REVIEW

Calcium Intake and Metabolism in Infants and Young Children: A Systematic Review of Balance Studies for Supporting the Development of Calcium Requirements

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ABSTRACT

Determining calcium requirements for infants and children is vital due to high calcium needs for growth. Balance studies enable comprehensive measurement of calcium metabolism and can support nutrient requirement development. This systematic review summarizes evidence from mass balance and isotopic studies in children aged 0–4 y to address key questions on calcium loss and absorption/retention identified by an expert group developing calcium requirements. Literature searches were implemented in multiple electronic databases to June 2020. Balance studies assessing calcium intake, loss, absorption, or retention in healthy children were eligible. A newly developed risk-of-bias assessment tool was used for balance studies, and a modified Grades of Recommendation, Assessment, Development, and Evaluation approach determined strength of evidence. Altogether, 23 studies (15 mass balance; 8 isotope) with 485 total participants were included. Only 3 studies were of children >6 mo. Mass balance studies suggested infant feed components may influence calcium balance. The random-effects model meta-regression on 42 mass balance study arms showed an average net calcium retention of 40.4% among infants aged 0–6 mo ($\beta = 0.404$ [95% CI: 0.302, 0.506]). Isotope studies suggested calcium intake of 240 to 400 mg/d may promote optimal calcium absorption with minimal loss, and intake from human milk may lead to greater absorption and retention efficacy than formula or solid foods. Most studies had low risk of bias. Strength of evidence was low due to variability in infant feedings, limited endogenous and dermal calcium loss measures, and few studies isolating calcium effects. To improve certainty of the body of evidence, more balance studies isolating effects of calcium intake in this age group are needed. Future work on calcium needs should incorporate both balance measures and biological endpoints of importance (e.g. bone mineral density or content) to determine adequate calcium intake for growth in infants and children. *Adv Nutr* 2022;13:1529–1553.

Statement of Significance: A systematic review on calcium balance studies was commissioned by the WHO/FAO to support an international expert group tasked with updating calcium requirements for infants and children aged 0–4 y. This review provides a comprehensive evidence base for setting calcium requirements, using the factorial approach, in this population and highlights the future work needed in pediatric calcium balance design.

Keywords: calcium, mass balance, infant, preschool children, nutritional requirements, systematic review

Introduction

Calcium (Ca) is an essential nutrient that serves a critical role in bone structure, particularly in stages of growth, such as infancy and childhood. Inadequate calcium intake during childhood may increase the risk of fractures and rickets and prevent the achievement of maximal peak bone mass later in life (1, 2). Despite the risks associated with low calcium intake, there is currently limited knowledge on calcium needs to meet physiological requirements in infants and young children. Measuring bone outcomes following calcium supplementation in dose-response randomized controlled trials (RCTs) is one approach to assess calcium requirements in this population. However, long study durations are necessary to observe sufficient changes in bone outcomes (1, 2), making RCTs somewhat infeasible, as the maintenance of costs and careful dietary control is difficult over numerous

years. Moreover, RCTs may fail to account for other potential influences on bone outcomes, such as calcium loss and confounding dietary and lifestyle factors.

Balance studies may serve as an alternate approach to assess calcium metabolism and model skeletal change. These studies can be conducted over a shorter duration with adequate dietary control, and comprehensive measures of calcium metabolism can be determined. In balance studies, the amount of a mineral absorbed and retained by the body can be measured as a proportion of the amount consumed, after consideration of losses. Therefore, measuring calcium balance (e.g. absorption, retention, and losses) in response to various levels of intake can help determine needs for total body adequacy, while compensating for mineral loss. In theory, the level of calcium intake where calcium balance is optimized allows for maximal calcium retention. The retained calcium can, therefore, be used for bone mineralization in children (1).

For calcium, 2 formative balance designs exist: mass balance measurements and isotopic techniques. In mass balance studies, one can determine the amount of calcium absorbed and retained by calculating the difference between dietary calcium input and total urinary and fecal calcium output. However, mass balance studies cannot distinguish between endogenous calcium and nonabsorbed dietary calcium in fecal matter. Additionally, as with RCTs, long-term dietary control and complete urine and fecal collections are difficult to manage and obtain from a mass balance design (3). Alternatively, stable-isotope tracers can be used to provide greater control and accuracy in measuring calcium balance. For example, isotope studies allow for the differentiation of endogenous and dietary fecal calcium loss to determine fractional absorption. In single isotope studies, the administration of an oral isotope is followed by fecal collections to calculate the fraction of the tracer absorbed (4). In dual isotope studies, the relative fraction of an oral compared with an intravenous isotope tracer in a 24-h urine sample can be determined. This technique controls for variations in calcium distribution pool size and eliminates the need for multiple fecal collections over relatively long durations (4).

Given the advantages of balance studies in assessing calcium metabolism, a systematic review of balance studies was commissioned by the FAO and WHO expert group, charged with updating calcium requirements for infants and children aged 0–4 y (5). Balance studies were used to address the following key questions (KQs) formulated by the expert group as part of this task:

- Calcium losses: What are the routes for endogenous losses and amounts of calcium lost through these various routes in children aged 0–4 y? (For example, fecal, urinary, and dermal losses.)
- Calcium absorption and retention: What is the efficiency of absorption and retention of calcium (i.e. what percentage of calcium consumed is absorbed by the body) in children aged 0-4 y? (Considering the source of calcium, including human milk, vitamin D deficiency, effects of other nutrients consumed together with calcium, etc. where possible.)

Methods

This article is largely based on a full evidence report submitted to the WHO. We followed the methodology for conducting a systematic review outlined in the Institute of Medicine's Standards for Systematic Reviews (6) and reported the study results according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (7). The study protocol was preregistered on the International Prospective Register of Systematic Reviews, PROSPERO (https://www.crd.york.ac.uk/prospero/) as CRD42020198843.

Literature search and study selection process

Literature search strategies were developed according to the formulated KQs. These searches were implemented in MEDLINE® (1946 to Week 3 in June 2020), Embase (1966 to 23 June, 2020), and Cochrane Central (1991 to May 2020) databases. Searches were limited to human studies but with no language restrictions, and details are included in the PROSPERO protocol. Additional reference mining was performed in relevant authoritative reports and systematic reviews, and full-text articles from a preliminary scoping review (5) were rescreened for eligibility in this systematic review. After duplicate citations were removed, abstracts were screened by 2 independent investigators using the Rayyan software for systematic reviews (8). Full-text articles of screened-in abstracts were retrieved and screened by 1 investigator. All rejected articles were reviewed by a second investigator to confirm or refute their exclusion. Disagreements were adjudicated by a third investigator or by group consensus. Abstracts and full-text articles were assessed for study eligibility criteria and are presented in **Table 1**.

Data extraction

Standardized data extraction forms were created to extract study design and population characteristics from each included study. Extracted study design data included sample size; assignment to a run-in diet or assessment of participants' habitual diets; calcium content of each study arm for mass balance studies, and calcium dosage as oral isotope, i.v. isotope, or i.v. fecal isotope for isotopic studies; durations of calcium consumption, urine collections, and

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Supplemental Appendices A and B, Supplemental Detailed Narratives, and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.
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Abbreviations used: Ca, calcium; GRADE, Grades of Recommendation, Assessment, Development, and Evaluation; KQ, key question; PA, palmitic acid; PROSPERO, International Prospective Register of Systematic Reviews; RCT, randomized controlled trial; RoB, risk of bias.

TABLE 1 Study eligibility criteria for the systematic review of calcium intake and metabolism in infants and children aged 0–4 y

Category	Inclusion criteria	Exclusion criteria
Study design	Balance studies[1] Mechanistic studies[2]	In vitro (cell) and animal studies Unpublished studies (e.g. conference abstracts, posters)
Population	Generally healthy[3] children aged 0–4 y	Critically ill children admitted to neonatal intensive care unit Studies that enrolled exclusively premature infants (≤32 weeks of gestational age) or very low birth weight infants (≤1500 g) Studies conducted exclusively in children with moderate or severe acute malnutrition
Interventions or exposures	Dietary calcium intake (with or without vitamin D) from foods, supplements (e.g. infant formula) or isotopic calcium dosage	Non-oral intake of calcium such as injections or peripheral parenteral nutrition
Comparators	Any	None
Outcomes	Routes and amount of endogenous calcium losses (e.g. urinary, fecal, and dermal losses[4] where applicable) Calcium absorption and retention	Maternal health-related outcomes Any outcome measured only at birth in mothers or in infants

[1] Study with measure of dietary calcium intake plus measure of calcium accretion, retention, and/or loss.
[2] A study "designed to understand a biological or behavioral process, the pathophysiology of a disease, or the mechanism of action of an intervention. Not all mechanistic studies are clinical trials, but many are" (9).
[3] "Generally healthy" populations are defined as having ≤20% of the study population with disease at the study's baseline. Nutrition deficiencies, overweight, and obesity are not considered diseases in this systematic review.
[4] Recent reports from authoritative bodies have noted a lack of data for children regarding dermal losses and therefore it may be necessary to extrapolate from adult data.

fecal collections; methods used to assess calcium; and specific calcium outcomes measured in the study. Extracted population characteristics included age, sex, race/ethnicity, and health status. Results for mean calcium intake, urinary and fecal calcium loss, and concentrations of calcium absorption and retention were also extracted. To extract study results for all outcomes of interest, separate forms were created for mass balance and isotope studies. Data was extracted by 1 investigator and independently assessed by another investigator.

Risk-of-bias assessment

No risk-of-bias (RoB) assessment tool currently exists for studies with a balance design. We developed a RoB tool for calcium balance studies (see **Supplemental Appendix A**). Specified domains were created to assess potential biases of a balance design. Calcium balance studies were further categorized by isotopic or mass balance measurements, with domain questions corresponding to the methodological underpinnings associated with each design. These domains were based on the standardization of calcium, appropriation of compounds and dosages, physiologic quantification and duration of biological sample collection, and analytical techniques utilized. Two investigators independently performed the RoB assessment for each included study. Disagreements were resolved through discussions between the investigators.

Data synthesis and strength of evidence rating

Data were synthesized by each KQ, balance design, and balance outcome. Summary tables were created to present key study features and results to facilitate qualitative synthesis. The Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach (10, 11) was utilized to determine the strength of evidence for each outcome. We developed a modified GRADE approach to grade the strength of evidence for the calcium balance studies. **Supplemental Appendix B** presents details of this modified GRADE approach. GRADE evidence profile tables (12), with minor modifications, were used to present synthesized data for each KQ.

Meta-regression

No meta-analyses were performed due to large heterogeneity in exposure and outcome definitions or ascertainment methods across included studies. Random-effects model meta-regression analysis was performed to examine the relation between daily mean calcium intake and mean concentrations of calcium retention by prespecified age groups (0-90 d, 91-180 d). The unit of the meta-regression analysis is each intervention arm. Analysis and plotting were conducted in Stata 16 (StataCorp. 2019. Stata Statistical Software: Release 16. StataCorp LLC).

Results

Altogether, 23 calcium balance studies ($n = 15$ mass balance, and $n = 8$ isotope design) were included in this systematic review. The literature search and study selection process are summarized in **Figure 1**. A list of excluded full-text articles with exclusion reasons is available upon request. Below, the study characteristics and KQ results are reported separately for mass balance and isotope studies. Summary paragraphs

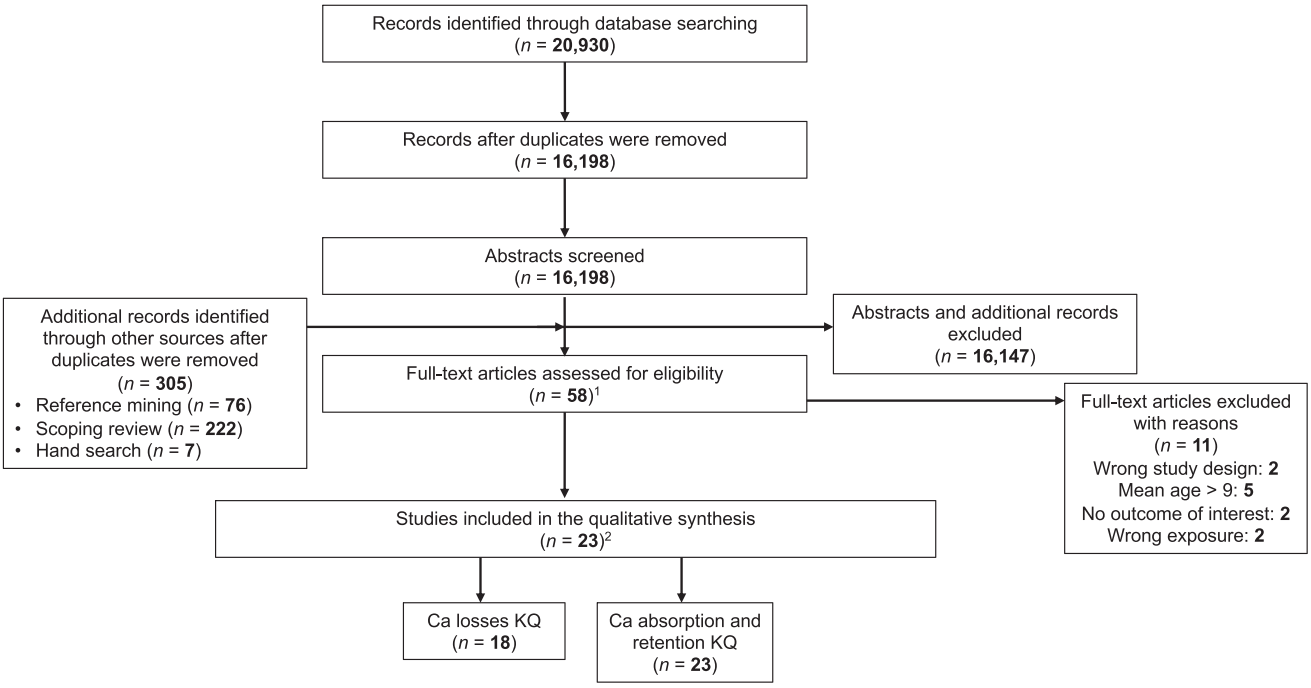

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart of the literature search and study selection process. [1]The abstract screening phase included both calcium and vitamin D articles, as the WHO/FAO commissioned both a calcium and vitamin D report to set requirements in children aged 0–4 y. Furthermore, the WHO/FAO expert panel developed additional calcium key questions, which included different study designs. This review only included calcium balance studies assessed in the calcium losses and absorption/retention KQs. [2]Included studies were often categorized into >1 key question. Studies included in each key question do not add up to the total number of studies included in the qualitative synthesis. Ca, calcium; KQ, key question.

describing the strength of evidence provide collective results from mass balance or isotope studies for each KQ. Detailed narratives of all balance studies addressing the calcium losses KQ and calcium absorption/retention KQ are found in **Supplemental Detailed Narratives**.

Study characteristics

Mass balance studies.

Fifteen studies in this review included mass balance measurements in the age group of interest. All 15 studies measured calcium intake, 10 measured urinary calcium loss, 14 measured fecal calcium loss, 14 measured absorption, and 12 measured retention. Eleven studies were in infants aged 0–90 d, but only 2 of these studies utilized interventions where the effects of calcium could be isolated (13, 14) (e.g. the only difference between arms is in the amount of dietary calcium). Five studies (15–19) were in infants aged 91–180 d. From these, only one was designed to isolate the effects of calcium (15). One study performed serial metabolic calcium balance measures across the first 6 mo of life (0–180 d) (19). No study reported calcium balance for ages >6 mo to <4 y.

Eleven studies included a run-in diet or otherwise standardized participants' calcium intake, and 3 studies measured habitual dietary intake prior to beginning the metabolic balance study. One study evaluated a single study arm diet (20). The remaining 14 studies compared multiple arms which varied in either calcium content of the total diet (14, 15, 19), calcium to phosphate ratios (13, 21), non-calcium nutrients such as blend of lipids (22, 23), both calcium content and lipids (16, 18, 24, 25), or presence of lactose (17, 26). Two studies compared infant formula to either transitional or mature human milk (24, 27). The duration of food consumption in these studies ranged from 3 to 180 d, and urinary and/or fecal collection periods ranged from 48 to 144 h. Atomic absorption spectrophotometry (AAS) was the commonly used method for measuring calcium content in food, urine, and/or feces. Study characteristics for all included mass balance studies are presented in **Table 2**.

Isotope studies.

Eight isotope studies (2 single isotope, 6 dual isotope studies) in the age group of interest were included in this review. Seven studies measured calcium intake, 4 reported losses in urine and feces, 8 measured absorption, and 3 measured retention. Four studies were conducted in infants aged 0–90 d, 1 study was in infants 91–180 d, 1 study was in infants 6–11 mo, and 2 studies were in children 12–36 mo. Six studies included a run-in diet or otherwise standardized participants' calcium intake prior to the start of the isotope balance study (13, 29–33). Three studies were either single arm studies, or only 1 study arm met inclusion criteria. Of the

TABLE 2 Characteristics of included mass balance studies reporting calcium outcomes in infants and children aged 0–4 y[1]

Author, year; country	Calcium outcomes	Age, mean ± SD, y (range, d)	Racial/ethnic background	Health status	Total enrolled *n*; % male	Run-in diet	Habitual diet assessment	Study arm: calcium content	Duration of food consumption, d	Duration of urine collections, h	Duration of fecal collections, h	Calcium assessment methods
Barnes et al., 1974 (22); USA	Intake, absorption, retention	0 ± 0 (NR)	NR	100% healthy	29; 100	Yes	Yes	Formula J (Ca/P 1.7): 0.53 mg/g Formula K (Ca/P 1.4): 0.70 mg/g Formula L (Ca/P 1.3): 0.65 mg/g	6	144	144	EDTA procedure
Barltrop et al., 1977 (13); United Kingdom	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (4–41)	NR	100% healthy	13; 92.3	Yes	Yes	Formula L (Ca/P 0.6); P supp.: NR Formula M (Ca/P 1.2); No supp.: NR Formula H (Ca/P 2.4); Ca supp.: NR	4–41	48	48	AAS
Carnielli et al., 1996 (23); Netherlands	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (NR)	NR	100% healthy	27; 100	Yes	NR	Beta (β) formula: 52.5 mg/100 mL Intermediate formula: 53 mg/100 mL Regular formula: 54 mg/100 mL	28	24	72	AAS
Clemente Yago et al., 1989[2] (20); Spain	Intake, urinary excretion, fecal excretion, absorption, retention	0.1 ± 0.1 (3–160)	NR	100% healthy	20; NR	NR	NR	Milk formula (Ca/P 1.6): 0.6 mg/mL (106.5 mg/[kg∗d])	3	72	72	AAS
DeVizia et al., 1985 (15); USA	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (22–237)	100% non-Hispanic white	100% healthy	6; 83.3	Yes	NR	Formula LCa (Ca/P 0.8): 0.39 mg/mL Formula MCa (Ca/P 1.4): 0.66 mg/mL Formula HCa (Ca/P 2.0): 1.02 mg/mL	3	72	72	AAS
Fomon et al., 1963 (19); USA	Intake, urinary excretion, fecal excretion, retention	0 ± 0 (8–182)	NR	100% healthy	28[3]; 64.3	Yes	NR	Human milk: 32.9 mg/100 mL Formula 22-3C: 41.9 mg/100 mL Formula 22-3D: 36.3 mg/100 mL Formula 22-3E: 45.8 mg/100 mL Formula S-26: 42.6 mg/100 mL Similac: 73.8 mg/100 mL	30–180[4]	72	72	(1912) McCrudden's method

(*Continued*)

TABLE 2 (*Continued*)

Author, year; country	Calcium outcomes	Age, mean ± SD, y (range, d)	Racial/ethnic background	Health status	Total enrolled *n*; % male	Run-in diet	Habitual diet assessment	Study arm: calcium content	Duration of food consumption, d	Duration of urine collections, h	Duration of fecal collections, h	Calcium assessment methods
Hanna et al., 1970 (24); USA	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (NR)	NR	100% healthy	38; 81.6	Yes	NR	Transitional breast milk (Ca/P 1.4): 0.26 mg/g Lyophilized mature human milk reconstitute (Ca/P 1.5): 0.21 mg/g Formula A (Ca/P 1.1): 0.47 mg/g Formula B (Ca/P 1.4): 0.42 mg/g	6	144	144	AAS
Manz et al., 1989[5] (14); Germany	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (13–54)	NR	Preterm infants	19; NR	NR	NR	Standard formula (Ca/P 1.4): 0.54 mg/mL Ca-L-lactate supp formula (Ca/P 2.0): 0.80 mg/mL	Mean (range) SF: 29 (13–54) CF: 15 (13–23)	8–12 (2x)	8–12 (2x)	AAS
Moya et al., 1982[5] (21); Spain	Intake, excretion (urine + fecal), absorption, retention	0 ± 0 (1–3)	NR	Low BW infants	26; NR	NR	NR	Formula A (Ca/P 2.4): 0.83 mg/mL Formula B (Ca/P 1.7): 0.73 mg/mL Formula C (Ca/P 4.2): 1.70 mg/mL	3	72	72	AAS
Moya et al., 1998 (26); Spain	Intake, fecal excretion, absorption, retention	0 ± 0 (2–8)	NR	100% healthy	19; NR	Yes	NR	Standard formula (Ca/P 1.5): 0.59 mg/g Lactose-free formula (Ca/P 1.6): 0.65 mg/g	3	72	72	AAS
Nelson et al., 1998 (25); USA	Intake, fecal excretion, absorption	0 ± 0 |(22–192)	NR	100% healthy	10; 60	Yes	NR	Palm olein formula: 580 mg/L High oleic safflower oil formula: 569 mg/L	3	—	72–96	AAS

(*Continued*)

TABLE 2 (*Continued*)

Author, year; country	Calcium outcomes	Age, mean ± SD, y (range, d)	Racial/ethnic background	Health status	Total enrolled *n*; % male	Run-in diet	Habitual diet assessment	Study arm: calcium content	Duration of food consumption, d	Duration of urine collections, h	Duration of fecal collections, h	Calcium assessment methods
Ostrom et al., 2002 (18); USA	Intake, fecal excretion, absorption	0 ± 0 (75–89)[6]	NR	100% healthy	35[7]; 48.6	Yes	NR	Casein hydrolysate + iron formula: 724 mg/L Casein hydrolysate + iron formula: 856 mg/L Soy protein + iron formula: 752 mg/L Soy protein + iron formula: 739 mg/L	3	—	72	AAS
Oliveira de Souza et al., 2017 (16); Brazil	Intake, urinary excretion, fecal excretion, absorption, retention	0.2 ± 0 (68–159)	NR[8]	100% healthy	33 (17)[9]; 53.1	Yes	NR	Formula PALM: 279 mg/100 g Formula NoPALM: 424 mg/100 g	14	72	72	AAS
Zannino et al., 1983 (27); Italy	Intake, fecal excretion, absorption	0 ± 0 (4)	NR	100% healthy	36; 100	NR	NR	Eulac formula: 43 mg/100 g Human milk: 33 mg/100 mL	4	—	72[10]	GEMENI self-analyzer
Ziegler et al., 1983[11] (17); USA	Intake, urinary excretion, fecal excretion, absorption, retention	0 ± 0 (27–382)	NR	100% healthy	6; 83.3	Yes	NR	Lactose formula: 669 mg/L Polycose and sucrose formula: 603 mg/L	11	72	72	AAS

[1] AAS, atomic absorption spectrophotometry; BW, birth weight; Ca, calcium; CF, Ca-L-lactate supp formula; EDTA, Ethylenediaminetetraacetic acid; HCa, high calcium formula;LCa, low calcium formula; MCa, moderate calcium formula; NoPALM, formula without olein palm or palm kernel oil; NR, not reported; P, phosphorus; PALM, formula with olein palm or palm kernel oil; SF, standard formula; supp, supplement.
[2] Some or all participants were given vitamin D supplementation.
[3] Study indicated only 25 participants had assessments of metabolic balance. However, 28 infants provided individual data on calcium and phosphorus balance, as shown in Table 3.
[4] Duration of formula and/or human milk consumption ranged from the first 4 wk to 6 mo of life.
[5] Health status was within systematic review acceptable parameters.
[6] Mean age at study entry ranged from 75 to 89 d. Thus, it can be assumed metabolic balance studies were conducted with infants who were aged between 91 and 181 d.
[7] 35 infants were enrolled in the study, however, only 22 infants provided data in the postmetabolic balance period.
[8] In the same clinical trial conducted by Leite et al. (28), 33 subjects were enrolled, of which 61% were referred to as "mulatto," 36% were black, and 3% were described as "brown," by the authors.
[9] 33 subjects were enrolled in the study. Of these, 17 subjects were included in the metabolic balance phase.
[10] 250 mg of carmine red in 5% glucose solution was administered. Stool collection began when the first marked stools appeared. After 36 h a second administration of carmine red was made. When the feces marked by the 2nd administration of carmine red appeared, the collection stopped, with the exclusion of this last sample.
[11] Mean age at study entry ranged from 27 to 382 d. Mean age at study completion ranged from 105 to 457 d.

5 studies comparing balance measures across multiple arms, the effects of dietary calcium could be isolated in 1 study (13). Oral doses of isotope ranged from 1.5 mg to 3 mg, with most studies using ^{44}Ca. Intravenous doses of isotope ranged from 10 ug to 15 ug, and ^{46}Ca was used in most studies. Duration of urine collections ranged from 24 to 120 h and fecal samples were collected from 48 to 336 h. Thermal ionization mass spectrometry (TIMS) was the most used method to quantify calcium in the urine and/or feces. Study characteristics for all included isotope studies are presented in **Table 3**.

KQ: What are the routes for endogenous losses and amounts of calcium lost through these various routes in children aged 0–4 y? (For example, fecal, urinary, and dermal losses.)

Detailed narrative syntheses of mass balance and isotope studies addressing this KQ are reported in **Supplemental Detailed Narratives**.

Urinary and fecal losses

Mass balance studies.

Fourteen mass balance studies (13–21, 23–27) reported urinary and fecal calcium loss in infants (**Table 4**). Nine studies assessed infants within the 0–90 d age range, while 5 studies assessed infants within the 91–180 d age range. In addition, Moya et al. (26) combined urinary and fecal excretions to quantify calcium loss, as urine output was low for infants in this study. The effects of dietary calcium on losses could be isolated in 3 of the 15 studies (13–15). The strength of evidence from mass balance studies on the routes and amount of calcium loss in relation to intake in subjects aged 0–4 y is low based on these 15 studies (**Table 5**). Three studies designed to isolate the effects of calcium suggest that increasing calcium intake from formula (93.8 mg/[kg∗d] to 176.0 mg/[kg∗d]) may increase fecal and urinary loss, though findings were variable. Studies in which the effects of calcium could not be isolated show that nutrients consumed with calcium may influence calcium loss. For instance, the presence of palm olein (18, 25) in formula resulted in significant increases in fecal calcium loss, irrespective of protein source (18). In a study using both infant formula and human milk, both fecal fatty acid loss (palmitic and stearic) and fecal calcium loss was lower in infants who consumed human milk than formula (24). When the fatty acid structure of an infant formula was modeled to resemble that of human milk (e.g. 66% of the available palmitic acid [PA] esterified at the β-position of the triglyceride [TG]), decreases in fecal calcium loss was observed, when compared to formulas with a lower degree of esterification at the β-position (22). The absence of carbohydrate (lactose, corn starch hydrolysate) in formula had no appreciable effect on calcium loss in infants aged 0–90 d (24),yet the presence of carbohydrate led to significant increases in calcium loss in infants aged 91–180 d (15). Consideration of the nutritional composition and quantity of nutrients in infant formula, to model that of human milk, may be critical to optimize intake of key nutrients for growth, development, and function, while minimizing losses. Ultimately, additional studies are necessary to confirm and better understand the contribution of calcium and intake of other nutrients (e.g. vitamin D, phosphorus, fatty acids) on overall calcium loss, and changes with intake in infants and young children.

Isotope studies.

Four isotope studies (2 single isotope [13, 30], 2 dual isotope [29, 34]) measured calcium losses in infants and young children. One study assessed infants within the 0–90 d age range consuming formula; 1 study assessed infants within the 6–11 mo age range consuming formula, human milk, and solid foods; and 2 studies assessed subjects in the 12–36 mo age range consuming postweaning foods. All 4 studies measured urinary losses, and 3 studies reported endogenous fecal losses (**Table 6**). The strength of evidence from isotope studies on the routes and amount of calcium loss in relation to intake in subjects 0–4 y is low based on 4 studies (**Table 5**). Limited data, variable units, minimal dietary calcium sources, and age discrepancies preclude any conclusions on the relations between calcium intake and losses in children aged 0–4 y. Future studies with multiple arms differing in calcium intake are necessary to better understand the contribution of urinary and fecal calcium excretion to overall calcium loss, and changes with intake in infants and young children. Additionally, studies on infants within the 91–180 d age range will provide insight on losses in this age group.

Dermal losses

Dermal calcium losses were not measured in the included balance studies. Lynch et al. (29) used a dermal loss value of 30 mg/d, estimated from data on prepubertal children in a balance model, to calculate required retention in subjects aged 1–4 y, which is described later in this article.

KQ: What is the efficiency of absorption and retention of calcium (i.e. what percentage of calcium consumed is absorbed by the body) in children aged 0–4 y? (Considering the source of calcium, including human milk, vitamin D deficiency, effects of other nutrients consumed together with calcium, etc. where possible.)

Detailed narrative syntheses of mass balance and isotope studies addressing this KQ are reported in **Supplemental Detailed Narratives**.

Absorption and retention

Mass balance studies.

Fifteen mass balance studies (13–27) reported absorption and retention outcomes in infants (**Table 4**). Nine studies assessed infants within the 0–90 d age range, 5 studies assessed infants within the 91–180 d age range, and 1 study assessed in infants aged 0–180 d. Of the 15 mass balance studies, 3 were designed to isolate the effects of calcium (13–15). The strength of evidence from mass balance studies on the efficiency of calcium absorption and retention in relation to intake in subjects 0–4 y is low based on 15 studies

TABLE 3 Characteristics of included isotopic studies reporting calcium outcomes in infants and children aged 0–4 y[1]

Author, year; country	Calcium outcomes	Mean age ± SD, y; (range, d)	Racial/ethnic background	Health status	Total *n* enrolled; % male	Run-in diet	Habitual diet assessment	Oral isotope, dosage	i.v. isotope, dosage	i.v. fecal isotope, dosage	Duration of urine collection, h	Duration of fecal collection, h	Calcium assessment method
Abrams et al., 1991[2] (30); USA	Urinary excretion, endogenous fecal excretion	3 ± NR	NR	100% healthy	1; 0	Yes	Yes	—	^{42}Ca, 0.5–0.6 mg/kg	—	120	240–336	Urine: TIMS; feces: QMS
Abrams et al., 1997 (34); USA	Intake, urinary excretion, endogenous fecal excretion, absorption, retention	0.5 ± 0.1 (164–226)	100% non-Hispanic white	100% healthy	14; 35.7	NR	Yes	^{44}Ca, 1.5 mg	^{46}Ca, 10 ug	—	24	—	TIMS
Abrams et al., 2002 (32); USA	Intake, absorption	0 ± 0 (56–84)	NR	100% healthy	18; 88.9	Yes	NR	^{44}Ca, 2 mg	^{46}Ca, 15 ug	—	24	—	NR
Barltrop et al., 1977[2] (13); United Kingdom	Intake, urinary excretion, fecal excretion, endogenous fecal excretion, absorption, retention	0 ± 0 (4–41)	NR	100% healthy	13; 92.3	Yes	Yes	^{46}Ca, 2 mg	—	—	48	48	AAS
Hicks et al., 2012[3] (31); USA	Intake, absorption	0 ± 0 (56–70)	NR	100% healthy	74; 59.5	Yes	NR	^{44}Ca, 3 mg	^{46}Ca, 0.01 mg	—	24	—	TIMS
Hillman et al., 1988[4] (35); USA	Absorption	0 ± 0 (14–21)	NR	Low BW and GA	7; NR	NR	Yes	^{44}Ca, 1.3 mg/kg	^{46}Ca, 7.5 ug/kg	—	24	—	TIMS
Lifschitz et al., 1998 (33); USA	Intake, absorption	0 ± 0	NR	100% healthy	14; 92.9	Yes	NR	^{44}Ca, 1.5 mg	^{46}Ca, 3 ug	—	24	—	TIMS
Lynch et al., 2007 (29); USA	Intake, urinary excretion, endogenous fecal excretion, absorption, retention	2.5 ± 0.2 (1–3 y)	46% non-Hispanic white, 29% Hispanic, 18% non-Hispanic black	100% healthy	28; 50	Yes	Yes	^{42}Ca, 2 mg	^{46}Ca, 15 ug	^{46}Ca, 40 ug	48	120	TIMS

[1] AAS, atomic absorption spectrophotometry; BW, birth weight; Ca, calcium; GA, gestational age; NR, not reported; QMS, quadruple mass spectrometer; TIMS, magnetic sector thermal ionization mass spectrometer/thermal ionization quadrupole mass spectrometer.
[2] Single isotope studies. The remaining studies used dual isotope designs.
[3] Run-in diet included the randomization to either a cow milk-based nonprebiotic containing control formula (CF) or the same formula with added prebiotics (PF). Human-milk-fed infants who had consumed human milk from birth were also included.
[4] Health status was within systematic review acceptable parameter.

TABLE 4 Results and overall risk-of-bias assessment of mass balance studies reporting calcium outcomes in infants and children aged 0–4 y[1]

Author, year	Isolated calcium effects[2]	Study arm	Total enrolled, *n*	Intake, mean ± SD mg/(kg∗d)	Urinary losses, mean ± SD mg/(kg∗d)	Fecal losses, mean ± SD mg/(kg∗d)	Absorption, mean ± SD mg/(kg∗d); mean ± SD %[3]	Retention, mean ± SD mg/(kg∗d); mean ± SD %[3]	Key findings, outcome: (comparisons)	Overall RoB
Infants (0–90 d)										
Barnes et al. 1974[4] (22)	No	Formula J (days 5–7)	10	90 ± NR	—	—	(32.4); 36 ± 12	(31.5); 35 ± 12	Absorption (%): (J > K)*	Low
									Absorption (%): (J > L)**	
		Formula K (days 5–7)	10	108 ± NR	—	—	(27); 25 ± 11	(27); 25 ± 9		
		Formula L (days 5–7)	9	114 ± NR	—	—	(22.8); 20 ± 7	(20.5); 18 ± 6		
		Formula J (days 8–10)	10	95 ± NR	—	—	(36.1); 38 ± 11	(35.2); 37 ± 12	Absorption (%): (J > K)**	
									Absorption (%): (J > L)***	
		Formula K (days 8–10)	10	113 ± NR	—	—	(30.5); 27 ± 8	(28.2); 25 ± 10		
		Formula L (days 8–10)	9	118 ± NR	—	—	(23.6); 20 ± 6	(21.2); 18 ± 5		
Barltrop et al. 1977[5] (13)	Yes	Formula L (Ca/P 0.6) -P supp	3	124 ± 12.0	1.3 ± 0.8	117 ± 22.1	2.25 ± 22; NR	6.45 ± 18.8; NR		Low
		Formula M (Ca/P 1.2) -No supp	5	112 ± 9.9	2.1 ± 1.1	123 ± 6.1	−13.5 ± 6.3; NR	−15.0 ± 5.6; NR		
		Formula H (Ca/P 2.4) -Ca supp	3	213 ± 16.0	1.5 ± 1.3	180 ± 26.4	32.4 ± 16.6; NR	30.9 ± 16.8; NR		
Carnielli et al. 1996 (23)	No	β-formula (β-F)	9	92.2 ± 10.1	6.2 ± 4.3	43.4 ± 18.1	(49.0); 53.1 ± 18.1	42.8 ± 23.1; 45.5 ± 21.3	Intake, urine, retention: (β-F vs. I-F vs. R-F), NS	SC
		Intermediate formula (I-F)	9	92.9 ± 8.5	5.4 ± 2.0	59.9 ± 15.1	(32.9); 35.4 ± 14.8	26.9 ± 16.0; 28.4 ± 15.6	Fecal: (β-F < I-F, R-F)*	
									Absorption (%): (β-F > I-F, R-F)*	
		Regular formula (R-F)	9	99.5 ± 13.9	3.7 ± 1.8	68.4 ± 22.3	(32.3); 32.5 ± 18.3	27.4 ± 14.8; 28.8 ± 18.5		
Clemente Yago et al. 1989[6] (20)	N/A	Milk formula + VD	20	106.5 ± 23.1	3.5 ± 3.3	50.6 ± 19.2	54.9 ± 18.8; NR	57.4 ± 20.6; NR		SC

(*Continued*)

TABLE 4 (*Continued*)

Author, year	Isolated calcium effects[2]	Study arm	Total enrolled, *n*	Intake, mean ± SD mg/(kg∗d)	Urinary losses, mean ± SD mg/(kg∗d)	Fecal losses, mean ± SD mg/(kg∗d)	Absorption, mean ± SD mg/(kg∗d); mean ± SD %[3]	Retention, mean ± SD mg/(kg∗d); mean ± SD %[3]	Key findings, outcome: (comparisons)	Overall RoB
Fomon et al. 1963 (19)	No	Age 8–30 d: Pooled human milk	6	72.8 ± 12.0	3.1 ± 2.4	45.0 ± 14.2	—	23.8 ± 12.3; 32.6 ± 16.7		Low
		Age 31–60 d: Pooled human milk	7	67.8 ± 22.3	3.7 ± 3.3	35.5 ± 15.6	—	28.6 ± 17.6; 40.5 ± 17.8		
		Age 61–90 d: Pooled human milk	6	50.4 ± 6.7	3.1 ± 2.5	23.3 ± 10.5	—	24.0 ± 9.9; 47.4 ± 16.6		
		Age 8–30 d: Formula S-26	1	85.0 ± NR	5.0 ± NR	63.0 ± NR	—	17.0 ± NR; NR		
		Age 31–60 d: Formula S-26	2	75.5 ± 9.1	3.0 ± 0.8	47.8 ± 6.1	—	24.8 ± 5.6; 32.5 ± 8.3		
		Age 61–90 d: Formula S-26	2	66.5 ± 8.7	1.8 ± 1.0	27.5 ± 6.6	—	37.2 ± 12.6; 54.7 ± 14.5		
		Age 8–30 d: Formula 22-3C	3	87.2 ± 7.9	6.0 ± 1.4	56.0 ± 9.0	—	25.2 ± 13.0; 28.2 ± 12.7		
		Age 8–30 d: Formula 22-3D	2	73.5 ± 4.9	4.5 ± 0.7	36 ± 2.8	—	32.5 ± 2.1; (44.2]		
		Age 31–60 d: Formula 22-3D	5	66.4 ± 11.8	4.1 ± 2.4	32.4 ± 11.1	—	29.9 ± 7.4; 45.8 ± 13.2		
		Age 61–90 d: Formula 22-3D	2	68.5 ± 20.5	7.5 ± 3.5	31.0 ± 4.2	—	30.0 ± 21.2; (43.8)		
		Age 61–90 d: Formula 22-3E	5	72.0 ± 4.3	8.7 ± 4.0	28.3 ± 11.7	—	35.0 ± 12.3; 48.2 ± 16.4		
		Age 8–30 d: Similac	5	140.7 ± 27.9	2.2 ± 3.0	102.2 ± 18.5	—	36.3 ± 17.5; 25.2 ± 8.2		
		Age 31–60 d: Similac	5	145.2 ± 34.7	1.0 ± 2.2	111.4 ± 57.5	—	32.9 ± 32.3; 23.0 ± 11.1		
		Age 61–90 d: Similac	5	142 ± 21.7	0.9 ± 1.1	77.3 ± 21.1	—	64.5 ± 27.6; 40.5 ± 15.3		
Hanna et al. 1970 (24)	No	Transitional breast milk (TBM)	11	40.4 ± 10.6	2.8 ± 2.5	16.4 ± 5.6	24 ± 8.7; 58.7 ± 14.5	21.2 ± 7.1; 52.4 ± 13.3	Intake and fecal: (A > TBM, B > TBM)**	Low
		Formula A	15	83.7 ± 15.2	2 ± 1.4	59.7 ± 14.9	24 ± 7.3; 29.3 ± 9	22 ± 7.1; 26.9 ± 8.9	Absorption/retention (%): (A < TBM, B < TBM)**	
		Formula B	6	75.6 ± 8.7	1.3 ± 0.6	55.6 ± 6	20 ± 11.4; 25.3 ± 12.8	18.7 ± 11; 23.6 ± 12.7	Absorption/retention (mg/(kg∗d)): (A vs. TBM, B vs. TBM), NS	
		Lyophilized mature human milk (LMM)	6	45.8 ± 9.7	2.7 ± 2.2	22.8 ± 10.3	22.9 ± 8.5; 51.4 ± 18.4	20.3 ± 6.7; 45.6 ± 15.4	All outcomes: (LMM vs. TBM), NS	

(*Continued*)

TABLE 4 (*Continued*)

Author, year	Isolated calcium effects[2]	Study arm	Total enrolled, *n*	Intake, mean ± SD mg/(kg∗d)	Urinary losses, mean ± SD mg/(kg∗d)	Fecal losses, mean ± SD mg/(kg∗d)	Absorption, mean ± SD mg/(kg∗d); mean ± SD %[3]	Retention, mean ± SD mg/(kg∗d); mean ± SD %[3]	Key findings, outcome: (comparisons)	Overall RoB
Manz et al. 1989[7] (14)	Yes	Standard formula	19	97.4 ± NR	1.8 ± 1.2	—	—	—	Urine: (Ca supp > SF)***	Low
		Ca-supp formula	19	140 ± NR	3.9 ± 2.5	—	—	—		
		Standard formula	8	93.8 ± 3.6	2.4 ± 1.2	59.2 ± 9.6	(34.7); 37 ± 10	32 ± 8.8; NR	Intake, urine, retention, and absorption: (Ca supp > SF)**	
		Ca-supp formula	8	145 ± 20	6.0 ± 2.4	65.4 ± 14	(81.2); 56 ± 7	74.2 ± 15; NR	Fecal: (Ca supp vs. SF), NS	
Moya et al. 1998[8] (26)	No	Lactose-free formula	9	121 ± 30	—	63 ± 25	(58.1); 48 ± 17	56 ± 23; NR	Intake, losses, retention: (LF vs. SF), NS	Low
		Standard formula	10	139 ± 26	—	67 ± 20	(68.1); 49 ± 14	68 ± 22; NR		
Moya et al. 1982[8] (21)	No	Formula A (Ca/P 2.4)	10	89.7 ± 13.8	0.3 ± 0.1	37 ± 11.5	(50.8); 56.6 ± NR	50.8 ± 15.9; NR	Retention: (A > B)*	Low
		Formula B (Ca/P 1.7)	8	71.1 ± 12.5	0.2 ± 0.1	29.6 ± 7.4	(39.3); 55.3 ± NR	39.3 ± 12.8; NR	Retention: (A, B < C)***	
		Formula C (Ca/P 4.2)	8	156.8 ± 19.8	0.5 ± 0.2	53.3 ± 7.4	(105.2); 67 ± NR	105.2 ± 21.9; NR		
Nelson et al. 1998 (25)	No	Formula PO	10	86.0 ± 15.9	—	53.4 ± 12.0	32.6 ± 12.2; 37.5 ± 11.5	—	Fecal: (PO > HOS)** Absorption (mg/(kg∗d)): (PO < HOS)***	Low
		Formula HOS	10	86.8 ± 14.2	—	37.4 ± 14.9	49.4 ± 14.4; 57.3 ± 14.9	—	Absorption (%): (PO < HOS)**	
Zannino et al. 1983 (27)	No	Eulac formula	18	48.4 ± 2.0	—	1.4 ± 1.1	47.1 ± 2.1; 97.1 ± 2.2	—	Intake: (Eulac > HM)*** Absorption (mg/kg∗d)): (Eulac > HM)***	Low
		Human milk	18	36.5 ± 3.1	—	0.4 ± 0.3	36.1 ± 3.1; 98.9 ± 0.8	—	Absorption (%): (Eulac vs. HM), NS Fecal: (Eulac vs. HM), NS	
Infants (91–180 d)										
DeVizia et al. 1985 (15)	Yes	LCa formula	6	65 ± 14	2 ± 1	28 ± 10	37 ± 10; 57 ± 10	35 ± 10; 54 ± 10	Urine: (HCa > MCa; HCa > LCa; MCa = LCa)**	Low
		MCa formula	6	117 ± 28	2 ± 2	62 ± 23	55 ± 18; 47 ± 11	53 ± 19; 45 ± 11	Fecal: (HCa > MCa > LCa)*** Absorption (mg/(kg∗d)): (HCa > MCa > LCa)***	
		HCa formula	6	176 ± 42	4 ± 2	109 ± 39	67 ± 20; 39 ± 10	64 ± 21; 37 ± 10	Retention (mg/(kg∗d)): (HCa = MCa; HCa > LCa; MCa > LCa)*** Absorption/retention (%): (LCa > MCa > HCa)***	

(*Continued*)

TABLE 4 (*Continued*)

Author, year	Isolated calcium effects[2]	Study arm	Total enrolled, *n*	Intake, mean ± SD mg/(kg∗d)	Urinary losses, mean ± SD mg/(kg∗d)	Fecal losses, mean ± SD mg/(kg∗d)	Absorption, mean ± SD mg/(kg∗d); mean ± SD %[3]	Retention, mean ± SD mg/(kg∗d); mean ± SD %[3]	Key findings, outcome: (comparisons)	Overall RoB
Fomon et al. 1963 (19)	No	Age 91–120 d: Pooled human milk	7	55.0 ± 10.7	4.1 ± 2.8	20.5 ± 9.3	—	30.3 ± 12.4; 54.1 ± 14.3		Low
		Age 121–150 d: Pooled human milk	8	46.0 ± 5.1	3.4 ± 4.2	21.4 ± 6.6	—	21.2 ± 4.4; 47.1 ± 12.2		
		Age 151–182 d: Pooled human milk	5	45.5 ± 8.7	5.1 ± 1.8	20.5 ± 10.7	—	22.1 ± 9.5; 46.9 ± 12.5		
		Age 91–120 d: Formula S-26	1	70.0 ± NR	3.0 ± NR	44.0 ± NR	—	23.0 ± NR; NR		
		Age 91–120 d: Formula 22-3E	7	67.3 ± 10.9	7.0 ± 3.3	27.9 ± 7.4	—	31.4 ± 10.3; 46.7 ± 13.0		
		Age 121–150 d: Formula 22-3E	4	76.8 ± 6.3	9.5 ± 6.3	28.3 ± 4.9	—	39.0 ± 4.8; 50.5 ± 3.3		
		Age 91–120 d: Similac	6	123.9 ± 16.5	1.7 ± 2.8	67.4 ± 18.5	—	54.8 ± 15.3; 44.2 ± 11.3		
		Age 121–150 d: Similac	9	105.9 ± 16.5	1.6 ± 3.4	65.4 ± 27.1	—	38.8 ± 16.4; 37.0 ± 25.3		
		Age 151–182 d: Similac	11	105.9 ± 18.1	1.6 ± 2.5	63.5 ± 20.5	—	40.9 ± 16.3; 39.1 ± 16.7		
Ostrom et al. 2002[9] (18)	No	Casein hydrolysate NUTR formula	10	100.0 ± 12.6	—	55.0 ± 19.0	41.0 ± 19.0; 41.0 ± 19.0	—	Fecal: (NUTR > AILM)** Absorption (mg/(kg∗d)): (NUTR < ALIM)** Absorption (%): (NUTR < ALIM)**	Low
		Casein hydrolysate ALIM formula	10	108.0 ± 22.1	—	30.0 ± 9.5	74.0 ± 28.5; 66.0 ± 15.8	—		
		Soy protein PRO formula	12	77.0 ± 13.9	—	58.0 ± 13.9	17.0 ± 10.4; 22.0 ± 10.4	—	Fecal: (PRO > ISO)* Absorption (mg/(kg∗d)): (PRO < ISO)* Absorption (%): (PRO < ISO)*	
		Soy protein ISO formula	12	78.0 ± 20.8	—	44.0 ± 13.9	29.0 ± 13.9; 37.0 ± 13.9	—		
Oliveria de Souza et al. 2017[10] (16)	No	PALM formula	17	50.2 ± 9.6	1.8 ± 0.8	29.3 ± 11.4	19.5 ± 10.3; 39.1 ± 20.6	18.2 ± 10.0; 42.2 ± 15.3	Intake: (NoPALM > PALM)*** Urine and fecal: (NoPALM vs. PALM), NS	Low

(*Continued*)

TABLE 4 (*Continued*)

Author, year	Isolated calcium effects[2]	Study arm	Total enrolled, *n*	Intake, mean ± SD mg/(kg∗d)	Urinary losses, mean ± SD mg/(kg∗d)	Fecal losses, mean ± SD mg/(kg∗d)	Absorption, mean ± SD mg/(kg∗d); mean ± SD %[3]	Retention, mean ± SD mg/(kg∗d); mean ± SD %[3]	Key findings, outcome: (comparisons)	Overall RoB
		NoPALM formula	17	71.9 ± 13.3	1.6 ± 0.7	28.8 ± 13.2	50 ± 18.7; 62.2 ± 18.3	48.2 ± 18.6; 60 ± 18.3	Absorption/ retention (mg/(kg∗d)): (NoPALM > PALM)***	
									Absorption/retention (%): (NoPALM > PALM)***	
Ziegler et al. 1983[2] (17)	No	Formula L	6	113 ± 22	4.0 ± 3.0	61 ± 30	52 ± 12; 48 ± 17	31 ± 8.0; 33 ± 11	Fecal: (SCS > L)***	Low
									Absorption (mg/(kg∗d)): (SCS < L)***	
									Absorption (%): (SCS < L)**	
		Formula SCS	6	97 ± 23	3.0 ± 2.0	66 ± 25	48 ± 12; 44 ± 16	28 ± 9.0; 30 ± 11	Retention (mg/(kg∗d)): (SCS < L)***	
									Retention (%): (SCS < L)*	

[1] β-F, β formula; ALIM, protein hydrolysate formula with iron; Ca, calcium; HCa, high calcium formula; HOS, formula with high-oleic safflower oil; HM, human milk; I-F, intermediate formula; ISO, soy protein formula with iron;L, lactose formula; LCa, low calcium formula; LF, lactose-free formula; LMM, lyophilized mature human milk; MCa, moderate calcium formula; N/A, not applicable; NoPALM, formula without olein palm or palm kernel oil; NR, not reported; NS, not significant; NUTR, hypoallergenic protein hydrolysate formula with iron; P, phosphorus; PALM, formula with olein palm or palm kernel oil; PO, formula with 45% palm olein; PRO, soy protein formula with iron; R-F, regular formula; RoB, risk of bias; SC, some concerns; SCS, polycose and sucrose formula; SF, standard formula; supp, supplemented; TBM, transitional breast milk; VD, vitamin D. *P<0.05; **P<0.01, ***P<0.001.

[2] Effects of calcium can be isolated when the only difference between the control and intervention is in the amount of dietary calcium (e.g. Formula X compared with Formula X + calcium supplement). Not applicable for studies with only 1 arm of interest.

[3] Total net absorption and retention values in parenthesis are means calculated by authors of this review by multiplying mean fractional absorption and mean calcium intake. Total net absorption and retention values without parenthesis were reported by study authors.

[4] It is not clear from the article if values in parentheses are SD or SE.

[5] Reported values in the table are based on individual data, with the following calculations: duplicates per subject were averaged, units converted from mg/d to mg/(kg∗d) based on individual weight, data from subjects 11 and 3b were excluded due to prematurity and incomplete collections, respectively. When needed, *n* values were adjusted according to available data. Urine losses were not counted in 1 individual taking Formula L nor were fecal losses in 3 individuals taking Formula M. Retention and absorption were not calculated for these subjects. Negative numbers were reported: calculations were conducted, not a result of error.

[6] Urine loss and retention were measured in 10 participants only.

[7] First comparison on all participants (19); the second had fecal collections on 8 infants only. Converted mmol/kg/d to mg/(kg∗d) where appropriate.

[8] According to authors, in all cases, values in urine losses were added to the fecal losses because of their low content in calcium and magnesium.

[9] SE of intake, urinary losses, and fecal losses were converted to SD using calculators proposed by Wan et al. (2014) (36).

[10] Median and IQR values were reported in the original article and were converted to mean and SD for this review using calculators proposed by Wan et al. (2014) (36).

TABLE 5 Grading of Recommendations, Assessment, Development and Evaluations (GRADE) evidence profile table: calcium requirements for infants and children aged 0–4 y[1]

	Quality assessment							
Study design	**Number of studies**	**Limitations**	**Inconsistency**	**Balance design**	**Imprecision**	**Isolated Ca effects and dose response[2]**	**Strength of evidence[3]**	**Justification**
Losses KQ Mass balance	14	No serious limitations: Overall RoB was rated as low for 86% of studies reporting urinary or fecal losses	Consistent: Losses for both urine and feces were reported in most studies (71%). Losses were reported as mg/(kg∗d) for infants. This reporting allowed for comparison of outcomes within age groups	Complete balance measures not possible by design: Although losses in both urine and feces were reported in most studies, the mass balance design limits the measure of endogenous fecal excretion	Some imprecision: 100% of studies reported SD or SE as their measure of variance with reasonable plausibility. Though, small sample sizes and incomplete balance design measures limit the accuracy of precise measures reported. Intrasubject variability and other components in milk or formula may affect calcium losses observed	29% of studies could isolate the effects of calcium by comparing losses in infants with different concentrations of calcium intake. Of these studies, 44% demonstrated a dose-response relation with respect to losses	⊕⊕◯◯ LOW	Evidence on the relation between calcium intake and losses in infants is low due to the inherent limitation in measuring endogenous fecal losses, some imprecision in the estimates reported, and the small quantity of studies where the effects of calcium could be isolated

(*Continued*)

TABLE 5 (*Continued*)

	Quality assessment							
Study design	**Number of studies**	**Limitations**	**Inconsistency**	**Balance design**	**Imprecision**	**Isolated Ca effects and dose response[2]**	**Strength of evidence[3]**	**Justification**
Isotope studies	4	No serious limitations: Overall RoB was rated as low for 75% of the studies and some concerns for the remaining 25%	Some inconsistency: All 4 studies reported both urinary and endogenous fecal losses. Losses were reported as percent of isotope intake in 1 study (13), and mg/(kg∗d) in 3 studies. However, infants in studies reporting losses as mg/(kg∗d) differed in age, limiting comparability across studies	Some indirect measures of calcium balance: Endogenous fecal calcium was measured in 50% of the studies but estimated in the remaining 50%. Urinary losses were directly measured in all studies reporting urinary calcium	Imprecise: 75% of studies reported SD or SE with reasonable plausibility. However, 1 study did not report precise calcium intake (30) and only 1 study reported power calculations (32). Additionally, the small sample sizes (n ≤ 28/group) and estimation of endogenous fecal calcium limit our confidence in the precision of estimates	One study (25%) (13) could isolate the effects of calcium by comparing direct losses in groups differing only in calcium intake but did not perform statistical comparisons or demonstrate a dose-response relation with respect to losses	⊕⊕◯◯ LOW	Evidence on the relation between calcium intake and losses in infants is low due to imprecision across studies, indirectness in the measurement of fecal losses, and a lack of studies designed to isolate the effects of calcium

(*Continued*)

TABLE 5 (*Continued*)

	Quality assessment							
Study design	**Number of studies**	**Limitations**	**Inconsistency**	**Balance design**	**Imprecision**	**Isolated Ca effects and dose response[2]**	**Strength of evidence[3]**	**Justification**
Absorption and retention KQ								
Mass balance	15	No serious limitations: Overall RoB was rated as low for 87% of studies reporting on absorption or retention	Consistent: Units were reported either as percent of intake or mg/(kg∗d) to allow for comparisons across studies with infants. Only 1 study reported unexplainably low or negative absorption findings (13)	Complete balance measures not possible by design: Absorption and retention were tabulated in all studies, though the mass balance design itself limits the measure of endogenous fecal excretion. Therefore, estimations for absorption and retention may be skewed	Some imprecision: 73% of studies reported SD or SE with reasonable plausibility. Small sample sizes and lack of endogenous measures limit the accuracy of precise measures reported	27% of studies could isolate the effects of calcium by comparing absorp-tion/retention in groups with different concentrations of calcium intake in infants <1 y. Of these, 75% demonstrated a dose-response relation	⊕⊕◯◯ LOW	Evidence on the relation between calcium intake on absorption and/or retention is low in infants, as absorption and retention were tabulated based on small sample sizes and lack of measures on both endogenous fecal and urinary losses in some or all studies. Findings varied, as calcium dosage and formula composition differed widely across diets

(*Continued*)

TABLE 5 (*Continued*)

	Quality assessment							
Study design	**Number of studies**	**Limitations**	**Inconsistency**	**Balance design**	**Imprecision**	**Isolated Ca effects and dose response[2]**	**Strength of evidence[3]**	**Justification**
Isotope studies	8	No serious limitations: Overall RoB was rated as low for 88% of the studies and some concerns for the remaining 13%	Some inconsistency: Absorption was reported as percent intake in all studies. Total net absorption (mg/d) was additionally reported or calculated by authors of this review in 88% of these studies. In 3 studies, retention was reported as percent intake (33%) or mg/d (66%). Dietary sources were variable with age, resulting in some inconsistency in the relation between intake and absorption/retention	Some indirect measures of calcium balance: Studies reporting retention used either estimates of endogenous fecal calcium (33%) or extrapolated values from a subset of the population (66%) for calculations. No concerns regarding indirectness in the measurement of absorption	Some imprecision: 100% of studies reported SD or SE with reasonable plausibility. However, 2 studies did not report calcium intake (33, 35) and only 1 study reported power calculations (32). Additionally, the small sample sizes ($n \leq$ 28/group) and estimation of endogenous fecal calcium limit our confidence in the precision of estimates	In the 1 study (13%) isolating the effects of calcium, no dose-response effect on absorption or retention was observed (13)	⊕⊕◯◯ LOW	Evidence on the relation between calcium intake and absorption/retention in infants is low due to inconsistency and imprecision across studies. Additionally, indirectness in the measurement of fecal losses limits SOE for retention

[1] For this strength of evidence evaluation, the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) was modified to accommodate the balance study design. Ca, calcium; KQ, key question; RCT, randomized-controlled trials; RoB, risk of bias; SOE, strength of evidence.

[2] Dose-response relation refers to a directional trend between calcium intake and the calcium balance measure of interest within a study.

[3] Symbols indicate the following strength of evidence: ⊕⊕⊕⊕ High (we are very confident that the true effect lies close to that of the estimate of the effect.); ⊕⊕⊕◯, Moderate (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 (our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.); and ⊕◯◯◯, Very low (we have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.).

TABLE 6 Results and overall risk-of-bias assessment of included isotopic studies reporting calcium outcomes in infants and children aged 0–4 y[1]

Author, year	Isolated calcium effects?[2]	Study arm[3]	Total enrolled, *n*	Intake, mean ± SD mg/d	Urinary losses, mean ± SD	Endogenous fecal losses, mean ± SD	Absorption, mean ± SD mg/d; mean ± SD %[4]	Retention, mean ± SD mg/d; mean ± SD %[4]	Key findings, outcome: (comparisons)	Overall RoB
Infants (0–90 d)										
Abrams et al. 2002 (32)	No	Lactose-containing formula	18	507 ± 105	—	—	339 ± 88; 66.5 ± 11.9	—	Intake: (Lac vs. No-Lac), NS	Low
		Lactose-free formula	18	500 ± 91	—	—	279 ± 85; 56.2 ± 15.3	—	Absorption (mg/d): (Lac > No-Lac)**	
									Absorption (%): (Lac > No-Lac)**	
Barltrop et al. 1977[5] (13)	Yes	Formula L (Ca/P 0.56) - P supp	4[6]	245 ± 23	0.14 ± NR %	5.4 ± NR %	(85.6); 35 ± NR	(68.8); 28 ± 10.8	All outcomes: (Formula L vs. M vs. H), NS	SC
		Formula M (Ca/P 1.2) - No supp	5[6]	241 ± 12	0.25 ± 0.2 %	3.4 ± NR %	(56.6); 23.5 ± NR	(96.4); 40 ± 19.2		
		Formula H (Ca/P 2.4) - Ca supp	4[6]	470 ± 12	0.13 ± 0.1 %	3.2 ± 2.4 %	(155); 33 ± 2.8	(141); 30 ± 2.6		
Hillman et al. 1988 (35)	No	Age 2 wk	5[7]	—	—	—	NR; 42.3 ± 10.5	—		Low
		Age 3 wk	2[7,8]	—	—	—	NR; 49.8 ± 5.4	—		
Hicks et al. 2012 (31)	No	Control formula	29	557 ± 16	—	—	328 ± 13; 59.2 ± 2.3	—	All outcomes: (CF vs. PF), NS	Low
		Prebiotic formula	20	543 ± 17	—	—	300 ± 14; 56.8 ± 2.6	—	Intake: (HM < CF vs. PF)*** Absorption (mg/d): (HM > CF vs. PF)***	
		Human milk	19	246 ± 20	—	—	187 ± 16; 76.0 ± 2.9	—	Absorption (%): (HM > CF vs. PF)***	
Infants (91–180 d)										
Lifschitz et al. 1998 (33)	No	Formula	9	473.1 ± NR[9]	—	—	273 ± 80; 57.7 ± 12.9	—	Absorption (mg/d): (F + RC > F)*	Low
									Absorption (%): (F + RC vs. F), NS	
		Formula with rice cereal	9	741.3 ± NR[9]	—	—	424 ± 180; 57.2 ± 18.4	—		

(*Continued*)

TABLE 6 (*Continued*)

Author, year	Isolated calcium effects?[2]	Study arm[3]	Total enrolled, *n*	Intake, mean ± SD mg/d	Urinary losses, mean ± SD	Endogenous fecal losses, mean ± SD	Absorption, mean ± SD mg/d; mean ± SD %[4]	Retention, mean ± SD mg/d; mean ± SD %[4]	Key findings, outcome: (comparisons)	Overall RoB
Infants (6–11 mo)										
Abrams et al. 1997 (34)	N/A	Infants, 5–7 mo	14	259 ± NR[10]	23.4 ± 17.2 mg/d	∼3 mg/(kg∗d)[11]	(158.7); 61.3 ± 22.7	68 ± 38; NR[12]		Low
Children (1–3 y)										
Lynch et al. 2007 (29)	N/A	Children, 1–4 y	28[13]	550.7 ± 218.6	2.2 ± 0.2 mg/(kg∗d), 27.4 ± NR mg/d	3.5 ± NR mg/(kg∗d)	(251.1); 45.6 ± 2.5	161 ± 17; NR		Low
Abrams et al. 1991 (30)	No	Subject, age 3 y	1	300–800 ± NR	2.8 ± NR mg/(kg∗d)	1.0 ± NR mg/(kg∗d), 25.9 ± NR mg/d	—	—		Low

[1] Ca, calcium; CF, control formula; F, formula; HM, human milk; Lac, lactose-containing formula; N/A, not applicable; NoLac, lactose-free formula; NR, not reported; NS, not significant; P, phosphorus; PF, prebiotic formula; RC, rice cereal; RoB, risk of bias; SC, some concerns; supp, supplemented. $^{*}P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$.

[2] Effects of calcium can be isolated when the only difference between the control and intervention is in the amount of dietary calcium (e.g. Formula X compared with Formula X + calcium supplement). Not applicable for studies with only 1 arm of interest.

[3] Studies with 1 study arm reported were either single arm studies, or only 1 study arm met inclusion criteria.

[4] Total net absorption and retention values in parenthesis are means calculated by authors of this review by multiplying mean fractional absorption and mean calcium intake. Total net absorption and retention values without parenthesis were reported by study authors.

[5] SD was calculated from SE using calculators proposed by Wan et al. (2014) (36).

[6] Formula L/M/H: intake, $n = 4/5/3$; urine, $n = 3/5/3$; fecal, $n = 4/5/4$; endogenous fecal, $n = 3/2/4$; absorption, $n = 3/2/4$; retention, $n = 4/5/4$.

[7] Mean absorption calculated using data reported for individual study participants.

[8] Restudied 2 of the 5 initial children aged 3 wk.

[9] Study authors reported that calcium intake was measured; however, values were not reported. Therefore, intake values were calculated by authors of this article by dividing total net absorption by fractional intake (F: 273/0.577 = 473.14 mg/d, F+R: 424/0.572 = 741.26 mg/d).

[10] 215 mg/d in breast milk plus 44 mg/d from beikost (solid food).

[11] Estimated endogenous fecal calcium used to calculate retention (i.e. endogenous excretion was not directly measured).

[12] Retention from human milk only (215 mg/d).

[13] Endogenous fecal, $n = 8$; value used as estimated endogenous excretion to calculate retention for the whole population, $n = 28$. Urinary excretion in mg/d was calculated using data from individual study participants.

(**Table 5**). Three studies designed to isolate the effects of calcium suggest that increasing calcium intake from formula (93.8 mg/[kg∗d] to 176.0 mg/[kg∗d]) may increase absorption or retention, though findings were variable. Studies in which the effects of calcium could not be isolated show that the quantity of nutrients consumed with calcium may influence calcium accrual. The addition of palm olein (16, 18, 25) to formula led to decreases in calcium absorption and retention, despite variabilities in the protein source (18) or calcium content (16). Modeling the fatty acid structure in an infant formula to resemble that of human milk (e.g., 66% of available PA esterified at the β-position of the TG) resulted in greater calcium absorption and retention, compared with conventional formulas (13). Moreover, consumption of infant formula with differences in micronutrient (vitamin D, phosphorus) (13, 20, 21) or carbohydrate (17) content, along with fatty acid blends may impact calcium absorption and retention. Unequivocally large calcium intakes from formula (60 to 140 mg/[kg∗d]) compared with human milk (40 to 70 mg/[kg∗d]) were observed, yet human milk consumption resulted in greater calcium absorption and retention in infants aged 0–180 d (19). Additional studies are needed to confirm and better understand the effects of calcium, other nutrient intakes (e.g. vitamin D, phosphorus, fatty acids), and food compositions (e.g. formula, human milk) on overall calcium accrual and changes with intake in infants and young children.

Isotope studies.

Eight isotope studies (1 single isotope (13), 7 dual isotope (29–35) reporting calcium absorption in infants and young children were included (**Table 6**). Three of these studies (13, 29, 34) also measured calcium retention. The source of dietary calcium differed, along with infant age, across studies: formula (4 studies) or exclusively human milk (1 study) in infants 0–90 d, formula with or without added rice cereal in infants 91–180 d (1 study), human milk and solid foods in infants 6–11 mo (1 study), and solid foods in young children 12–36 mo (2 studies). The strength of evidence from isotope studies on the efficiency of absorption and retention of calcium in relation to intake in subjects aged 0–4 y is low based on 8 studies reporting absorption and 3 studies reporting retention (**Table 5**). Findings across studies were variable. At intakes between 241 mg/d and 741.1 mg/d, fractional absorption ranged from 23.5% to 76.0%, and total net absorption (reported by study authors or calculated by authors of this review) ranged from 56.6 mg/d to 328 mg/d. At intakes between 241 mg/d and 550.7 mg/d, retention efficacy ranged from 28% to 40%, and total retention ranged from 68 mg/d to 161 mg/d. Overall, findings suggest that calcium intakes of 241 mg/d to 259 mg/d result in greater fractional absorption, but lower total net absorption than calcium intakes of 470 mg/d to 740 mg/d, regardless of dietary source. At similar calcium intakes, absorption efficacy from human milk may be greater than that from formula or solid food, and lactose may enhance absorption efficacy from formula. Controlled studies designed to isolate the effects of calcium and use of consistent dietary sources of calcium in infants would strengthen the proposed relations. Additional studies on older infants (91 d to 1 y) are necessary to determine changes in absorption throughout infancy. Findings on calcium retention were limited, and additional studies using direct measures of endogenous fecal calcium, rather than estimates, are necessary to determine associations with intake and age.

RoB assessment

The overall RoB was low across most mass balance studies (**Supplemental Table 1**). Only 2 studies (20, 23) were rated as having some concerns for bias due to the lack of information available on a validated technique for quantification of calcium in formula samples.

The overall RoB was low in 7 out of the 8 isotope studies (**Supplemental Table 2**). One study (13) was rated as having some concerns for bias primarily due to lack of reporting on sterility and pyrogenicity testing of the isotopes administered.

Meta-regression

Infants <12 mo.

Random-effects meta-regression was performed to examine the relation between daily mean calcium intake and retention concentrations in infants aged 0–6 mo. Of note, no studies in infants aged 6 mo to 1 y reported sufficient data for the meta-regression. In total, 43 study arms from 10 publications were included in the analysis (14–17, 19–21, 23, 24, 26). All included studies used mass balance measurements. The meta-regression results showed that every 10 mg/(kg∗d) increase in mean calcium intake was associated with an average calcium retention of 4.04 mg/(kg∗d) (β-coefficient = 0.404 [95% CI: 0.302, 0.506], $P<0.0001$). In other words, on average, the net retention of calcium was 40.4% (95% CI: 30.2–50.6%). However, the residual heterogeneity was very large ($I^2 = 86.18\%$, $P<0.0001$) (**Figure 2**).

Children >12 mo.

The existing data was insufficient to perform meta-regression in children >12 mo.

Discussion

Balance studies provide a controlled and comprehensive understanding of calcium metabolism in response to various concentrations of calcium intake. To our knowledge, this is the first systematic review on calcium balance studies that will be used to inform calcium requirements in infants and children aged 0–4 y set by the FAO/WHO. The 15 mass balance studies and 8 isotope studies included in this systematic review provide insight on calcium absorption, retention, and losses in infants and young children consuming calcium in various quantities and from various sources. Overall, the included mass balance studies suggest the nutrient content of infant feedings may negatively (e.g. fatty acid structure and composition) or positively (e.g. carbohydrate source) influence calcium balance. The included isotopic

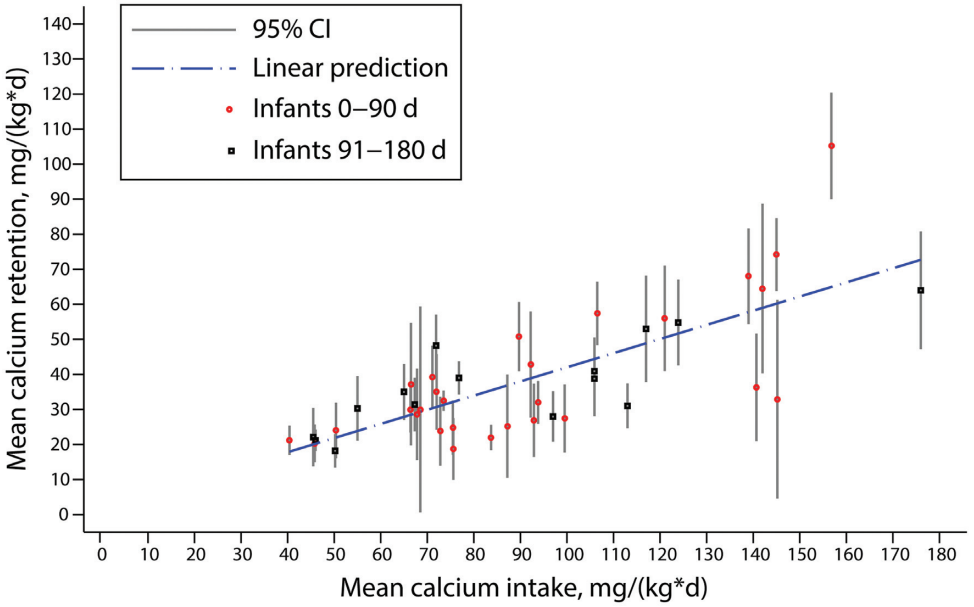

FIGURE 2 Random-effects meta-regression of the relation between daily mean calcium intake and retention concentrations in infants aged 0–6 mo.

studies suggest that specified calcium intake ranges (240 to 400 mg/d) may result in optimal calcium absorption with minimized calcium loss. Additionally, findings suggest that similar calcium intakes from human milk, compared with formula or solid foods, may lead to greater absorption and retention efficacy. Of note, inherent differences in the composition of human milk relative to formula, including immunological factors and other bioactive compounds, limit comparability based on calcium intake alone. Additionally, although calcium intake from human milk is generally on the lower end of the 240–400 mg/d range, there is no current data to support the benefit of achieving a higher bone mass using formula, than that of infants fed human milk (37). Furthermore, the WHO recognizes that human milk provides sufficient calcium to support bone growth from 0 to 6 mo. Therefore, future balance studies on human milk should be used to set the standard target for infant formulas (37).

Despite the findings from balance studies, the strength of evidence from the reviewed studies is low and limitations exist. As discussed, the mass balance studies included in this review were designed to assess how compositional differences in formula affect calcium loss, absorption, and/or retention in young infants (aged 0–180 d). The available isotopic studies included infants (aged 0–11 mo) and young toddlers (aged 1–3 y) and were designed to quantify calcium balance following controlled nutrient feedings. Overall, of the 23 balance studies included, only 2 studies assessed infants beyond the age of 1 y. Most studies included formula as the primary intervention, yet large nutrient variability existed across the formulations given. Few studies assessed calcium balance in response to variations in calcium dose. Taken together, constraints on subject age, differences in infant feedings, and restricted doses of calcium consumed limit the comparability of findings across studies.

Consideration of mineral loss is a critical component in determining calcium balance and needs. Routes of loss may vary but are typically unique to the mineral. For calcium, it is acknowledged that excretory pathways primarily lie in the urine and feces. Other bodily fluids and tissue, such as sweat and mucosal cells, can further contribute to total calcium loss (38). Calcium excreted in the feces is comprised of both unabsorbed dietary calcium and calcium secretions from the digestive system (e.g. saliva, gastric and pancreatic juices, bile), the latter of which is referred to as endogenous fecal calcium (30, 38). Although it is crucial to measure endogenous calcium loss to determine net absorption, measuring such losses cannot be done in a mass balance design (39), as the 2 forms of fecal calcium are not readily distinguishable (30). In this review, 15 out of the 23 included studies were described as mass balance studies. As such, complete balance measures (e.g. endogenous fecal loss) was not possible by design, which limits the interpretation of findings from these studies. Out of the 8 isotopic studies included in this review, 4 studies reported endogenous fecal losses. Of these, only 2 studies directly measured endogenous loss. Balance data on dermal calcium loss (e.g. sweat) is notably absent in infants and young children. Historically, dermal loss data is extrapolated from adults (40) for quantifying calcium accretion in children. Although direct measures of dermal calcium loss in infants and young children (aged 0–9 y) would be the ideal approach, measuring such losses in these age groups may be impractical, considering the conditions necessary to induce sweating and perform collection (e.g.

skin washing and weighing, use of cotton suits and skin patches over multiple days) (40). As a result, some degree of estimation from adolescents or adults may be necessary.

Studying and quantifying nutrient needs poses numerous challenges, one of which is designing controlled feedings where the nutrient in question can be isolated from all possible dietary and extraneous confounding factors. Nutrition research has acknowledged the importance of studying the combined effect of nutrients on health, as individuals do not consume single nutrients or specific foods in isolation, and nutrition-related disease is likely linked to the synergistic effects of multiple dietary components (41, 42). Assessing nutrient needs in younger populations, however, should involve a controlled, single-nutrient approach, as the focus is to optimize long-term health outcomes (e.g. bone accretion and growth) rather than reduce the risk for disease. Optimizing such outcomes in children requires an understanding of how these nutrients, individually, confer their benefits across early life stages. In this review, the effects of calcium could only be isolated in 3 out of the 23 included studies. An additional 3 studies (20, 29, 34) were described as single-arm interventions and were not included in this assessment. Overall, most studies with multiple comparators could not isolate the effects of calcium. Thus, interpretation of calcium balance outcomes from these studies is difficult, as calcium accretion may not depend on calcium intake alone but on the variability of other nutrients within the dietary feedings given. Studying calcium intake in isolation across the life stage would provide a stronger evidence base for directly linking calcium intake on bone accretion and growth.

The interactions between nutrients and the alteration of mutual requirements based on such interactions are commonplace in the study of nutritional needs. For calcium, there is an inherent lack of efficient conservation mechanisms in humans; thus, this nutrient is particularly sensitive to various nutrient-nutrient interactions (43). Excess consumption of sodium, for example, may lead to excess urinary calcium loss, as both nutrients share a common pathway for resorption in the kidney, whereby increased filtration of one mineral leads to excess loss of the other (43). In this systematic review, numerous mass balance studies in infants have demonstrated that fatty-acid composition (PA) negatively affects calcium absorption, as unabsorbed PA has the tendency to complex with calcium and form insoluble calcium soaps (16, 18, 25). Despite the compelling evidence on the relation between fatty acid intake on calcium balance reported, there are a limited number of studies in younger populations (0–9 y) assessing the effects of mineral, vitamin, and macronutrient consumption on calcium balance. Ideally, including studies where inhibitory or enhanced calcium-nutrient interactions have been identified could strengthen the quantification of calcium needs across the lifespan.

Future directions

Design of balance studies.

Based on the low strength of available evidence for this systematic review, and the variability and gaps among included studies, the following future directions may help guide the design and implementation of calcium balance studies in younger populations.

1. The quantity of calcium balance studies in infants and young children are limited.
   a. No mass balance studies were reported for the age range of 6 mo to 3 y.
   b. No isotope studies were reported for the age range of 91 to 180 d.

   Growth rates vary considerably from birth through childhood (38). Therefore, extrapolating data from older or younger age groups, even within pediatric populations, may not provide accurate estimates of calcium needs. Therefore, future work should focus on studying the above-mentioned age groups.
2. Studies using *larger sample sizes*, designed to *isolate the effects of calcium* (e.g. the only difference between intervention and control group is in the amount of dietary calcium), and/or *using a range of calcium doses* to demonstrate a dose-response effect will provide greater confidence in the relation between intake and relevant measures.
3. For greater comparability across studies, standardized units (e.g. mg/[kg∗d]) and dietary sources (e.g. formula or human milk in infants) are necessary.
4. *Direct measures of endogenous fecal losses* rather than estimates are needed to determine accurate measures of calcium retention and accretion.
5. Future balance studies should further assess *the interactions between calcium and other nutrients* (e.g. iron, magnesium, zinc, sodium, vitamin D, fatty acids, protein).

Determination of calcium needs.

Decades of work on mass balance and isotope studies have characterized calcium absorption, retention, and loss to understand and assess calcium metabolism in healthy pediatric populations (44, 45). Much of this available balance data has served as valuable evidence for establishing DRIs in young children (38). It is compelling to recommend the exclusive use of balance studies to determine an optimal calcium intake to meet needs across the first years of life. However, the sole use of balance studies may not be practical, given the identified gaps in current evidence and general limitations in infantile balance design (e.g. difficulties in measuring endogenous and dermal calcium loss and cross-sectional nature of measures), the latter of which may not be easily rectified with additional studies alone.

Alternatively, data from balance studies could act as complementary evidence to surrogate endpoints of bone mineral density and content, serum values, and clinical outcomes (e.g. rickets) for determining calcium needs. This approach, commonly referred to as the factorial method/calculation, uses both balance measures (e.g. calcium fractional absorption and losses) and whole-bone mineral density data (as measured by DXA) to determine average calcium retention and skeletal accretion, respectively (38, 44) (**Figure 3**).

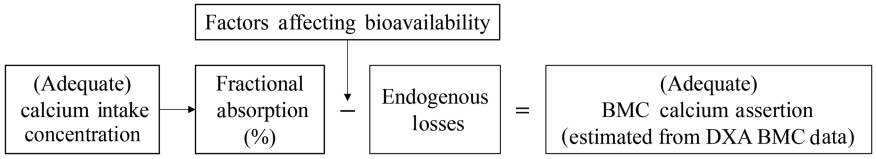

FIGURE 3 Theoretical framework for computing calcium needs using the factorial approach. This framework assumes the vitamin D status is adequate. BMC, bone mineral content; DXA, dual-energy x-ray absorptiometry.

Although there are limitations to using factorial calculations (e.g. variability in data across studies) (44), the combined use of balance data and surrogate endpoints provides a sound strategy for establishing calcium needs in age groups or populations where data may be limited, such as infants and young children. In further support of incorporating bone-related outcomes, the Institute of Medicine (IOM) Committee tasked with updating calcium DRIs in 2010 reviewed existing evidence to validate indicators of calcium adequacy; bone health was found to satisfy the criteria as an indicator for calcium needs (e.g. causality was established with sufficient dose-response evidence) (46). Furthermore, the committee concluded that during periods of bone calcium accretion (e.g. growth), bone calcium accretion/retention is informative when combined with a factorial approach. These findings continue to highlight the use of complementary evidence (e.g. DXA and balance study data) for understanding needs and setting requirements for specific nutrients or populations.

Future work determining calcium needs in infants and young children would greatly benefit from well-designed balance studies that measure all pertinent outcomes (intake, losses [endogenous and dermal], absorption, and retention) to model skeletal change. However, from a practical standpoint, the use of measured or extrapolated balance outcomes, along with surrogate endpoints, should continue to be used in factorial calculations to estimate calcium needs in infants and young children.

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References

1. Baker SS, Cochran WJ, Flores CA, Georgieff MK, Jacobson MS, Jaksic T, Krebs NF. American Academy of Pediatrics. Committee on Nutrition. Calcium requirements of infants, children, and adolescents. Pediatrics 1999;104(5 Pt 1):1152–7.
2. Greer FR, Krebs NF, American Academy of Pediatrics Committee on Nutrition. Optimizing bone health and calcium intakes of infants, children, and adolescents. Pediatrics 2006;117(2):578–85.
3. Abrams SA. Calcium turnover and nutrition through the life cycle. Proc Nutr Soc 2001;60(2):283–9.
4. Abrams SA. Clinical studies of mineral metabolism in children using stable isotopes. J Pediatr Gastroenterol Nutr 1994;19(2):151–163.
5. Chung M, Ruan M, Cara KC, Yao Q, Penkert LP, Chen J. Vitamin D and calcium in children 0–36 months: a scoping review of health outcomes. J Am Coll Nutr 2020;[in press].
6. Institute of Medicine. In: Eden J, Levit L, Berg A, Morton S, eds. Finding What Works in Health Care: Standards for Systematic Reviews. Washington (DC): 2011.
7. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6(7):e1000097.
8. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan – a web and mobile app for systematic reviews. Systematic Reviews 2016;5(1):210.
9. National Institute of Allergy and Infectious Disease "What is a mechanistic study?" 2020. https://www.niaid.nih.gov/grants-contracts/what-mechanistic-study#:~:text=NIH%20deflnes%20mechanistic%20studies%20as,of%20action%20of%20an%20intervention
10. Guyatt GH, Oxman AD, Kunz R, Vist GE, Falck-Ytter Y, Schunemann HJ, Group GW. What is "quality of evidence" and why is it important to clinicians? BMJ 2008;336(7651):995998.
11. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, Schunemann HJ, Group GW. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 2008;336(7650):924–6.
12. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, Norris S, Falck-Ytter Y, Glasziou P, DeBeer H, et al. GRADE guidelines: 1. Introduction – GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64(4):383–94.
13. Barltrop D, Mole RH, Sutton A. Absorption and endogenous faecal excretion of calcium by low birthweight infants on feeds with varying contents of calcium and phosphate. Arch Dis Child 1977;52(1):41–9.
14. Manz F, Diekmann L, Stock GJ. Effect of calcium supplementation on calcium and phosphorus balance and renal net acid excretion in preterm infants fed a standard formula. Acta Paediatr 1989;78(4):525–31.
15. DeVizia B, Fomon SJ, Nelson SE, Edwards BE, Ziegler EE. Effect of dietary calcium on metabolic balance of normal infants. Pediatr Res 1985;19(8):800–6.
16. Souza CO, Leite MEQ, Lasekan J, Baggs G, Pinho LS, Druzian JI, Ribeiro TCM, Mattos AP, Menezes-Filho JA, Costa-Ribeiro H. Milk protein-based formulas containing different oils affect fatty acids balance in term infants: a randomized blinded crossover clinical trial. Lipids in Health and Disease 2017;16(1):78.
17. Ziegler EE, Fomon SJ. Lactose enhances mineral absorption in infancy. J Pediatr Gastroenterol Nutr 1983;2(2):288–94.
18. Ostrom KM, Borschel MW, Westcott JE, Richardson KS, Krebs NF. Lower calcium absorption in infants fed casein hydrolysate- and soy protein-based infant formulas containing palm olein versus formulas without palm olein. J Am Coll Nutr 2002;21(6):564–9.

19. Fomon SJ, Owen GM, Jensen RL, Thomas LN. Calcium and phosphorus balance studies with normal full term infants fed pooled human milk or various formulas. Am J Clin Nutr 1963;12(5):346–57.
20. Clemente Yago F, Cortes Castell E, Moya Benavent M, Ballester Galindo I, Herrero Galiana A, Campello Oltra MJ. Mineral metabolism of calcium, magnesium and phosphorus in the normal newborn infant. An Esp Pediatr 1989;31(6):533–6.
21. Moya M, Domenech E. Role of calcium-phosphate ratio of milk formulae on calcium balance in low weight infants during the first three days of life. Pediatr Res 1982;16(8):675–81.
22. Barnes LA, Morrow G, 3rd, Silverio J, Finnegan LP, Heitman SE. Calcium and fat absorption from infant formulas with different fat blends. Pediatrics 1974;54(2):217–21.
23. Carnielli VP, Luijendijk IHT, Van Goudoever JB, Sulkers EJ, Boerlage AA, Degenhart HJ, Sauer PJJ. Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. Journal of Pediatric Gastroenterology & Nutrition 1996;23(5):553–60.
24. Hanna FM, Navarrete DA, Hsu FA. Calcium fatty acid absorption in term infants fed human milk and prepared formulas simulating human milk. Pediatrics 1970;45(2):216–24.
25. Nelson SE, Frantz JA, Ziegler EE. Absorption of fat and calcium by infants fed a milk-based formula containing palm olein. J Am Coll Nutr 1998;17(4):327–32.
26. Moya M, Cortes E, Juste M, Vera A, Gonzalez de Dios J. Absorptive pattern of individual fatty acids and total fat in full term babies. Its stability in the absence of lactose. An Esp Pediatr 1998;48(5): 515–21.
27. Zannino L, Bini P, Moretto R, Viano I, Ferro R, Crosato M. Calcium and fat balance in low birth weight newborn infants fed an adapted formula and mother's milk. Minerva Pediatr 1983;35(4):193–9.
28. Leite ME, Lasekan J, Baggs G, Ribeiro T, Menezes-Filho J, Pontes M, Druzian J, Barreto DL, de Souza CO, Mattos Â, et al. Calcium and fat metabolic balance, and gastrointestinal tolerance in term infants fed milk-based formulas with and without palm olein and palm kernel oils: a randomized blinded crossover study. BMC Pediatrics 2013;13(1):215.
29. Lynch MF, Griffin IJ, Hawthorne KM, Chen Z, Hamzo M, Abrams SA. Calcium balance in 1–4-y-old children. Am J Clin Nutr 2007;85(3): 750–4.
30. Abrams SA, Sidbury JB, Muenzer J, Esteban NV, Vieira NE, Yergey AL. Stable isotopic measurement of endogenous fecal calcium excretion in children. J Pediatr Gastroenterol Nutr 1991;12(4):469–73.
31. Hicks PD, Hawthorne KM, Berseth CL, Marunycz JD, Heubi JE, Abrams SA. Total calcium absorption is similar from infant formulas with and without prebiotics and exceeds that in human milk-fed infants. BMC Pediatrics 2012;12(1):118.
32. Abrams SA, Griffin IJ, Davila PM. Calcium and zinc absorption from lactose-containing and lactose-free infant formulas. Am J Clin Nutr 2002;76(2):442–6.
33. Lifschitz CH, Abrams SA. Addition of rice cereal to formula does not impair mineral bioavailability. Journal of Pediatric Gastroenterology & Nutrition 1998;26(2):175–8.
34. Abrams SA, Wen J, Stuff JE. Absorption of calcium, zinc, and iron from breast milk by five- to seven-month-old infants. Pediatr Res 1997;41(3):384–90.
35. Hillman LS, Tack E, Covell DG, Vieira NE, Yergey AL. Measurement of true calcium absorption in premature infants using intravenous 46Ca and oral 44Ca. Pediatr Res 1988;23(6):589–94.
36. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Method 2014;14(1):135.
37. Abrams SA. Building bones in babies: can and should we exceed the human milk-fed infant's rate of bone calcium accretion? Nutr Rev 2006;64(11):487–94.
38. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press Washington (DC): 2011.
39. Heaney RP, Skillman TG. Secretion and excretion of calcium by the human gastrointestinal tract. J Lab Clin Med 1964;64:29–41.
40. European Food Safety Authority. Scientific Opinion on Dietary Reference Values for calcium. EFSA Journal 2015;13(5):4101.
41. Tapsell LC, Neale EP, Satija A, Hu FB. Foods, nutrients, and dietary patterns: interconnections and implications for dietary guidelines. Adv Nutr 2016;7(3):445–54.
42. Cespedes EM, Hu FB. Dietary patterns: from nutritional epidemiologic analysis to national guidelines. Am J Clin Nutr 2015;101(5): 899–900.
43. Heaney RP. Chapter 79: Calcium. Edition ed. In: Bilezikian P, Raisz L, Martin J, eds. Principles of Bone Biology(Third Edition): Academic Press, Cambridge (MA) 2008, 1697–710.
44. O'Brien KO, Abrams SA. Using stable isotope tracers to study bone metabolism in children. J Physiol 2019;597(5):1311–9.
45. Abrams SA. Calcium absorption in infants and small children: methods of determination and recent findings. Nutrients 2010;2(4): 474–80.
46. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. The Journal of Clinical Endocrinology & Metabolism 2011;96(1): 53–8.