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Impact of nutritional supplementation on environmental enteric dysfunction (EED) in children living in rural areas: a systematic review

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HIGHLIGHTS

- The lack of impact from multiple interventions toward undernutrition reflects a strong reason to believe that EED is the missing link that sustains undernutrition in low-to-middle-income countries (LMICs).
- There is currently no protocol for the diagnosis and treatment of EED, hence EED is widely believed to be highly prevalent and underdiagnosed in LMICs.
- To our knowledge, this is the first systematic review to study the impact of nutritional interventions on EED.
- The systematic review illustrates that nutritional interventions have a minimal impact on EED biomarkers and linear growth and reflects the importance of understanding better the mechanisms causing EED and its consequences.

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ABSTRACT – Background – A staggering 99% of infant undernutrition mortality comes from Sub-Saharan Africa and South Asia. Despite multiple interventions focusing on nutrition adequacy, 2.7 million children worldwide remain associated with undernutrition-related mortality. The lack of impact from multiple interventions toward undernutrition reflects a strong reason to believe that EED is the missing link that sustains undernutrition in low-to-middle-income countries (LMICs). EED is a sub-clinical condition caused by repeated oral enteropathogenic and non-pathogenic fecal microbes exposure that causes intestinal villous malformation, multi-omics changes, chronic intestinal and systemic inflammation, and gut dysbiosis. EED impacts the absorptive capacity and the integrity of the gut, causing a cycle of undernutrition in children. There is currently no protocol for the diagnosis and treatment of EED, hence EED is widely believed to be highly prevalent and underdiagnosed in LMICs. **Objective –** To our knowledge, this is the first systematic review to study the impact of nutritional interventions on EED. Previous studies yielded inconsistent results, hence the synthesis of this information is essential in attaining a deeper understanding of EED to formulate new targets of intervention against child undernutrition. **Methods –** This systematic review is registered to PROSPERO (CRD42022363157) in accordance to PRISMA, using keywords referring to nutrient supplementation, EED, and child growth failure. **Results –** Eleven articles were eligible for review, comprising randomized controlled trials performed mainly in the African continent, with a total of 5689 healthy children eligible for analysis. **Conclusion –** The systematic review illustrates that nutritional interventions have a minimal impact on EED biomarkers and linear growth and reflects the importance of understanding better the mechanisms causing EED and its consequences. It appears that the anabolic contribution of nutrition intervention to child growth is negated by EED.

Keywords – Gastrointestinal absorption; intestinal diseases; child malnutrition, dietary supplements; gastrointestinal microbiome; inflammation; environment, preventive medicine and public health.

INTRODUCTION

Undernutrition is a persistent public health challenge in low-to-middle-income countries (LMICs). The Joint Malnutrition Estimate revealed there is a significant lack of progress in meeting the 2025 target by the World Health Assembly and the 2030 Sustainable Development Goals (SDG)[1]. Undernutrition comprises stunting and wasting. The number of stunting has progressively declined in the last 20 years, however, at this rate, it would not be sufficient to meet the 2030 SDG. On the other hand, the incidence of wasting in children has persisted for the past 20 years, hence a reversal trajectory is needed to achieve the 2030 SDG[1]. Stunting has declined worldwide except in Africa, while more than 50% of wasted children live in South Asia. Undernutrition is responsible for 45% of mortality, and a staggering 99% of infant undernutrition mortality comes from the LMICs in Sub-Saharan Africa and South Asia[2]. In addition, these numbers have not reflected the impact of the COVID-19 pandemic. Undernutrition has serious consequences that impact human capital and robs children of their full physical and cognitive potential[3].

Undernutrition is a multifactorial disease. The focus on interventions should be multi-sectoral and include all undernutrition aspects, while it is also important to note that adequate maternal and infant nutrition is identified as the primary determinant[4]. The first 1000 days represent a critical period in preventing all forms of undernutrition, especially after the age of 6 months, where complementary feeding is required to optimize infant growth and development[4,5]. Despite multiple interventions focusing on nutrition adequacy, at present time, around 2.7 million children worldwide are associated with undernutrition-related mortality, around 250 million children are at risk for stunting due to chronic undernutrition, while the rates of wasting are progressively increasing[1,5] The lack of impact from multiple interventions toward undernutrition reflects a strong reason to believe that environmental enteric dysfunction (EED) may be the missing link that sustains and amplifies undernutrition in low-to-middle income countries (LMICs).

Recent studies have discovered that EED is a complex, sub-clinical condition believed to be caused by environmental drivers such as repeated oral enteropathogenic and non-pathogenic fecal microbes' exposure, as well as impaired gut microbiome composition[6]. This leads to severe villous malformation, multi-omics changes, chronic intestinal and systemic inflammation and gut dysbiosis. EED significantly impacts the absorptive capacity and the integrity of the gut and consequently causes a cycle of undernutrition in children[7]. There is currently no protocol for the diagnosis and treatment of EED[8]. This is why EED is widely believed to be highly prevalent and underdiagnosed in LMICs[6]. The combination of chronic inflammation of the gut, gut dysbiosis, gut histopathologic damage, and multi-omics changes decreases the gut's ability to utilize the nutrition for optimal growth and development[9]. Hence, we postulate that the administration of nutrition that improves gut integrity, function, and structure, and that has anti-inflammatory capability may positively impact EED and consequently child growth, especially when combined with water, sanitation, and hygiene (WASH) interventions. Recent trials used zinc[10-12], breast milk proteins[13], micronutrient powders[14], common beans[15,16], eggs, and lipid-based supplements[17,18] have been used to ameliorate EED in children, but the results were inconsistent. We believe the synthesis of this information is essential in attaining a deeper understanding of EED and formulating new targets of intervention in the future against child undernutrition.

METHODS

Registration of systematic review and search strategy

This systematic review is registered on PROSPERO with registration number: CRD42022363157. The search strategy was in accordance with the Preferred Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines using keywords referring to nutrient supplementation, EED, and child growth failure (TABLE 1). Using the PICOS principle, the inclusion criteria were: 1) population: infants aged 0–36 months living in low-middle income countries, 2) intervention: the use of nutritional supplementation with the aim of ameliorating EED, 3) comparison: the use of control groups, 4) primary outcome change

TABLE 1. Search strategy.

Database	Search Terms	Hits
PUBMED	(Supplementation OR intervention) AND (environmental enteropathy OR environmental enteric dysfunction) AND (stunting OR malnutrition OR growth failure)	51
Cochrane	(Supplementation OR intervention) AND (environmental enteropathy OR environmental enteric dysfunction) AND (stunting OR malnutrition OR growth failure)	59
Total		110

in EED biomarkers and secondary outcome: linear growth. 5) study design: randomized-controlled trials and/or meta-analyses were considered eligible. Linear growth was measured as a growth outcome in this study because it is the chronic manifestation of malnutrition. To restrict our literature search, we focused our search on articles in English, published between 2013 to 2023, and trials with human participants (FIGURE 1). The search was conducted electronically on the 13th of November 2023.

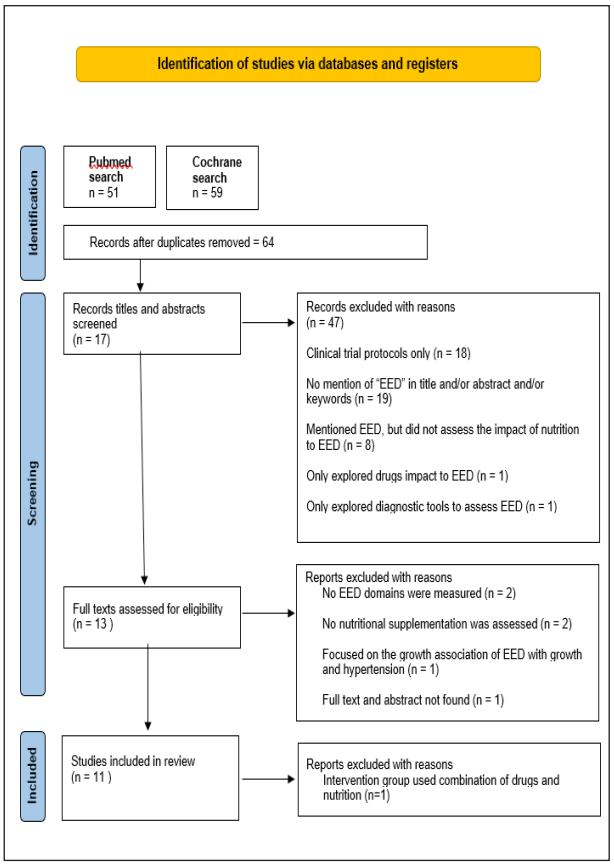

FIGURE 1. Search strategy.

Data extraction

All identified articles were downloaded, and all duplicates were excluded. The title and abstract of each article were checked for relevance. Then the inclusion criteria were inserted to filter and screen the available reports. The remaining eligible articles were assessed for full-text accessibility. There were two reviewers (RAR, AY) who performed the study selection and extracted information from the eligible studies. Any disagreements and discrepancies were decided by discussion. To extract information from the eligible studies, we used a standardized data abstraction form which comprises of study design, study population, sample size, geographical location, type of nutrient supplement used, duration of intervention, and method of administering the intervention, measured EED domains and *P*-values when possible.

Study quality assessment

The two reviewers who performed the study selection and extraction also performed the risk of bias assessment of the included studies by utilizing the seven domains as cited from the Cochrane Handbook for randomized controlled trials. A study is considered highly susceptible to bias if there were two or more domains that scored high susceptibility, when there were three unclear domains or when there was one highly susceptible domain with two domains with unclear risk.

RESULTS

Description of the included studies

The synthesis of reports and intervention characteristics were summarized in TABLE 2. The included reports were 11 randomized controlled trials (RCT). Six were double-blinded, two were cluster RCTs, one was single-blinded, and one was a non-blinded RCT. Several rural regions were represented: Malawi (n=6), Laos (n=2), Nicaragua (n=1), Mali (n=1), Zimbabwe (n=1), and Bangladesh (n=1). The studies included 5689 healthy children, mostly of African ethnicity. The most represented age group was 6–35 months old infants. The duration of the included studies ranged from 48 days to 18 months. The used nutrient supplementation was Zinc (n=3), multiple micronutrient powders (MNP) (n=4), Fish oil (n=1), small quantity

TABLE 2. Summary of findings.

Name of Study	Number of subjects	Age range	Study Design	Duration	Nutrition Intervention	Effect on EED	Effect on Linear Growth	Association Between EED and Linear Growth	Risk of Bias
Cheng et al.[13]	214	12–23 months	RCT	16 weeks	Lactoferrin and Lysozyme	Significant change of L% over 8 weeks when compared to the control group (0.23% vs 0.14% *P*=0.04). But not sustained after 16 weeks of study (0.16% vs 0.11% *P*=0.17)	After 16 weeks of intervention, there was no significant change in linear growth when compared to the control (0.028 mm/d and 0.030 mm/d respectively; *P*=0.12) I-		Low
Agapova et al.[16]	331	12–23 months	RCT	48 weeks	Cowpea and common bean	The dual sugar absorption test, measuring %L and L:M, were not significantly different between the intervention and control groups after 48 weeks. However, only the common bean group had a significant effect estimate on %L (–0.073396, SD 0.029887; *P*=0.0073)	Change in LAZ score after 48 weeks of intervention was not significant compared to the control group. The changes in LAZ score was 0.14±0.49, -0.11±0.54 (*P*=0.67) and -0.07±0.51 (*P*=0.31) in the control, cowpea and common bean group respectively after 48 weeks	The parameter was not studied	Low
Wessells et al.[10]	359	6–23 months	RCT	36 weeks	Zinc and MNP	The intervention after 36 weeks did not have a significant effect estimate on citrulline, kynurenine, tryptophan and KT ratio	After 36 weeks, there was no significant difference of LAZ score between the intervention and control groups. The LAZ score at the end of the study were: preventive zinc group –1.93±0.97, micronutrient powder group –1.94±1.00, theraupetic zinc group –1.95±1.02 and control group –1.93±1.00 (*P*=0.58) → obtained from parent trial (Balffour 2019)	Citrulline, Kynurenine, Tryptophan and KT ratio was not associated with changes in LAZ score after 36 weeks	Low
Hinnouho et al[12]	720	6-23 months	RCT	36 weeks	Zinc and MNP	The intervention had no significant impact on endline MPO, NEO and CAL after 36 weeks	No data	MPO, but not NEO and CAL, was associated with change in LAZ score after 16–20 weeks ((–0.029, (–0.054, –0.003); *P*= 0.027)). However, after 32–40 weeks of intervention, MPO, NEO and CAL were not associated with the change in LAZ score	Low
Bierut et al.[20]	275	9 months	RCT	36 weeks	Bovine Colostrum and Egg	Using L%<0.20 as a cut-off for EED, after 36 months of intervention, the study found resolution of EED in 50% (10/20 subjects) of patients, while in the control group EED resolution was found in 13.3% (2/15 subjects) of patients (*P*<0.05)	After 36 weeks of intervention, there was a significant difference of change in LAZ score between the intervention and control group (0.12 z-scores, *P*=0.0011). However, the study observed the two groups until 5 months post-intervention, and at that point the difference of change of LAZ score between the two groups was no longer significant. On the other hand, 5 months post intervention, the prevalence of stunting in the intervention group was significantly smaller (47 ((35)); RR 0.70; CI95%=0.52–0.94; *P*=0.018)) compared to the control group (62 ((49))	The parameter was not studied	Low
Ryan et al.[11]	222	12–36 months	RCT	48 days	Zinc	Change in L:M and the prevalence of subjects with L:M >0.30 was significantly lower in the zinc group compared to placebo (*P*<0.02 and *P*<0.04 respectively). However, there was no significant difference between groups for mannitol excretion after the intervention	There was no significant change in length (cm) between groups after 48 days. The change in length was 1.1±0.9 in the zinc group and 1.1±1.6 in the placebo group	The parameter was not studied	Low
Lin et al.[18]	1783	recruited since birth	Cluster RCT	18 months	SQ-LNS	NEO was lower in the intervention group at 3 months and 14 months. However, by 28 months there were no differences in NEO in between all groups At 3 and 14 months, there was no difference in MPO in between groups, however by 28 months, MPO was higher in the WASH group and nutrition group compared to controls At 3 months, Lactulose was lower in all intervention groups compared to the control group. However, at 14 months there were no differences in all groups. And at 28 months, lactulose was higher in the WASH group compared to controls At 3 months, mannitol was lower in all intervention groups compared to control groups. However, at 14 and s8 months, there were no difference in mannitol in all groups L:M at 3 months was no different in all groups. At 28 months, L:M was higher in all intervention groups compared to control group There was no difference in AAT in all groups at 3, 14 and 28 months	Linear growth was an assessed parameter in the parent trial; however, the results are not yet published	Association between Linear growth and EED was an assessed parameter in the parent trial, however, the results are not yet published	Low
Smith et al.[14]	230	12-35 months	RCT	24 weeks	MNP and Fish Oil	L:M after 24 weeks significantly improved from baseline in intervention groups and control group (*P*<0.05), however there was no significant difference between the intervention groups and the control group. In terms of L% excreted. there was no significant difference between groups after 24 weeks	The parameter was not studied	The parameter was not studied	Low
Gough et al.[17]	1169	recruited since birth	Cluster RCT	18 months	SQ-LNS	There was no consistent evidence that the intervention interacted with any biomarker in this study Stool biomarkers of inflammation decreased at the end of the study, MPO was consistent from months 1–6 7518 ng/mL (95%CI:7014–8059) then rapidly declined to 2825ng/mL (95%CI:2669–2991) at 18 months. NEO increased in the first six months (from 980 nmol/L (95%CI:908–1057) to 1283 nmol/L (95%CI:1230–1337)) then decreased at 18 months to 383 nmol/L (95%CI:362–407) Intestinal damage biomarkers had no discernible pattern. Stool REG-1β increased in the first 12 months from 40.6µg/mL (95%CI:36.4–45.1) to 154.6 µg/mL (95%CI:145.5–164.2) then decreased to 132.9µg/mL (95%CI:123.1–143.6). Plasma citrulline increased from 2744 ng/mL (95%CI:2658–2834) at month 6 to 3150 ng/mL (95%CI:3075–3227) by 12 months. I-FABP had a U-shaped pattern, starting with a decline in the first 6 months from 1031 pg/mL (95%CI:993–1021) to 913 pg/mL (95%CI:892–934, then increased to 1198 pg/mL (95%CI:1160–1237) at 18 months Intestinal permeability biomarkers overall decreased during the 18 month follow-up, showed by AAT from 0.47 mg/mL (95%CI:0.44–0.51) to 0.24 mg/mL (95%CI:0.22–0.25) at 18 months and urinary LM ratio declined from 0.84 (95%CI:0.71–0.8) at 6 months to 0.49 (95%CI:0.43,0.53) at 18 month Plasma systemic inflammation markers showed opposite patterns, where CRP steadily increased rom 0.63mg/L (95%CI:0.55–0.71) to 1.28mg/L (95%CI:1.14–1.43) in 18 months, KTR declined from 64.8 (95%CI: 62.2,67.6) at 1 month to 44.9 (95%CI: 43.6,46.3) between 6–12 months Marker of microbial translocation, Soluble CD14 increased from 769,192 pg/mL (95%CI: 741,069–798,383) to 1,334,175 pg/mL (95%CI: 1,292,950–1,3767,16) in 18 months Meanwhile IGF-1 decreased from 27.1 ng/mL (26.0–28.2) to 17.9 ng/mL at 12 months (17.4–18.5)	The parameter was not studied	The parameter was not studied	Low
Vilander et al.[21]	95	6 months	RCT	6 months	Rice bran	There was no significant difference of total fecal sIgA between rice bran and control groups after intervention. Total fecal sIgA is significantly correlated with NEO, AAT, CAL and MPO over a 6 month period of study. Malian rice bran groups observed significantly decreased EED scores compared to control group, but this was not observed in the Nicaragua rice-bran group. NEO, AAT, CAL and MPO were not correlated with EED scores	The parameter was not studied	The parameter was not studied	Low
Stephenson et al.[15]	291	6 months	RCT	6 months	Cowpea and common bean	After 6 months of intervention, there was no significant difference of L:M, Lactulose excretion and mannitol secretion compared to the control group	The cowpea group observed a significant decrease in LAZ score ((-0.14±0.52 (111); *P*=0.048) compared to common bean and placebo in the first 3 months of the study. However no significant change of LAZ score was observed in all groups after 6 months of intervention. At the end of the study, the change of LAZ score was -0.13±0.68 (103), -0.17±0.66 (106), -0.14±0.60 (98) for cowpea, common bean and the control group respectively (*P*>0.05)	The parameter was not studied	Low

*Risk of bias of included RCTs were critically appraised using the Revised Cochrane risk-of-bias tools for randomized trials.

lipid-based nutrients (SQ-LNS) (n=2), Lactoferrin added with lysozyme (n=1), bovine colostrum and egg powder (n=1), cowpea (n=2), common beans (n=2) and rice bran (n=1). In two studies, (WASH) interventions were assessed in combination and in separately with nutritional supplementation[17,18].

Impact of nutritional intervention to EED biomarkers

The impact of nutrition supplementation was assessed against EED biomarkers in all included studies (n=11). The EED biomarkers can be classified into several domains[19]: intestinal inflammation (serum IgA, fecal myeloperoxidase (MPO), neopterin (NEO) and calprotectin (CAL)) (n=4), intestinal damage and repair (regenerating protein 1 beta (REG1B), intestinal fatty acid binding protein (I-FABP), plasma citrulline) (n=3), growth axis (insulin-like growth factor 1 (IGF-1)) (n=1), intestinal permeability (lactulose to mannitol ratio (L:M ratio), percentage of lactulose excreted (L%) (n=8) and microbial translocation (C-reactive protein (CRP), Kynurenine to tryptophan ratio (K:T ratio), Alpha-1 acid glycoprotein (AGP), and soluble CD14) (n=2).

The impact of nutritional intervention against EED yielded inconsistent results. Six studies demonstrated that the intervention had an impact on EED biomarkers. It appears that the EED biomarkers representing gut permeability were the most affected. Common bean supplementation showed a significant effect estimate on L% compared to the control group[16]. A zinc supplementation study found that there was a significant decrease in L:M ratio in the intervention group compared to the control group[11]. Bovine colostrum and egg supplementation decreased the prevalence of EED (using the EED cutoff L:M >0.2) significantly compared to the control group[20]. Rice-bran supplementation caused a significant decrease in EED composite score compared to the control group[21]. Interestingly in two trials, nutritional supplementation did not have a sustainable impact on EED biomarkers. In the WASH benefits trial, during the 3rd month NEO, MPO, lactulose and mannitol was lower in the intervention group, but by the end of the study after 28 months, no difference of these biomarkers was seen when compared to the control group[18]. A similar finding, zinc supplementation study found that the intervention decreased L% more significantly than the control group, but this impact was not sustained until the end of the study[13]. There was no observed impact of nutrient supplementation on microbial translocation and intestinal damage and repair and on the growth axis biomarker[10,12,17].

Impact of nutritional intervention on linear growth in children with EED

Six studies evaluated the outcome of linear growth after nutritional supplementation. Four of those reported no impact of supplementation on linear growth among children with EED[10,11,13,16]. On the other hand, study by Bierut et al. observed that Length-to-age Z scores (LAZ scores) decreased in the group that received supplementation as well as in the control group, even though the decrease was significantly less in children in the former group[20]. A similar result was also described by a study by Stephenson et al. which observed that LAZ score decreased in all groups at 9 months of age, with children receiving cowpea experiencing the least decrease. However, at the age of 12 months, the decrease of LAZ in the cowpea group was no longer significant compared to the control group[15]. The correlation between linear growth and EED biomarkers was assessed in two studies with no correlation found at the end of these studies[10,12].

DISCUSSION

We found that nutritional supplementations alone had a limited and non-sustainable impact on EED and linear growth in children with EED. To our knowledge, this is the first systematic review that sheds light on the impact of nutritional administration on children with EED. This surprising lack of impact may be caused by 1) the complexity and multifactorial nature of EED, and 2) EED biomarkers were not sufficiently sensitive.

The impact of nutritional supplementation on EED

Nutrition interventions alone may not have an adequate impact on EED because of their multifactorial and complex nature. Recent studies have uncovered that EED causes histopathological damage[22-24], chronic inflammation[22], gut dysbiosis[25], and multi-omics mechanisms[23], caused by multiple environmental insults[25]. All these aforementioned factors

may influence the level of EED biomarkers in the respective studies and may exacerbate EED despite nutritional supplementation. Recent studies have found core gut transcriptomic changes that include the up-regulation of antimicrobial detoxification, and lymphocyte activation genes, as well as the down-regulation of metabolism and mucin genes in children with EED[23,26]. Other than multi-omics changes, histopathological studies in EED, revealed extensive villous remodelling, epithelial detachment, goblet and Paneth cell depletion, intraepithelial lymphocytic infiltration[22], and impaired amino acid and fatty acid absorption[26]. In combination, the nutrition deficiency, histopathological changes, and multi-omics changes lead to net energy loss and refractory undernutrition, which most likely cannot be solved by food-based interventions alone. This finding supports other studies that warrant multi-sectoral and inter-collaborative efforts in treating EED[17,18].

From our systematic review, we found inconsistent results on the impact of nutrition supplementation against EED biomarkers. This may be caused by the different domains of EED biomarkers are representing. For example, in our systematic review there are three zinc studies, one studied the domain of systemic inflammation[10] (kynurenine, tryptophan, K:T ratio and plasma citrulline), one studied biomarkers of intestinal inflammation[12] (MPO, NEO, CAL and Alpha-1 anti-trypsin (AAT)), and one studied biomarker of gut integrity and permeability[13] (L;M ratio, lactulose and mannitol excretion). We found zinc supplementation significantly improved gut permeability and integrity[13] (by significantly decreasing L:M ratio after intervention), but did not impact other domains of EED[10,12]. This can be reflected by the use of zinc in acute diarrhoea in order to induce reepithelization of the gut, which improves gut integrity. We should consider which domains of EED a certain biomarker is representing, because if the biomarker does not represent the pathway between the intervention and the outcome, then the result would likely be negative. Other included trials in our systematic reviews also discovered that the gut permeability biomarkers were the most affected by nutrition supplementation[13,20], while the EED biomarkers of microbial translocation, gut, and systemic inflammation, intestinal damage, and repair were not affected[17,18]. Different nutritional supplementations may also have different active components that may impact different domains of EED. We highly recommend trials to study EED holistically, by including biomarkers that represent all domains of EED because EED is a complex and multifactorial process.

Unexpectedly, we found in several trials, that nutritional supplementation initially had an impact on improving EED biomarkers, but the impact was not sustained until the end of the trial[13,18]. From our systematic review, a trial using supplementation of Lactoferrin and Lysozyme observed a significant change of L% over 8 weeks when compared to the control group (0.23% vs 0.14% P=0.04)[13]. But it was not sustained after 16 weeks of study (0.16% vs 0.11% P=0.17), a similar pattern was observed in Lin et al., where initially during the 3rd month of intervention NEO, MPO, Lactulose and Mannitol were lower in the intervention group, but by the end of the study after 28 months, no difference of these biomarkers was seen when compared to the control group[18]. This may be caused by increased mobility and dietary diversity of the child and weaning from breastfeeding as they age above 6 months, making these children at greater risk and susceptibility towards continuous enteropathogenic exposure, hence sustaining EED and negating the positive impact of the nutritional supplementation.

Surprisingly, only one study in our systematic review found that nutritional supplementation positively impacts EED biomarkers until the end of the study. The supplementation of bovine colostrum and egg significantly decreased the prevalence of EED (using a cut-off of L:M ratio <0.20 for no EED and L:M ratio >0.20) compared to the control group at the end of the study[20]. This may be caused by the immunoreactive contained in bovine colostrum and egg decreasing chronic gut inflammation[20]. However, this finding needs to be interpreted with caution, because there is currently no standardized cut-off values for any EED biomarkers, including L:M ratio.

It is difficult to ascertain whether the changes of EED biomarkers were caused by nutritional intervention or other environmental insults[27] or the guts adaptive changes to impoverished conditions[19]. This is further complicated by the fact that as of now, there is no clear definition of EED and no published

reference values of EED biomarkers[28]. To our knowledge, there is currently one trial that aims to validate EED biomarkers[8]. Histopathology remains the gold standard in diagnosing EED, however, due to ethical and safety reasons, this is not a practical method, especially in LMICs[8]. Hence most trials have resorted to non-invasive measures of EED biomarkers. A very common method of non-invasive EED measure was the dual-sugar absorption test. This involves the patient receiving a dose of lactulose and mannitol, followed by a timed urine collection. Dual-sugar testing has several advantages as it is non-invasive, able to simultaneously assess two physiologic processes (absorption and permeability) and several studies have shown that dual-sugar absorption tests correlate with linear growth[29], however it is performed with variable subject preparation, data collection, and data representation[29]. Another recently developed diagnostic method was the use of fecal biomarkers of EED such as MPO, AAT and NEO[27]. These fecal biomarkers assess intestinal inflammation which is theoretically activated by translocated microbial products and LPS caused by impaired gut integrity in EED[19]. However, it is important to note that fecal biomarkers were also correlated with other chronic GI diseases and poor WASH practices such as mouthing of contaminated materials, open defecation, food and water contamination, and intestinal parasites[27]. This highlights the urgency of forming a case definition of EED and validation of currently used non-invasive EED biomarkers.

The addition of WASH interventions to nutrition interventions using SQ-LNS based on Infant and Young Child Feeding (IYCF) did not result in a meaningful impact against EED. To our knowledge, there are currently no other published trials studying the combination of WASH and nutritional interventions on EED. The EED biomarkers were similar in all groups at the end of these two trials. This suggests that neither WASH interventions nor IYCF practices prevented EED. The lack of impact is likely caused by persistent enteropathogen exposure. The addition of nutrition supplementation unlikely affects enteropathogen exposure, while the WASH intervention in these two studies may not be comprehensive enough to prevent further enteropathogen exposure to the children, consequently leading to the persistence of EED[17,18].

The impact of nutrition supplementation to linear growth in children with EED

Nutrition intervention has minimum impact on linear growth in children with EED in our systematic review. Three studies observed no impact on linear growth[13,14,16], while one study found a modest and transient impact on linear growth[15]. This is different from Zhang et al. who observed significant linear growth in children aged 9–12 months using oral nutrition supplementations (ONS)[30] and from Panjwani et al.[31], who found complementary food supplementation has a positive impact on linear growth. The difference can be explained by the different forms of nutrition used. In Zhang et al. and Panjwani et al., they focused on studies using cow-based polymeric ONS or complementary food supplementations to provide balanced calories, while in this review, additional nutrition was used mainly due to anti-inflammatory, gut microbiota altering, and gut epithelial regeneration ability to ameliorate EED. Our study also differs from Roberts et al.[32], who found that the administration of zinc and multiple micronutrients has a positive impact on linear growth in children above 2 years old. The age difference may have caused different results, as in older children, the gut structure and microbiome may have adapted and developed optimally compared to the infant counterpart[33]. It is important to note that none of these studies assessed EED biomarkers, hence the children subjects in their cohort may not have EED[5,30,31]. It appears that administering nutrition that contains a balanced blend of macronutrients and micronutrients along with anti-inflammatory, gut healing and gut microbiome altering properties would ameliorate EED and consequently improve child growth.

EED is likely not correlated with linear growth. In two included studies, fecal EED biomarkers, markers of intestinal damage, and systemic inflammation were not correlated with linear growth. It is also interesting to note, that in several of our included studies, the findings between the impact of nutrition on EED and linear growth were contradicting, where there is an impact on EED biomarkers but no impact on linear growth or vice versa[13-16,20]. This is slightly different from a recent systematic review attempting to establish the link between EED para-

meters and stunting, which found supporting evidences that systemic and intestinal inflammation in EED may correlate with linear growth[19]. In theory, chronic inflammation may suppress the production of IGF-1, which leads to decreased growth hormone production, consequently causing linear growth failure[18,19] These differences may be caused by various confounding factors that may contribute to EED and linear growth such as the study subject selection and population, severity of undernutrition, food security, climate, WASH facilities, and exposure to enteric pathogens[19]. As of now, there is no firm establishment that EED will always consequently affect linear growth. In fact, EED may be an adaptive mechanism in impoverished conditions.

Intriguingly, most of the study population had linear growth faltering, including the intervention groups[15,16,20]. Even more surprising, the included study population in our included studies had adequate nutrition intake obtained from 24-hour food recalls[11,14-16]. This strongly suggests that many factors other than nutrition play a part in linear growth. This is similar to growth patterns in LMICs around the world, where children were born with baseline LAZ below zero followed by a gradual decline in LAZ up until 2 years old[34].

Strengths and limitations

This was the first systematic review that summarized evidence of nutritional intervention against EED that compiled mostly high-quality RCTs, exploring multiple domains of EED and its unclear relationship with linear growth failure. Some limitations of our review are notable. First, most of the studies were conducted in a specific region of Africa, hence the results of this review may not be directly applicable to LMICs in other continents, however, the population and the impoverished conditions were relevant to the study of EED interventions. Second, we observed heterogeneity in the nutrition used to ameliorate EED. The different components of these nutrients may influence the EED biomarkers differently.

Implications for the future

Despite insignificant results, this review sheds light on the future of EED studies. We have gained insight that our knowledge regarding EED remains superficial at best. This review highlights the importance of establishing a case definition of EED and reference values for non-invasive EED biomarkers. In addition, we recommend multi-omics in vitro studies and further studies on the gut microbiome, which appear to be two significant, yet underexplored domains of EED. A deeper understanding of multi-omics and microbiomes in EED may unlock new targets of intervention and eventually help solve persistent child growth failure in LMICs.

CONCLUSION

Due to the heterogenous nature of the methodologies used and parameters assessed in evaluating EED, it is difficult to conclude the impact of nutritional supplementation on EED biomarkers. This indicates that our current understanding of EED remains superficial and this highlights the importance of establishing a case definition of EED and reference values for non-invasive EED biomarkers This review also demonstrated that EED is complex and multi-factorial, hence EED would require a multifaceted intervention. and should be of great concern to the intervention of undernutrition worldwide.

Authors' contribution

Rachmadi RA contributed to the conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft, writing – review & editing, visualization, and project administration of the study. Ariani Y contributed to the conceptualization, methodology, formal analysis, investigation, data curation, writing – review & editing and supervision of the study. Alatas FS contributed to the methodology, validation, formal analysis, resources, writing – review & editing and supervision of the study.

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Rachmadi RA, Ariani Y, Alatas FS. Impacto da suplementação nutricional na disfunção entérica ambiental (DEA) em crianças que vivem em áreas rurais: uma revisão sistemática. Arq Gastroenterol. 2024;61:e23159.

RESUMO – Contexto – Um número impressionante de 99% da mortalidade por desnutrição infantil provém da África Subsaariana e do Sul da Ásia. Apesar de múltiplas intervenções focadas na adequação nutricional, 2,7 milhões de crianças em todo o mundo permanecem associadas à mortalidade relacionada à desnutrição. A falta de impacto de múltiplas intervenções em direção à desnutrição reflete uma forte razão para acreditar que a disfunção entérica ambiental (DEA) é o elo perdido que sustenta a desnutrição em países de baixa e média renda. A DEA é uma condição subclínica causada pela exposição repetida a micróbios fecais enteropatogênicos e não patogênicos por via oral, que causa malformação vilosa intestinal, alterações multiômicas, inflamação intestinal e sistêmica crônica, e disbiose intestinal. A DEA impacta a capacidade absortiva e a integridade do intestino, causando um ciclo de desnutrição em crianças. Atualmente, não existe protocolo para o diagnóstico e tratamento da DEA, portanto, acredita-se amplamente que a DEA seja altamente prevalente e subdiagnosticada em países de baixa e média renda. **Objetivo** – Até onde sabemos, esta é a primeira revisão sistemática para estudar o impacto das intervenções nutricionais na DEA. Estudos anteriores apresentaram resultados inconsistentes, portanto, a síntese dessas informações é essencial para obter uma compreensão mais profunda da DEA e formular novos alvos de intervenção contra a desnutrição infantil. **Métodos** – Esta revisão sistemática está registrada no PROSPERO (CRD42022363157) de acordo com o PRISMA, utilizando palavras-chave referentes à suplementação de nutrientes, DEA e falha no crescimento infantil. **Resultados** – Onze artigos foram elegíveis para revisão, compreendendo ensaios clínicos randomizados realizados principalmente no continente africano, com um total de 5689 crianças saudáveis elegíveis para análise. **Conclusão** – A revisão sistemática ilustra que as intervenções nutricionais têm um impacto mínimo nos biomarcadores da DEA e no crescimento linear, e reflete a importância de entender melhor os mecanismos que causam a DEA e suas consequências. Parece que a contribuição anabólica da intervenção nutricional para o crescimento infantil é negada pela DEA.

Palavras-chave – Absorção gastrointestinal; doenças intestinais; desnutrição infantil, suplementos dietéticos; microbioma gastrointestinal; inflamação; meio ambiente, medicina preventiva e saúde pública.

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