NATIONAL ACADEMIES Sciences Engineering Medicine

Protein Quality and Growth Monitoring Studies to Satisfy Quality Factor Requirements for Infant Formula

Evidence Scan Search Protocol

July 15, 2024

Background:

Infant formula is often used as the sole source of nutrition for a vulnerable population during a critical period of growth and development. In general, the laws and regulations that apply to food also apply to infant formula, but additional requirements specific to infant formula appear in section 412 of the Food Drug &Cosmetic Act and in the US Food and Drug Administration regulations 21 CFR parts 106 and 107.

In this consensus study, a committee convened by the National Academies of Sciences, Engineering, and Medicine is charged to examine and report on the state of the science regarding methodologies for assessing biological quality of protein in an infant formula and the state of the science regarding methodologies for assessing the ability of an infant formula to support normal physical growth. As part of its task, the committee will conduct evidence scans based on the following protocol.

Research Questions:

1) In animal or in-vitro studies, what is the state of the science regarding methodologies for assessing the biological quality of protein in infant formulas with altered quality and/or quantity compared to standard protein composition in infant formula or human milk and the extent to which the methods have been validated?

2) In healthy term infants to one year of age, what study designs and methods, including benefits and limitations, are used to examine infant formula quality factors, including physical growth and body composition.

Population:

Question 1: Animal models or *in vitro* models.
Question 2: Infants (birth to 12 months), full-term and healthy.

Protocol Development:

A separate protocol was developed to answer each research question. The method or study design being used (including reporting on their strengths and weaknesses and whether they have been validated and examine whether methods vary globally) is incorporated into each protocol. Both protocols are limited to primary articles published in English, in peer-reviewed journals between January 2000 and June 2024. A cut-off year of 2000 was selected to ensure that articles using the Protein Efficiency Ratio (PER) method were captured.

For Research Question 1, the protocol methodology is limited to studies enrolling healthy animal models or *in-vitro* models, examining protein quality measures in animal-based, plant-based, or partially hydrolyzed infant formula compared to animals fed standard protein content or human milk amino acid pattern or infant amino acid requirements. Studies included are limited to validity or reliability studies, experiments with and without randomization to

treatment, in-vitro digestibility studies, and meta-analyses and systematic reviews conducted worldwide.

For Research Question 2, the protocol methodology is limited to studies enrolling full-term and healthy infants up to 12 months of age, examining physical growth, along with body composition and biomarkers of growth, in infants fed animal-based, plant-based, or partially hydrolyzed infant formula compared to infants fed standard infant formula or human milk. Studies included are limited to randomized and non-randomized control trials, cohort and case-control studies, and meta-analysis and systematic reviews conducted in high-income countries (based on the UN/WHO index).

PECOD Framework and Inclusion/Exclusion Criteria

The research questions served as an organizational tool for identifying search terms and prespecified inclusion and exclusion criteria to inform eligibility of the search results for subsequent data extraction and tabulation.

Analytic Framework – Protein Quality

Research Question 1 included the following sub-questions:

1. What study designs are used to assess protein quality?
2. What methods and measures are used to assess protein quality?
3. Do methods and measures vary globally?

These questions informed the analytic framework shown in Figure 1.

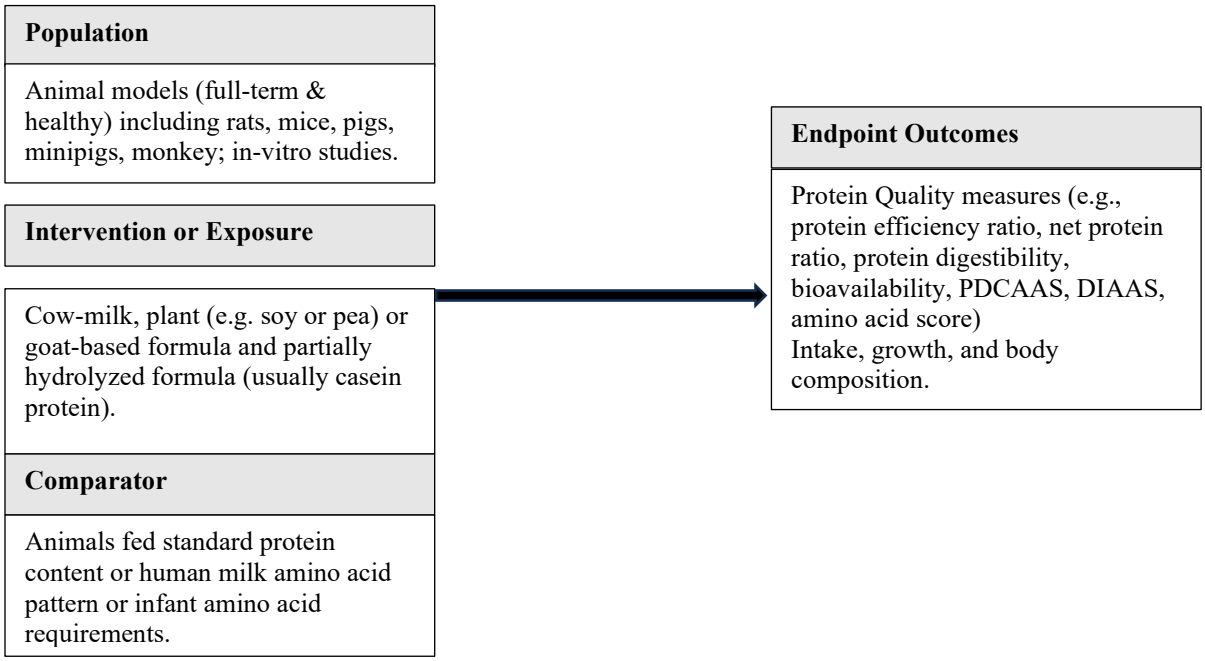

FIGURE 1 Analytic framework to examine evidence from animal studies.

Inclusion/Exclusion Criteria

A set of pre-specified criteria to support the screening of retrieved articles in the evidence scan are shown in Table 1.

TABLE 1 Inclusion and exclusion criteria for Research Question #1

Category	Inclusion Criteria	Exclusion Criteria
Publication status	Articles published in peer-reviewed journals	Articles that have not been peer reviewed and are not published in peer-reviewed journals, including unpublished data, manuscripts, pre-prints, reports, abstracts, and conference proceedings
Date of publication	January 2000 or after	Articles published prior to January 2000
Language of publication	Articles published in English	Articles published in languages other than English
Study design	Validity or reliability studies (compared to gold standard method) Experiments with randomization to treatments. Non-randomized experiments, including quasi-experimental and controlled before-and-after studies. In-vitro digestibility studies Meta-Analyses/ Systematic reviews	Editorials Abstracts/conference abstracts Study protocols Narrative reviews
Interventions/ exposures	Cow-milk, plant (e.g. soy or pea) or goat-based formula and partially hydrolyzed formula (usually casein protein)	All other specialized formulas (non-exempt formulas by FDA such as fully hydrolyzed, amino acid or formulas for metabolic diseases), or toddler formulas.
Comparators	Animals fed standard protein content or human milk amino acid pattern or infant amino acid requirements	Exempt formulas by FDA such as fully hydrolyzed, amino acid or formulas for metabolic diseases, toddler formulas.

Category	Inclusion Criteria	Exclusion Criteria
Outcomes	Protein quality measures (e.g., protein efficiency ratio, net protein ratio, protein digestibility, bioavailability, PDCAAS, DIAAS, amino acid score) Intake, growth, and body composition	Fecal microbiota, metabolomics, neural development, Caco-2 cells/CaCO2, fatty acid profile, folate, niacin, minerals, zinc, prebiotics, flavonoids, oligosaccharides, phospholipids
Study participants	Animal models In-vitro studies	
Health status of study participants	Studies that enroll healthy animal models or in-vitro models	Studies that exclusively enroll: - Animals born prematurely - Metabolic conditions or disease
Country	Studies conducted in any country	

Keywords:
Animal model keywords: animal, animal model, mouse, rat, rat model, mice, guinea pig, swine, pig, monkey

Infant formula, infant nutrition, infant food, artificial milk

Protein content; dairy protein; plant protein; milk protein; hydrolyzed milk proteins; protein determination, protein quality, protein analysis, dietary protein; in vitro digestion; proteolysis; ileal; endogenous; protein quality; amino acids; digestible indispensable amino acid scores (DIAAS); protein digestibility; digestible indispensable amino acid scores (DIAAS); protein digestibility-corrected amino acid scores (PDCAAS); protein energy ratio (PER); protein bioavailability; apparent protein digestibility; true ileal protein digestibility (tipd); standardized ileal digestibility (sid); true digestibility; amino acid bioavailability; amino acid score; essential amino acid quantification; standardized total tract digestibility (STTD); endogenous nitrogen flow (ENFL); endogenous amino acid flow (EAAFL); gastric protein coagulation; N conversion factor.

Analytic Framework – Infant Formula Quality Factors

Research Question 2 included the following sub-questions:

1. What are the strengths and weaknesses of study designs and methods used to measure quality of infant formula in human infants?
2. Have the various study designs been validated?
3. Do methods and measures to assess 'quality of infant formula' vary globally?

These questions informed the analytic framework shown in Figure 2.

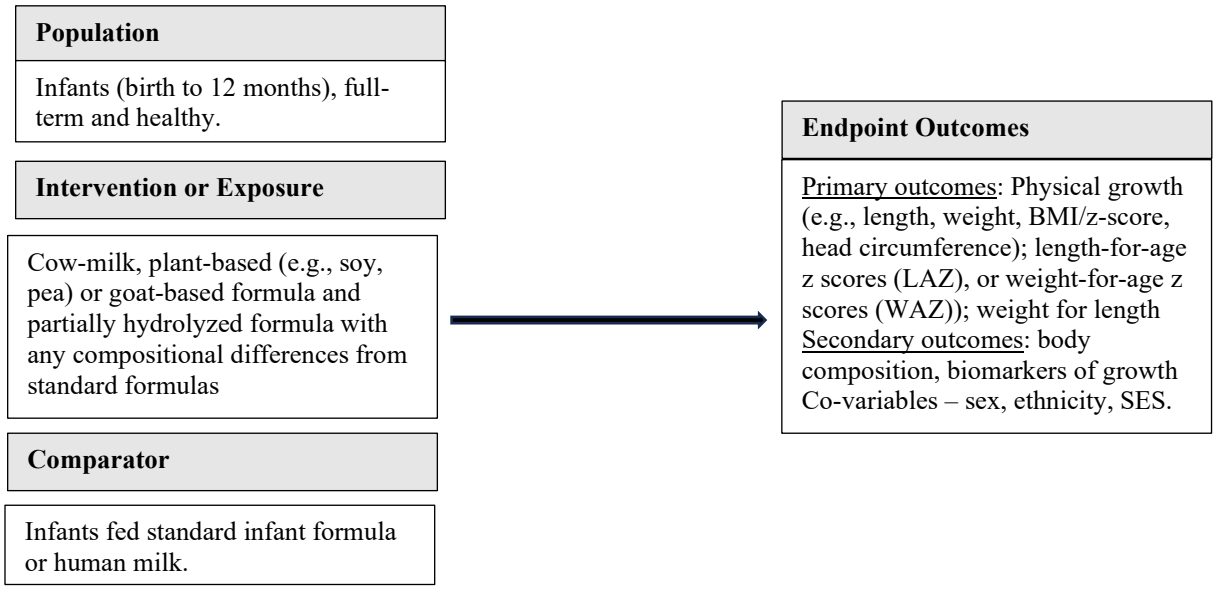

FIGURE 2 Analytic framework to examine quality factors of infant formulas.

Inclusion/Exclusion Criteria

A set of prespecified criteria, developed to support the screening of retrieved articles in the evidence scan, are shown in Table 2.

Table 2 – Inclusion and Exclusion Criteria for Research Question #2

Category	Inclusion Criteria	Exclusion Criteria
Publication status	Articles published in peer-reviewed journals	Articles that have not been peer reviewed and are not published in peer-reviewed journals, including unpublished data, manuscripts, pre-prints, reports, abstracts, and conference proceedings
Date of publication	January 2000 or after	Articles published prior to January 2000
Language of publication	Articles published in English	Articles published in languages other than English
Study design	Randomized controlled trials. Non-randomized controlled trials, including quasi-experimental and controlled before-and-after studies, observational. Prospective cohort studies Retrospective cohort studies Meta-Analyses/ Systematic reviews Case-control studies	Editorials Narrative reviews Abstracts/conference abstracts Study protocols.
Interventions/ exposures	Cow-milk, plant-based (e.g., soy, pea) or goat-based formula and partially hydrolyzed formula with any compositional differences from standard formulas	All other specialized formulas (non-exempt formulas by FDA such as fully hydrolyzed, amino acid or formulas for metabolic diseases), or toddler formulas.
Comparators	Infants fed standard infant formula or human milk	Same as for Interventions/Exposures

Category	Inclusion Criteria	Exclusion Criteria
Outcomes	Primary outcomes: Physical growth (e.g., length, weight, BMI/z-score, head circumference); length-for-age z scores (LAZ), or weight-for-age z scores (WAZ)); weight for length Secondary outcomes: body composition, biomarkers of growth Co-variables – sex, ethnicity, SES	Fecal microbiota, metabolomics, neurodevelopment, other general health outcomes
Study participants	Full-term infants up to 12 months of age	
Health Status of study participants	Studies that enroll full-term and healthy infants	Studies that exclusively enroll: - pre-term or premature infants - infants with high birth weight or low birth weight - infants diagnosed with a disease or condition
Country	Studies conducted in high-Income countries (e.g. UN/WHO index)	

Keywords:
Infant keywords: infant, newborn, neonate

Infant formula keywords: infant formula, infant nutrition, infant food, artificial milk

Infant growth key words: anthropometry, infant growth, growth, growth and development, growth charts, body length, weight gain, weight for length, weight growth rate, weight velocity standards, growth rate, growth performance, physiology, z-score, weight gain / physiology, body length / physiology, child development / physiology, infant nutrition physiology, body mass index, body composition, fat mass, lean mass, lean body mass, head circumference; length-for-age z scores (LAZ), or weight-for-age z scores (WAZ); head circumference for age z-score, body composition as lean or fat mass by DXA, skinfolds, air-displacement plethysmography.