World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Nutritional interventions and BMI dynamics: A comprehensive analysis of growth outcomes in pediatric populations

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World Journal of Advanced Research and Reviews, 2024, 22(02), 020–030

Publication history: Received on 20 March 2024; revised on 29 April 2024; accepted on 01 May 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.2.1318

Abstract

Introduction: This review article emphasizes the significance of Body Mass Index (BMI) and insulin-like growth factor-1 (IGF1) in evaluating pediatric growth outcomes, particularly for those at risk of undernutrition. The review explores the long-term health consequences of early BMI trajectories, especially their association with cardiometabolic profiles in adulthood. The article also examines the correlation between BMI, insulin-like growth factor 1 (IGF-1), and growth velocity, focusing on their complex interactions in malnourished and obese pediatric populations.

Methods: A comprehensive literature search was conducted using PubMed, Google Scholar, and Scopus from 1985 to 2024, focusing on BMI, height growth velocity, IGF1 levels, and the effects of nutritional interventions on pediatric growth outcomes. Studies were included if they focused on children aged 0-18 years and evaluated the impact of BMI and/or nutritional interventions on growth outcomes, with English language articles being prioritized.

Review Results: A total of 15 studies involving 4737 participants were analyzed. The review highlights significant findings including the normalization of growth hormone (GH)-IGF axis post nutritional rehabilitation in malnourished children, the rapid response of IGF-1 levels to nutritional interventions, and the influence of protein-rich diets on catch-up growth. Studies also showed that early nutritional support, particularly through human milk, significantly affects IGF-1 levels and that dietary protein intake correlates with IGF-I concentrations and growth in toddlers. Long-term studies indicated that severe malnutrition can have enduring metabolic effects, necessitating sustained nutritional strategies.

Discussion: The review highlights the significance of early nutritional support and its impact on IGF-1 levels, which are crucial for healthy growth trajectories. The findings advocate for careful monitoring of early growth rates to optimize long-term health outcomes and stress the importance of adequate protein intake during early childhood.

Conclusions: A balanced approach to nutrition, focusing on the interplay between BMI, growth velocities, and IGF1 levels, is crucial in pediatric care. Strategic nutritional interventions, especially those rich in protein, can effectively manage growth outcomes across different BMI categories and improve health trajectories in pediatric populations. This review underscores the need for comprehensive strategies to address nutritional deficiencies and manage growth effectively from infancy through adolescence

Keywords: Nutrition; growth; Protein intake; BMI; IGFI; Malnutrition.

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1. Introduction

The development of the WHO growth reference for children and adolescents aims to provide a consistent standard that aligns with the WHO Child Growth Standards, emphasizing the importance of BMI in evaluating pediatric growth outcomes. Studies have shown that changes in BMI during infancy and childhood are significantly associated with cardiometabolic profiles in adulthood, indicating the long-lasting effects of early BMI trajectories on health outcomes [1,2].

Research highlights substantial heritability of BMI and central adiposity in children, suggesting a significant genetic component to pediatric obesity, which underscores the complexity of addressing this issue solely through nutritional interventions [3]. Additionally, a study on the Utah Population Database found significant genetic contributions to the low BMI phenotype, supporting the idea that genetics play a role in weight regulation across the BMI spectrum [4].

The identification of six new loci associated with BMI highlights a significant neuronal influence on body weight regulation, suggesting that genes expressed in the central nervous system play a critical role in predisposition to obesity [5].

The link between BMI and insulin-like growth factor 1 (IGF-1) level in children has been explored in a few studies. A study aimed to analyze the correlation between serum IGF-1 and body weight among children with a height less than the 10th percentile finds a strong correlation between body weight and serum IGF-1 level (r=0.718), implying that body weight, as reflected in BMI, and serum IGF-1 levels are correlated in children with short stature [6]. Another study found that higher infant peak weight velocity (PWV) and BMI at adiposity peak (BMIAP) were associated with higher childhood BMI and body fat percentage, suggesting that rapid weight gain in infancy could lead to increased general and abdominal adiposity at school age [7]. A study examining the growth velocity among school-aged children with different BMI categories found that obesity was associated with higher annual weight gain and height gain between 6 and 13 years of age. However, overweight and obese girls between 9 and 13 years of age had less linear growth velocity than underweight girls at the same interval, suggesting that puberty may have a greater influence on growth velocity than BMI in girls entering pubertal age [8].

A study showed a significant correlation between spontaneous growth hormone (GH) secretion, IGF-I serum levels, IGF-binding protein 3 (IGFBP-3) levels, and height in healthy children and adolescents. This correlation was significant across various groups, including females, males, prepubertal, and pubertal children. The findings imply that IGF-I and IGFBP-3 serum levels reflect spontaneous GH secretion in healthy individuals, and that IGF-I levels are more sensitive to GH regulation than IGFBP-3 [9].

In healthy twin children, Kao et al. reported a marked genetic influence on IGF-I levels, with a high within-pair correlation for monozygotic pairs. There was a significant correlation between IGF-I level and height, providing clear evidence that IGF-I levels correlate with height, which is genetically controlled [10].

Few studies investigate the apparently complex interaction between BMI, weight gain, height, height growth velocity, and IGF1 level in underweight and wasted infants and children and their response to nutritional intervention [11,12]. Given the complex interplay between BMI dynamics, genetic predispositions, and the impact of nutritional interventions on pediatric growth outcomes, it is evident that there exists a substantial gap in our understanding of these relationships. The role of IGF-1 and its interaction with BMI and growth velocity in both healthy and undernourished populations warrant deeper investigation to uncover potential therapeutic targets.

2. Methods

Literature Search Strategy: A comprehensive literature search was conducted on PubMed, Google Scholar, and Scopus databases to identify relevant studies published between 1985 and 2024. The search strategy was designed to capture studies examining the impact of Body Mass Index (BMI), height growth velocity, IGF1 Level and nutritional interventions, and their effects on growth outcomes in pediatric populations. Keywords and phrases used in the search included "pediatric population," "BMI," "nutritional interventions," "growth outcomes," "underweight," "obese," "basal BMI," "HtSDS," "weight gain," "IGF1," and "nutritional rehabilitation." The search was limited to articles published in English.

Inclusion and Exclusion Criteria: Studies were included in the review if they met the following criteria:

- Focused on pediatric populations aged 0-18 years.
- Evaluated the impact of BMI and/or nutritional interventions on growth outcomes.
- Included measures of growth such as height-for-age, weight-for-age, BMI, Height Standard Deviation Scores (HtSDS), and weight gain.
- Assessed outcomes before and after nutritional rehabilitation, which could include counseling, nutritional supplementation, or both.
- Reported on biomarkers of nutritional and growth status, including but not limited to IGF1 levels. Studies were excluded if they:
- Were not conducted on human subjects.
- Did not provide clear outcomes related to growth or nutritional status.
- Were case reports, editorials, commentaries, or reviews without original data.
- Focused solely on populations with specific diseases or conditions that could independently affect growth outcomes, unless the study specifically addressed the impact of nutritional interventions in these contexts.

Data Extraction and Synthesis: Relevant data were extracted from each included study, focusing on study design, patient demographics, inclusion criteria, details of nutritional interventions (type, duration, and intensity), growth and nutritional outcome measures, and key findings. The synthesis of data aimed to highlight the relationship between BMI, nutritional interventions, pediatric growth outcomes, and IGF1 level with a particular focus on the effects of nutritional rehabilitation on underweight pediatric populations.

3. Results

We reviewed and analyzed a total of 15 studies involving 4737 infants and children. (Figure 1) The studies collectively assess the impact of nutritional interventions on growth outcomes, focusing on the changes in IGF-1 levels and growth hormone dynamics within pediatric populations under varying nutritional statuses. (table 1).

Table 1 Impact of Nutritional Rehabilitation on IGF1 Levels and Growth Outcomes in Undernourished Infants and Children: A Summary of Key Research Findings

Author(s)	Year	Title	Main Findings	Characteristics of Patients	Number of Patients
Soliman et al.(13)	1986	Serum insulin-like growth factors I and II concentrations and growth hormone and insulin responses to arginine infusion in children with protein-energy malnutrition before and after nutritional rehabilitation	Serum insulin, GH, IGFs I and II concentrations were measured in children with severe malnutrition before and after nutritional rehabilitation. IGF-I and II concentrations were reduced in malnourished children but returned to normal after refeeding. Basal GH levels were high in malnourished groups and normalized after refeeding. GH and insulin responses to arginine were depressed in malnourished groups but improved after nutritional rehabilitation.	51 children with kwashiorkor, marasmic-kwashiorkor, marasmus before and after nutritional rehabilitation, as well as in 10 underweight and eight normal Egyptian children.	51
Donahue and Phillips (14)	1989	Response of IGF-1 to nutritional support in malnourished hospital patients: a possible indicator of short-term changes in nutritional status.	Found that IGF-1 levels responded rapidly to nutritional intervention, suggesting its utility as an index for guiding nutritional therapy.	Malnourished hospital patients	15

Gallaher et al.(15)	1998	Fetal programming of insulin-like growth factor (IGF)-I and IGF-binding protein-3: evidence for an altered response to undernutrition in late gestation following exposure to periconceptual undernutrition in the sheep.	Found that periconceptual maternal undernutrition influences fetal IGF axis, altering its response to undernutrition in late gestation.	Sheep fetuses	-
Kabir et al.(16)	1992	Effects of a protein-rich diet during convalescence from shigellosis on catch-up growth, serum proteins, and insulin-like growth factor-I	Children with shigellosis showed greater weight gain, increases in mid-arm circumference, and serum protein concentrations when fed a high-protein diet during convalescence. Serum IGF-I increased more in children on the high-protein diet.	22 children aged 2 to 4 years with shigellosis assigned to high-protein or control diets after antibiotic treatment.	22
Tovar et al.(17)	1999	Effect of nutritional rehabilitation of undernourished rats on serum insulin-like growth factor (IGF)-I and IGF-binding proteins.	Studied the effect of various protein concentrations in diets on serum IGF-I levels in previously undernourished rats, finding that higher protein content was more effective in raising IGF-I levels.	Undernourished rats	-
Lo et al. (18)	2002	Cord IGF-1 levels show a positive correlation with gestational age and an inverse relationship with birth weight. Nutrition, especially human milk feeding, influences IGF-1 levels in newborns.	Cord IGF-1 levels positively correlate with gestational age and inversely with birth weight. Human milk feeding influences IGF-1 levels.	38 preterm and 43 term neonates with umbilical cord blood samples at birth.	81
Ong et al.(19)	2002	Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate	Early growth rates influence IGF-I levels, which are related to body composition in childhood.	-	497
Vásquez-Garibay et al.(20)	2006	Effect of nucleotide intake and nutritional recovery on insulin-like growth factor I and other hormonal biomarkers in severely malnourished children.	Demonstrates the impact of nucleotide intake and nutritional support on IGF1 and other hormonal biomarkers, suggesting improved catch-up growth.	Severely malnourished children	26
Hoppe et al.(21)	2004	Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children	Animal protein intake, particularly milk, is positively associated with sIGF-I concentrations and height in children.	90 children, 2.5 years old, with detailed diet records and sIGF-I measurements.	90
Kon' et al. (22)	2014	The Study of Breast Milk IGF-1, Leptin, Ghrelin and Adiponectin Levels as Possible Reasons of High	Indicated breast milk levels of IGF-1, among other hormones, might influence infant growth and weight gain.	103 mother-infant pairs	103

		Weight Gain in Breast-Fed Infants			
Bourdon et al.(23)	2019	Metabolomics in plasma of Malawian children 7 years after surviving severe acute malnutrition: "ChroSAM" a cohort study	Metabolite profiles of SAM survivors were similar to siblings and community controls 7 years after treatment. IGF1, creatinine, and FGF21 were highlighted but did not differ significantly between SAM survivors and controls. Current stunting was associated with IGF1, which could be influenced by early childhood SAM.	352 children treated for SAM in 2006-2007 at Queen Elizabeth Central Hospital, Malawi, with a subset of 69 SAM survivors, 44 siblings, and 37 community controls studied.	150
Gonzales et al.(24)	2020	Childhood Malnutrition and Association of Lean Mass with Metabolome and Hormone Profile in Later Life	Metabolites including lipids, amino acids, and hormones were associated with LMI among survivors of child SM and controls in Malawi. Plasma FGF21 and tryptophan were specifically associated with LMI among cases but not controls, indicating altered metabolic mechanisms in SM survivors.	Survivors of child severe malnutrition (SM) (n=69) and controls (n=77) in Malawi, 7 years after treatment.	146
Barffour et al.(25)	2023	Insulin-like Growth Factor 1 (IGF1), IGF Binding Protein-3 (IGFBP3) and Growth Response to Daily Zinc Supplementation: A Randomized Trial in Rural Laotian Children	Baseline IGF1 and IGFBP3 significantly modified the impact of zinc supplementation on linear and ponderal growth, suggesting IGF1 bioavailability may drive catch-up growth in zinc-supplemented children.	Laotian children 6–23 months old participating in a double-blind, placebo-controlled trial (N=419).	419
Soliman A, et al.(11)	2021	Children aged 2-10 years	Nutritional interventions led to improved growth outcomes across BMI categories. Positive impact of interventions on growth in underweight and obese categories.	-	-
Yang Li, et al. (26)	2023	Chinese children aged 2–18 years	Inverted J-shaped relationship between BMI SDS and IGF-1 levels, with highest levels in the overweight group. Both lower and higher BMI SDS increase odds of low IGF-1 levels.	3227 children aged 2-18 years	3227

3.1. Summary of the results

- IGF-I and II concentrations were reduced in malnourished children but normalized following nutritional rehabilitation. (11,13)
- IGF-1 levels responded rapidly to nutritional therapy, serving as a potential indicator for short-term changes in nutritional status. (14)
- Periconceptual maternal undernutrition alters the fetal IGF axis, affecting responses to later undernutrition. (15)
- High-protein diets during recovery from shigellosis increased weight, serum proteins, and IGF-I levels in children. (16)
- Higher protein content in diets raised IGF-I levels effectively in previously undernourished rats. (17)
- Human milk feeding influenced IGF-1 levels in newborns, showing positive correlations with gestational age and inverse relations with birth weight. (18)
- Early growth rates affected IGF-I levels related to body composition in childhood. (19)

- Nucleotide intake and nutritional support improved IGF1 and other hormonal biomarkers, aiding catch-up growth. (20)
- Dietary animal protein intake, particularly milk, was positively associated with sIGF-I concentrations and height in toddlers. (21)
- Breast milk levels of IGF-1 and other hormones influenced infant growth and weight gain. (22)
- Metabolite profiles in Malawian children showed no significant differences between SAM survivors and controls years later; however, current stunting was associated with lower IGF1 levels. (23)
- Metabolites and hormones related to lean mass index indicated altered metabolic mechanisms in survivors of severe malnutrition. (24)
- Zinc supplementation influenced IGF1 and IGFBP3 levels, suggesting that micronutrient bioavailability is crucial for catch-up growth. (25)
- Nutritional interventions improved growth outcomes across various BMI categories in children aged 2-10 years. (11)
- Observed an inverted J-shaped relationship between BMI standard deviation scores and IGF-1 levels, with the highest levels in the overweight group. (26).

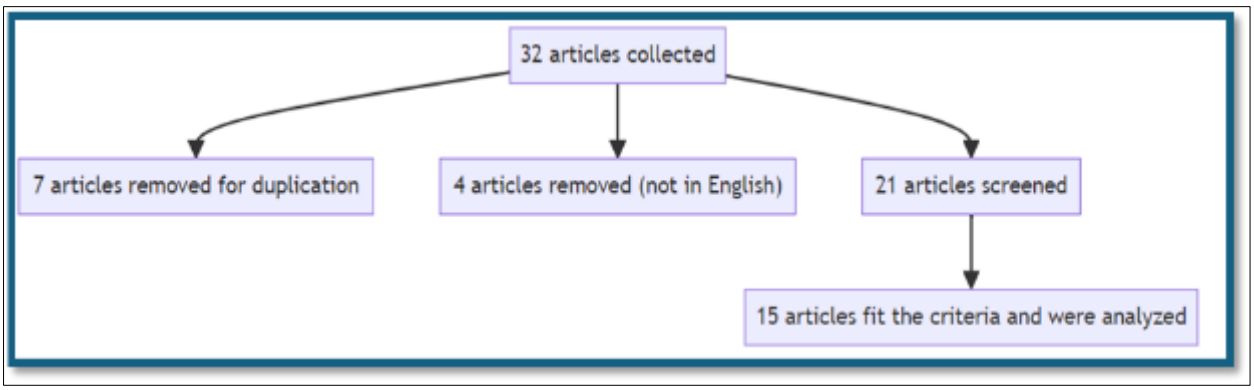

Figure 1 PRISMA Flow Diagram of the study

4. Discussion

These diverse studies on the nutritional, hormonal, and growth dynamics in pediatric populations under various conditions provide compact information that can be useful on clinical grounds.

4.1. Restoration of Growth Hormones through Nutritional Rehabilitation

Nutritional rehabilitation plays a crucial role in the restoration of essential growth hormones in malnourished children, enabling them to achieve normal growth factor levels. Soliman et al. (1986) highlighted that appropriate nutritional intervention could normalize Growth Hormone (GH) and Insulin-like Growth Factors I and II (IGF-I and II), which are vital for proper development and health. Subsequent research, including studies by Nabwera et al. (2017), corroborated these findings, demonstrating improvements in hormone levels and physical recovery post-intervention. Nabwera et al. (2017) identified hormonal profiles, such as insulin and cortisol, as key predictors of nutritional recovery, stressing the importance of these biomarkers in rehabilitation strategies. Additionally, Vasquez-Garibay et al. (2006) showed that targeted nutrient supplementation, like nucleotides, profoundly impacts IGF-I levels, significantly promoting catch-up growth in malnourished children [13,20,27].

4.2. Protein-Rich Diets Promoting Catch-Up Growth

The role of protein-rich diets in facilitating catch-up growth is well-established, particularly in children recovering from nutritional deficiencies and illnesses such as shigellosis. Kabir et al. (1992) documented that a high-protein diet led to notable improvements in body weight, serum proteins, and IGF-I levels, underlining the importance of protein intake during recovery. Further investigations have continued to reinforce the necessity of protein in dietary interventions aimed at promoting growth. A follow-up study by Kabir et al. (1998) demonstrated that such diets not only enhance weight gain but also height over extended periods, underscoring the enduring benefits of protein. Contemporary research by Gat-Yablonski and Phillip (2015) and Arsenault and Brown (2017) has explored the mechanisms through which nutrition influences systemic growth factors, confirming that protein supplementation significantly aids the "catch-up" growth process, especially following acute malnutrition [16,28,29,30].

4.3. Early Nutritional Support and IGF-1 Levels

Early nutritional support, particularly through human milk feeding, has been shown to significantly influence IGF-1 levels, a critical determinant of neonatal and infant growth. Lo et al. (2002) established foundational knowledge by illustrating how human milk correlates with higher IGF-1 levels associated with better gestational outcomes and reduced birth weight complications. This area of research has expanded with studies like those by Büyükkayhan et al. (2003) and Yumani et al. (2020), which have emphasized the positive correlation between macronutrient intake and IGF-1 levels, particularly noting significant benefits in preterm infants. These studies suggest that optimal nutrition from an early stage can profoundly impact the hormonal environment, thus influencing growth trajectories and overall health outcomes in newborns [18,31,32].

4.4. Long-Term Benefits of Early Growth Rates

The implications of early growth rates on long-term health outcomes have garnered significant attention. Research initiated by Ong et al. (2002) linked early growth rates with subsequent levels of IGF-I, which correlate strongly with later body composition and metabolic health. This line of inquiry has been extended by studies such as those by Wells et al. (2007) and Criscuolo et al. (2008), which have explored how early life growth patterns associate with the risk of metabolic syndrome in adulthood and how nutritional interventions can modify these risks. These findings advocate for vigilant monitoring and management of early growth rates to optimize health outcomes throughout life, particularly in relation to body composition and metabolic health [19,33,34].

4.5. Dietary Protein and Growth in Toddlers

In toddlers, dietary protein intake is essential for influencing levels of IGF-I, crucial for development and growth. Hoppe et al. (2004) showed a positive association between dietary animal protein intake and increased IGF-I concentrations. Subsequent research supports these findings, with Pucilowska et al. (1993) and Putet et al. (2015) demonstrating that varying protein levels in diets can significantly affect IGF-I levels and overall growth outcomes in young children. These studies collectively highlight the foundational role of adequate protein intake in promoting robust growth and development during the critical early years of life [21, 35,36].

4.6. Breastfeeding and Infant Growth Hormones

The benefits of breastfeeding extend beyond basic nutrition, significantly impacting infant growth hormones. Kon' et al. (2014) emphasized the role of hormones such as IGF-1 in breast milk, which are pivotal for infant growth and weight gain. This complex composition of breast milk, including hormones like leptin and ghrelin, as reviewed by Mazzocchi et al. (2019), helps regulate appetite and energy balance, potentially protecting against obesity in later life as discussed by Savino et al. (2009). These insights reinforce the critical nature of breastfeeding for its comprehensive benefits on infant health and developmental outcomes [22, 37,38].

4.7. Long-Term Metabolic Outcomes after Severe Malnutrition

Addressing severe childhood malnutrition is crucial not only for immediate survival but also for preventing long-term metabolic disorders. Bourdon et al. (2019) and Gonzales et al. (2020) highlight that children can exhibit normal metabolic profiles years after malnutrition, though some may experience altered metabolic mechanisms that link to later life health issues. Comprehensive reviews by Grey et al. (2021) and specific studies by Dalvi et al. (2018) on the long-term metabolic effects of malnutrition suggest a heightened risk of non-communicable diseases, necessitating proactive and sustained nutritional interventions from an early age [23,24,39,40].

4.8. Impact of Nutritional Strategies on BMI and Growth Factors

The strategic application of tailored nutritional interventions can effectively manage and improve growth outcomes across different BMI categories. Research by Soliman A et al. (2021), Barffour et al. (2023), and Yang Li et al. (2023) has demonstrated that specific dietary adjustments, including micronutrient supplementation like zinc, can significantly influence BMI and growth factors such as IGF-1, offering potential strategies for enhancing growth in malnourished and underweight children [11, 25,41].

In summary, the collective evidence from the reviewed studies underscores the complex interplay between nutritional status, BMI, HtSDS, and IGF1 in determining growth patterns in pediatric populations. these studies together emphasize the multifaceted approach needed in pediatric care to address nutritional deficiencies, support recovery from illness, and manage growth and development. Nutritional interventions, especially those rich in animal protein such as milk, have been shown to positively affect growth velocities and IGF-1 levels. The findings also reveal a nuanced relationship between BMI and IGF-1 levels, suggesting that undernutrition can disrupt normal growth patterns, this review

emphasizes the need for a balanced nutritional approach and maintaining a healthy BMI as pivotal factors in supporting the normal growth trajectories of children.

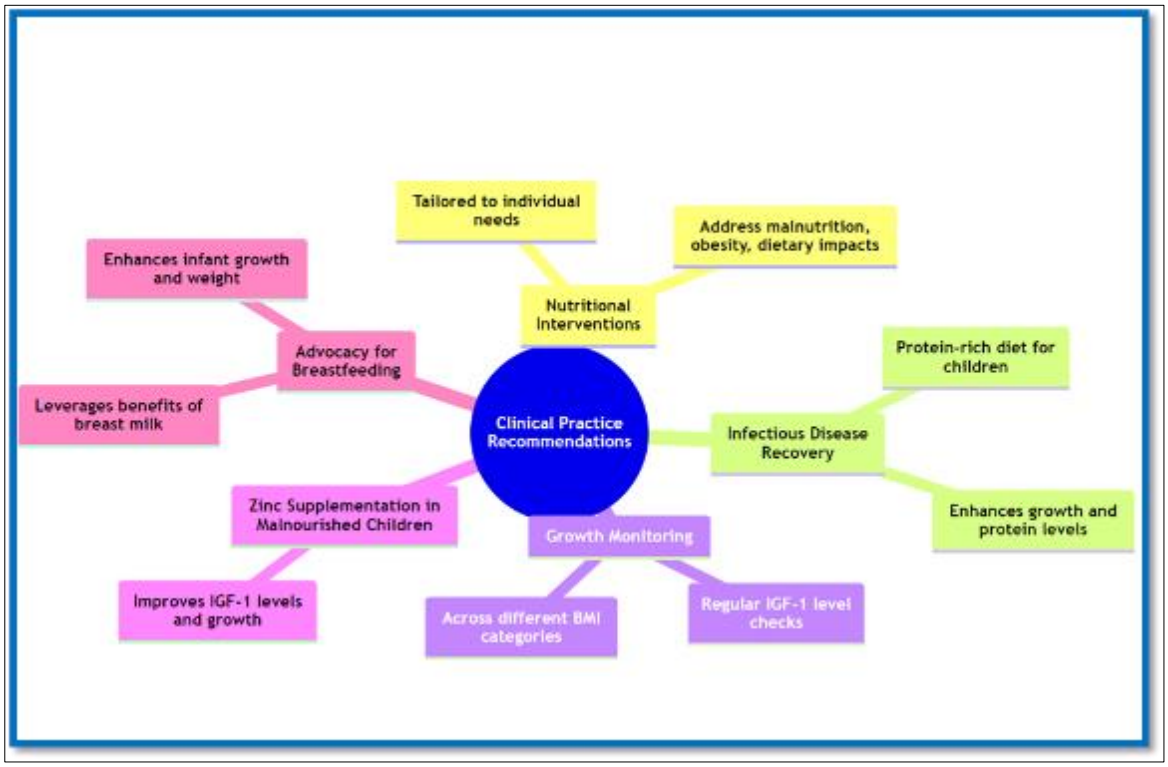

Figure 2 Clinical recommendations suggested by this review

Recommendations

- Clinical Practice Recommendations: (figure 2)
  - Implement nutritional interventions tailored to individual needs, considering the diverse effects of malnutrition, obesity, and specific dietary components on growth and hormonal profiles.
  - For children recovering from infectious diseases like shigellosis or severe acute malnutrition, a protein-rich diet can enhance catch-up growth and improve serum protein levels.
  - Regularly monitor IGF-1 levels in children across different BMI categories to assess growth and development status, particularly in those with nutritional deficiencies or at risk of obesity.
  - Consider zinc supplementation in malnourished children, keeping in mind its potential to improve IGF-1 levels and support linear and ponderal growth, especially in zinc-deficient regions.
  - Advocate for breastfeeding to leverage the beneficial effects of breast milk components, including IGF-1, on infant growth and weight gain.
- Public Health Policy Recommendations:
  - Develop and promote public health programs focused on balanced nutrition in early childhood, emphasizing the importance of animal protein and breastfeeding in supporting optimal growth.
  - Implement strategies to prevent malnutrition and obesity in children, recognizing the long-term health implications of abnormal BMI and nutritional status on hormonal levels and growth.
  - Comprehensive nutritional rehabilitation programs for malnourished children to address not only immediate recovery needs but also long-term growth and metabolic health.
- Recommendations for Further Research
  - To enhance our understanding of pediatric growth and development, further research is crucial in several key areas. Longitudinal studies are needed to evaluate the enduring impacts of nutritional interventions on children's growth, hormonal balance, and metabolic health, providing a clearer picture of how early dietary choices influence long-term outcomes.
  - Investigating the effects of specific dietary components, like animal protein and micronutrients, on serum IGF-1 levels and growth will help refine nutritional guidelines and recommendations.

    - Exploring the long-term health implications of early nutritional status, especially in preventing chronic diseases linked to malnutrition and obesity from a young age, will offer insights into improving public health strategies and interventions aimed at fostering healthier future generations.
- Strength points

This review study offers a comprehensive overview of the impacts of nutrition and BMI on pediatric growth and hormonal levels, guiding evidence-based recommendations for clinical and public health practices.

- Weakness points

The weak points in the review include variability in study designs and potential bias, which may impact result comparability and conclusions. Its broad overview might not fully explore specific interventions, risking oversimplification.

5. Conclusion

This review provides compelling evidence that strategic nutritional interventions, particularly those rich in proteins, are essential in managing pediatric growth dynamics and improving health outcomes. It emphasizes the importance of early nutritional support and its role in modulating growth factors like IGF-1, crucial for pediatric populations. As the global community continues to address pediatric undernutrition and obesity, the insights from this study advocate for a balanced nutritional strategy to ensure optimal growth and long-term health.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict between the authors regarding the study. All authors have participated, reviewed and approved the publication of the study.

Author Contributions

AS was responsible for the conceptualization of the review study, setting the stage for the research with a clear outline of the scope and objectives. All authors actively participated in the data collection, screening, and analysis process, ensuring a comprehensive and meticulous evaluation of the research findings. The original draft preparation was undertaken by AS, who integrated the collected data and articulated the study's key insights. NS significantly contributed to refining the manuscript, providing expert review and editing to enhance the intellectual content and clarity. All authors have given their final approval of the version to be published, collectively ensuring the manuscript's accuracy and integrity, and have agreed to the published version, thus upholding rigorous scholarly standards, and ensuring the work's credibility and reliability.

Funding

No funding was required or obtained for this study, ensuring the independence and impartiality of the research findings and conclusions.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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