researchers collected data on growth rates, as well as on serum preprandial essential amino acids and urea and prealbumin concentrations. We excluded this study because fortification arms were not designed to provide individualized fortification based on infant laboratory values or human milk analysis.

#### Kanmaz 2013

This RCT performed by Kanmaz and colleagues at a single-center NICU in Turkey enrolled infants at  $\leq$  32 weeks' gestation and with birth weight  $\leq$  1500 grams between November 2010 and August 2011. Researchers randomized infants to three groups.

1. A standard fortification group, with estimated protein intake of 3 g/kg/d.
2. A moderate fortification group, with estimated protein intake of 3.3 g/kg/d.
3. An aggressive fortification group, with estimated protein intake of 3.6 g/kg/d.

Objectives of this study were to assess the effects of varying amounts of protein fortification on short-term growth and feeding intolerance, and metabolic effects based on blood urea nitrogen, calcium, phosphorus, and alkaline phosphatase levels. We excluded this study because investigators used a blind-fortification approach, assigning protein fortification in three fixed amounts. Fortification in study groups was not individualized to study infants based on laboratory values or human milk analysis.

#### Maas 2017; Mathes 2018

These reports were the result of a single-center, randomized controlled, partially blinded trial performed in Germany between October 2012 and October 2014. Included infants were  $\leq$  32 weeks' gestation and had birth weight  $\leq$  1500 grams. Infants were randomized to one of three groups.

1. A lower-protein, standard fortification group administered 5 g/100 mL of milk fortifier (FM-85, Nestlé Nutrition) to yield an estimated 3.5 g/kg/d of protein.

2. A higher-protein group administered a fixed amount of investigational multi-component fortifier aimed at achieving a goal protein of 4.5 g/kg/d.
3. A higher-protein group that received individualized fortification on top of standard fortification based on analysis of human milk macronutrient concentration to achieve protein content of 4 to 4.5 g/kg/d based on birth weight above or below 1500 grams. We excluded these studies because included infants may not have received an exclusive human milk diet. Breast milk feeding was supplemented with standard preterm formula (Beba preterm infant formula, Nestlé Nutrition) if the breast milk supply of the infant's mother did not meet the infant's enteral feeding volume.

**Maas 2017:** the primary outcome of the original study was weight gain (g/kg/d) measured from birth to end of intervention. Secondary outcomes were head circumference from birth to end of intervention; weight, length, and head circumference at discharge; and lower leg longitudinal growth (mm/week).

**Mathes 2018:** this report was the result of analysis of secondary outcomes of the RCT originally reported by [Maas 2017](#). The aim of this arm of the study was to determine the impact of increased enteral protein intake on plasma urea concentration and urine urea/creatinine ratio and to determine if the urine urea/creatinine ratio represents plasma urea concentration and the enteral protein supply. Secondary outcomes reported in this publication included analysis of urine urea/creatinine ratio and plasma urea concentration.

#### [McLeod 2016](#)

McLeod and colleagues reported on a randomized controlled, single-center study out of Western Australia conducted between January 2009 and June 2009. Infants at < 30 weeks' gestation were included in the study if they had no congenital anomalies, if mothers planned to provide human milk, and if living remotely would not prevent participation in all assessments. Infants were randomized to one of two groups.

1. Routine practice to provide fortification based on assumed composition targeting 3.8 to 4.4 grams of protein/kg/d and 130 to 150 kcal/kg/d.
2. Intervention group providing individualized fortification based on measured milk composition analyzed with mid-infrared spectrophotometry (Miris).

The goal of this study was to test the hypothesis that growth and body composition of preterm infants better match intrauterine growth if fortification is individualized based on human milk analysis. We excluded this study because an intervention infant did

not receive an exclusive human milk diet and was transitioned to preterm formula due to lack of donor human milk.

#### [Morlacchi 2016](#)

This prospective interventional single-center study was performed in Italy between October 2014 and March 2015. Infants at < 32 weeks' gestation, weighing < 1500 grams, and at ≥ 10th percentile of weight based on Fenton growth who were receiving a diet consisting exclusively of human milk were eligible for inclusion in this study. A cohort of infants treated in the same NICU during the six months before study intervention who fulfilled inclusion criteria for the study and received standard fortification according to nursery feeding guidelines was considered the control group. Analysis of human milk for the intervention group was performed using mid-infrared spectroscopy (Miris). Individualized fortification was targeted to achieve fat, protein, and carbohydrate levels recommended by ESPGHAN. The primary aim of this study was to determine whether targeted breast milk fortification improved growth among very low birth weight infants. We excluded this study because it was not a randomized or quasi-randomized controlled trial.

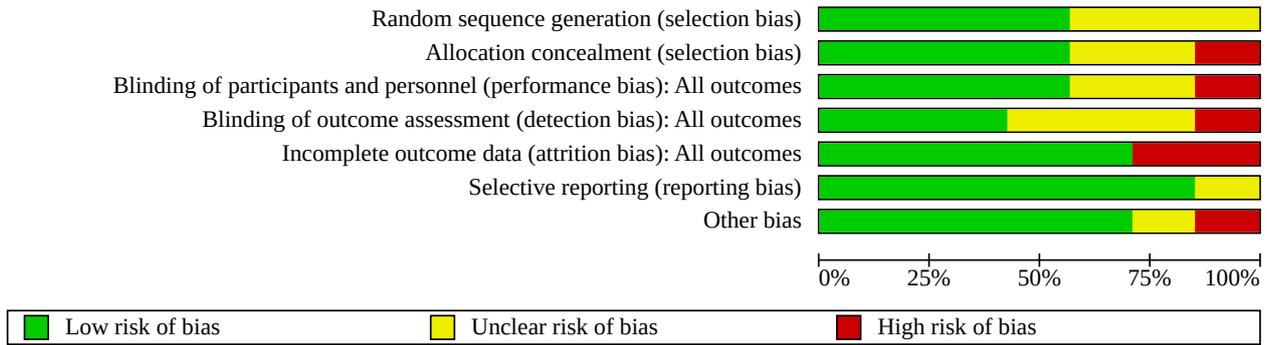
#### [Quan 2019](#)

Quan and colleagues completed a prospective, randomized controlled, single-center study between September 2012 and August 2016. Infants were included in this study if gestational age was < 34 weeks, birth weight was between 800 and 1800 grams, and infants received a diet exclusively of human milk defined as own mother's milk comprising ≥ 80% of total enteral feeding. Infants who received ≤ 80% of mother's own milk were excluded from the study. For infants in the individualized fortification group, the macronutrient composition of human milk was measured using a mid-infrared milk analyzer (Miris), and blood urea nitrogen levels were evaluated twice weekly along with measurement of body weight. Based on protein level determined from milk analysis and blood urea nitrogen levels, fortifier was added via a defined level-based system. Primary outcomes were protein intake from parenteral nutrition and enteral nutrition and weight gain velocity per week and throughout the study. Secondary outcomes were weekly protein intake, protein/energy ratio, growth Z scores, length of stay, and time for body weight to reach 1800 to 2000 grams. We excluded this study because infants did not receive an exclusive human milk diet; up to 20% of enteral feeding volume could comprise formula, as donor human milk was not available in the investigators' NICU.

#### **Risk of bias in included studies**

A "Risk of bias" graph is provided in [Figure 2](#) and a summary is provided in [Figure 3](#).

**Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.**



**Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.**

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias): All outcomes	Blinding of outcome assessment (detection bias): All outcomes	Incomplete outcome data (attrition bias): All outcomes	Selective reporting (reporting bias)	Other bias
Agakidou 2019	+	?	+	?	+	+	+
Arslanoglu 2006	?	+	+	-	+	+	+
Bulut 2019	+	-	+	+	-	?	+
Hair 2014	?	?	-	?	+	+	-
Kadioglu Simsek 2019	+	+	?	+	+	+	?
Moro 1995	?	+	?	?	-	+	+
Rochow 2020	+	+	+	+	+	+	+

## Allocation

Agakidou 2019 and Kadioglu Simsek 2019 allocated participants using a computer-generated randomization list; Arslanoglu 2006, Bulut 2019, Moro 1995, and Rochow 2020 used sealed envelopes. In Hair 2014, the details of random sequence generation were unclear.

## Blinding

Agakidou 2019 did not provide details on allocation concealment, although outcome assessment appeared adequately blinded. Bulut 2019 reported that it was not possible to blind investigators to study group assignment. Arslanoglu 2006 also reported that investigators were not blinded to study group assignment but noted that caregivers responsible for infants' care and feeding were not involved in the investigation. Hair 2014 was unable to blind investigators to group assignment at one of two sites. Kadioglu Simsek 2019 and Moro 1995 did not provide details regarding blinding of personnel, and Arslanoglu 2006, Hair 2014, and Moro 1995 did not provide details regarding blinding of outcome assessment.

## Incomplete outcome data

In Bulut 2019, seven deaths and four cases of NEC occurred, leading to exclusion "during the course of the study"; however it is unclear if these occurred before or during the study intervention; if the latter, it is unclear if these occurred equally in the two study arms.

In Hair 2014, three enrolled infants were excluded from the analyses presented in Hair 2016 (secondary analysis of Hair 2014) that were not excluded from the intention-to-treat analysis in the Hair 2014 initial report. It is unclear whether these post-hoc exclusions affected the statistical significance of findings of the Hair 2016 report, or whether this was a factor in their exclusion.

Attrition was unbalanced among the three groups in Moro 1995 (0/14, 2/14, and 4/14), and data for withdrawals were not reported.

In Rochow 2020, 76 randomized infants were excluded before the study intervention was initiated due to early transfer before completing 14 study days, deviation from the feeding protocol, or use of steroids or diuretics; exclusions occurred equally in the two study arms. Clinical outcomes, but not growth outcomes, were reported for excluded infants.

## Selective reporting

None of the included studies revealed selective outcome reporting.

## Other potential sources of bias

In Hair 2014, the study sponsor, Prolacta Bioscience, provided the product for the study and assisted in data analysis. Two study authors received financial support and speaker honoraria from Prolacta Bioscience, and two other study authors were employees of Prolacta Bioscience.

Kadioglu Simsek 2019 did not provide case definitions for clinical sepsis, NEC, BPD, ROP, or osteopenia.

## Effects of interventions

See: **Summary of findings 1 Targeted or adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth**

**weight infants receiving human milk; Summary of findings 2 Targeted individualized compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk; Summary of findings 3 Adjustable individualized fortification compared to standard non-individualized fortification for promoting growth and development in very low birth weight infants receiving human milk; Summary of findings 4 Targeted individualized fortification compared to adjustable individualized fortification for promoting growth and development in very low birth weight infants receiving human milk**

## Comparison 1. Individualized (targeted or adjustable) versus standard fortification

See **Summary of findings 1**.

Six of the included studies measured the primary outcome of growth velocity of weight (Agakidou 2019; Arslanoglu 2006; Hair 2014; Kadioglu Simsek 2019; Moro 1995; Rochow 2020), and five of those studies measured growth velocity of length and head circumference at end of the intervention (Agakidou 2019; Arslanoglu 2006; Hair 2014; Kadioglu Simsek 2019; Moro 1995). In addition, one study that investigated this comparison collected data on retinopathy of prematurity and osteopenia (Kadioglu Simsek 2019), and four studies included bronchopulmonary dysplasia, defined as respiratory support at 36 weeks' postmenstrual age (PMA) (Agakidou 2019; Hair 2016 [secondary analysis of Hair 2014]; Kadioglu Simsek 2019; Rochow 2020). One study investigated targeted, adjustable, and standard fortification in three study arms (Kadioglu Simsek 2019); the study arms were combined as described earlier in Methods. The Hair 2016 report (secondary analysis of Hair 2014) was included in **Analysis 1.4**, and no other outcomes were included in this comparison because it included only the subgroup of infants with BPD from the prior study in 2014, and therefore could be included only in the BPD analysis for this comparison.

### Growth velocity, weight in g/kg/d, end of intervention

Among the six included studies, there was an estimated mean difference of 1.88 (95% confidence interval [CI] 1.26 to 2.50; 345 participants) favoring individualized fortification for improved weight growth velocity. The results show high heterogeneity ( $I^2 = 87%$ ). We assessed the certainty of evidence as moderate for this outcome, downgrading by one level because of inconsistency due to high heterogeneity in the estimate of effect (**Analysis 1.1**).

### Growth velocity, length in mm/d, end of intervention

The estimated mean difference for length growth velocity was 0.43 (95% CI 0.32 to 0.53; 5 studies, 242 participants) favoring individualized fortification for improved length growth velocity. The results show high heterogeneity ( $I^2 = 88%$ ). We assessed the certainty of evidence as low for this outcome, downgrading by one level for inconsistency due to high heterogeneity in estimate of effect, and by one level for imprecision due to wide confidence intervals that included both clinically significant and clinically insignificant effects (**Analysis 1.2**).

### Growth velocity, head circumference in mm/d, end of intervention

The estimated mean difference for head circumference growth velocity was 0.14 (95% CI 0.06 to 0.23; 5 studies, 242 participants), again favoring individualized fortification. The results show high

heterogeneity ( $I^2 = 75\%$ ). We assessed the certainty of evidence as low for this outcome, downgrading by one level for inconsistency due to high heterogeneity in estimate of effect, and by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects ([Analysis 1.3](#)).

### **Bronchopulmonary dysplasia**

There appeared to be no difference in bronchopulmonary dysplasia, with an estimated risk ratio of 0.89 (95% CI 0.71 to 1.12; 4 studies, 391 participants). We assessed the certainty of evidence as very low for this outcome, downgrading by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control, by one level for inconsistency due to variation among studies in case definition of the outcome, and by one level for imprecision due to wide confidence intervals that included both clinically significant and clinically insignificant effects ([Analysis 1.4](#)).

### **Retinopathy of prematurity**

There appeared to be no difference in retinopathy of prematurity, with an estimated risk ratio of 0.79 (95% CI 0.36 to 1.72; 1 study, 60 participants). We assessed the certainty of evidence as very low for this outcome, downgrading by one level because of imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects, and by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control ([Analysis 1.5](#)).

### **Osteopenia of prematurity**

Osteopenia of prematurity did not appear significantly different between groups, with an estimated risk ratio of 0.86 (95% CI 0.40 to 1.84; 1 study, 60 participants). We assessed the certainty of evidence as very low for this outcome, downgrading by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects, by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control, and by one level for indirectness due to use of the surrogate outcome of osteopenia rather than patient-important outcomes of fracture ([Analysis 1.6](#)).

## **Comparison 2. Targeted versus standard fortification**

See [Summary of findings 2](#).

Four studies compared targeted fortification to standard fortification and provided data on growth velocity of weight at the end of the intervention ([Agakidou 2019](#); [Hair 2014](#); [Kadioglu Simsek 2019](#); [Rochow 2020](#)). Three of these studies included data on growth velocity of length and growth velocity of head circumference at the end of the intervention ([Agakidou 2019](#); [Hair 2014](#); [Kadioglu Simsek 2019](#)). Many of the outcomes in this comparison were collected in only one study, although different studies collected different outcomes (thus all of the data were not derived from the same study). One study collected growth parameter data at 40 weeks' PMA, and at 3, 6, 9, and 12 months' corrected age (CA) ([Agakidou 2019](#)). That same study also investigated change in body mass index (BMI) at those respective time points. In-hospital mortality was compared in three studies ([Agakidou 2019](#); [Hair 2014](#); [Rochow 2020](#)), data on necrotizing enterocolitis and culture-proven late-onset bacterial sepsis were collected in two studies ([Hair 2014](#); [Rochow 2020](#)), and BPD was

analyzed in four studies ([Agakidou 2019](#); [Hair 2016](#) [secondary analysis of [Hair 2014](#)]; [Kadioglu Simsek 2019](#); [Rochow 2020](#)). There was also a subgroup analysis of infants with BPD that analyzed the following outcomes: in-hospital mortality, length of hospital stay, and PMA at discharge ([Hair 2016](#), from a prior study [Hair 2014](#)).

### **Growth velocity, weight in g/kg/d, end of intervention**

The estimated mean difference was 1.87 g/kg/d (95% CI 1.15 to 2.58; 4 studies, 269 participants), suggesting that targeted fortification yields improved growth velocity of weight when compared to standard fortification. The results show high heterogeneity ( $I^2 = 91\%$ ). We assessed the certainty of evidence as moderate for this outcome, downgrading by one level for inconsistency due to high heterogeneity in the estimate of effect ( $I^2 \geq 75\%$ ) ([Analysis 2.1](#)).

### **Growth velocity, weight in g/kg/d, start of fortification to 40 weeks' PMA**

Only one study collected data on growth velocity of weight from start of intervention to 40 weeks' PMA. The estimated mean difference was -0.03 (95% CI -1.19 to 1.13; 46 participants) ([Analysis 2.2](#)).

### **Growth velocity, weight in g/kg/d, start of fortification to three months' CA**

The same study collected growth velocity of weight data from start of intervention to three months' CA. The estimated mean difference was -0.31 (95% CI -1.11 to 0.49; 46 participants) ([Analysis 2.3](#)).

### **Growth velocity, weight in g/kg/d, start of fortification to six months' CA**

The same study analyzed growth velocity of weight data from start of intervention to six months' CA. The estimated mean difference was 0.09 (95% CI -0.31 to 0.49; 45 participants) ([Analysis 2.4](#)).

### **Growth velocity, weight in g/kg/d, start of fortification to 12 months' CA**

The same study analyzed growth velocity of weight data from start of intervention to 12 months' CA. The estimated mean difference was -0.04. (95% CI -0.36 to 0.28; 45 participants) ([Analysis 2.5](#)).

### **Growth velocity, length in mm/d, end of intervention**

There was an estimated mean difference of 0.45 (95% CI 0.32 to 0.57, 3 studies, 166 participants), suggesting that growth velocity of length at the end of the intervention is positively affected in the targeted fortification group compared to the standard fortification group. Heterogeneity was high ( $I^2 = 91\%$ ). We assessed the certainty of evidence as low for this outcome, downgrading by one level for inconsistency due to high heterogeneity in estimate of effect ( $I^2 \geq 75$ ), and by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects ([Analysis 2.6](#)).

### **Growth velocity, length in mm/d, start of fortification to 40 weeks' PMA**

Only one study collected data on growth velocity of length from start of intervention to 40 weeks' PMA. The estimated mean difference was 0.02 (95% CI -0.16 to 0.20; 48 participants) ([Analysis 2.7](#)).

### **Growth velocity, length in mm/d, start of fortification to three months' CA**

The same study collected growth velocity of length data from start of intervention to three months' CA. The estimated mean difference was -0.02 (95% CI -0.12 to 0.08; 46 participants) ([Analysis 2.8](#)).

### **Growth velocity, length in mm/d, start of fortification to six months' CA**

The same study collected growth velocity of length data from start of intervention to six months' CA. The estimated mean difference was 0.07 (95% CI 0.00 to 0.14; 45 participants) ([Analysis 2.9](#)).

### **Growth velocity, length in mm/d, start of fortification to 12 months' CA**

The same study collected growth velocity of length data from start of intervention to 12 months' CA. The estimated mean difference was 0.00 (95% CI -0.07 to 0.07; 44 participants) ([Analysis 2.10](#)).

### **Growth velocity, head circumference in mm/d, end of intervention**

Head circumference growth velocity was not statistically significantly different between fortification groups, with an estimated mean difference of 0.08 (95% CI -0.01 to 0.18; 3 studies, 166 participants). The results show high heterogeneity ( $I^2 = 79\%$ ). We assessed the certainty of evidence as low for this outcome, downgrading by one level for inconsistency due to heterogeneity in estimate of effect, and by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects ([Analysis 2.11](#)).

### **Growth velocity, head circumference in mm/d, start of fortification to 40 weeks' PMA**

Only one study collected data on growth velocity of head circumference from start of intervention to 40 weeks' PMA. The estimated mean difference was -0.07 (95% CI -0.16 to 0.02; 48 participants) ([Analysis 2.12](#)).

### **Growth velocity, head circumference in mm/d, start of fortification to three months' CA**

The same study collected growth velocity of head circumference data from start of intervention to three months' CA. The estimated mean difference was 0.00 (95% CI -0.06 to 0.06; 46 participants) ([Analysis 2.13](#)).

### **Growth velocity, head circumference in mm/d, start of fortification to six months' CA**

The same study collected growth velocity of head circumference data from start of intervention to six months' CA. The estimated mean difference was 0.01 (95% CI -0.03 to 0.05; 45 participants) ([Analysis 2.14](#)).

### **Growth velocity, head circumference in mm/d, start of fortification to 12 months' CA**

The same study collected growth velocity of head circumference data from start of intervention to 12 months' CA. The estimated mean difference was -0.01 (95% CI -0.04 to 0.02; 45 participants) ([Analysis 2.15](#)).

### **Change in BMI, end of intervention**

The same study collected change in BMI data at end of intervention. The estimated mean difference was -0.08 (95% CI -0.28 to 0.12; 48 participants) ([Analysis 2.16](#)).

### **Change in BMI, start of fortification to 40 weeks' PMA**

The same study collected change in BMI data from start of fortification to 40 weeks' PMA. The estimated mean difference was -0.05 (95% CI -0.18 to 0.08; 48 participants) ([Analysis 2.17](#)).

### **Change in BMI, start of fortification to three months' CA**

The same study collected change in BMI data from start of fortification to three months' CA. The estimated mean difference was -0.04 (95% CI -0.11 to 0.03; 46 participants) ([Analysis 2.18](#)).

### **Change in BMI, start of fortification to six months' CA**

The same study collected change in BMI data from start of fortification to six months' CA. The estimated mean difference was -0.02 (95% CI -0.05 to 0.01; 45 participants) ([Analysis 2.19](#)).

### **Change in BMI, start of fortification to 12 months' CA**

The same study collected change in BMI data from start of fortification to 12 months' CA. The estimated mean difference was -0.02 (95% CI -0.05 to 0.01; 44 participants) ([Analysis 2.20](#)).

### **Length of hospital stay in days**

Length of hospital stay in days was collected in one study, with an estimated mean difference of -12.00 days (95% CI -26.38 to 2.38; 75 participants). We assessed the certainty of evidence as very low for this outcome, downgrading by one level due to risk of bias, and by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control ([Analysis 2.21](#)).

### **Postmenstrual age at discharge in weeks**

Postmenstrual age at discharge in weeks was collected in one study, with an estimated mean difference of -1.70 (95% CI -3.47 to 0.07; 75 participants). We downgraded the evidence by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects, and by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control ([Analysis 2.22](#)).

### **In-hospital mortality**

The estimated risk ratio for in-hospital mortality was 0.14 (95% CI 0.02 to 1.14; 3 studies, 334 participants), suggesting no differences between fortification groups. We assessed the certainty of evidence as very low for this outcome, downgrading by one level for inconsistency due to variation among studies in fortification procedures, and by two levels for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects and due to the small number of events ([Analysis 2.23](#)).

### **Necrotizing enterocolitis**

The estimated risk ratio for necrotizing enterocolitis was 0.40 (95% CI 0.08 to 1.99; 2 studies, 257 participants), revealing no differences between fortification groups. We assessed the certainty of evidence as very low for this outcome, downgrading by one level

for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects, and by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control (Analysis 2.24).

#### **Culture-proven late-onset bacterial sepsis**

The estimated risk ratio for culture-proven late-onset bacterial sepsis was 1.29 (95% CI 0.76 to 2.17; 2 studies, 257 participants), revealing no differences between groups. We assessed the certainty of evidence as low for this outcome, downgrading by one level for imprecision due to wide confidence intervals that include both clinically significant and clinically insignificant effects, and by two levels for imprecision due to total enrollment insufficient for 50% power to detect 20% change compared to control (Analysis 2.25).

#### **Retinopathy of prematurity, any**

Only one study collected data on retinopathy of prematurity. The risk ratio was 1.00 (95% CI 0.43 to 2.33; 40 participants) (Analysis 2.26).

#### **Osteopenia of prematurity**

Only one study collected data on osteopenia of prematurity. The risk ratio was 0.86 (95% CI 0.35 to 2.10; 40 participants) (Analysis 2.27).

#### **Bronchopulmonary dysplasia**

The estimated risk ratio for bronchopulmonary dysplasia was 0.88 (95% CI 0.70 to 1.11; 4 studies, 371 participants), revealing no differences between fortification groups (Analysis 2.28).

#### **BPD subgroup – in-hospital mortality**

In a subgroup analysis from one prior study, in-hospital mortality of patients with BPD was analyzed in the two fortification groups for a total of 21 participants, but the odds ratio could not be estimated (Analysis 2.29).

#### **BPD subgroup – length of hospital stay in days**

That same subgroup analysis collected data on in-hospital mortality. The estimated mean difference was -17.00 (95% CI -48.53 to 14.53; 21 participants) (Analysis 2.30).

#### **BPD subgroup – postmenstrual age at discharge in weeks**

That same subgroup analysis collected data on PMA at discharge. The mean difference was -2.90 (95% CI -6.78 to 0.98; 21 participants) (Analysis 2.31).

### **Comparison 3. Adjustable versus standard fortification**

See [Summary of findings 3](#).

Three studies provided end of intervention outcome data for growth velocity outcomes, weight in g/kg/d, length in mm/d, and head circumference in mm/d (Arslanoglu 2006; Kadioglu Simsek 2019; Moro 1995). Two studies provided end of intervention outcome data for the outcome growth velocity in weight g/d (Arslanoglu 2006; Moro 1995). One study provided end of intervention data for any retinopathy of prematurity, osteopenia, and bronchopulmonary dysplasia (Kadioglu Simsek 2019).

#### **Growth velocity, weight in g/kg/d, end of intervention**

The estimated mean difference for the outcome of growth velocity and weight at end of intervention was 2.86 (95% CI 1.69 to 4.03; 3 studies, 96 participants), favoring adjustable over standard fortification for improved weight growth velocity at end of intervention. Heterogeneity between studies was high ( $I^2 = 94%$ ). We assessed the certainty of evidence as moderate for this outcome, downgrading by one level for inconsistency due to high heterogeneity in the estimate of effect ( $I^2 \geq 75%$ ) (Analysis 3.1).

#### **Growth velocity, length in mm/d, end of intervention**

Adjustable fortification improved linear growth velocity in preterm infants when compared to standard fortification practices. The estimated mean difference for growth velocity of length at end of intervention was 0.54 (95% CI 0.38 to 0.70; 3 studies, 96 participants). Heterogeneity between studies was high ( $I^2 = 92%$ ). We assessed the certainty of evidence as moderate for this outcome, downgrading by one level for inconsistency due to high heterogeneity in the estimate of effect ( $I^2 \geq 75%$ ) (Analysis 3.2).

#### **Growth velocity, head circumference in mm/d, end of intervention**

Adjustable fortification improved velocity of head growth in preterm infants when compared to standard fortification practices. The estimated mean difference for growth velocity of head circumference at end of intervention was 0.36 (95% CI 0.21 to 0.50; 3 studies, 96 participants). Heterogeneity between studies was moderate ( $I^2 = 50%$ ). We assessed the certainty of evidence as high for this outcome (Analysis 3.3).

#### **Growth velocity, weight in g/d, end of intervention**

The estimated mean difference for growth velocity of weight in g/d at end of intervention in preterm infants was 3.26 (95% CI 1.17 to 5.34; 2 studies, 56 participants), favoring adjustable over standard fortification for improved weight growth velocity at end of intervention (Analysis 3.4).

#### **Retinopathy of prematurity, any**

The estimated risk ratio for retinopathy of prematurity was 0.57 (95% CI 0.20 to 1.65; 1 study, 40 participants) (Analysis 3.5).

#### **Osteopenia**

The estimated risk ratio for osteopenia was 1.00 (95% CI 0.39 to 2.58; 1 study, 40 participants) (Analysis 3.6).

#### **Bronchopulmonary dysplasia**

The estimated risk ratio for bronchopulmonary dysplasia was 1.20 (95% CI 0.44 to 3.30; 1 study, 40 participants) (Analysis 3.7).

### **Comparison 4. Targeted versus adjustable fortification**

See [Summary of findings 4](#).

Two studies provided end of intervention outcome data for growth velocity, weight in g/kg/d, length in mm/d, and head circumference in mm/d (Bulut 2019; Kadioglu Simsek 2019). One study provided end of intervention data for the following outcomes: any retinopathy of prematurity, osteopenia, and bronchopulmonary dysplasia (Kadioglu Simsek 2019).

### **Growth velocity, weight in g/kg/d, end of intervention**

The estimated mean difference for growth velocity of weight at end of intervention was 2.49 (95% CI 0.44 to 4.54; 2 studies, 72 participants), suggesting no difference in velocity of weight gain for infants who received targeted fortification compared to adjustable fortification. We assessed the certainty of evidence as moderate for this outcome, downgrading by one level for imprecision due to wide confidence intervals that include both clinically significant benefit and clinically insignificant effects ([Analysis 4.1](#)).

### **Growth velocity, length in mm/d, end of intervention**

The estimated mean difference for growth velocity of length at end of intervention was 0.07 (95% CI -0.06 to 0.20; 2 studies, 72 participants). This suggests no difference in linear growth velocity when the effects of targeted practices were compared with the effects of adjustable fortification practices. We assessed the certainty of evidence as low for this outcome, downgrading by two levels for imprecision due to total enrollment insufficient for 50% power to detect a 20% change compared to control ([Analysis 4.2](#)).

### **Growth velocity, head circumference in mm/d, end of intervention**

No difference in velocity of head growth was evident when effects of targeted practices were compared with effects of adjustable fortification practices. The estimated mean difference in growth velocity of head circumference in mm/d at end of intervention was 0.04 (95% CI -0.10 to 0.17; 2 studies, 72 participants). We assessed the certainty of evidence as low for this outcome, downgrading by two levels for imprecision due to total enrollment insufficient for 50% power to detect a 20% change compared to control ([Analysis 4.3](#)).

### **Retinopathy of prematurity, any**

The estimated risk ratio for retinopathy of prematurity was 1.75 (95% CI 0.61 to 5.05; 1 study, 40 participants) ([Analysis 4.4](#)).

### **Osteopenia**

The estimated risk ratio for osteopenia was 1.00 (95% CI 0.39 to 2.58; 1 study, 40 participants) ([Analysis 4.5](#)).

### **Bronchopulmonary dysplasia**

The estimated risk ratio for bronchopulmonary dysplasia was 1.00 (95% CI 0.39 to 2.58; 1 study, 40 participants) ([Analysis 4.6](#)).

### **Subgroup analyses and investigation of heterogeneity**

None of the studies included in this review subgrouped their study cohort either by birth weight or by donor breast milk versus mother's own milk; therefore planned subgroup analyses could not be performed.

Studies included in this review differed with respect to standard feeding and fortification regimens, pre-intervention regimens, fortifiers used, duration of the intervention, reported outcomes, and timing of outcome measurements. This variation among studies was reflected in nine analyses that showed high heterogeneity in the estimate of effect. When inspection of forest plots suggested that one outlier study was a primary cause of heterogeneity, we performed a sensitivity analysis to assess the impact of this upon the result of excluding the outlier study.

Analysis 1.1: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased but the overall result of the analysis was not changed, continuing to significantly favor individualized fortification (mean difference [MD] 1.47, 95% confidence interval [CI] 0.84 to 2.11).

Analysis 1.2: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased and the overall result of the analysis continued to favor individualized fortification; however this finding was no longer statistically significant (MD 0.14, 95% CI 0.00 to 0.28; P = 0.06).

Analysis 1.3: no single study appeared to be an outlier compared to other studies.

Analysis 2.1: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased but the overall result of the analysis was not changed, continuing to significantly favor individualized fortification (MD 1.47, 95% CI 0.74 to 2.20).

Analysis 2.6: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased and the overall result of the analysis continued to favor individualized fortification; however this finding was no longer statistically significant (MD 0.16, 95% CI -0.01 to 0.34; P = 0.06).

Analysis 2.11: no single study appeared to be an outlier compared to other studies.

Analysis 3.1: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased but the overall result of the analysis was not changed, continuing to significantly favor individualized fortification (MD 1.48, 95% CI 0.22 to 2.75).

Analysis 3.2: [Kadioglu Simsek 2019](#) appeared prominent compared to other studies in its effects favoring individualized fortification. When this study was excluded, heterogeneity was decreased and the overall result of the analysis continued to favor individualized fortification; however this finding was no longer statistically significant (MD 0.08, 95% CI -0.18 to 0.33; P = 0.55).

Analysis 4.3: no single study appeared to be an outlier compared to other studies.

### **Sensitivity analyses**

We planned to perform sensitivity analyses by excluding unblinded trials and those without adequate treatment allocation concealment. Of seven included studies, one was assessed as having high risk of bias due to lack of allocation concealment ([Bulut 2019](#)), one due to lack of blinding of personnel ([Hair 2014](#)), and one due to lack of blinding of outcome assessment ([Arslanoglu 2006](#)). In sensitivity analyses, Analysis 1.3 (Comparison: Targeted or adjustable vs standard; Outcome: growth velocity, head circumference in mm/d, end of intervention) became non-significant (MD 0.10, 95% CI -0.03 to 0.23; P = 0.13) with exclusion of [Arslanoglu 2006](#) and [Hair 2014](#). Analysis 3.4 (Comparison:

Adjustable vs standard; Outcome: growth velocity, weight in g/d, end of intervention) became non-significant (MD 2.30, 95% CI -0.23 to 4.83;  $P = 0.07$ ) with exclusion of [Arslanoglu 2006](#). Analysis 4.1 (Comparison: Targeted vs adjustable; Outcome: growth velocity, weight in g/kg/d, end of intervention) became non-significant (MD 0.78, 95% CI -2.04 to 3.60;  $P = 0.59$ ) with exclusion of [Bulut 2019](#). Exclusion of [Arslanoglu 2006](#), [Bulut 2019](#), and [Hair 2014](#) did not change the statistical significance of any other analyses when they were included. [Hair 2014](#) was the only study reporting outcomes for Analyses 2.21, 2.22, 2.29, 2.30, and 2.31.

## DISCUSSION

### Summary of main results

We assessed the comparison between individualized (adjustable and/or targeted) and standard fortification of human breast milk in very preterm infants using growth velocity of weight as the primary outcome. We included seven studies and eight publications in this analysis. Six studies provided data on the primary outcome and compared individualized versus standard fortification for a total of 345 participants, two reported data on the primary outcome and compared type of individualization (targeted versus adjustable) for a total of 72 participants, and one was a follow-up analysis of a specific cohort of patients from one of the other studies, for a total of 21 participants. One study consisted of three arms and compared standard versus targeted versus adjustable. In addition, the study that was a follow-up subgroup analysis was not included in either of these comparisons but was included separately in the targeted versus standard fortification analysis, as it gave additional information on a bronchopulmonary dysplasia (BPD) subgroup. Individualized versus standard fortification studies were further categorized and analyzed by specific type of individualization: targeted versus standard (4 studies, 269 participants) and adjustable versus standard (3 studies, 96 participants).

#### I. Individualized (adjustable/targeted) versus standard fortification

When compared to standard fortification, individualized fortification led to improved growth velocities among all three growth parameters measured: weight, length, and head circumference at end of intervention (low- to moderate-certainty evidence).

Retinopathy of prematurity (ROP), osteopenia, and BPD were not different between groups, but data on these outcomes were limited; only one study assessed ROP and osteopenia, and four studies assessed BPD. All reported a small or very small number of events (very low-certainty evidence).

#### II. Targeted versus standard fortification

Targeted fortification resulted in improved growth velocities of weight and length but no statistically significant difference in head circumference (low- to moderate-certainty evidence).

One study followed all three growth parameters and calculated body mass index (BMI) at end of intervention, at 40 weeks' postmenstrual age (PMA), and at 3, 6, and 12 months' corrected age (CA), and found no significant differences among groups in any of these outcomes at any of the time points ([Agakidou 2019](#)). The only study that evaluated these outcomes beyond end of intervention

did not observe a significant difference at end of intervention among groups fed targeted versus standard fortification.

In a BPD subgroup analysis of in-hospital mortality, length of hospital stay, and PMA at discharge in one study ([Hair 2016](#)) (secondary analysis of [Hair 2014](#)), no differences were evident.

No differences were evident between fortification groups for the following clinical outcomes.

1. Length of hospital stay in days and PMA at discharge in weeks; however data were collected in only one study (very low-certainty evidence).
2. In-hospital mortality, necrotizing enterocolitis, and culture-proven late-onset bacterial sepsis, but these outcomes were evaluated in only two of the included studies (very low-certainty evidence).
3. Retinopathy of prematurity and osteopenia, but these were evaluated in only one study.
4. BPD, collected in three studies.

#### III. Adjustable versus standard fortification

Adjustable fortification resulted in significant improvement in growth velocity of weight, length, and head circumference at end of intervention when compared to standard fortification (moderate- to high-certainty evidence). When growth velocity of weight was expressed in grams/d as opposed to grams/kg/d, a significant difference was evident, in which infants receiving adjustable fortification showed improved growth.

Retinopathy of prematurity, osteopenia, and BPD did not appear to be different between the two groups but were assessed in only one study.

#### IV. Targeted versus adjustable fortification

When methods of individualized fortification were compared, targeted fortification resulted in improved growth velocity of weight when compared to adjustable fortification, but length and head circumference growth velocities were not different between the two groups. These methods were compared in only two studies (low- to moderate-certainty evidence).

Retinopathy of prematurity, osteopenia, and BPD were not significantly different between the two groups, but again, these data were collected in only one of the studies.

#### Overall completeness and applicability of evidence

Included studies were conducted in similar populations in neonatal intensive care units (NICUs) in several different countries, including Greece, Italy, Turkey, USA, and Canada. Accordingly, our synthesized observed findings are likely generalizable to NICU populations with the resources available to individualize fortification. Further, these studies included detailed feeding regimen information to promote replicability and clinical implementation.

Although feeding regimen details were reported, site-specific regimens varied. These observed inconsistencies contributed to the heterogeneity of findings, and thus decreased the overall quality of evidence. Specifically, differences existed among studies with respect to standard regimens, pre-intervention

regimens, duration of the intervention, timing of corresponding measurements, and reported outcomes. The design and implementation of interventions and the use of fortifiers varied. The data leave a very important clinical question unanswered: what is an optimal fortification practice? Although it appears that individualized fortification is better in the short term for multiple growth parameters, the optimal regimen with which to individualize fortification remains unidentified. Studies of adjustable fortification differed in details of the adjustment algorithms. Two of the studies used the same strategy for testing blood urea nitrogen (BUN) but used different protein supplements (Arslanoglu 2006; Kadioglu Simsek 2019), and one of the studies used a calculation for “corrected serum nitrogen” and used a different upper threshold for when to hold on adding fortifier - 12 as opposed to 14 (Moro 1995). Studies of targeted fortification differed in the macronutrients fortified. Some targeted protein only (Agakidou 2019; Kadioglu Simsek 2019), one focused only on kcal/oz (Hair 2014), and one targeted goals for all three components: protein, carbohydrate, and fat (Rochow 2020).

Studies consistently assessed short-term growth outcomes including weight, length, and head circumference growth velocities at end of intervention. Evidence was insufficient to justify conclusions on other clinical or outpatient growth outcomes. The safety of the interventions remains unknown. Further, no studies evaluated neurodevelopmental outcomes including severe neurodevelopmental impairment.

### Quality of the evidence

Identified evidence addressing our review question was of very low to moderate certainty. There was heterogeneity, sometimes substantial, due to differences between studies in standard feeding regimens, pre-intervention regimens, duration of intervention, timing of end of intervention measurements, reported outcomes, and case definitions of some reported outcomes. Studies of adjustable fortification differed in the details of their adjustment algorithms. Studies of targeted fortification differed in the macronutrients they fortified (e.g. protein only [Agakidou 2019]; protein, carbohydrate, and fat [Rochow 2020]). Included studies were often imprecise due to relatively small enrollment or small numbers of events.

### Potential biases in the review process

We attempted to minimize bias in our review process as feasible. The literature search included searches of major literature databases, clinical trial registries, and Cochrane databases of clinical trials. Two review authors screened each abstract for further review based on the inclusion criteria, and a third review author adjudicated disagreement. Once an abstract was chosen, two review authors reviewed the full article and extracted data. Risk of bias was also assessed by two review authors. Study authors were contacted by email for clarification or to request unpublished data when necessary.

### Agreements and disagreements with other studies or reviews

We found no other reviews of this topic during our search. The Cochrane Library includes reviews of non-individualized nutritional fortification of breast milk feedings in preterm infants, including reviews of multi-nutrient fortification versus no fortification (Brown 2020), human- versus bovine-derived milk fortifier (Premkumar

2019), fortification versus no fortification following hospital discharge (Young 2013), protein fortification versus no protein fortification (Amissah 2018a), carbohydrate fortification versus no carbohydrate fortification (Amissah 2018b), fat fortification versus no fat fortification (Amissah 2018c), and early versus late fortification (Thanigainathan 2020). In contrast, this review examines the strategy of individualized versus non-individualized fortification, irrespective of the specific macronutrient fortified. Thus this review is not directly comparable to reviews of non-individualized versus no fortification or of different non-individualized fortification regimens. However, the authors of reviews of non-individualized nutritional fortification of breast milk feedings in preterm infants have found, as we did, that the literature addressing their review question was often noteworthy for small sample sizes, low precision, and other causes of low certainty of evidence (Amissah 2018a).

## AUTHORS' CONCLUSIONS

### Implications for practice

We found moderate- to low-certainty evidence suggesting that individualized (targeted or adjustable) fortification of enteral feeds in very low birth weight infants increases growth velocity of weight, length, and head circumference during the intervention compared with standard non-individualized fortification. Evidence examining important in-hospital and post-discharge clinical outcomes was sparse and of low or very low certainty, precluding inferences regarding safety or clinical benefits beyond short-term growth. The optimal regimen for individualizing fortification remains unknown.

### Implications for research

The best approach for individualized fortification remains largely unexplored. Findings from this review suggest that targeted or adjustable approaches may improve short-term growth, but data were insufficient to establish which method, if either, is superior. Current data also lack conclusive evidence regarding which macronutrients the individualized fortification should be directed toward when such practices are implemented. Thus, does the addition of protein, fat, or carbohydrates, or a combination of those macronutrients, yield the best growth when individualized fortification strategies are used? In addition, further research is warranted to evaluate safety with respect to important clinical outcomes, including mortality, necrotizing enterocolitis, bronchopulmonary dysplasia, sepsis, neurodevelopmental outcomes, and growth beyond NICU discharge in this population.

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\* Indicates the major publication for the study

**CHARACTERISTICS OF STUDIES**
**Characteristics of included studies** [ordered by study ID]

**Agakidou 2019**
**Study characteristics**

Methods	Randomized double-blind study with parallel design (2 treatment groups) and allocation ratio 1:1. performed at 1 center in Greece to compare the effects of a protein-targeting fortification protocol vs standard fortification on growth up to 12 months' corrected age. Allocation was performed through a computer-generated randomization list, with randomization clustered based on birth weight below and equal to/over 1200 grams. In both groups, fortification started as soon as enteral nutrition reached 100 mL/kg/d (T1). All infants in both intervention groups were fed exclusively own mother's milk (OMM) fortified with a cow's milk-based, multi-nutrient HMF (PreNAN FM-85; Nestlé, Vevey, Switzerland) containing 0.20 grams of protein, 0.66 grams of carbohydrates, 0.004 grams of fat, and 3.48 kcal per 1 gram of fortifier. During the week preceding OMM fortification initiation, eligible neonates were randomly allocated to either targeted or standard groups. Milk analysis was performed with mid-infrared spectrometry, using the Milkoscan TM Minor (FOSS Analytical A/S, Hillerod, Denmark)
Participants	Eligible were appropriate-for-gestational-age preterm infants at 25 to 32 weeks' gestation, birth weight < 1500 grams, admitted within the first 24 hours of life to the study NICU between March 2013 and March 2016, whose mothers intended to provide them with their own breast milk. Excluded were in-

**Agakidou 2019** (Continued)

infants with evidence of maternal health problems precluding breast-feeding, congenital infection, metabolic/genetic syndromes, early death, intraperiventricular hemorrhage of grade III to IV, sepsis and/or necrotizing enterocolitis, and consent refusal. Post-randomization exclusion criteria included death before the 40th week PMA, interruption of enteral or exclusive own mother's milk feeding for longer than 3 days for various reasons (i.e. inadequate OMM supply, feeding intolerance, sepsis, and/or necrotizing enterocolitis), moderate/severe bronchopulmonary dysplasia, and withdrawal of parental consent. 77 were randomized - 39 and 38 in standard and targeted groups, respectively; 29 infants were excluded following randomization - 16 from the standard group (4 moderate/severe bronchopulmonary dysplasia, 2 sepsis/necrotizing enterocolitis, 1 sepsis-related death, 3 feeding intolerance, 6 inadequate milk supply), and 13 from the targeted group (3 moderate/severe bronchopulmonary dysplasia, 1 sepsis/necrotizing enterocolitis, 2 feeding intolerance, 7 inadequate milk supply)

Interventions	Intervention was maintained until 35 weeks' PMA <ul style="list-style-type: none"> <li>• Standard: the fixed fortification group received 5 grams of HMF per 100 mL of OMM, providing 1 gram of protein per 100 mL OMM</li> <li>• Targeted: fortifier was added based on protein content of OMM, birth weight, and daily amount of milk intake to attain the recommended daily protein intake (4 to 4.5 g/kg-1 for infants with birth weight &lt; 1200 grams and 3.5 to 4.0 g/kg-1 for infants with birth weight of 1200 to 1500 grams). Lactose, fat, and energy content of OMM and HMF were not taken into account when the amount of HMF given to the targeted group was calculated. Adjustment of fortification to OMM protein content and daily volume of milk intake continued until the 35th week PCA (T2); then fortification was switched to the standard protocol</li> </ul>
Outcomes	Growth outcomes <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: yes</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: yes</li> </ol> In-hospital clinical outcomes: yes Neurodevelopmental outcomes: no Randomized infants who were excluded due to adverse events were included in the review for counts of BPD and death. No case definition was provided for NEC; sepsis and NEC were treated as a single adverse outcome
Notes	Also examined postnatal IGF-1 and ghrelin plasma levels in the 2 fortification arms

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "allocation was performed through a computer-generated randomization list"
Allocation concealment (selection bias)	Unclear risk	Insufficient detail to assess
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Only a member of the nursing staff who was not involved in the infants' care and in clinical/laboratory assessment was aware of group assignment. The same person was responsible for precise measurement of the quantity of HMF and distribution of the proper portion (divided into 8 feeds) for each participant
Blinding of outcome assessment (detection bias)	Unclear risk	Insufficient detail to assess

**Agakidou 2019** (Continued)

## All outcomes

Incomplete outcome data (attrition bias) All outcomes	Low risk	Data available for all infants; no withdrawals from the study
Selective reporting (reporting bias)	Low risk	Outcomes seem to be reported in full
Other bias	Low risk	Nothing to indicate any other source of bias

**Arslanoglu 2006**
**Study characteristics**

Methods	Randomized controlled trial performed at 1 center in Italy designed to explore the individualized adjustable approach to fortification of feedings in VLBW infants. Predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes. Randomization used stratification by birth weight (< 1250, 1251 to 1500, and 1501 to 1750 grams). Infants were enrolled and randomized to 1 of the feeding groups - adjustable or standard - if and when they reached a feeding volume of 90 mL/kg/d. The actual study began when the feeding volume reached 150 mL/kg/d with full-strength standard fortification. The study ended when the infant reached a weight of 2000 grams. Infants received the regimen to which they were randomized throughout the study
Participants	Infants with birth weight between 600 and 1750 grams and gestational age between 24 and 34 weeks were eligible if they reached a feeding volume of 90 mL/kg/d before DOL (day of life) 21. Excluded were infants with major congenital abnormalities, chromosomal aberrations, systemic disease, sepsis, necrotizing enterocolitis or intraventricular hemorrhage, ventilator dependent on DOL 21, and multiple births. 36 participants were enrolled and 32 infants completed the study (16 in each study arm)
Interventions	<p>The intervention was maintained until a weight of 2 kg was achieved or for a minimum 14 days</p> <ul style="list-style-type: none"> <li>• Standard: infants in the standard fortification arm received human milk fortified with HMF in the standard amount (5 g/100 mL of HM) throughout the study. The HMF provided (per 100 mL of breast milk) 0.8 grams of protein in the form of hydrolyzed bovine whey proteins and 18 calories (from protein and maltodextrins)</li> <li>• Adjustable: infants in the adjustable fortification arm started out with standard fortification, but then adjustments to fortification were made at 6 levels, differing in the amount of HMF and additional protein added based on 2 times-weekly (Monday and Thursday) determinations of blood urea nitrogen (BUN). If BUN was between 9 and 14 mg/dL (3.2 to 5.0 mmol/L), no adjustment was made. Every time the BUN was &lt; 9 mg/dL (&lt; 3.2 mmol/L), fortification was increased by 1 level. If BUN was &gt; 14 mg/dL (&gt; 5.0 mmol/L), a decrease in fortification by 1 level was made</li> </ul>
Outcomes	<p>Growth outcomes</p> <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: no</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: no</li> </ol> <p>In-hospital clinical outcomes: no</p> <p>Neurodevelopmental outcomes: no</p>

**Arslanoglu 2006** (Continued)

Weight gain in grams/d was calculated as the difference between initial and final weight, divided by the number of days elapsed, and in g/kg/d by dividing gain in grams/d by average weight during the observation period

Notes Single center, non-blinded, small sample size

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient detail to assess (no details about how randomization was done)
Allocation concealment (selection bias)	Low risk	Quote: "predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "caregivers responsible for infants' care and feeding were not involved in the investigation"
Blinding of outcome assessment (detection bias) All outcomes	High risk	Quote: "it was not possible to blind investigators to study group assignment"
Incomplete outcome data (attrition bias) All outcomes	Low risk	1 infant in each group withdrew after randomization due to reaching 2000 grams before 14 days, and their data were excluded. Not likely to lead to attrition bias (1 in each group)
Selective reporting (reporting bias)	Low risk	Outcomes seem to be reported in full
Other bias	Low risk	Nothing to indicate any other source of bias

**Bulut 2019**
**Study characteristics**

**Methods**

Randomized controlled trial performed at 1 center in Turkey; to compare effects of targeted and adjustable protein fortification on early growth of breast-fed VLBW preterm infants. The study was a prospective, single-center, randomized trial in which infants received either the targeted or adjustable protein fortification regimen during 4 weeks. Predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes. It was not possible to blind investigators to study group assignment, but caregivers responsible for infant care and feeding were not involved in the investigation. Assessment of growth included measurement of daily weight gain (g/d and g/kg/d) and weekly increases in head circumference (mm) and length (cm). Weight gain in g/d was calculated as the difference between initial and final weights, divided by the number of days elapsed, and in g/kg/d by dividing gain in g/d by average weight during the observation period. All anthropometric measurements were taken by nurses who were blinded to the study. Growth status was evaluated by determining Z scores and the end of study extrauterine growth restriction (EUGR) ratio of the total population. Growth Z scores were calculated at birth, at the beginning of the study, and at the end of the study using the LMS method, based on Fenton growth charts. EUGR was defined as a decrease > 1 Z score (severe EUGR > 2) in weight between birth and other measures taken during the hospital stay. Parenteral nutrition was initiated on the first day of life at 70 to 80 mL/kg/d, including 3 g/kg/d protein and 1 g/kg/d lipid; this was increased to 150 to 160 mL/kg/d, including 3.5 g/kg/d protein and 2 g/kg/d lipid within the first week. Minimal enteral nutrition commenced as soon as colostrum was produced. The daily volume of enteral nutrition was increased in increments of 10 to 20 mL/kg, as tolerated. When the feeding

**Bulut 2019** (Continued)

volume reached 80 mL/kg/d, breast milk was enriched with a commercially available fortifier (Aptamil Eoprotin; Milupa, Fulda, Germany), first at 1 unit/80 mL, then at 3 units/90 mL, and last at 4 units/100 mL milk (standard fortification). Infants were randomized to targeted ( $n = 16$ ) and adjustable ( $n = 16$ ) protein fortification groups when the volume of fortified breast milk given reached 150 mL/kg/d, which was the commencement day of the trial. The study ended after growth of all infants was monitored for 4 weeks

Participants	VLBW preterm infants $\leq 32$ weeks' gestational age who were hospitalized at our NICUs between September 2013 and February 2014, and who were exclusively fed fortified breast milk Excluded was any congenital abnormality, metabolic disease, necrotizing enterocolitis, moderate to severe bronchopulmonary dysplasia, or feeding with formula or formula plus breast milk. 49 participants were enrolled and 32 completed the study, with 16 in each study arm
Interventions	<p>Intervention maintained for 4 weeks</p> <ul style="list-style-type: none"> <li>• Targeted: breast milk was analyzed daily with a mid-infrared spectrophotometer (Miris, Uppsala, Sweden). When protein intake was <math>&lt; 4.5</math> g/kg/d in the targeted protein fortification group, additional protein (Protifar; Nutricia, Fulda, Germany) supplement was given to maintain the target protein intake at 4.5 g/kg/d</li> <li>• Adjustable: the protein content of breast milk with standard fortification was presumed to be 2.2 g/100 mL. BUN values were measured weekly, and at levels <math>&gt; 5</math> mg/dL, protein intake was considered sufficient. At <math>&lt; 5</math> mg/dL, additional protein was given to reach a maximum estimated amount of 4.5 g/kg/d</li> </ul>
Outcomes	<p>Growth outcomes</p> <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: no</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: no</li> </ol> <p>In-hospital clinical outcomes: no</p> <p>Neurodevelopmental outcomes: no</p> <p>Weight gain in g/d was calculated as the difference between initial and final weight, divided by the number of days elapsed, in g/kg/d, by dividing gain in g/d by average weight during the observation period</p>
Notes	ClinicalTrials.gov NCT03324126

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes
Allocation concealment (selection bias)	High risk	It was not possible to blind investigators to study group assignment
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Caregivers responsible for infant care and feeding were not involved in the investigation
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All anthropometric measurements were taken by nurses who were blinded to the study

**Bulut 2019** (Continued)

Incomplete outcome data (attrition bias) All outcomes	High risk	7 deaths, 4 cases of NEC occurred, leading to exclusion (quote: "during the course of the study"); unclear if these occurred before or during the study intervention; if the latter, unclear if these occurred equally in the 2 study arms
Selective reporting (reporting bias)	Unclear risk	Insufficient detail to assess (comparative clinical outcomes, safety data not reported)
Other bias	Low risk	Nothing to indicate any other source of bias

**Hair 2014**
**Study characteristics**

Methods	<p>Randomized controlled trial performed at 2 centers in USA (quote: "to evaluate whether premature infants who received an exclusive human milk [HM]-based diet and an HM-derived cream supplement [cream] would have weight gain [g/kg/d] at least as good as infants receiving a standard feeding regimen [control]"). Infants were randomized to 1 of 2 groups via blocks of 4. Fortification began by the time infants were tolerating 100 mL/kg/d of enteral feeds, if not sooner. The cream group was defined as the intent-to-treat group. Fortification began by the time infants were tolerating 100 mL/kg/d of enteral feeds, if not sooner. Once fortified feeds were tolerated, the caloric content of HM was determined daily from a 24-hour batch sample, using a commercially available near-infrared milk analyzer (Spectratar 2400RTW; Unity Scientific, Brookfield, CT, USA). This caloric information was available only to study investigators and was not part of routine care at either study site</p>
Participants	<p>Included infants were 750 to 1250 grams BW, with reasonable expectation of survival for study duration through 36 weeks' postmenstrual age (PMA), weaned from fortification, adherence to a feeding protocol providing an exclusive HM-based diet and potentially a donor HM-derived cream supplement, achievement of enteral feeds by 21 days of life, and informed consent from parent or legal guardian. Excluded were infants with "major congenital anomalies or clinically significant congenital heart disease, low expectation for survival, high potential for early transfer to a non-study institution, enrollment in another clinical study that affected nutritional management, failure to start minimum enteral feeds before 21 days of life, presence of intestinal perforation or stage 2 NEC before tolerating fortified feeds, or inability to participate in the study for any reason based on the decision of the study investigator." 78 participants were randomized, with 39 in each study arm. None were excluded after randomization</p> <p><b>For the Hair 2016 secondary analysis:</b> 3 of these infants were excluded from analysis (1 due to sepsis and a subsequent bowel obstruction before the start of milk analysis, 1 due to clinically significant congenital heart disease and chromosomal abnormality, and 1 due to intestinal perforation before the start of fortified feeds), as their underlying condition placed undue influence on the primary outcomes of this study. Thus, 75 infants (Control n = 37, Cream n = 38) were evaluated</p>
Interventions	<p>Intervention was maintained until 36 weeks' PMA or weaned from fortifier, whichever occurred first</p> <ul style="list-style-type: none"> <li>• Standard: control group received fortification based on the assumption that the HM was 20 kcal/oz. Human milk and human milk-derived fortifier were provided according to the institutional standard of care, and there was no use of milk analysis (mother's own or donor)</li> <li>• Targeted: cream group received the same standard feeding regimen with the addition of a donor HM-derived cream supplement if the HM they were receiving was found to be &lt; 20 kcal/oz after analysis. The donor HM-derived cream supplement, Prolact CR (Prolacta Bioscience, City of Industry, CA, USA), was standardized to 25% lipids and contained 2.5 kcal/mL. The appropriate amount of cream was added to HM to bring the caloric content to approximately 20 kcal/oz. Donor HM or own mother's milk was fortified with cream supplement to a target level of 20 kcal/oz because it is generally assumed that mother's milk is 20 kcal/oz</li> </ul>
Outcomes	Growth outcomes

**Hair 2014** (Continued)

- End of intervention: yes
- 35/36 weeks' PMA: yes
- End of NICU stay: no
- Post-NICU stay: no

In-hospital clinical outcomes: yes

Neurodevelopmental outcomes: no

Weight gain velocity (g/kg/d) was calculated using the Patel Method. If a study subject failed to complete the requisite study period (through 36 weeks' PMA or weaned from fortifier), then the rate of change in weight was calculated for time on the study

Notes ClinicalTrials.gov NCT01487928. Funded by the US Department of Agriculture (USDA)/Agricultural Research Service (ARS) (58-6250-6-001) and the National Center for Research Resources General Clinical Research for Children (RR00188). Prolacta Bioscience provided the product for the study and assisted in data analysis

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient detail to assess (quote: "[I]nfants were randomized into 1 of 2 groups via blocks of 4, the size of which was blinded")
Allocation concealment (selection bias)	Unclear risk	Insufficient detail to assess
Blinding of participants and personnel (performance bias) All outcomes	High risk	Quote: "because of the nature of the interventions by which the nutrition was prepared and delivered, masking of the study groups was not possible at 1 site. The cream supplement mixes readily with HM and its addition does not change the composition or consistency of the HM. At 1 site, we were unable to prepare the milk and deliver it to the infant in a blinded fashion for logistical reasons"
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient detail to assess
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data available for all infants; no withdrawals from the study (for <a href="#">Hair 2014</a> )  <b>Unclear risk for Hair 2016:</b> secondary analysis: 3 enrolled infants were excluded from the analyses presented in this paper but were not excluded from the intention-to-treat analysis in the 2014 initial report. It is unclear whether these post-hoc exclusions affected the statistical significance of findings of the current study
Selective reporting (reporting bias)	Low risk	All outcomes seem to be reported in full
Other bias	High risk	Prolacta Bioscience provided the product for the study and assisted in data analysis. 2 study authors received financial support and received speaker honoraria from Prolacta Bioscience. 2 other study authors are employees of Prolacta Bioscience

**Kadioglu Simsek 2019**
**Study characteristics**

Methods	<p>Randomized controlled trial performed at 1 center in Turkey to compare effects of adjustable fortification (AF), targeted fortification (TF), and standard fortification (SF) methods on early growth of very low birth weight infants. Milk was unfortified initially. SF was commenced when milk intake reached 100 mL/kg/d of enteral feeding in all study infants. Infants were randomized to 3 fortification groups when feeding volumes reached 160 mL/kg/d (full enteral feeding). In Group 2 (AF), fortification was based on BUN levels tested 2 times a week and was performed according to <a href="#">Arslanoglu 2006</a>. Protein supplement (Milupa Aptamil protein supplement; Nutricia, Fulda, Germany) was added to HM according to BUN results. Fortification started at level 0 (0.8 grams protein/100 mL) and was reduced by 1 level when BUN level was &gt; 14 mg/dL, or was increased by 1 level when BUN was &lt; 9 mg/dL. In Group 3 (TF), breast milk analyses were performed at 2 different days of the week in the morning from batches collected by mothers to measure the protein content of breast milk for each infant. Milk samples were analyzed using an HMA (Miris, Uppsala, Sweden)</p>
Participants	<p>Clinically stable infants with birth weight (BW) &lt; 1500 grams and gestational age &lt; 32 weeks who were fed only human milk (HM) were included in the study. Excluded were Infants with significant congenital anomalies, respiratory support requirement, or sepsis, and those who underwent cardiac and intestinal surgery, or who were receiving mixed feeding (preterm formula/breast milk). 60 infants were randomized, with 20 in each study arm</p>
Interventions	<p>Intervention maintained for 4 weeks</p> <ul style="list-style-type: none"> <li>• Standard: in Group 1 (SF), 1 gram (1 scoop) of HMF Eoprotin (Milupa) was added to every 25 mL of HM. Infants in the SF group received HM fortified with human milk fortifier (HMF) in the standard amount (2.3 g/100 mL of HM). HMF provided 0.8 grams of protein and 10 calories per 100 mL of breast milk</li> <li>• Adjustable: in Group 2 (AF), infants were also fed an SF regimen at the beginning. AF was based on BUN levels tested 2 times a week and was performed according to the <a href="#">Arslanoglu 2006</a> study. Protein supplement (Milupa Aptamil protein supplement) was added to HM according to BUN results. Fortification was started at level 0 (0.8 grams protein/100 mL) and was reduced 1 level when BUN level was &gt; 14 mg/dL, or was increased 1 level when BUN was &lt; 9 mg/dL</li> <li>• Targeted: an appropriate amount of protein supplement was added right before the milk was consumed to achieve target protein intake of 3.5 to 4.5 g/kg</li> </ul>
Outcomes	<p>Growth outcomes</p> <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: no</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: no</li> </ol> <p>In-hospital clinical outcomes: yes</p> <p>Neurodevelopmental outcomes: no</p> <p>Weight gain in grams per day was calculated as the difference between initial and final weight, divided by the number of days elapsed; this was converted to grams/(kilogram per day) by dividing gain in grams per day by average weight during the observation period</p>
Notes	<p>Continuous outcomes reported as median (IQR) converted to mean (SD) (<a href="#">Luo 2018</a>; <a href="#">Median to Mean Calculator 2020</a>). Case definitions not provided for clinical sepsis, NEC, BPD, ROP, or osteopenia. 4 cases of NEC occurred in the study cohort; however none were observed after randomization</p>

**Risk of bias**

Bias	Authors' judgement	Support for judgement
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**Kadioglu Simsek 2019** (Continued)

Random sequence generation (selection bias)	Low risk	Quote: "sequential numbers generated at the computer center of the NICU"
Allocation concealment (selection bias)	Low risk	Quote: "the allocations were contained in opaque sequentially numbered sealed envelopes"
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Insufficient detail to assess
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All measurements were performed by trained nurses...and they were blind to the study group
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data available for all participants
Selective reporting (reporting bias)	Low risk	No study protocol, but outcomes seem to be reported in full
Other bias	Unclear risk	Insufficient detail to assess (case definitions not provided for clinical sepsis, NEC, BPD, ROP, or osteopenia)

**Moro 1995**
**Study characteristics**

Methods	<p>Randomized controlled trial performed at 1 center in Italy to test adjustable fortification and to compare it to a standard fortification scheme where fortifier is added in fixed proportions. Feeding of (unfortified) breast milk was initiated at the discretion of the attending physicians. Most of the infants were fed their own mother's expressed breast milk, but ~ 25% of infants received predominantly or exclusively pasteurized breast milk from a local milk bank. The proportion of infants receiving banked milk was similar in the 3 feeding groups. When milk volume reached 150 mL/kg/d and intravenous fluids were discontinued, infants whose parents consented were enrolled in the study, and fortification with the assigned regimen was started. Separate randomization schedules were used for AGA and SGA infants. As soon as feedings reached the respective target volume, which usually occurred 3 days later, the study began (day 1)</p>
Participants	<p>Infants were eligible if their birth weight was between 900 and 1500 grams, if they were no longer receiving intravenous fluids, and if they were free of major congenital malformations and systemic illness. 42 participants were enrolled and 36 infants completed the study, with 12 in each study arm</p>
Interventions	<p>Intervention was maintained until hospital discharge at a body weight of ~ 2200 grams</p> <ul style="list-style-type: none"> <li>• Standard: in the standard fortification arm, infants were fed breast milk fortified with an experimental bovine milk protein-based fortifier (EBMF), added in a fixed amount (3.5 grams to each 100 mL of breast milk)</li> <li>• Adjustable: in the adjustable arm, infants were fed breast milk that was also fortified with EBMF, except that the amount of fortifier was added at 7 levels, differing in the amount of fortifier, on the basis of 2 times-weekly determinations of corrected serum urea nitrogen (CSUN). "Correction" of serum urea to a normal serum creatinine concentration was used because the low glomerular filtration rate of young preterm infants leads to elevation of serum urea nitrogen (SUN) independently from the level of protein intake. CSUN was calculated as <math>SUN \times 0.5/SCr</math>, where 0.5 is the "normal" serum creatinine concentration, and SCr is the serum creatinine concentration determined at the same time as SUN. In this</li> </ul>

**Moro 1995** (Continued)

way, by correcting the SUN to a creatinine concentration of 0.5 mg/dL, the "renal" component of the SUN value was in effect removed. If the CSUN was between 9.1 and 12.0 mg/dL, fortification was not changed from the standard 3.5 grams per 100 mL of milk. If it was outside this range, the amount of fortifier was changed by not more than 1 level at a time

Outcomes	<p>Growth outcomes</p> <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: no</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: no</li> </ol> <p>In-hospital clinical outcomes: no</p> <p>Neurodevelopmental outcomes: no</p> <p>Gains in weight, length, and head circumference were calculated in the customary fashion as the difference between values at the beginning and at the end, divided by the number of days in the interval. Expression of weight gain per unit of body weight (kg) was accomplished using average weight for the interval</p>
Notes	<p>Infants continued to receive the assigned regimen until hospital discharge at a body weight of &gt;2200 grams; however growth outcomes were reported only for 3 weeks after the beginning of the intervention</p>

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient detail to assess (quote: "randomization schedules"; no details given)
Allocation concealment (selection bias)	Low risk	Assignment of infants to 1 of 3 fortification regimens was done via sealed envelopes
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Insufficient detail to assess
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Insufficient detail to assess
Incomplete outcome data (attrition bias) All outcomes	High risk	Imbalanced attrition (0/14, 2/14, and 4/14) and data not reported for withdrawals
Selective reporting (reporting bias)	Low risk	Outcomes seem to be reported in full
Other bias	Low risk	No indication of any other bias

**Rochow 2020**
**Study characteristics**

**Rochow 2020** (Continued)

Methods	<p>Randomized controlled trial performed at 1 center in Canada to compare growth of preterm infants fed targeted or standard fortification. Randomization was stratified by gestational age (&lt; 28 weeks vs &gt; 28 weeks) with variable block sizes of 2, 4, and 6, to minimize bias in patient allocation. For each stratum, a series of opaque, sealed, and consecutively numbered envelopes were generated and opened by dietary assistants in their offices outside the NICU. After reaching total fluid intake of 120 mL/kg/d, standard fortification was introduced for all study participants using half of the full concentration for 2 days and the final concentration of standard fortification thereafter. In cases of elevated blood urea nitrogen levels (&gt; 100 mmol/L), triglycerides (&gt; 3 mmol/L), or glucose (&gt; 12 mmol/L), the fortification dosage was halved and the study period was discontinued, as long as these conditions were present. After normalization, the study was resumed when infants were expected to complete the minimum study period. The intervention was discontinued for infants who required fluid restriction (&lt; 140 mL/kg/d for &gt; 3 days), had hepatic disease (total serum bilirubin &gt; 10 mg/dL), or developed NEC (Bell stage <math>\geq</math> 2) or Gram-negative sepsis</p>
Participants	<p>Infants at &lt; 30 weeks' gestational age at birth with anticipated length of stay &gt; 21 days and receiving fortified BM were eligible. Excluded were infants with gastrointestinal malformation, major congenital anomalies, stage 2 NEC, abdominal surgery, and Gram-negative sepsis. 179 participants were enrolled and 103 infants were included in the final analysis. 76 were excluded before initiating study intervention or due to early transfer before completing 14 study days, deviation of the feeding protocol, or use of steroids or diuretics. This left 51 and 52 infants in the 2 study arms for final analysis</p>
Interventions	<p>Intervention maintained for minimum 21 days to be completed before 36 weeks' postmenstrual age (PMA)</p> <ul style="list-style-type: none"> <li>• Standard: infants in the standard fortification arm received standard fortifier (Enfamil HMF; Mead Johnson, Cleveland, OH, USA) powder at recommended dosage of 1 package per 25 mL, providing an additional 1 gram of fat, 1.1 gram of protein, and 0.4 gram of carbohydrates per 100 mL of BM. Infants on donor milk received an additional 0.4 gram of whey protein powder per 100 mL (Beneprotein)</li> <li>• Targeted: infants in the targeted fortification arm received standard fortification similar to that of infants in the standard arm and had additional modular components added after human milk analysis. Macronutrients of BM were measured using a calibrated and validated near-infrared milk analyzer (SpectraStar; Unity Scientific, Brookfield, CT, USA). Lactose content was measured using an established reference method. Osmolality of native and fortified BM was measured using a freezing point osmometer (3320 MicroOsmometer; Advanced Instruments, Norwood, MA, USA). Analysis of mother's own milk and of donor milk was done 3 times per week. The amount of additional fortification required to reach ESPGHAN targets was calculated for each macronutrient using a standardized study recipe sheet. After milk analysis, fortifier was added to achieve BM contents of 4.4 grams fat, 8.3 grams carbohydrates, and 3.0 grams protein per 100 mL to reach ESPGHAN recommended intakes, assuming an average fluid intake of 150 mL/kg/d, leading to total daily intake of 6.6, 12.5, and 4.5 g/kg/d for fat, carbohydrates, and protein, respectively. To prepare feeds, standard fortifier was first added to native BM at the recommended dosage as per standard fortification practices. Then, individual modular components were added to achieve target concentrations according to the fortification recipe. In the intervention group, standard fortification was introduced similarly to the control group (i.e. over two days once intake of 120 mL/kg/d was reached). Thereafter, the modular components were introduced over a 3-day period</li> </ul>
Outcomes	<p>Growth outcomes</p> <ol style="list-style-type: none"> <li>1. End of intervention: yes</li> <li>2. 35/36 weeks' PMA: yes</li> <li>3. End of NICU stay: no</li> <li>4. Post-NICU stay: no</li> </ol> <p>In-hospital clinical outcomes: yes</p> <p>Neurodevelopmental outcomes: no</p> <p>Growth velocity was calculated as average rate of weight gain (g/kg/d) during the 21-day study period with a generalized reduced gradient method, starting on study day 2 after full introduction of targeted</p>

**Rochow 2020** (Continued)

fortification. Clinical outcomes were reported for all randomized infants, including those excluded post randomization

Notes ClinicalTrials.gov NCT01609894.

**Risk of bias**

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomization was stratified by gestational age with variable block sizes of 2, 4, and 6 to minimize bias in patient allocation
Allocation concealment (selection bias)	Low risk	For each stratum, a series of opaque, sealed, and consecutively numbered envelopes were generated and opened by dietary assistants in their offices outside the NICU
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All investigators, research assistants, parents, and healthcare providers, except dietary assistants, were blinded to randomization and nutritional intervention
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All investigators, research assistants, parents, and healthcare providers, except dietary assistants, were blinded to randomization and nutritional intervention
Incomplete outcome data (attrition bias) All outcomes	Low risk	76 randomized subjects were excluded before initiating study intervention or due to early transfer before completing 14 study days, deviation of feeding protocol, or use of steroids or diuretics; exclusions occurred equally in the 2 study arms. Clinical outcomes but not growth outcomes were reported for excluded infants
Selective reporting (reporting bias)	Low risk	Growth outcomes were not available for infants excluded after randomization
Other bias	Low risk	Nothing to indicate any other source of bias

AF: adjustable fortification; AGA: appropriate for gestational age; BPD: bronchopulmonary dysplasia; BUN: blood urea nitrogen; BW: birth weight; CSUN: corrected serum urea nitrogen; DOL: day of life; EBMF: experimental bovine milk protein-based fortifier; ESPGHAN: European Society of Paediatrics Gastroenterology, Hepatology and Nutrition; EUGR: extrauterine growth restriction; HM: human milk; HMF: human milk fortifier; IGF-1: insulin-like growth factor-1; IQR: interquartile range; NEC: necrotizing enterocolitis; NICU: neonatal intensive care unit; OMM: own mother's milk; PCA: postconceptual age; PMA: postmenstrual age; ROP: retinopathy of prematurity; SCR: serum creatinine concentration; SD: standard deviation; SF: standard fortification; SGA: small for gestational age; SUN: serum urea nitrogen; TF: targeted fortification; VLBW: very low birth weight.

**Characteristics of excluded studies** [ordered by study ID]

Study	Reason for exclusion
<a href="#">Boehm 1993</a>	Fortification not individualized in any study arm
<a href="#">Kanmaz 2013</a>	Fortification not individualized in any study arm
<a href="#">Maas 2017</a>	Participants did not receive human milk exclusively
<a href="#">Mathes 2018</a>	Participants did not receive human milk exclusively

Study	Reason for exclusion
<a href="#">McLeod 2016</a>	Participants did not receive human milk exclusively
<a href="#">Morlacchi 2016</a>	Assignment was not randomized or quasi-randomized
<a href="#">Quan 2019</a>	Participants did not receive human milk exclusively

### Characteristics of studies awaiting classification *[ordered by study ID]*

#### Brion 2020

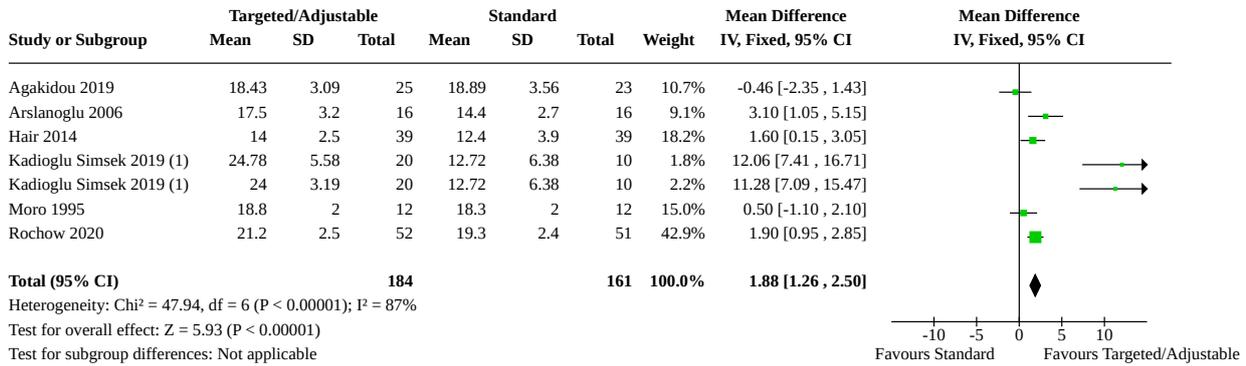
Methods	
Participants	
Interventions	
Outcomes	
Notes	This study is awaiting classification because it was published after our literature review was completed

## DATA AND ANALYSES

### Comparison 1. Targeted or adjustable vs standard

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
<a href="#">1.1 Growth velocity, weight, g/kg/d, end of intervention</a>	6	345	Mean Difference (IV, Fixed, 95% CI)	1.88 [1.26, 2.50]
<a href="#">1.2 Growth velocity, length, mm/d, end of intervention</a>	5	242	Mean Difference (IV, Fixed, 95% CI)	0.43 [0.32, 0.53]
<a href="#">1.3 Growth velocity, head circumference, mm/d, end of intervention</a>	5	242	Mean Difference (IV, Fixed, 95% CI)	0.14 [0.06, 0.23]
<a href="#">1.4 Bronchopulmonary dysplasia</a>	4	391	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.71, 1.12]
<a href="#">1.5 Retinopathy of prematurity, any</a>	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.79 [0.36, 1.72]
<a href="#">1.6 Osteopenia</a>	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.40, 1.84]

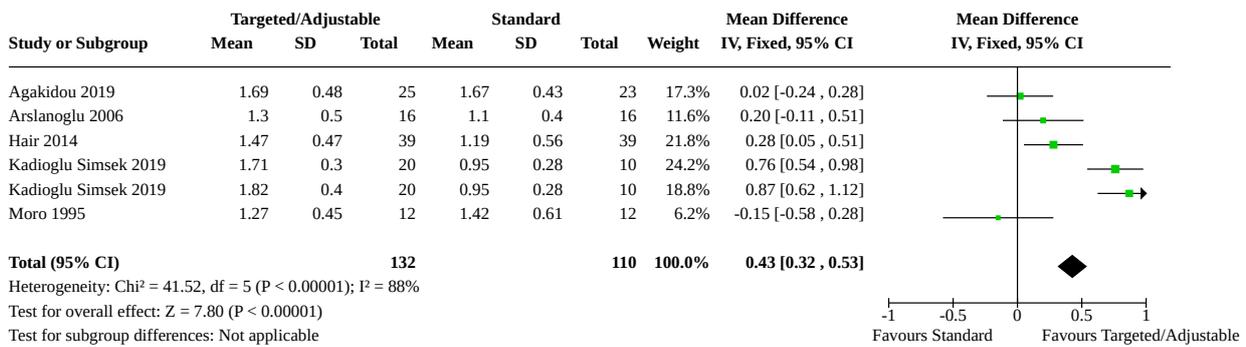
**Analysis 1.1. Comparison 1: Targeted or adjustable vs standard, Outcome 1: Growth velocity, weight, g/kg/d, end of intervention**



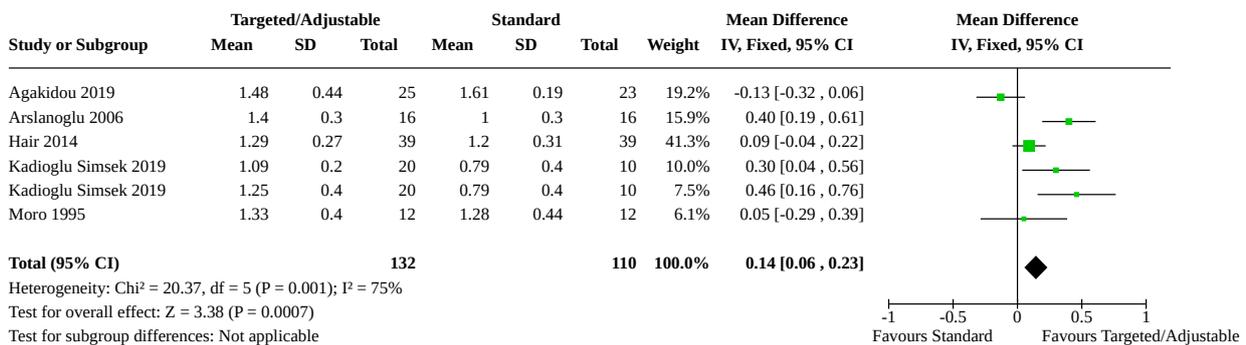
**Footnotes**

(1) This is a multi-arm study. To include each of the comparison arms in the analysis, we have divided the infants reported in the control group against each comparison, with the means an

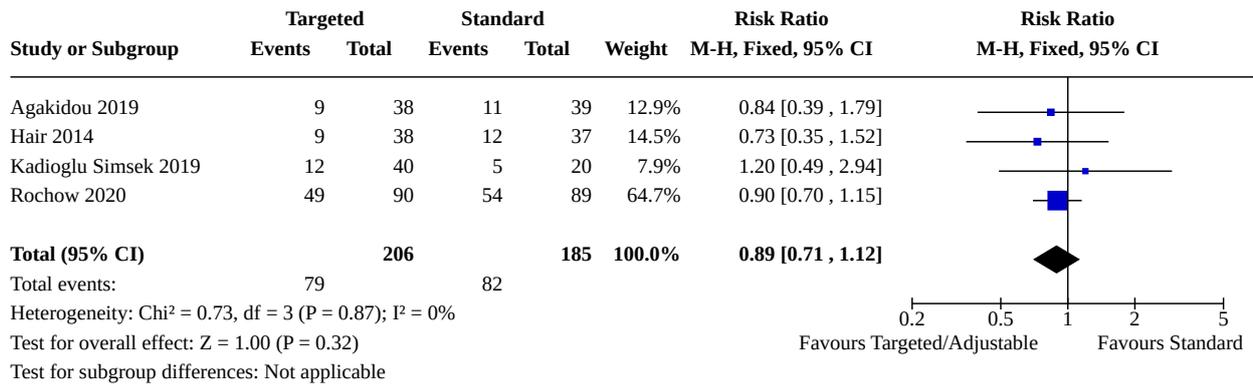
**Analysis 1.2. Comparison 1: Targeted or adjustable vs standard, Outcome 2: Growth velocity, length, mm/d, end of intervention**



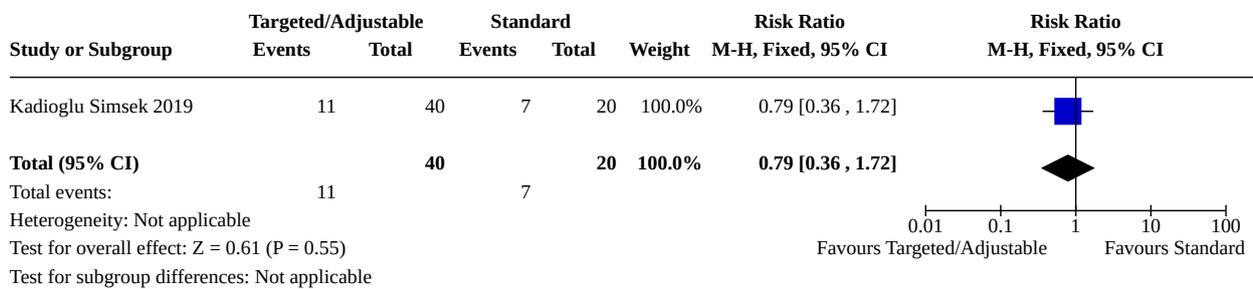
**Analysis 1.3. Comparison 1: Targeted or adjustable vs standard, Outcome 3: Growth velocity, head circumference, mm/d, end of intervention**



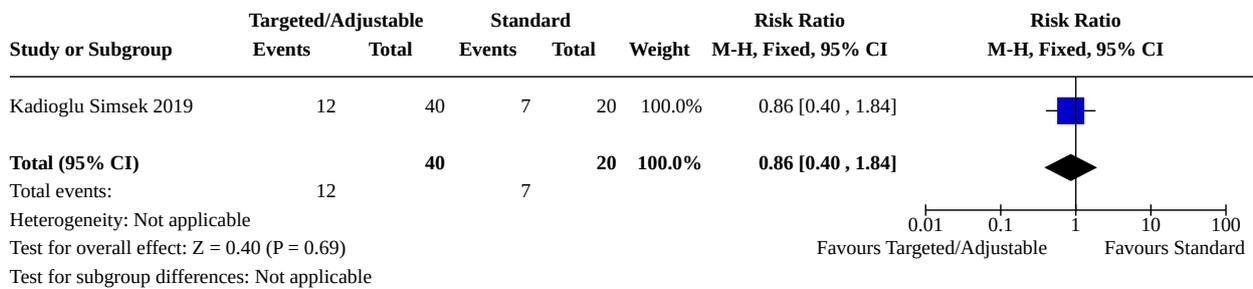
**Analysis 1.4. Comparison 1: Targeted or adjustable vs standard, Outcome 4: Bronchopulmonary dysplasia**



**Analysis 1.5. Comparison 1: Targeted or adjustable vs standard, Outcome 5: Retinopathy of prematurity, any**



**Analysis 1.6. Comparison 1: Targeted or adjustable vs standard, Outcome 6: Osteopenia**



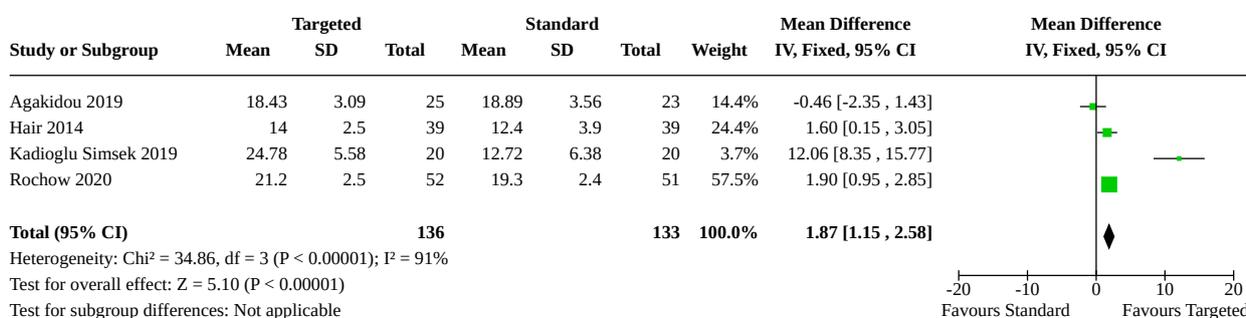
**Comparison 2. Targeted vs standard fortification**

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Growth velocity, weight, g/kg/d, end of intervention	4	269	Mean Difference (IV, Fixed, 95% CI)	1.87 [1.15, 2.58]
2.2 Growth velocity, weight, g/kg/d, start of fortification to 40 weeks' PMA	1	47	Mean Difference (IV, Fixed, 95% CI)	-0.03 [-1.19, 1.13]

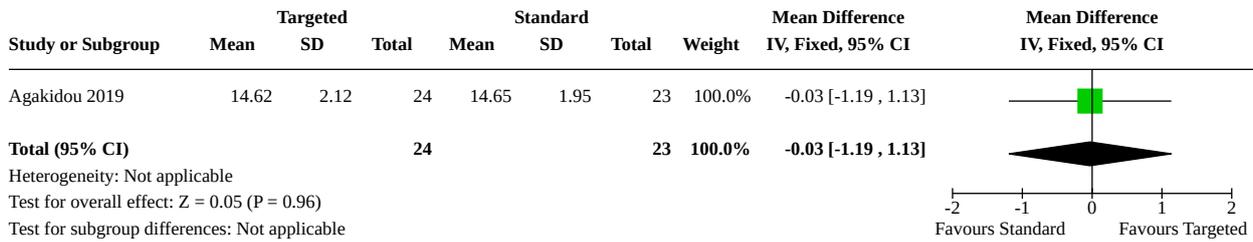
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.3 Growth velocity, weight, g/kg/d, start of fortification to 3 months' CA	1	46	Mean Difference (IV, Fixed, 95% CI)	-0.31 [-1.11, 0.49]
2.4 Growth velocity, weight, g/kg/d, start of fortification to 6 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	0.09 [-0.31, 0.49]
2.5 Growth velocity, weight, g/kg/d, start of fortification to 12 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	-0.04 [-0.36, 0.28]
2.6 Growth velocity, length, mm/d, end of intervention	3	166	Mean Difference (IV, Fixed, 95% CI)	0.45 [0.32, 0.57]
2.7 Growth velocity, length, mm/d, start of fortification to 40 weeks' PMA	1	48	Mean Difference (IV, Fixed, 95% CI)	0.02 [-0.16, 0.20]
2.8 Growth velocity, length, mm/d, start of fortification to 3 months' CA	1	46	Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.12, 0.08]
2.9 Growth velocity, length, mm/d, start of fortification to 6 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	0.07 [0.00, 0.14]
2.10 Growth velocity, length, mm/d, start of fortification to 12 months' CA	1	44	Mean Difference (IV, Fixed, 95% CI)	0.00 [-0.07, 0.07]
2.11 Growth velocity, head circumference, mm/d, end of intervention	3	166	Mean Difference (IV, Fixed, 95% CI)	0.08 [-0.01, 0.18]
2.12 Growth velocity, head circumference, mm/d, start of fortification to 40 weeks' PMA	1	48	Mean Difference (IV, Fixed, 95% CI)	-0.07 [-0.16, 0.02]
2.13 Growth velocity, head circumference, mm/d, start of fortification to 3 months' CA	1	46	Mean Difference (IV, Fixed, 95% CI)	0.00 [-0.06, 0.06]
2.14 Growth velocity, head circumference, mm/d, start of fortification to 6 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	0.01 [-0.03, 0.05]
2.15 Growth velocity, head circumference, mm/d, start of fortification to 12 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	-0.01 [-0.04, 0.02]
2.16 Change in BMI, end of intervention	1	48	Mean Difference (IV, Fixed, 95% CI)	-0.08 [-0.28, 0.12]
2.17 Change in BMI, start of fortification to 40 weeks' PMA	1	48	Mean Difference (IV, Fixed, 95% CI)	-0.05 [-0.18, 0.08]
2.18 Change in BMI, start of fortification to 3 months' CA	1	46	Mean Difference (IV, Fixed, 95% CI)	-0.04 [-0.11, 0.03]
2.19 Change in BMI, start of fortification to 6 months' CA	1	45	Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.05, 0.01]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.20 Change in BMI, start of fortification to 12 months' CA	1	44	Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.05, 0.01]
2.21 Length of hospital stay, days	1	75	Mean Difference (IV, Fixed, 95% CI)	-12.00 [-26.38, 2.38]
2.22 Postmenstrual age at discharge, weeks	1	75	Mean Difference (IV, Fixed, 95% CI)	-1.70 [-3.47, 0.07]
2.23 In-hospital mortality	3	334	Risk Ratio (M-H, Fixed, 95% CI)	0.14 [0.02, 1.14]
2.24 Necrotizing enterocolitis	2	257	Risk Ratio (M-H, Fixed, 95% CI)	0.40 [0.08, 1.99]
2.25 Culture-proven late-onset bacterial sepsis	2	257	Risk Ratio (M-H, Fixed, 95% CI)	1.29 [0.76, 2.17]
2.26 Retinopathy of prematurity, any	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.43, 2.33]
2.27 Osteopenia	1	40	Risk Ratio (M-H, Fixed, 95% CI)	0.86 [0.35, 2.10]
2.28 Bronchopulmonary dysplasia	4	371	Risk Ratio (M-H, Fixed, 95% CI)	0.88 [0.70, 1.11]
2.29 BPD subgroup - in-hospital mortality	1	21	Odds Ratio (M-H, Fixed, 95% CI)	Not estimable
2.30 BPD subgroup - length of hospital stay, days	1	21	Mean Difference (IV, Fixed, 95% CI)	-17.00 [-48.53, 14.53]
2.31 BPD subgroup - postmenstrual age at discharge, weeks	1	21	Mean Difference (IV, Fixed, 95% CI)	-2.90 [-6.78, 0.98]

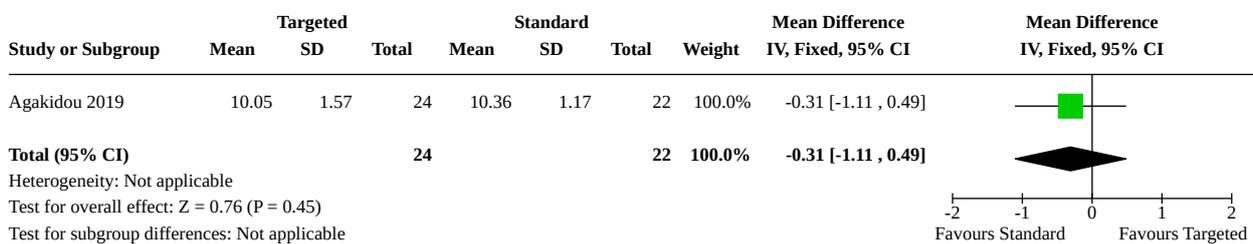
**Analysis 2.1. Comparison 2: Targeted vs standard fortification, Outcome 1: Growth velocity, weight, g/kg/d, end of intervention**



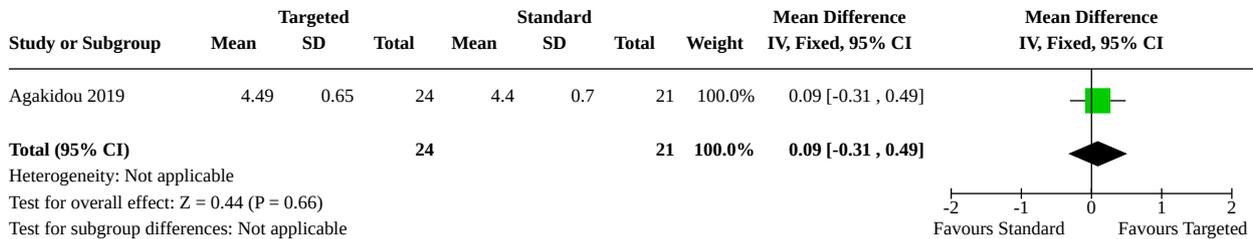
**Analysis 2.2. Comparison 2: Targeted vs standard fortification, Outcome 2: Growth velocity, weight, g/kg/d, start of fortification to 40 weeks' PMA**



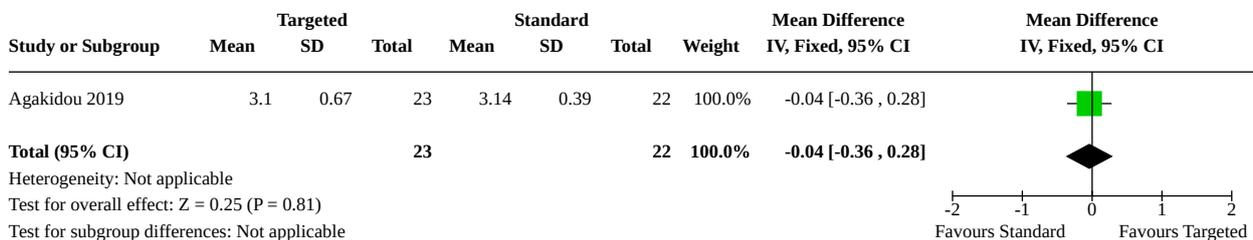
**Analysis 2.3. Comparison 2: Targeted vs standard fortification, Outcome 3: Growth velocity, weight, g/kg/d, start of fortification to 3 months' CA**



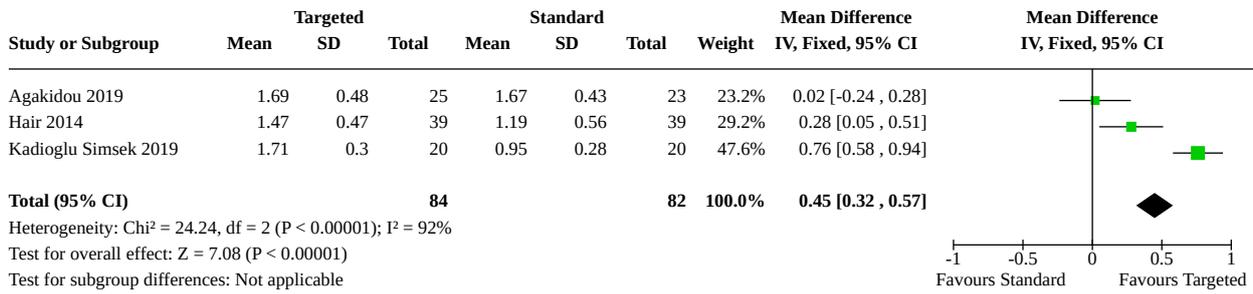
**Analysis 2.4. Comparison 2: Targeted vs standard fortification, Outcome 4: Growth velocity, weight, g/kg/d, start of fortification to 6 months' CA**



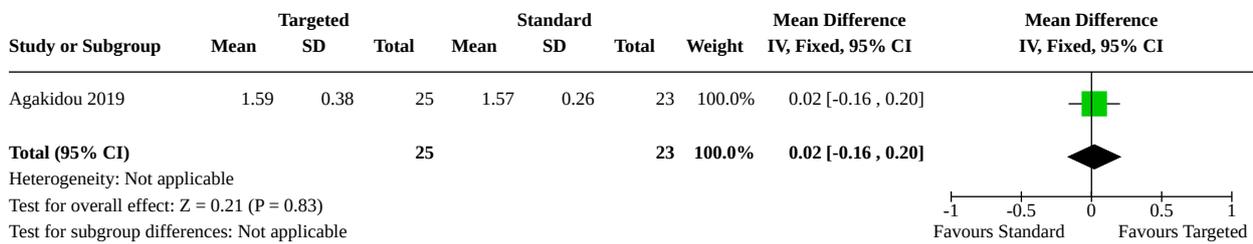
**Analysis 2.5. Comparison 2: Targeted vs standard fortification, Outcome 5: Growth velocity, weight, g/kg/d, start of fortification to 12 months' CA**



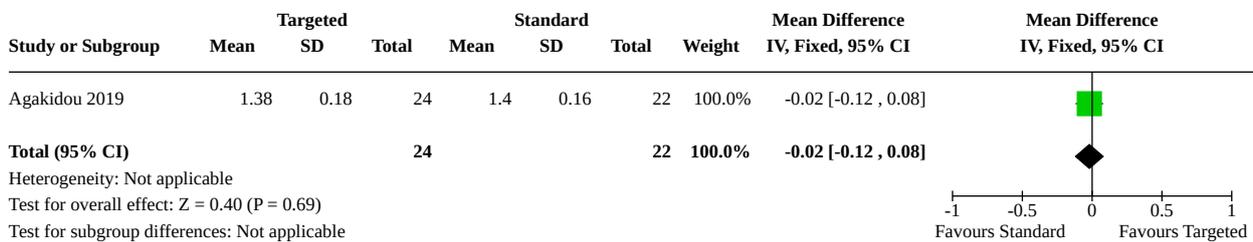
**Analysis 2.6. Comparison 2: Targeted vs standard fortification, Outcome 6: Growth velocity, length, mm/d, end of intervention**



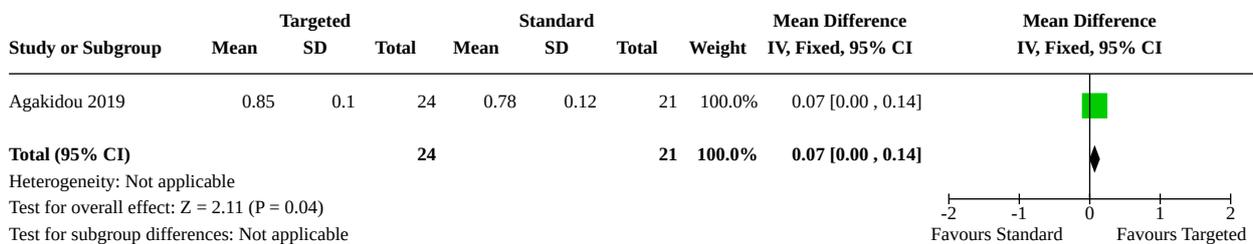
**Analysis 2.7. Comparison 2: Targeted vs standard fortification, Outcome 7: Growth velocity, length, mm/d, start of fortification to 40 weeks' PMA**



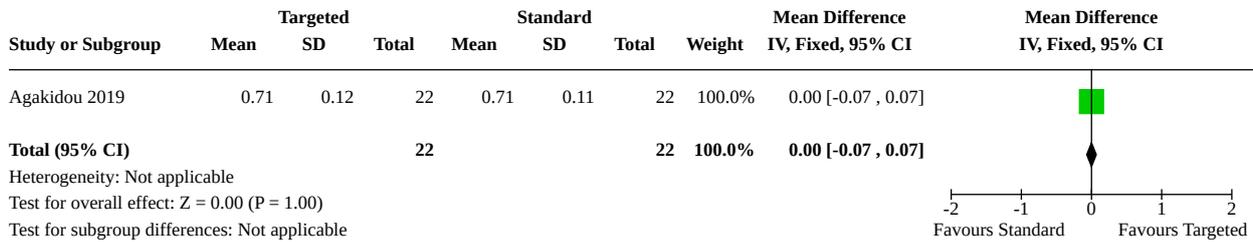
**Analysis 2.8. Comparison 2: Targeted vs standard fortification, Outcome 8: Growth velocity, length, mm/d, start of fortification to 3 months' CA**



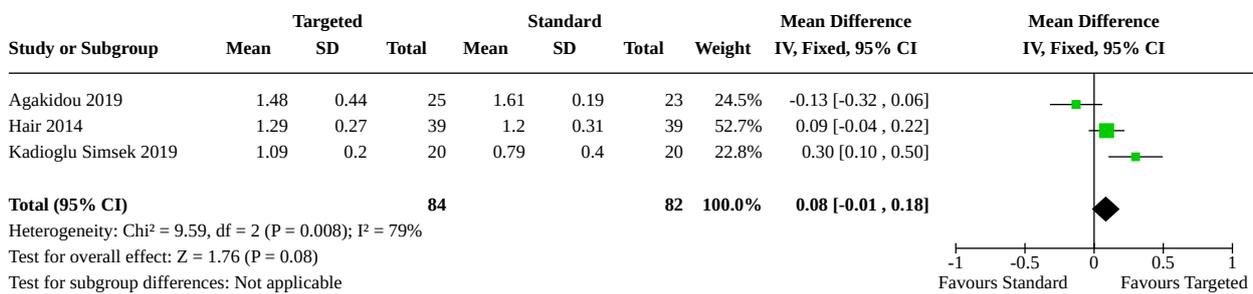
**Analysis 2.9. Comparison 2: Targeted vs standard fortification, Outcome 9: Growth velocity, length, mm/d, start of fortification to 6 months' CA**



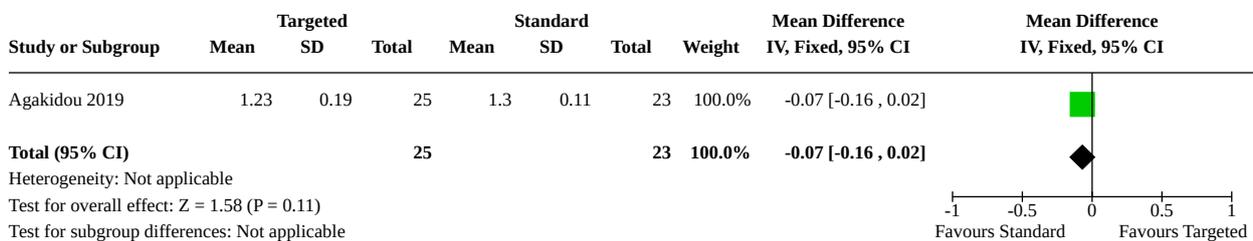
**Analysis 2.10. Comparison 2: Targeted vs standard fortification, Outcome 10: Growth velocity, length, mm/d, start of fortification to 12 months' CA**



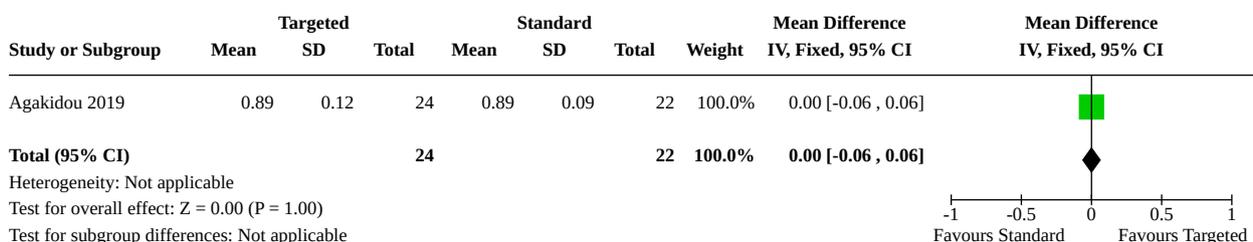
**Analysis 2.11. Comparison 2: Targeted vs standard fortification, Outcome 11: Growth velocity, head circumference, mm/d, end of intervention**



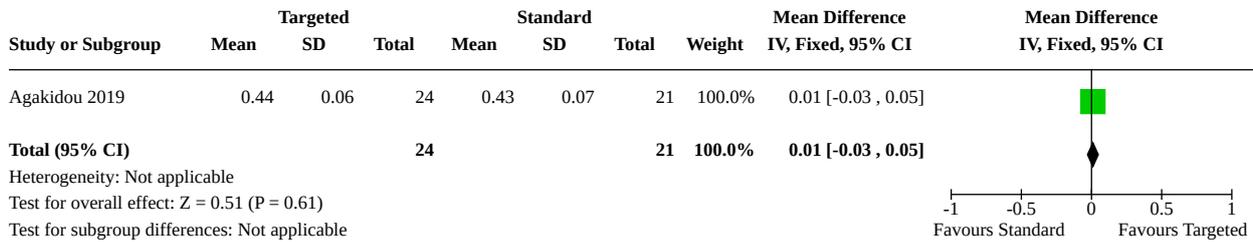
**Analysis 2.12. Comparison 2: Targeted vs standard fortification, Outcome 12: Growth velocity, head circumference, mm/d, start of fortification to 40 weeks' PMA**



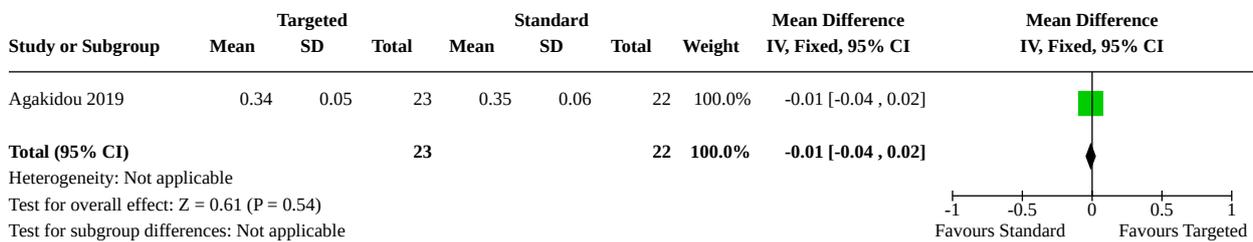
**Analysis 2.13. Comparison 2: Targeted vs standard fortification, Outcome 13: Growth velocity, head circumference, mm/d, start of fortification to 3 months' CA**



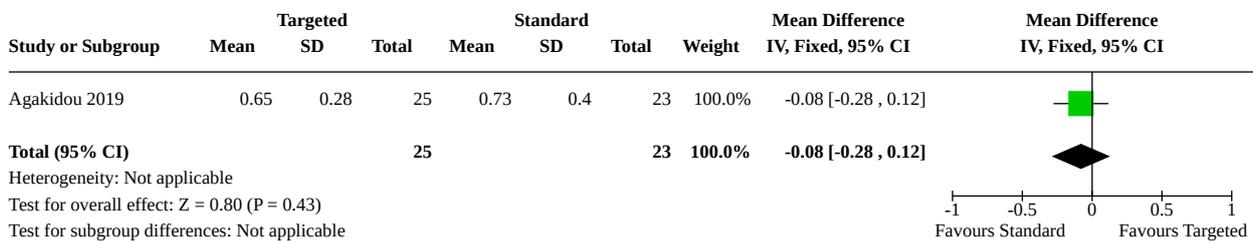
**Analysis 2.14. Comparison 2: Targeted vs standard fortification, Outcome 14: Growth velocity, head circumference, mm/d, start of fortification to 6 months' CA**



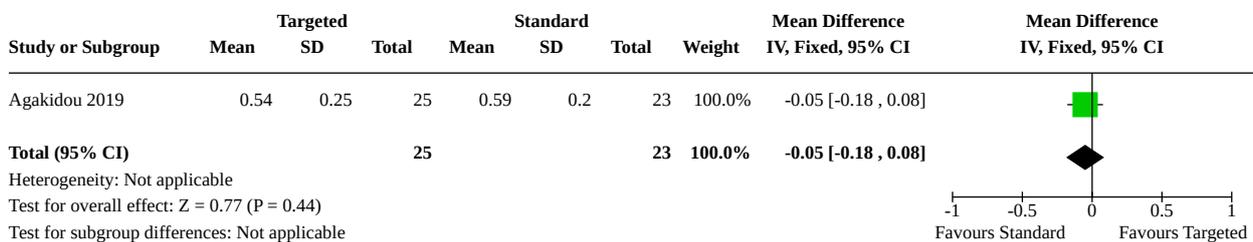
**Analysis 2.15. Comparison 2: Targeted vs standard fortification, Outcome 15: Growth velocity, head circumference, mm/d, start of fortification to 12 months' CA**



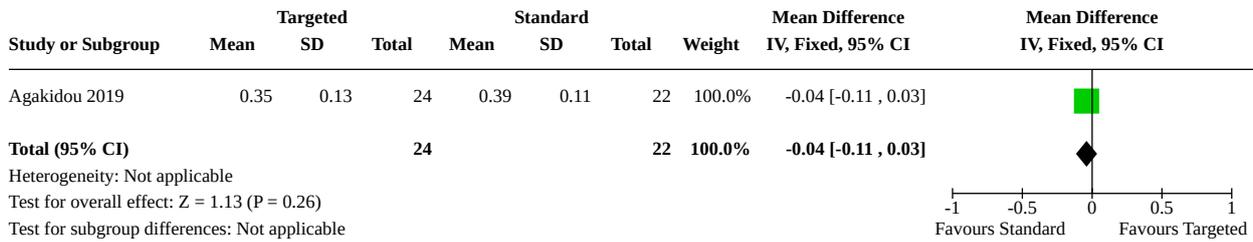
**Analysis 2.16. Comparison 2: Targeted vs standard fortification, Outcome 16: Change in BMI, end of intervention**



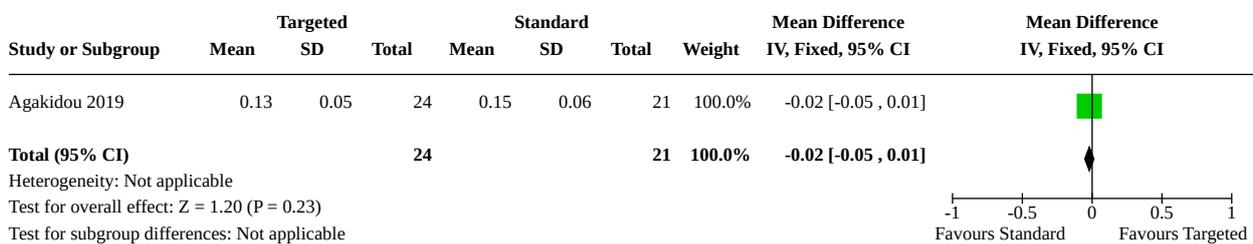
**Analysis 2.17. Comparison 2: Targeted vs standard fortification, Outcome 17: Change in BMI, start of fortification to 40 weeks' PMA**



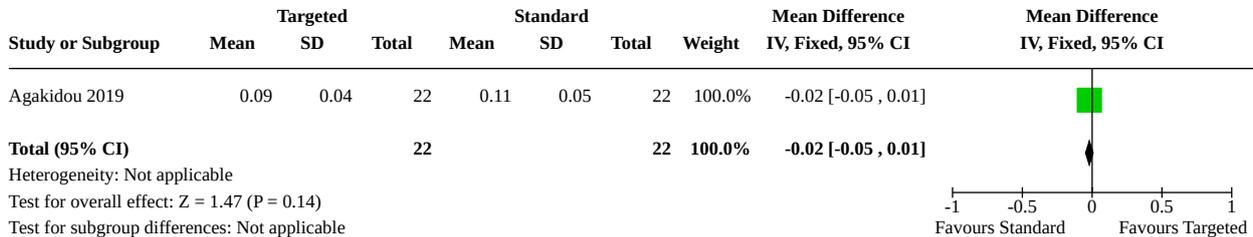
**Analysis 2.18. Comparison 2: Targeted vs standard fortification, Outcome 18: Change in BMI, start of fortification to 3 months' CA**



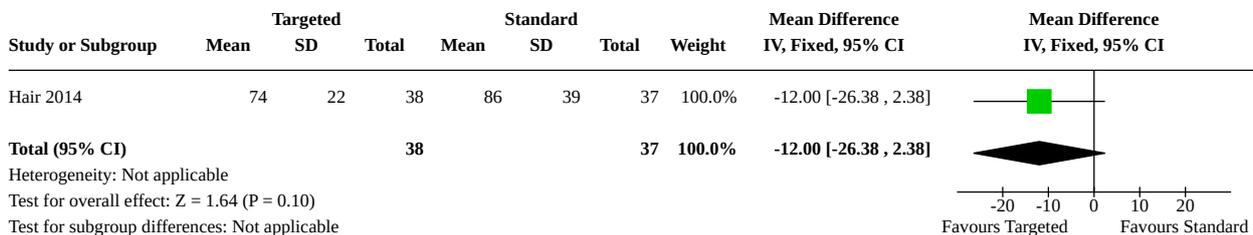
**Analysis 2.19. Comparison 2: Targeted vs standard fortification, Outcome 19: Change in BMI, start of fortification to 6 months' CA**



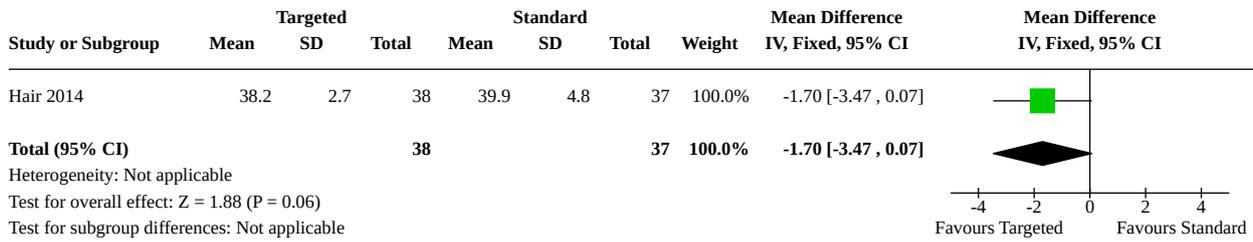
**Analysis 2.20. Comparison 2: Targeted vs standard fortification, Outcome 20: Change in BMI, start of fortification to 12 months' CA**



**Analysis 2.21. Comparison 2: Targeted vs standard fortification, Outcome 21: Length of hospital stay, days**



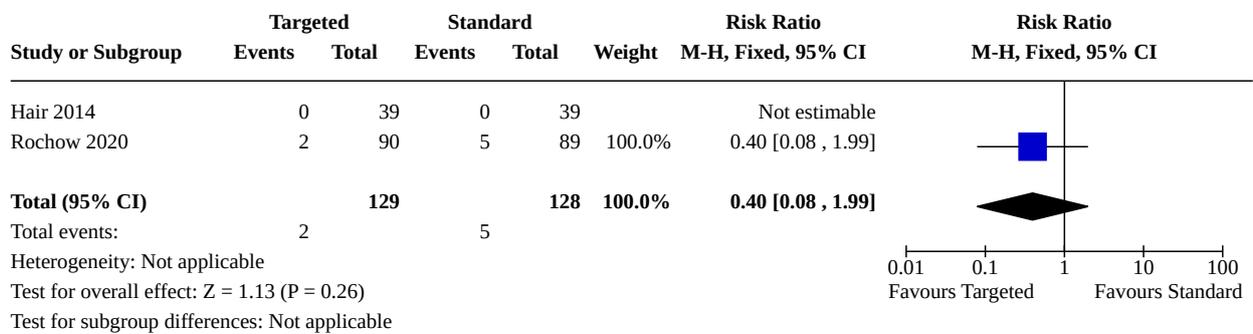
**Analysis 2.22. Comparison 2: Targeted vs standard fortification, Outcome 22: Postmenstrual age at discharge, weeks**



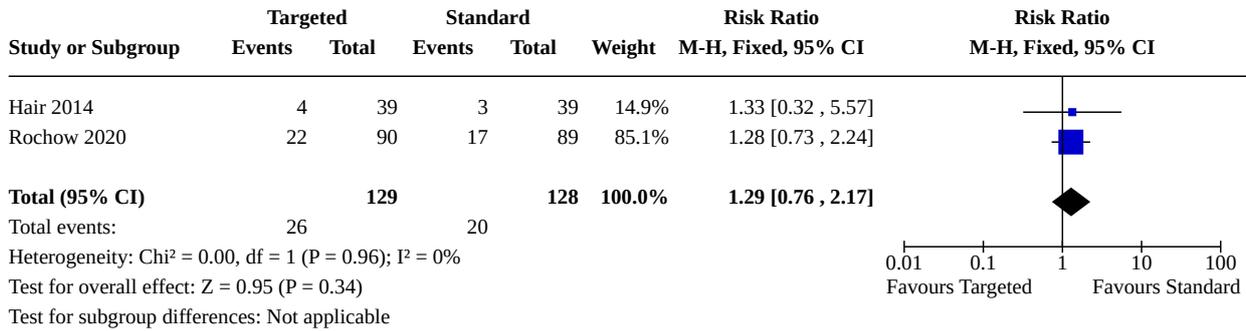
**Analysis 2.23. Comparison 2: Targeted vs standard fortification, Outcome 23: In-hospital mortality**



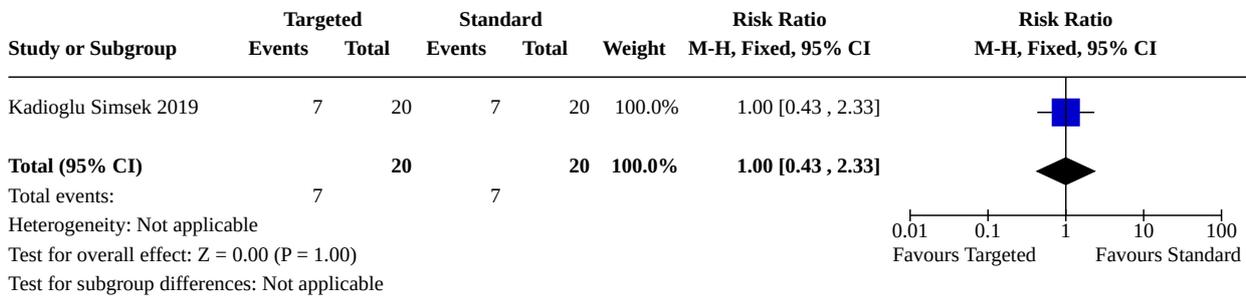
**Analysis 2.24. Comparison 2: Targeted vs standard fortification, Outcome 24: Necrotizing enterocolitis**



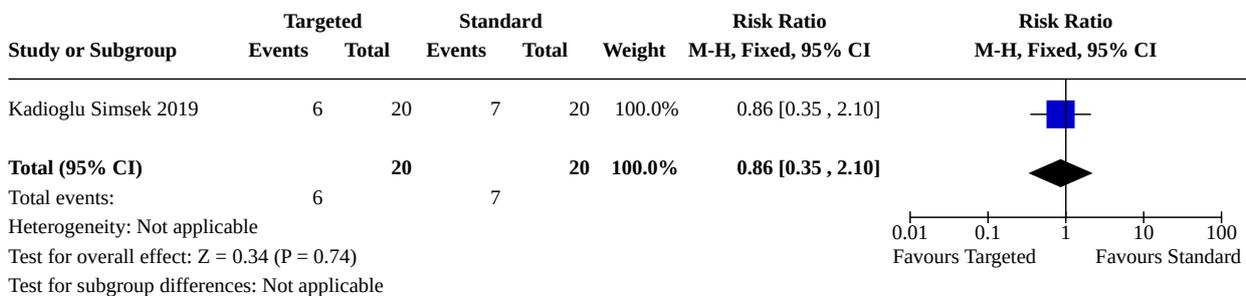
**Analysis 2.25. Comparison 2: Targeted vs standard fortification, Outcome 25: Culture-proven late-onset bacterial sepsis**



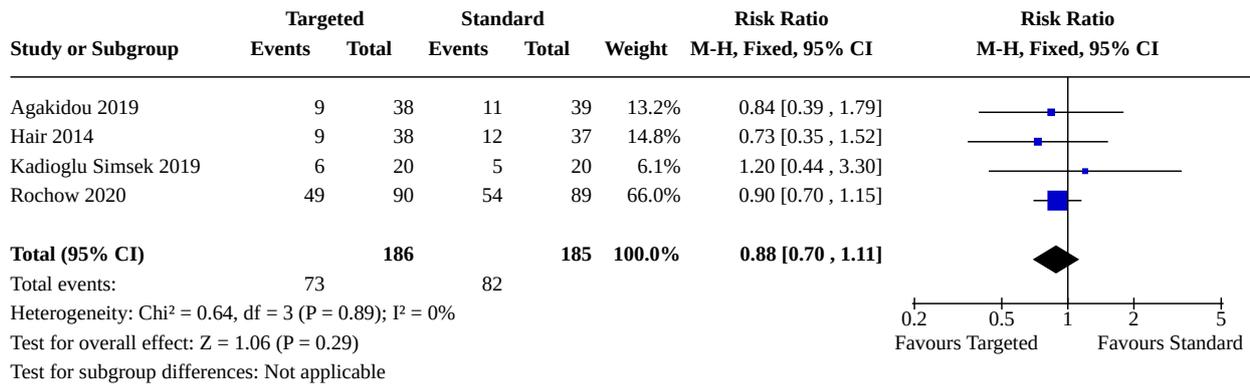
**Analysis 2.26. Comparison 2: Targeted vs standard fortification, Outcome 26: Retinopathy of prematurity, any**



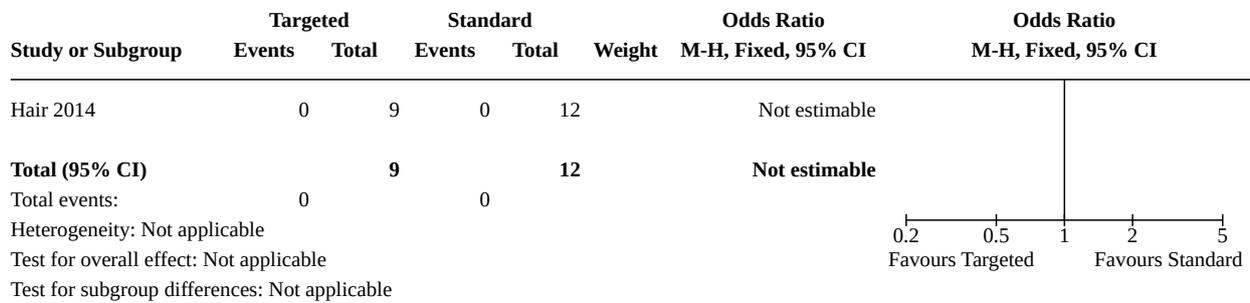
**Analysis 2.27. Comparison 2: Targeted vs standard fortification, Outcome 27: Osteopenia**



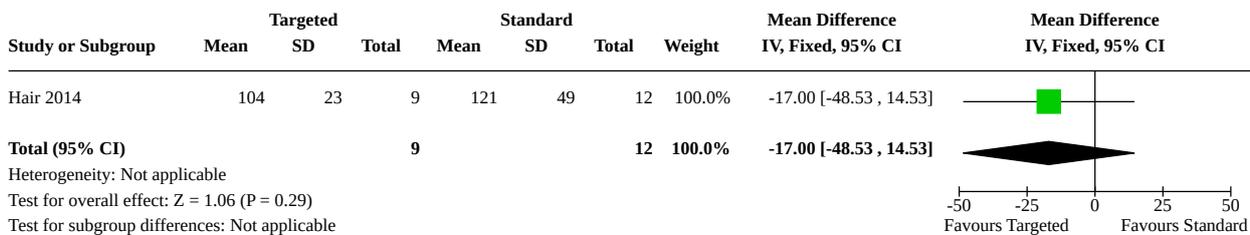
**Analysis 2.28. Comparison 2: Targeted vs standard fortification, Outcome 28: Bronchopulmonary dysplasia**



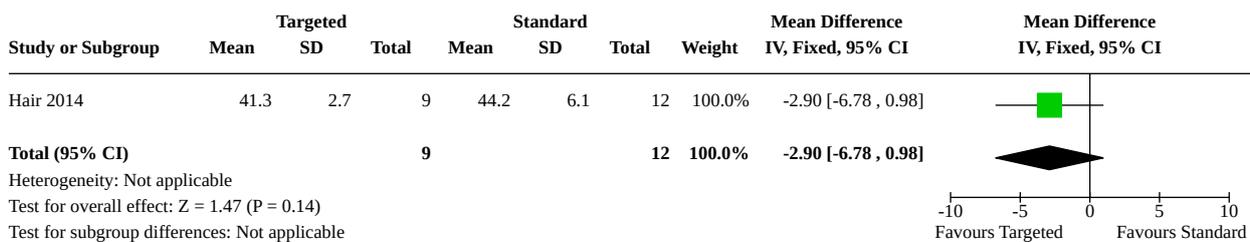
**Analysis 2.29. Comparison 2: Targeted vs standard fortification, Outcome 29: BPD subgroup - in-hospital mortality**



**Analysis 2.30. Comparison 2: Targeted vs standard fortification, Outcome 30: BPD subgroup - length of hospital stay, days**



**Analysis 2.31. Comparison 2: Targeted vs standard fortification, Outcome 31: BPD subgroup - postmenstrual age at discharge, weeks**



**Comparison 3. Adjustable vs standard fortification**

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.1 Growth velocity, weight, g/kg/d, end of intervention	3	96	Mean Difference (IV, Fixed, 95% CI)	2.86 [1.69, 4.03]
3.2 Growth velocity, length, mm/d, end of intervention	3	96	Mean Difference (IV, Fixed, 95% CI)	0.54 [0.38, 0.70]
3.3 Growth velocity, head circumference, mm/d, end of intervention	3	96	Mean Difference (IV, Fixed, 95% CI)	0.36 [0.21, 0.50]
3.4 Growth velocity, weight, g/d, end of intervention	2	56	Mean Difference (IV, Fixed, 95% CI)	3.26 [1.17, 5.34]
3.5 Retinopathy of prematurity, any	1	40	Risk Ratio (M-H, Fixed, 95% CI)	0.57 [0.20, 1.65]
3.6 Osteopenia	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.39, 2.58]
3.7 Bronchopulmonary dysplasia	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.20 [0.44, 3.30]

**Analysis 3.1. Comparison 3: Adjustable vs standard fortification, Outcome 1: Growth velocity, weight, g/kg/d, end of intervention**

Study or Subgroup	Adjustable			Standard			Weight	Mean Difference IV, Fixed, 95% CI	Mean Difference IV, Fixed, 95% CI
	Mean	SD	Total	Mean	SD	Total			
Arslanoglu 2006	17.5	3.2	16	14.4	2.7	16	32.5%	3.10 [1.05, 5.15]	
Kadioglu Simsek 2019	24	3.19	20	12.72	6.38	20	14.0%	11.28 [8.15, 14.41]	
Moro 1995	18.8	2	12	18.3	2	12	53.5%	0.50 [-1.10, 2.10]	
<b>Total (95% CI)</b>			<b>48</b>			<b>48</b>	<b>100.0%</b>	<b>2.86 [1.69, 4.03]</b>	

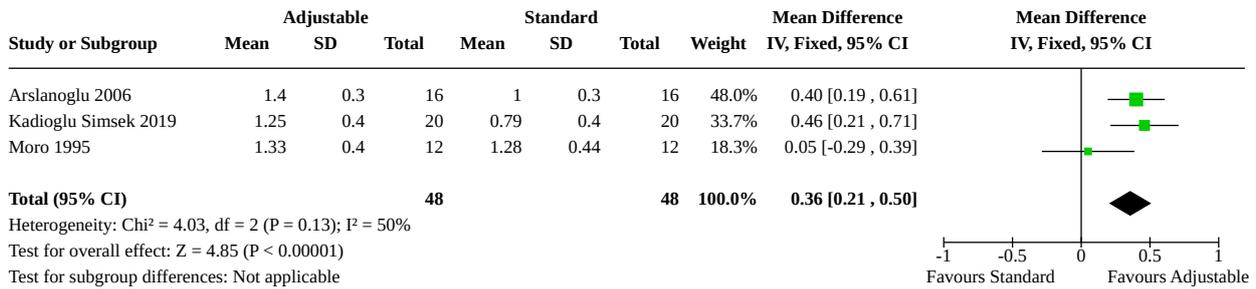
Heterogeneity: Chi<sup>2</sup> = 36.27, df = 2 (P < 0.00001); I<sup>2</sup> = 94%  
 Test for overall effect: Z = 4.78 (P < 0.00001)  
 Test for subgroup differences: Not applicable

**Analysis 3.2. Comparison 3: Adjustable vs standard fortification, Outcome 2: Growth velocity, length, mm/d, end of intervention**

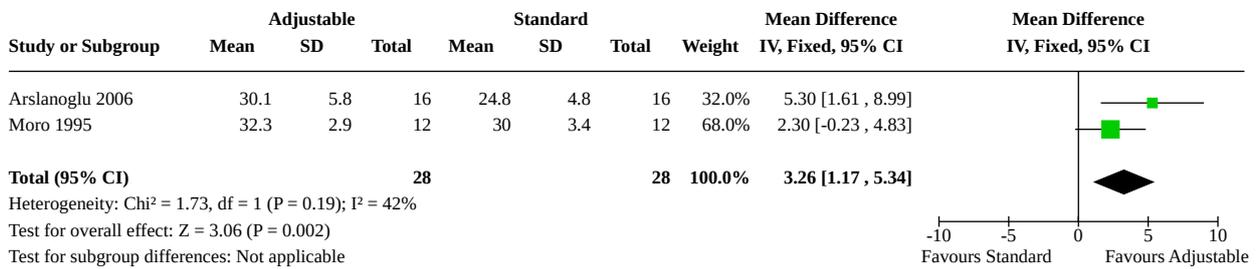
Study or Subgroup	Adjustable			Standard			Weight	Mean Difference IV, Fixed, 95% CI	Mean Difference IV, Fixed, 95% CI
	Mean	SD	Total	Mean	SD	Total			
Arslanoglu 2006	1.3	0.5	16	1.1	0.4	16	27.1%	0.20 [-0.11, 0.51]	
Kadioglu Simsek 2019	1.82	0.4	20	0.95	0.28	20	58.3%	0.87 [0.66, 1.08]	
Moro 1995	1.27	0.45	12	1.42	0.61	12	14.5%	-0.15 [-0.58, 0.28]	
<b>Total (95% CI)</b>			<b>48</b>			<b>48</b>	<b>100.0%</b>	<b>0.54 [0.38, 0.70]</b>	

Heterogeneity: Chi<sup>2</sup> = 23.59, df = 2 (P < 0.00001); I<sup>2</sup> = 92%  
 Test for overall effect: Z = 6.48 (P < 0.00001)  
 Test for subgroup differences: Not applicable

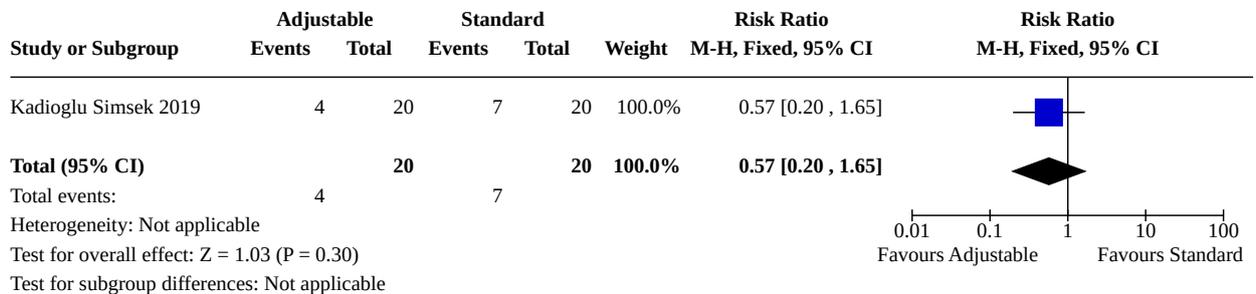
**Analysis 3.3. Comparison 3: Adjustable vs standard fortification, Outcome 3: Growth velocity, head circumference, mm/d, end of intervention**



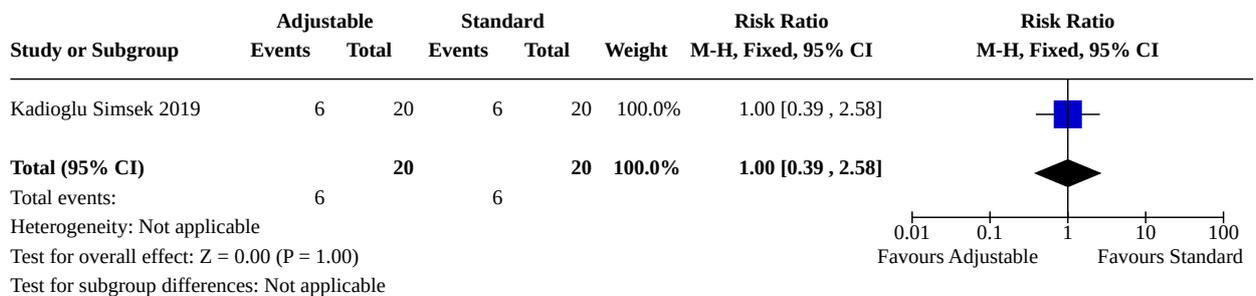
**Analysis 3.4. Comparison 3: Adjustable vs standard fortification, Outcome 4: Growth velocity, weight, g/d, end of intervention**



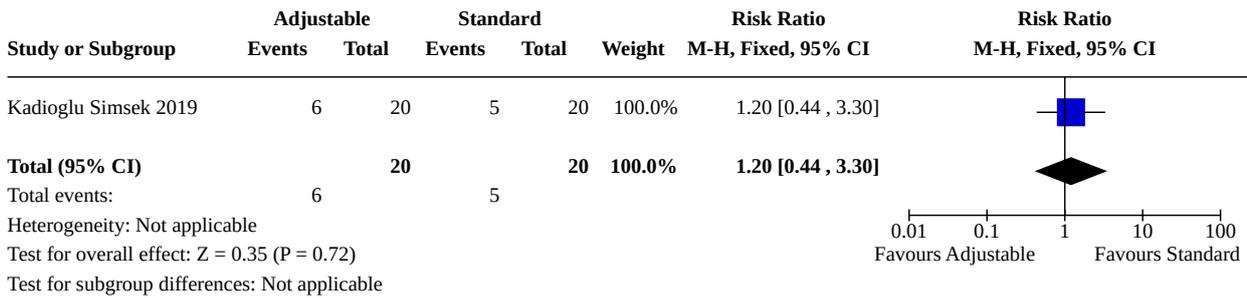
**Analysis 3.5. Comparison 3: Adjustable vs standard fortification, Outcome 5: Retinopathy of prematurity, any**



**Analysis 3.6. Comparison 3: Adjustable vs standard fortification, Outcome 6: Osteopenia**



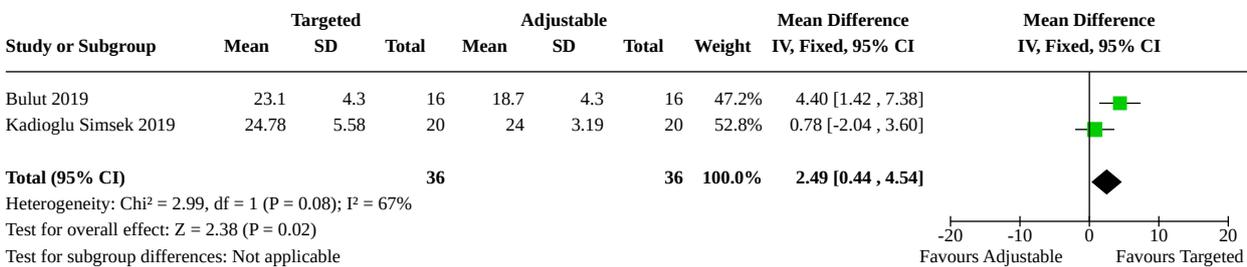
**Analysis 3.7. Comparison 3: Adjustable vs standard fortification, Outcome 7: Bronchopulmonary dysplasia**



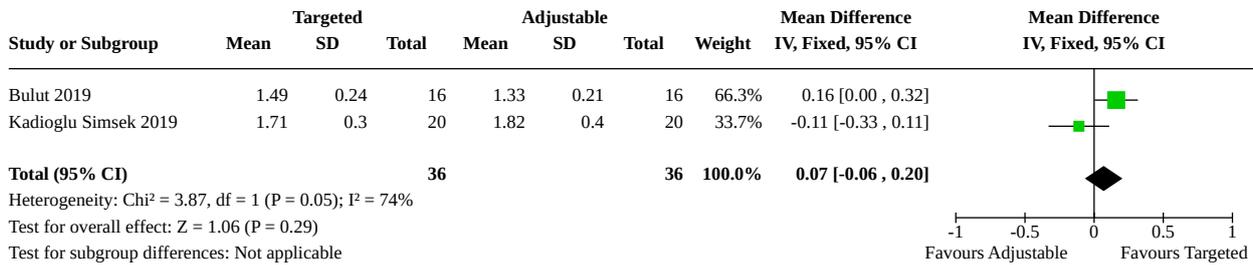
**Comparison 4. Targeted vs adjustable fortification**

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Growth velocity, weight, g/kg/d, end of intervention	2	72	Mean Difference (IV, Fixed, 95% CI)	2.49 [0.44, 4.54]
4.2 Growth velocity, length, mm/d, end of intervention	2	72	Mean Difference (IV, Fixed, 95% CI)	0.07 [-0.06, 0.20]
4.3 Growth velocity, head circumference, mm/d, end of intervention	2	72	Mean Difference (IV, Fixed, 95% CI)	0.04 [-0.10, 0.17]
4.4 Retinopathy of prematurity, any	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.75 [0.61, 5.05]
4.5 Osteopenia	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.39, 2.58]
4.6 Bronchopulmonary dysplasia	1	40	Risk Ratio (M-H, Fixed, 95% CI)	1.00 [0.39, 2.58]

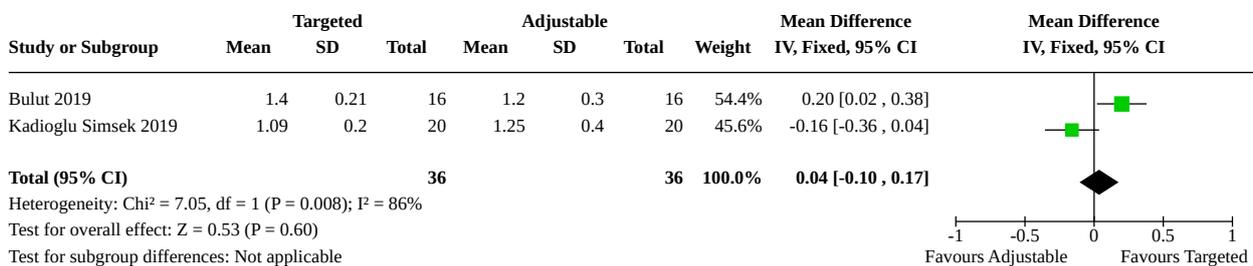
**Analysis 4.1. Comparison 4: Targeted vs adjustable fortification, Outcome 1: Growth velocity, weight, g/kg/d, end of intervention**



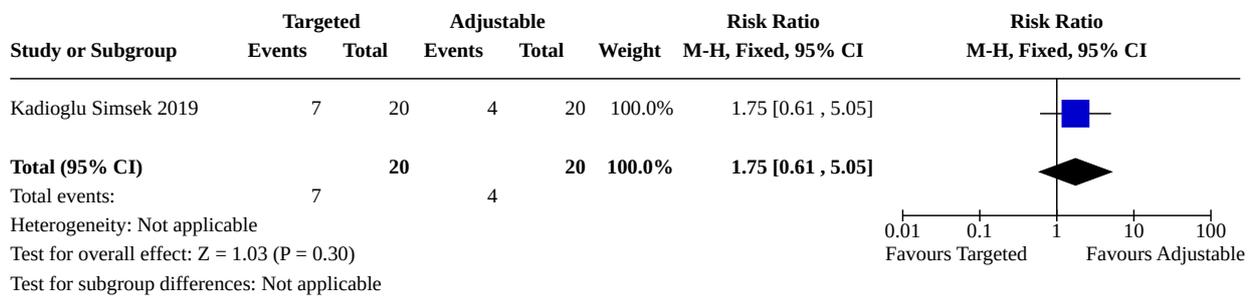
**Analysis 4.2. Comparison 4: Targeted vs adjustable fortification, Outcome 2: Growth velocity, length, mm/d, end of intervention**



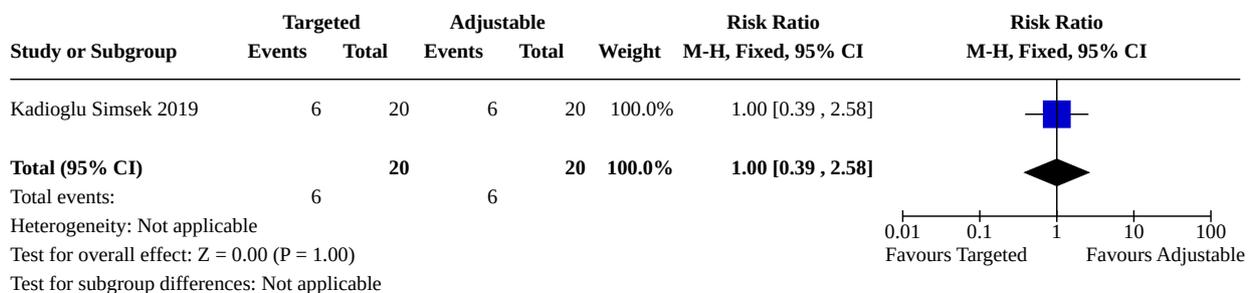
**Analysis 4.3. Comparison 4: Targeted vs adjustable fortification, Outcome 3: Growth velocity, head circumference, mm/d, end of intervention**



**Analysis 4.4. Comparison 4: Targeted vs adjustable fortification, Outcome 4: Retinopathy of prematurity, any**



**Analysis 4.5. Comparison 4: Targeted vs adjustable fortification, Outcome 5: Osteopenia**



**Analysis 4.6. Comparison 4: Targeted vs adjustable fortification, Outcome 6: Bronchopulmonary dysplasia**

Study or Subgroup	Targeted		Adjustable		Weight	Risk Ratio M-H, Fixed, 95% CI	Risk Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total			
Kadioglu Simsek 2019	6	20	6	20	100.0%	1.00 [0.39, 2.58]	
<b>Total (95% CI)</b>		<b>20</b>		<b>20</b>	<b>100.0%</b>	<b>1.00 [0.39, 2.58]</b>	
Total events:	6		6				
Heterogeneity: Not applicable							
Test for overall effect: Z = 0.00 (P = 1.00)							
Test for subgroup differences: Not applicable							

**APPENDICES**

**Appendix 1. Search strategies**

The RCT filters have been created using Cochrane's highly sensitive search strategies for identifying randomised trials (Higgins 2011b). The neonatal filters were created and tested by the Cochrane Neonatal Information Specialist.

**CENTRAL via CRS Web**

1MESH DESCRIPTOR Milk, Human EXPLODE ALL AND CENTRAL:TARGET

2MESH DESCRIPTOR Food, Fortified EXPLODE ALL AND CENTRAL:TARGET

3MESH DESCRIPTOR Dietary Supplements EXPLODE ALL AND CENTRAL:TARGET

4#3 OR #2 AND CENTRAL:TARGET

5#1 AND #4 AND CENTRAL:TARGET

6(fortif\* OR supplement\* OR enrich\*) ADJ4 (human OR breast OR expressed OR mother\* OR maternal OR donor\*) ADJ2 milk\* AND CENTRAL:TARGET

7(fortif\* OR supplement\* OR enrich\*) ADJ4 (DHM OR HM OR breastmilk\*) AND CENTRAL:TARGET

8#5 OR #6 OR #7 AND CENTRAL:TARGET

9MESH DESCRIPTOR Infant, Newborn EXPLODE ALL AND CENTRAL:TARGET

10infant or infants or infant's or "infant s" or infantile or infancy or newborn\* or "new born" or "new borns" or "newly born" or neonat\* or baby\* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW or ELBW or NICU AND CENTRAL:TARGET

11#10 OR #9

12#11 AND #8

**MEDLINE via Ovid**

1. exp Milk, Human/

2. exp Food, Fortified/

3. exp Dietary Supplements/

4. 2 or 3

5. 1 and 4

6. (fortif\* adj4 ((human or breast or expressed) adj2 milk\*)).mp.

7. (fortif\* adj4 ((mother\* or maternal or donor\*) adj2 milk\*)).mp.

8. (supplement\* adj4 ((human or breast or expressed) adj2 milk\*)).mp.
9. (supplement\* adj4 ((mother\* or maternal or donor\*) adj2 milk\*)).mp.
10. (enrich\* adj4 ((human or breast or expressed) adj2 milk\*)).mp.
11. (enrich\* adj4 ((mother\* or maternal or donor\*) adj2 milk\*)).mp.
12. ((fortif\* or supplement\* or enrich\*) adj4 DHM).mp.
13. ((fortif\* or supplement\* or enrich\*) adj4 HM).mp.
14. ((fortif\* or supplement\* or enrich\*) adj4 breastmilk\*).mp.
15. 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16. 5 or 15
17. exp infant, newborn/
18. (newborn\* or new born or new borns or newly born or baby\* or babies or premature or prematurity or preterm or pre term or low birth weight or low birthweight or VLBW or LBW or infant or infants or "infant s" or infant's or infantile or infancy or neonat\*).ti,ab.
19. 17 or 18
20. randomized controlled trial.pt.
21. controlled clinical trial.pt.
22. randomized.ab.
23. placebo.ab.
24. drug therapy.fs.
25. randomly.ab.
26. trial.ab.
27. groups.ab.
28. or/20-27
29. exp animals/ not humans.sh.
30. 28 not 29
31. 19 and 30
32. 16 and 31

#### MEDLINE via PubMed

Terms: (((("Milk, Human"[Mesh] AND ("Food, Fortified"[Mesh] OR "Dietary Supplements"[Mesh]))) OR ((fortif\*[TW] OR supplement\*[TW] OR enrich\*[TW]) AND (human[TW] OR breast[TW] OR expressed[TW] OR mother\*[TW] OR maternal[TW] OR donor\*[TW]) AND milk\*[TW])) OR ((fortif\*[TW] OR supplement\*[TW] OR enrich\*[TW]) AND (DHM[TW] OR HM[TW] OR breastmilk\*[TW]))) AND (((infant, newborn[MeSH] OR newborn\*[TIAB] OR "new born"[TIAB] OR "new borns"[TIAB] OR "newly born"[TIAB] OR baby\*[TIAB] OR babies[TIAB] OR premature[TIAB] OR prematurity[TIAB] OR preterm[TIAB] OR "pre term"[TIAB] OR "low birth weight"[TIAB] OR "low birthweight"[TIAB] OR VLBW[TIAB] OR LBW[TIAB] OR infant[TIAB] OR infants[TIAB] OR infant's[TIAB] OR "infant s"[TIAB] OR infantile[TIAB] OR infancy[TIAB] OR neonat\*[TIAB]) AND (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab]) NOT (animals[mh] NOT humans[mh]))) Filters: Publication date from 2018/09/01

#### CINAHL via EBSCOhost

S1MH milk, human

S2MH Food, Fortified

S3MH Dietary Supplementation

S4S2 OR S3

S5S1 AND S4

S6(fortif\* OR supplement\* OR enrich\*) AND (human OR breast OR expressed OR mother\* OR maternal OR donor\*) AND milk\*

S7(fortif\* OR supplement\* OR enrich\*) AND (DHM OR HM OR breastmilk\*)

S8S5 OR S6 OR S7

S9 ((infant or infants or infant's or infantile or infancy or newborn\* or "new born" or "new borns" or "newly born" or neonat\* or baby\* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW)) AND ((randomized controlled trial OR controlled clinical trial OR randomized OR randomised OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial))

S10S8 AND S9

## ISRCTN

milk AND Interventions: fortification AND Participant age range: Neonate

milk AND Interventions: supplementation AND Participant age range: Neonate

## Appendix 2. 'Risk of bias' tool

### Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we categorized the method used to generate the allocation sequence as:

1. low risk (any truly random process, e.g. random number table; computer random number generator);
2. high risk (any non-random process, e.g. odd or even date of birth; hospital or clinic record number); or
3. unclear risk.

### Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we categorized the method used to conceal the allocation sequence as:

1. low risk (e.g. telephone or central randomization; consecutively numbered, sealed, opaque envelopes);
2. high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
3. unclear risk.

### Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we categorized the methods used to blind study participants and personnel from knowledge of which intervention a participant received. We assessed blinding separately for different outcomes or classes of outcomes. We categorized the methods as:

1. low risk, high risk, or unclear risk for participants; and
2. low risk, high risk, or unclear risk for personnel.

### Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we categorized the methods used to blind outcome assessment. We assessed blinding separately for different outcomes or classes of outcomes. We categorized the methods as:

1. low risk for outcome assessors;
2. high risk for outcome assessors; or
3. unclear risk for outcome assessors.

### Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomized participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorized the methods as:

1. low risk (< 20% missing data);
2. high risk ( $\geq$  20% missing data); or
3. unclear risk.

**Selective reporting bias. Are reports of the study free of the suggestion of selective outcome reporting?**

For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found. We assessed the methods as:

1. low risk (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
2. high risk (where not all of the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified outcomes of interest and are reported incompletely and so cannot be used; the study fails to include results of a key outcome that would have been expected to have been reported); or
3. unclear risk.

**Other sources of bias. Was the study apparently free of other problems that could put it at high risk of bias?**

For each included study, we described any important concerns we had about other possible sources of bias (e.g. whether there was a potential source of bias related to the specific study design, whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:

1. low risk;
2. high risk; or
3. unclear risk.

If needed, we planned to explore the impact of the level of bias by undertaking sensitivity analyses.

**HISTORY**

Protocol first published: Issue 11, 2019

Review first published: Issue 11, 2020

**CONTRIBUTIONS OF AUTHORS**

Drs Fabrizio, Trzaski, and Hagadorn drafted this review, and all review authors reviewed and revised the final draft and take responsibility for its contents.

**DECLARATIONS OF INTEREST**

VF on the Mothers' Milk Bank Northeast Advisory Board in a voluntary capacity. This is a non-profit community milk bank that provides donated, pasteurized human milk.

JMT has no interests to declare.

EAB has no interests to declare.

PE has no interests to declare.

SL has no interests to declare.

MML has no interests to declare.

JIH has no interests to declare.

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**SOURCES OF SUPPORT****Internal sources**

- No sources of support supplied

**External sources**

- Vermont Oxford Network, USA

Cochrane Neonatal Reviews are produced with support from Vermont Oxford Network, a worldwide collaboration of health professionals dedicated to providing evidence-based care of the highest quality for newborn infants and their families.

- The Gerber Foundation, USA

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## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the following changes to the published protocol ([Fabrizio 2019](#)).

1. Inclusion criteria for birth weight were broadened to include all preterm infants at < 37 weeks' gestation or < 2500 grams birth weight.
2. We updated the "Risk of bias" tool.
3. Outcomes included in the protocol, but not included in the review, were Ponderal Index, incidence of growth at < 10th percentile for corrected age, time to regain birth weight, time to establishment of full enteral feedings, duration of parenteral nutrition, feeding intolerance, and neurodevelopmental outcomes. These were not included because they were not addressed in the included studies.
4. The primary outcome in the protocol was in-hospital growth; however due to available data in the included studies, the primary outcome in the review is growth velocity at end of study intervention.
5. We included "any retinopathy of prematurity," which was listed in our protocol, but not the additionally defined treated ROP, due to available data in the included studies.
6. We included "any BPD" as opposed to the more rigorous definition of BPD as provided in our protocol due to available data in the included studies.
7. Osteopenia was included in the review but had not been included in the protocol because it was one of the outcomes provided in one of the included studies.
8. "Sepsis" or "late-onset sepsis" in the protocol became "culture-proven sepsis" in the review.
9. "Length of hospitalization" in the protocol was called "length of hospital stay" in the review.

## INDEX TERMS

### Medical Subject Headings (MeSH)

Bias; Blood Urea Nitrogen; Body Height; Bone Diseases, Metabolic [epidemiology]; Child Development [\*physiology]; Confidence Intervals; Enteral Nutrition; Enterocolitis, Necrotizing [epidemiology]; \*Food, Fortified; Head [anatomy & histology] [growth & development]; \*Infant Formula; Infant Nutritional Physiological Phenomena; Infant, Premature [\*growth & development]; Infant, Very Low Birth Weight [\*growth & development]; \*Milk, Human; Randomized Controlled Trials as Topic; Retinopathy of Prematurity [epidemiology]; Weight Gain

### MeSH check words

Humans; Infant, Newborn