Received: 14 February 2021 | Revised: 25 July 2021 | Accepted: 2 August 2021

DOI: 10.1111/mcn.13266

ORIGINAL ARTICLE

Maternal & Child Nutrition WILEY

# Prenatal nutrition supplementation and growth biomarkers in preadolescent Bangladeshi children: A birth cohort study

Towfida Jahan Siddiqua[1] | Anjan Kumar Roy[1] | Evana Akhtar[1] | Md. Ahsanul Haq[1] | Yukiko Wagatsuma[2] | Eva-Charlotte Ekström[3] | Md. Nure Alam Afsar[1] | Md. Iqbal Hossain[1] | Tahmeed Ahmed[1] | Shams El Arifeen[1] | Rubhana Raqib[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

[2]Department of Clinical Trial and Clinical Epidemiology, University of Tsukuba, Tsukuba, Japan

[3]International Maternal and Child Health, Womens and Childrens Health, Uppsala University, Uppsala, Sweden

**Correspondence**
Dr. Towfida Jahan Siddiqua, Assistant Scientist, Nutrition and Clinical Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), 68, Shaheed Tajuddin Ahmed Sharani, Dhaka 1212, Bangladesh.
Email: towfida@icddrb.org

**Funding information**
Swedish International Development Cooperation Agency, Grant/Award Number: 54100089; icddrb, Grant/Award Number: 384, SWE-2008-065; Japan Society for the Promotion of Science, Grant/Award Number: 18256005

## Abstract

Little is known about the usefulness of biomarkers to study the influence of prenatal nutrition supplementation in improving child growth. Anthropometry is not always straightforward to understand how nutrition might impact growth, especially in settings with high rates of malnutrition and infections. We examined the effects of prenatal supplementation on growth and growth biomarkers and the relationship between anthropometric measures and growth biomarkers of children at 4.5 and 9 years of age. Children were enrolled from a longitudinal cohort, where mothers were randomized into daily supplementation with either early-food (≤9 gestation week [GW]) or usual-food (∼20 GW) (608 kcal 6 days/week); they were further randomized to receive 30-mg or 60-mg iron with 400-μg folic acid, or multiple micronutrients (MM) in rural Bangladesh. Anthropometric data were collected from mothers at GW8 and children at 4.5 ($n = 640$) and 9 years ($n = 536$). Fasting blood was collected from children at each age. Early-food supplementation showed reduced stunting and underweight at 4.5 and 9 years age respectively compared to usual-food. Prenatal supplementations did not have any effect on growth biomarkers except for STAT5b expression which was lower in the early-food compared to the usual-food group ($\beta = -0.21$; 95 CI% $= -0.36, -0.07$). Plasma concentrations of 25-hydroxy vitamin D and calcium were both inversely associated with weight-for-age and body mass index-for-age Z-scores at 9 years, particularly in early-food and MM groups. Although there was minimal effect on child growth by prenatal supplementations, the associations of biomarkers with anthropometric indices were predominantly driven by timing of food or MM supplementations.

**Abbreviations:** 25(OH)D, 25-hydroxy vitamin D; Ca, Calcium; DOHaD, Developmental Origins of Health and Disease; GH, Growth hormone; IGF-1, Insulin-like growth factor 1; IGF-BP3, IGF-binding protein 3; Mg, Magnesium; MINIMat, Maternal and Infant Nutrition Interventions in Matlab; MM, Maternal multiple micronutrient; PBMC, Peripheral blood mononuclear cells; PTH, Parathyroid hormone; SOCS2, Suppressor of cytokine signalling-2; STAT5b, Signal transducer and activator of transcription 5B.

**KEYWORDS**
anthropometric indices, growth biomarkers, MINIMat, preadolescent children, prenatal supplementation

## 1 | INTRODUCTION

**Key messages**

- No overall effect was observed on biomarkers of growth among children born to mothers who were supplemented with food and different micronutrient alternatives.
- This study demonstrated for the first time in humans that expression of STAT5b, a molecular marker was affected in 9 years old children through in utero nutrition supplementation.
- The associations of biomarkers with child anthropometric indices were predominantly driven by timing of prenatal nutrition interventions.

Maternal malnutrition in early pregnancy remains the single most preventable risk factor for all aspects of child growth and development (Black et al., 2013; Ekstrom et al., 2016). Maternal micronutrient as well as chronic energy deficiency is widespread in South Asia (Osmani & Sen, 2003). The Developmental Origins of Health and Disease (DOHaD) hypothesis suggests that improving the nutritional status of malnourished mothers may have favourable impact on the offspring's health outcomes (Uauy et al., 2011). Most studies explored the effect of prenatal nutritional supplementation on improving weight gain in children (Devakumar et al., 2016) with limited focus on linear growth. Even fewer studies have investigated effects beyond the neonatal period since long-term follow-up studies are more difficult to conduct (Lanou et al., 2014; Lu et al., 2014). Most of the studies are cross-sectional surveys (Shrimpton et al., 2001; Victora et al., 2010), only few randomized controlled trials have assessed effects of nutritional supplementation on long-term growth outcomes (Ekstrom et al., 2016; Svefors et al., 2016). Again, none of these studies specifically assessed combination of growth biomarkers in relation to growth indicators in children. Deficits in early growth have substantial health implication leading to increased mortality as well as morbidity. Hence, in light of the lack of an evidence base on the role of nutrition in rural Bangladesh, it is important to investigate how the timing of the nutritional supplementation may influence the individual's linear growth and related outcomes. Measuring children's weight and height have several advantages due to noninvasiveness and available accepted standards. However, these are limited by lower sensitivity and accuracy and unlikely to provide appropriate warning with a decrease in growth unless conducted over a prolonged period of time (Harkare et al., 2021; Raiten et al., 2013). Indeed, anthropometry alone is insufficient to predict specific differences in patterns between children growing normally and those who are malnourished. Despite the fact that the extent of the problem is well understood, no longitudinal study has been conducted to advance our understanding to explain how nutritional supplementation might impact growth biomarkers. It is also important to acquire in-depth knowledge of the association between growth biomarkers and anthropometric indices among children to achieve cost-effectiveness of future intervention strategies.

Three large-scale trials of nutrition interventions in pregnancy in Bangladesh suggest that maternal multiple micronutrient (MM) and food supplements can improve child growth under certain circumstances (Christian et al., 2016; Islam Khan, 2013; Mridha et al., 2016). A previous report from the Maternal and Infant Nutrition Interventions in Matlab (MINIMat) (Persson et al., 2012) trial has shown that early food supplementation in pregnancy reduced the occurrence of stunting during 0–54 months in children especially in boys. However, prenatal MM supplementation increased stunting (Khan et al., 2011) as well as decreased the insulin-like growth factor 1 (IGF-1) levels up to 5 years of age in the MINIMat cohort (Ekstrom et al., 2016). Whether an early timing of prenatal food and micronutrient supplementation positively influence biomarkers of child growth and if these interventions in pregnancy can have a significant impact on these outcomes during the later period of rapid growth is however not clear.

Growth hormone (GH) stimulates the growth of all tissues in children and adolescents. It stimulates secretion of IGF-1 that promotes bone growth and is also responsible for the growth-promoting effects of GH (Vance, 1990). Activation of signal transducer and activator of transcription 5B(STAT5b), a part of a GH-regulated somatic growth pathway modulates transcription of IGF-1 and IGF-binding protein 3 (IGF-BP3) (Eugster & Pescovitz, 2003; Rotwein & Chia, 2010; Woelfle et al., 2007). IGF-1 and IGF-BP3 have strong role in bone growth of children. Suppressor of cytokine signalling 2 (SOCS2) is a key negative regulator of GH-dependent body growth (Greenhalgh et al., 2005). It also inhibits GH signalling, including reduction of STAT5 activation. Thus, assessment of both positive (STAT5b) and negative (SOCS2) regulators of GH activity is required to obtain a comprehensive picture to better elucidate the mechanism of growth regulation among undernourished preadolescent children. Enhanced understanding of such regulation is a way to predict the physiological consequences of growth hormone signalling associated with the nutrition intervention.

From early childhood to late adolescence, the bone formation predominates on bone resorption and puberty has a fundamental role in the acquisition of bone mass (Stagi et al., 2013). The hormones, calcitonin and parathyroid hormone (PTH) play important roles in bone remodelling through their actions on osteoclasts and osteoblasts, respectively (Carter & Schipani, 2006). Adequate vitamin D status is

important for optimal growth and bone health; low levels of vitamin D are associated with elevated levels of PTH and consequently inadequate bone mineralization (Pekkinen et al., 2012). A number of studies observed that a combination of bone health nutrients is preferable to any single nutrient alone. However, there are no reports on the status of growth biomarkers during pre-adolescence in relation to nutrition interventions in pregnancy.

We aimed to evaluate the long-term effects of prenatal nutritional supplementation on growth and growth biomarkers and the relationship between anthropometric measures and growth biomarkers of children at 4.5 and 9 years of age. The MINIMat was a randomized factorial design population based trial of prenatal supplementation with food and micronutrients in rural Bangladesh (Persson et al., 2012); the mother–child cohort was followed longitudinally at various age intervals to study diverse outcomes (Arifeen et al., 2018). Here we studied biochemical markers in plasma [25-hydroxy vitamin D (25(OH)D), calcium (Ca), magnesium (Mg), PTH, calcitonin, GH, IGF-1, IGF-binding protein 3 (IGF-BP3)] and molecular markers (STAT5b and SOCS2) in peripheral blood mononuclear cells (PBMC) in children at 4.5 and 9 years of age.

## 2 | METHODS

### 2.1 | Study area, population and intervention

icddr,b, a global health research institute, operates a health and demographic surveillance system (HDSS) in Matlab, a rural area in Bangladesh with a target population of ~220,000. The MINIMat trial (2001–2003) enrolled 4,436 pregnant women with 3,591 live births (Persson et al., 2012). The primary objectives of the trial were to evaluate effects of prenatal nutrition on health outcomes of the women and their newborn infants.

In a randomized trial, locally produced energy–protein food supplement (608 kcal 6 days/week) was given to pregnant women; the timing of introduction of food was either (i) immediately after confirmation of pregnancy, around GW8–9 (early-food), or (ii) at the time of their choosing (usual-food), around GW20 (Table S1a). The enrolled women at GW14 were also randomly allocated to one of three different micronutrient supplementations in a double-blinded manner: (i) 30 mg iron and 400 μg folic acid (Fe30F), (ii) 60 mg iron and 400 μg folic acid (Fe60F) or (iii) the UNICEF preparation of 15 different micronutrients (MM) including 30 mg iron and 400 μg folic acid (Table S1b). It is important to mention that pregnant women receive a daily dose of 60 mg iron and 400 μg folic acid (iron-folate) as part of routine care provided by the Health, Nutrition and Population Sector Program of Bangladesh.

### 2.2 | Data collection

The present study was nested in the MINIMat cohort that predominantly involved studies on immune function and toxicant exposures in a subgroup of children who were born between June 2003 to June 2004 and followed at 4.5 and 9 years of age (Ahmed et al., 2013; Mannan et al., 2016; Raqib et al., 2009) (Figure 1). Using a screening list, field workers initially surveyed the field site for availability of 9 years old children who had previously participated in studies at 4.5 years of age. Children who did not have anthropometric measurements taken at birth were not included in this study. Baseline (age, body mass index [BMI], parity, socio-economic status [SES)] and educational level) and follow-up data on mothers (urinary metals) were available (Persson et al., 2012).

The SES was updated at 4.5 and 9 years of age based on household assets using data from the HDSS database (Ahmed et al., 2013; Mannan et al., 2016; Raqib et al., 2009). Anthropometric data was collected from mothers at GW8 and children at 4.5 and 9 years; Mid-Upper Arm Circumference (MUAC) was measured as described earlier (Islam Khan, 2013). The sitting height was measured adapting a removable box (60 cm) to the stadiometer that allowed the child to sit and place both feet flat on the floor. World Health Organization 2006 Child Growth Standards were used to determine weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ) and body mass index-for-age Z-score (BAZ).

### 2.3 | Biomarkers assays

Fasting blood and urine was collected from children early in the morning. Plasma and PBMC were separated, and stored in ultralow temperature (−80°C) for analysis in icddr,b, Dhaka. Plasma 25(OH)D, PTH, GH and calcitonin were measured by electrochemiluminescence immunoassay with Cobas e601 using kits (Roche Diagnostics, GmbH, Mannheim, Germany). Plasma Ca and Mg were measured by photometric method in automated analyser Cobas c311 (Roche) and adjusted with plasma albumin concentration. Plasma IGF-1 and IGFBP3 were measured by sandwich enzyme immunoassay using immunoassay kits (Quantikine ELISA, R&D Systems, Inc., Minneapolis, MN). Concentrations of calcitonin, IGFBP3 and GH were measured at 9 years of age only. For all analytes, the within- and between-assay coefficients of variation for plasma samples were ~10%.

### 2.4 | TaqMan-based gene expression assay

Total RNA was isolated from PBMC from a subset of sample ($n = 209$) at 9 years, using RNeasy plus Mini kit (Qiagen, Germany). RNA concentrations and purity were measured by NanoDrop 2000 (Thermo Fisher Scientific, Massachusetts, USA). For single-stranded cDNA synthesis, 1,000 ng of total RNA was transcribed with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific).

Gene expression for STAT5b and SOCS2 was measured using TaqMan gene expression assay (Applied Biosystems TaqMan Assays) based on primer-probe hydrolysis in CFX96 Real-Time System (BioRad California, USA). A reference gene, GAPDH, was used for

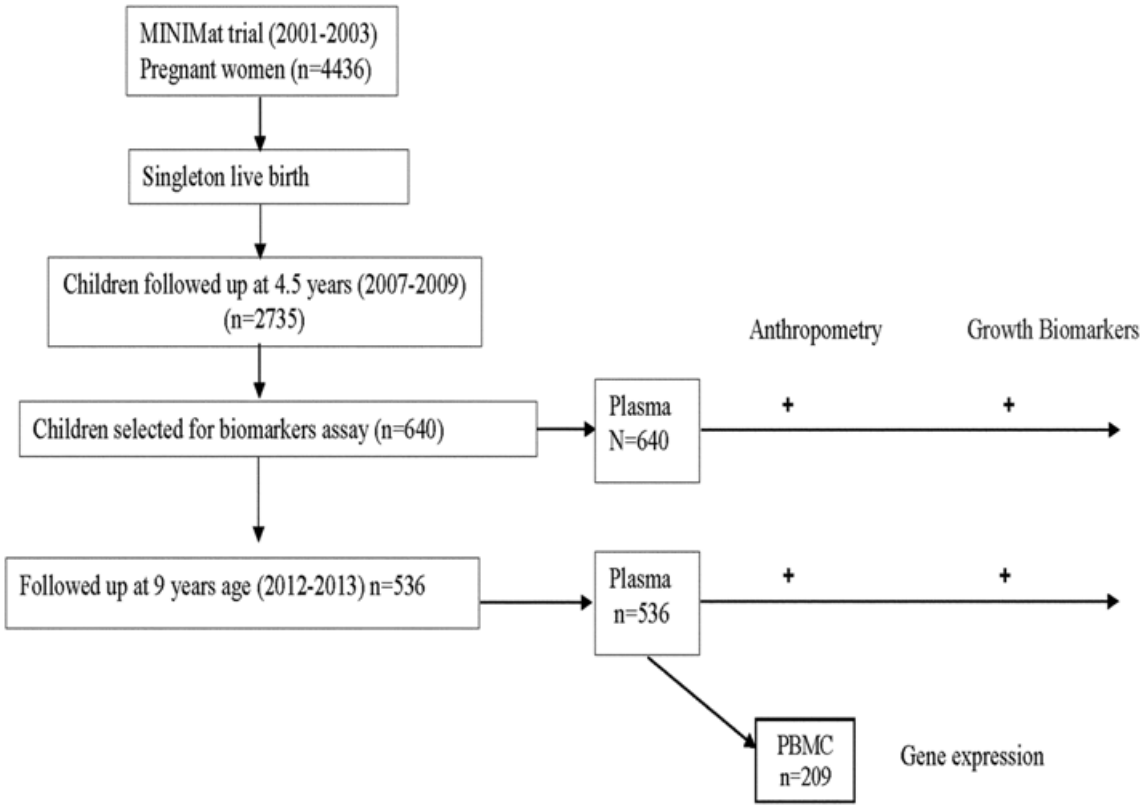


**FIGURE 1** Flowchart of the children enrolled and followed up

normalization of target genes. Both target and reference genes were designed with FAM as reporter dye on its 5′ end and MGB-NFQ dye as quencher on its 3′ end. Cycle threshold (Ct) values of STAT5b and SOCS2 were obtained at the end of the reaction and fold changes were calculated through ΔΔCt method

## 2.5 | Data analyses

The statistical analyses were performed with Stata 13 (StataCorp, LP, College Station, Texas, USA) and SPSS (version 22.0).

For data visualization, several statistical plots such as histogram, box plot, normal k-density curve, probability of skewness and kurtosis and q-q plots and scatter diagram were used. Quantitative data were transformed when not normally distributed, and analyses were performed on the transformed variables. Data were expressed using proportion for categorical data, mean and standard deviation and median with interquartile range where appropriate. Multivariate regression model was used to see the between group difference in Fe30F and MM compared to Fe60F (reference) and in early compared to usual food supplementation group (reference). Correlation and linear regressions were used to assess the relationship between two or more variables. Forward method was applied to fit the best model and select the covariates. Covariates that influenced the model $R^2$ by 5% or more, were selected to avoid collinearity. Thus, maternal BMI, child sex, age, birth weight and SES score at 4.5 years and 9 years were included in the final regression models. Since arsenic was inversely associated with IGF-1 and IGFBP3 in this cohort, all models were also adjusted for urinary arsenic at both time points (Ahmed et al., 2013; Gliga et al., 2018). For inferential statistics, 95% CI and *p* value were used.

The association between growth parameters (HAZ, WAZ and BAZ) and the outcome variables (Ca, Mg, 25(OH)D, PTH, IGF-1, IGF-BP3, calcitonin, GH, STAT5B and SOCS2) in study participants were evaluated by multivariate regression analyses. The same analysis was also performed after stratifying the study participants in respective food (usual and early) and micronutrient supplementation (Fe30F and MM) groups compared to the reference group (Fe60F)

## 2.6 | Ethical considerations

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was reviewed and approved by the Ethical Review Committee of the icddr,b, Bangladesh.

# 3 | RESULTS

## 3.1 | Demography and anthropometric indices

The recruitment and participant flow has been described in detail elsewhere (Ahmed et al., 2013; Mannan et al., 2016; Raqib

et al., 2017). In short, among 640 children who participated at 4.5 years of age, 89 were not available for various reasons (Figure 1). A total of 551 children were enrolled at 9 years of age, of whom 536 blood samples were available for analysis in the present study. The demographic characteristics of mothers and children are presented in Table S2 and Table 1 respectively. About 24% children were of low birth weight (<2,500 g). The family SES score did not differ between the three time points (GW8, 4.5 years and 9 years). Sitting height was weakly associated with HAZ as assessed by bivariate analyses ($r = 0.092$).

## 3.2 | Growth biomarkers during childhood at 4.5 and 9 years of age

Concentrations of the various biomarkers were found to be within age-specific reference range (Table S3). Plasma 25(OH)D levels decreased in 9 years old compared to 4.5 years (Table 1). According to the classification of Institute of Medicine, 25(OH)D deficiency (<30 nmol/L) was found in 3 children among 4.5 years old and 5 among 9 years old. Again, vitamin D insufficiency (30–47.5 nmol/L) was noted in 54 4.5 years old (10.2%) and 84 9 years old (15.6%) children. Similarly, PTH levels decreased from 4.5 to 9 years of age. Plasma PTH levels were inversely correlated with plasma 25(OH)D at 9 years of age ($r = -0.16$, $p < 0.001$) only.

Plasma Ca and IGF-1 levels increased in 9 years old children compared to 4.5 years old (Table 1). Girls (71.38 ± 2.74 μg/L) had higher IGF-1 levels than boys (64.02 ± 2.33 μg/L) at 4.5 years ($P = 0.041$). Similarly, both IGF-1 and IGF-BP3 levels were higher in girls than boys at 9 years (Figure S1).

## 3.3 | Effect of prenatal intervention on growth biomarkers

None of the micronutrient interventions affected growth defined in the standard way as increase in HAZ and WAZ (data not shown) or the growth biomarkers (Table S4). When food groups were compared, usual-food showed higher proportion of stunted (57.6% < −2 SD HAZ) and underweight children (55.1% < −2SD WAZ) in 4.5 and 9 years old respectively compared to early -food (Figure 2). MM supplementation was positively associated with BAZ in children at

**TABLE 1** Demographic status of the study participants

| Features | Children at 4.5 years ($n = 536$) | Children at 9 years ($n = 536$) | *P* value |
|---|---|---|---|
| Age, months | 55.92 ± 1.38 | 106.38 ± 1.42 | <0.001 |
| Females | 274 (51%) | 274 (51%) | |
| BMI, kg/m$^2$ | 13.82 ± 0.95 | 14.28 ± 1.63 | <0.001 |
| Stunting (HAZ < −2SD) | 158 (30%) | 118 (22%) | 0.123 |
| Underweight (WAZ < −2SD) | 212 (40%) | 214 (40%) | 0.910 |
| Sitting height ratio | - | 0.54 ± 0.21 | |
| MUAC, cm | - | 25.22 ± 4.1 | |
| SES tertiles | | | |
| Lowest, *n* (%) | 179(33.4%) | 179 (33.46%) | 1.000 |
| Middle, *n* (%) | 179(33.4%) | 178 (33.27%) | 1.000 |
| Highest, *n* (%) | 178(33.2%) | 178 (33.27%) | 1.000 |
| Biomarkers | | | |
| 25(OH)D, nmol/L | 71.74 ± 23.31 | 63.95 ± 17.01 | <0.001 |
| Ca, mg/dl | 7.67 ± 2.87 | 9.08 ± 0.55 | <0.001 |
| Mg, mmol/L | - | 0.82 ± 0.07 | - |
| PTH, ng/L | 44.26 ± 20.80 | 38.02 ± 13.04 | <0.001 |
| IGF-1, μg/L | 67.81 ± 41.00 | 98.39 ± 34.29 | <0.001 |
| IGF-BP3, ng/ml | - | 2,863.7 ± 856 | |
| Calcitonin, pg/ml | - | 5.05 ± 3.38 | |
| GH, pg/ml | - | 1,266 ± 1,869.5 | |
| STAT5B | - | 1.00 ± 0.52 | |
| SOCS2 | - | 0.95 ± 0.50 | |

*Note:* Data were given by mean ± SD or number with percentage in parenthesis. Sitting height ratio is the ratio between sitting height and total height. The sitting height ratio, MUAC, IGF-BP3, Calcitonin, GH, STAT5B and SOCS2 were measured in 9 year old children only.
Abbreviations: BMI, body mass index; MUAC, Mid-Upper Arm Circumference; 25(OH)D, 25-hydorxy vitamin D; Ca, calcium; Mg, magnesium; PTH, parathyroid hormone; GH, calcitonin, growth hormone; IGF-1, insulin-like-growth factor 1; IGF-BP3, IGF-binding protein 3; STAT5b, signal transducer and activator of transcription 5B; SOCS2, suppressor of cytokine signalling-2.

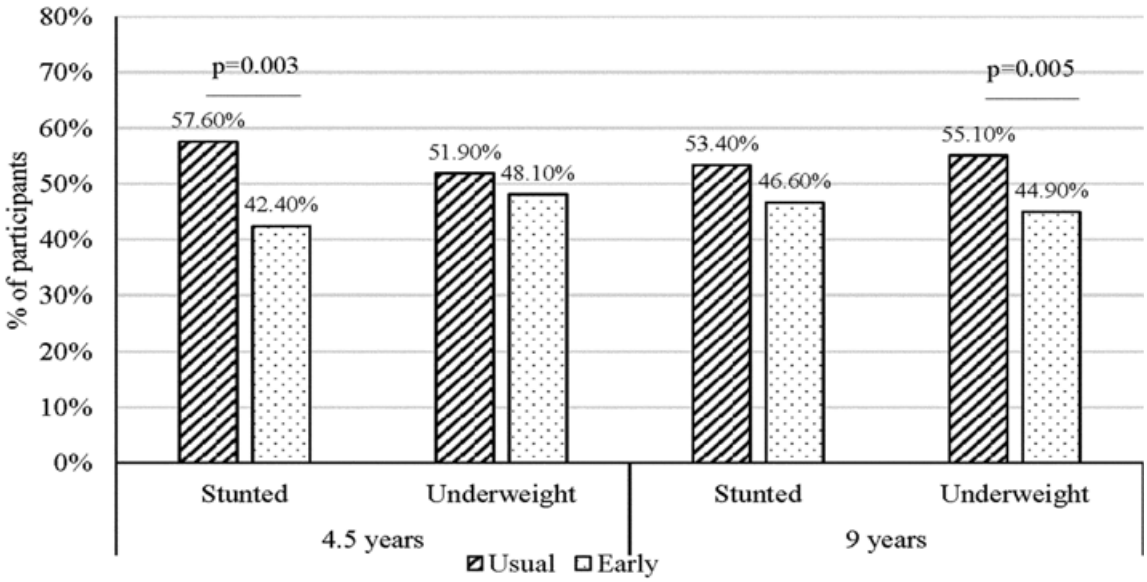


**FIGURE 2** Stunted and underweight children (%) in the usual and early supplementation groups

both 4.5 ($\beta = 0.10$; 95% CI $= -0.12, 0.48$; $P = 0.089$) and 9 years of age ($\beta = 0.08$; 95% CI $= -0.14, 0.31$; $P = 0.091$) compared to Fe60F; however, the associations were not strong.

When children were categorized according to the timing of introduction of food or micronutrient supplementation types, the early-food group showed lower levels of STAT5b compared to the usual-food ($\beta = -0.21$; 95% CI $= -0.36, -0.07$; $P = 0.005$) at 9 years. We found no differential effect by timing of food supplementation groups on other biomarkers ((25(OH)D, Ca, Mg, PTH, calcitonin, GH, IGF-1, IGF-BP3). No significant difference was obtained in the concentrations of biomarkers between the micronutrient groups (Table 3).

### 3.4 | Growth biomarkers and anthropometric-z scores

Multivariate adjusted regression analysis showed that plasma 25(OH)D concentration was inversely associated with WAZ and BAZ at both 4.5 and 9 years of age (Table 2). When stratified by sex, the estimates were generally found to be stronger in girls both at 4.5 and 9 years.

Plasma Ca levels at 4.5 years did not show any associations with anthropometric z scores. At 9 years, plasma Ca levels showed an inverse association with BAZ ($\beta = -0.05$; 95% CI $= -0.10, -0.01$) (Table 2). The inverse associations with WAZ and BAZ were prominent in boys only. Plasma Mg concentration was positively associated with BAZ among 9 years old boys ($P = 0.012$).

No associations were noted between anthropometric-z scores and PTH at 4.5 years. However at 9 years, PTH concentration was positively associated with all anthropometric indices.

At 4.5 years a positive association of IGF-1 levels was found with HAZ and WAZ in all children. Stratification by sex showed positive association of IGF-1 with HAZ in girls and all anthropometric z-scores in boys. At 9 years, IGF-1 levels in all children exhibited positive associations with all the anthropometric indices, irrespective of sex. Concentrations of IGF-BP3 were associated with HAZ and WAZ in all children with stronger estimates evident in boys.

An inverse association was obtained between plasma calcitonin and HAZ in all children and the estimate was stronger in girls (Table 2). With every 1 unit decrease in HAZ score, there was 0.43 pg/ml increase in calcitonin in all children and 0.9 pg/ml increase in girls. In contrast, with every 1 unit increase in BAZ score, there was 0.31 pg/ml increase in calcitonin concentration in all children. We did not observe any association between GH and anthropometric z-scores (data not shown).

A positive association between STAT5b expression and BAZ was observed in boys only. However, SOCS2 did not show any association with anthropometric z scores (data not shown).

### 3.5 | Growth indicators and biomarkers stratified by food and micronutrient supplementation

There was no interaction by treatment group for the relationship between anthropometry and growth biomarkers. Assessment of growth biomarkers in children are given in Table S4, stratified by micronutrient supplementation status or by usual and early food supplementation. Multivariate adjusted regression analysis showed strong negative associations between 25(OH)D levels and BAZ in both the early and usual food groups as well as in the Fe60F group among 4.5 years old (Figure 3). Inverse association also existed between 25(OH)D and WAZ in the usual-food, Fe30F and MM groups. However at 9 years, negative association of 25(OH)D with both WAZ and BAZ were strongly evident only in the early-food and MM groups (Figure 3). Of note, the associations between 25(OH)D and WAZ/BAZ in the Fe30F or Fe60F disappeared at 9 years.

**TABLE 2** Regression analysis of growth biomarkers with anthropometric indices at 4.5 and 9 years of the children stratified by sex

| | All children | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| [a]4.5 years | β (95% CI) | *p* value | β (95% CI) | *p* value | β (95% CI) | *p* value |
| 25(OH)D | | | | | | |
| HAZ | 0.35(−2.38, 3.07) | 0.803 | 0.23(−3.65, 4.12) | 0.905 | 1.80(−3.66, 7.25) | 0.517 |
| WAZ | −3.63(−6.52, −0.74) | 0.014 | −3.05(−7.10, 1.01) | 0.140 | −5.66(−10.58, −0.75) | 0.024 |
| BAZ | −5.29(−8.05, −2.54) | 0.0004 | −4.95(−9.0, −0.92) | 0.016 | −6.03(−9.86, −2.20) | 0.002 |
| Ca | | | | | | |
| HAZ | −0.06(−0.39, 0.28) | 0.742 | −0.02(−0.46, 0.42) | 0.937 | 0.09(−0.63, 0.81) | 0.807 |
| WAZ | −0.27(−0.63, 0.08) | 0.135 | −0.30(−0.76, 0.16) | 0.198 | −0.19(−0.84, 0.47) | 0.573 |
| BAZ | −0.31(−0.65, 0.03) | 0.076 | −0.45(−0.91, 0.01) | 0.056 | −0.21(−0.72, 0.30) | 0.422 |
| PTH | | | | | | |
| HAZ | −0.32(−2.81, 2.16) | 0.798 | −0.92(−4.40, 2.56) | 0.604 | 1.12(−4.10, 6.35) | 0.672 |
| WAZ | 0.29(−2.36, 2.94) | 0.830 | 1.07(−2.58, 4.71) | 0.565 | −0.77(−5.53, 4.0) | 0.750 |
| BAZ | 0.91(−1.64, 3.46) | 0.483 | 2.89(−0.74, 6.53) | 0.118 | −1.23(−5.0, 2.5) | 0.516 |
| IGF-1 | | | | | | |
| HAZ | 16.0(11.43, 20.57) | 0.0001 | 16.08(9.48, 22.66) | 0.0001 | 13.91(4.7, 23.13) | 0.003 |
| WAZ | 13.08(8.09, 18.07) | 0.0008 | 17.04(10.12, 24.0) | 0.0005 | 2.73(−5.81, 11.27) | 0.530 |
| BAZ | 0.94(−4.0, 5.86) | 0.709 | 7.81(0.60, 15.03) | 0.034 | −4.70(−11.37, 2.0) | 0.167 |
| [b]9 years | | | | | | |
| 25(OH)D | | | | | | |
| HAZ | 0.19(−1.61, 2.0) | 0.838 | 0.79(−1.79, 3.37) | 0.548 | −0.68(−4.85, 3.49) | 0.748 |
| WAZ | −2.05(−3.64, −0.46) | 0.011 | −1.96(−4.13, 0.22) | 0.077 | −3.38(−6.40, −0.37) | 0.028 |
| BAZ | −2.75(−4.20, −1.29) | 0.0002 | −3.19(−5.21, −1.18) | 0.002 | −2.32(−4.47, −0.17) | 0.034 |
| Ca | | | | | | |
| HAZ | 0.01(−0.05, 0.06) | 0.820 | −0.08(−0.16, 0.01) | 0.068 | 0.10(−0.03, 0.23) | 0.114 |
| WAZ | −0.04(−0.09, 0.01) | 0.118 | −0.08(−0.15, −0.02) | 0.017 | −0.03(−0.12, 0.07) | 0.550 |
| BAZ | −0.05(−0.10, −0.01) | 0.025 | −0.07(−0.13, −0.003) | 0.040 | −0.05(−0.11, 0.02) | 0.183 |
| [c]Mg | | | | | | |
| HAZ | 0.0002(−0.01, 0.01) | 0.958 | −0.003(−0.01, 0.01) | 0.557 | 0.0003(−0.02, 0.02) | 0.973 |
| WAZ | 0.004(−0.002, 0.01) | 0.194 | 0.01(−0.003, 0.01) | 0.184 | 0.001(−0.01, 0.02) | 0.864 |
| BAZ | 0.01(−0.0004, 0.01) | 0.067 | 0.01(0.002, 0.02) | 0.012 | 0.001(−0.01, 0.01) | 0.873 |

(Continues)

**TABLE 2** (Continued)

| | All children | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| [a]4.5 years | β (95% CI) | *p* value | β (95% CI) | *p* value | β (95% CI) | *p* value |
| PTH | | | | | | |
| HAZ | 1.40(0.01, 2.79) | 0.049 | 1.57(−0.19, 3.32) | 0.079 | 0.35(−3.22, 3.92) | 0.848 |
| WAZ | 1.53(0.33, 2.74) | 0.012 | 1.72(0.27, 3.17) | 0.020 | 1.41(−1.10, 3.91) | 0.271 |
| BAZ | 1.09(−0.03, 2.21) | 0.057 | 1.16(−0.23, 2.55) | 0.100 | 1.03(−0.77, 2.84) | 0.261 |
| IGF-1 | | | | | | |
| HAZ | 16.62(13.52, 19.71) | 0.00009 | 14.22(10.30, 18.15) | 0.0005 | 10.53(2.68, 18.38) | 0.009 |
| WAZ | 8.86(5.12, 12.61) | 0.0006 | 12.36(9.06, 15.67) | 0.0007 | 12.27(6.76, 17.78) | 0.0003 |
| BAZ | 7.41(4.69, 10.13) | 0.00002 | 7.10(3.79, 10.42) | 0.0002 | 6.57(2.55, 10.59) | 0.001 |
| IGF-BP3 | | | | | | |
| HAZ | 224.8(136.2, 313.5) | 0.0007 | 230.7(106.5, 354.9) | <0.001 | 53.0(−158.6, 264.6) | 0.622 |
| WAZ | 169.7(90.5, 248.8) | 0.0009 | 172.2(66.50, 277.9) | 0.002 | 38.4(−113.8, 190.6) | 0.619 |
| BAZ | 56.9(−17.0, 130.7) | 0.131 | 74.9(−25.7, 175.6) | 0.144 | 8.86(−100.3, 118.02) | 0.873 |
| Calcitonin | | | | | | |
| HAZ | −0.43(−0.75, −0.12) | 0.007 | −0.17(−0.70, 0.36) | 0.535 | −0.91(−1.51, −0.31) | 0.003 |
| WAZ | 0.01(−0.28, 0.29) | 0.956 | 0.18(−0.27, 0.63) | 0.428 | 0.07(−0.38, 0.51) | 0.767 |
| BAZ | 0.31(0.05, 0.57) | 0.018 | 0.37(−0.05, 0.79) | 0.084 | 0.28(−0.03, 0.60) | 0.080 |
| STAT5B[†] | | | | | | |
| HAZ | 0.01(−0.08, 0.10) | 0.894 | 0.03(−0.10, 0.16) | 0.676 | −0.04(−0.23, 0.16) | 0.698 |
| WAZ | 0.07(−0.03, 0.17) | 0.179 | 0.10(−0.01, 0.21) | 0.067 | −0.02(−0.16, 0.11) | 0.746 |
| BAZ | 0.05(−0.02, 0.12) | 0.163 | 0.12(0.02, 0.22) | 0.023 | −0.01(−0.10, 0.09) | 0.876 |

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; Ca, calcium; Mg, magnesium; PTH, parathyroid hormone; GH, calcitonin, growth hormone; IGF-1, insulin-like-growth factor 1; IGF-BP3, IGF-binding protein 3; STAT5b, Signal transducer and activator of transcription 5B.

[a]The model was adjusted by age, birth weight, sex, setting height, mother BMI, Gestational week (GW), SES at 4.5 and urinary arsenic at 4.5 years.

[b]The model was adjusted by age, birth weight, sex, sitting height, mother BMI, Gestational week (GW), SES at 9 years and urinary arsenic at 9 years.

[c]data is log transformed.

[c]Plasma Mg was log transformed.

[†]Subset analysis for $n = 209$ and boys $= 94$ and girls $= 115$.

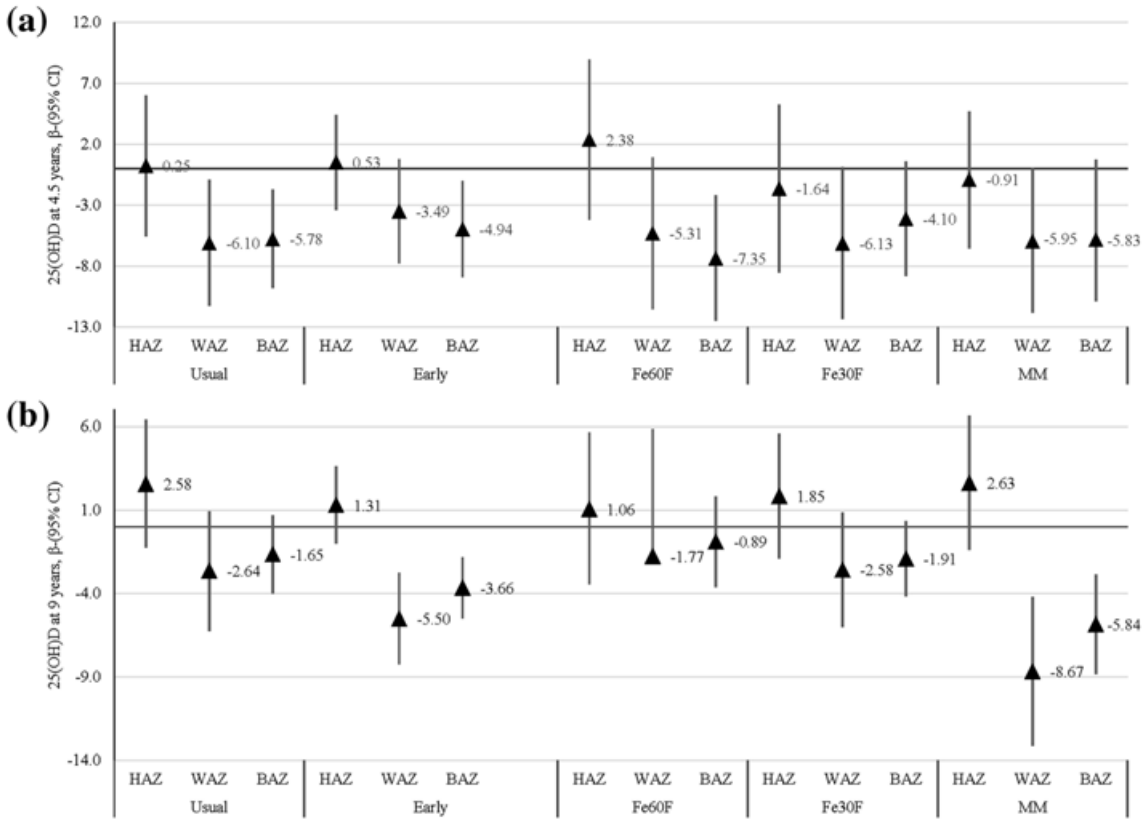


**FIGURE 3** Regression analysis of 25(OH)D with anthropometric indices at 4.5 and 9 years stratified by foods and micronutrient supplementation

No changes were observed in Ca and PTH levels at 4.5 years. Plasma Ca at 9 years showed inverse associations with WAZ and BAZ in the usual-food and MM groups (Table 3). In contrast, Mg concentrations increased with increasing WAZ and BAZ in the Fe60F groups and PTH levels increased with increasing WAZ and BAZ in the usual-food and MM groups. However, these associations were generally weak.

At 4.5 years, we found a positive association of IGF-1 levels with HAZ in all groups except for Fe30F, while with WAZ the association was evident in the early-food and MM groups. Interestingly, at 9 years, plasma IGF-1 was found positively associated with almost all anthropometric z-scores (HAZ, WAZ and BAZ) (Table 3) irrespective of timing of introduction of food or the types of micronutrients supplementation. At 9 years IGF-BP3 concentrations increased with increasing HAZ in the early-food, Fe30F and MM groups.

Calcitonin levels were inversely associated with HAZ in early-food, the Fe30F and MM groups. However, positive associations between calcitonin and WAZ and BAZ were observed in the early-food group as well as in the Fe60F group (Figure 4a,b).

## 4 | DISCUSSION

This study reports the long-term effects of prenatal nutritional interventions on growth in relation with various growth biomarkers as well as their association with anthropometric indices in children, followed from early childhood through preadolescence. Prenatal MM supplementation had a tendency to impact positively on body mass index of children at both 4.5 and 9 years while early food supplementation were related to reduced stunting and underweight in children, consistent with the findings of the larger cohort of MINIMat children at 5 years of age (Islam Khan, 2013). The positive associations of IGF-1 at both 4.5 and 9 years and IGF-BP3 in 9 years old on growth parameters were not influenced by timing of introduction of prenatal food or by types of micronutrient supplementation.

The existing method to evaluate child growth is limited to anthropometric measurement which is not always useful to reflect the accurate role of nutritional supplementation in improving growth (Raiten et al., 2013). Instruments are expensive, are of poor sensitivity and nonspecific for detecting impaired growth in nutritionally disadvantaged populations. Thus, it is critically important to identify usefulness of biomarkers in the assessment of the optimal child growth in response to nutrition. This trial was carried out in a population where undernutrition is prevalent and we observed no overall effect on biochemical parameters of growth among children born to mothers who received combination of the different food and micronutrient alternatives. Most studies that provided an antenatal MM versus iron and/folate supplement, found no or minimal effect on various outcomes including birth weight, and mortality in children (Persson et al., 2012; Ramakrishnan et al., 2012). Notably, this study

**TABLE 3** Mean change in bio-markers in Fe30F and MM group compared to Fe60F and early compared to usual food supplementation group

| | Fe60F ($n = 184$) | Fe30F ($n = 189$) | MM ($n = 162$) | Usual ($n = 256$) | Early ($n = 280$) |
|---|---|---|---|---|---|
| | | β(95% CI) | β(95% CI) | | β(95% CI) |
| [a]25(OH)D at 4.5 years | Ref. | 2.19(−2.63, 7.01) | 1.05(−4.0, 6.11) | Ref. | −3.36(−7.39, 0.67) |
| [b]25(OH)D at 9 years | Ref. | 3.0(−0.42, 6,36) | 1.23(−2.31, 4.78,) | Ref. | 0.06(−2.13, 2.25) |
| [a]Calcium at 4.5 years | Ref. | −0.15(−0.74, 0.44) | −0.14(−0.76, 0.48) | Ref. | 0.33(−0.17, 0.82) |
| [b]Calcium at 9 years | Ref. | −0.01(−0.12, 0.09) | 0.01(−0.10, 0.13) | Ref. | 0.04(−0.05, 0.13) |
| [a]PTH at 4.5 years | Ref. | 0.89(−3.43, 5.20) | 0.46(−4.07, 5.0) | Ref. | −1.85(−5.47, 1.76) |
| [b]PTH at 9 years | Ref. | −0.56(−3.17, 2.06) | −0.70(−3.44, 2.03) | Ref. | 0.06(−2.13, 2.25) |
| [a]IGF-1 at 4.5 years | Ref. | −5.95(−14.0, 2.07) | 2.56(−5.88, 11.0) | Ref. | 0.73(−6.05, 7.51) |
| [b]IGF-1 at 9 years | Ref. | −1.79(−7.70, 4.13) | 1.08(−5.10, 7.26) | Ref. | 1.59(−3.37, 6.55) |
| [a]mg at 4.5 years | Ref. | 0.003(−0.01, 0.02) | 0.01(−0.01, 0.03) | Ref. | 0.01(−0.01, 0.02) |
| [b]mg at 9 years | Ref. | −0.01(−0.02, 0.01) | 0.0003(−0.01, 0.02) | Ref. | −0.003(−0.02, 0.01) |
| [b]IGF-BP3 at 9 years | Ref. | 24.1(−143, 191) | −5.06(−180, 170) | Ref. | 12.9(−127, 153) |
| [b]Calcitonin at 9 years | Ref. | −0.28(−0.94, 0.37) | 0.15(−0.54, 0.84) | Ref. | 0.07(−0.49, 0.62) |
| [b]GH at 9 years | Ref. | 36.8(−343, 417) | 51(−348, 450) | Ref. | 60.4(−259, 380) |
| [b]STAT5B at 9 years | Ref. | 0.02(−0.15, 0.18) | 0.01(−0.18, 0.18) | Ref | −0.21(−0.36, −0.07) |
| [b]SOCS2 at 9 years | Ref. | −0.07(−0.23, 0.09) | −0.001(−0.18, 0.18) | Ref. | −0.11(−0.25, 0.04) |

*Note:* Multivariate regression model was used to estimate the *p* value.

Abbreviations: Fe30F, 30 mg iron and 400 μg of folic acid; Fe60F, 60 mg iron and 400 μg of folic acid; MMS, multiple micronutrients, 15 micronutrients including 30 mg iron and 400 μg of folic acid.

[a]The model was adjusted by age, birth weight, sex, sitting height, mother BMI, Gestational week (GW), SES at 4.5 and urinary arsenic at 4.5.

[b]The model was adjusted by age, birth weight, sex, setting height, mother BMI, Gestational week (GW), SES at 9 years and urinary arsenic at 9 years.

demonstrated for the first time in humans that expression of STAT5b, a molecular marker was affected in 9 years old children by in utero nutrition supplementation. While there is a biological justification for expecting a change in STAT5b, it is uncertain whether our finding could also represent a false positive. Several studies have shown that GH receptor-activated STAT5b has an important role in regulation of key enzymes involved in lipid and energy balance and support our finding of positive association between STAT5b and BAZ (Moller & Jorgensen, 2009). However, the role of early food supplementation on reducing STAT5b expression is not clear. Recent evidence from human studies has demonstrated that STAT5b is associated with metabolic disorders (Flores-Morales et al., 2006). Our group has earlier shown that chronic exposure to arsenic impaired IGF-1 expression in these MINIMat children at 4.5 years of age (26); whether arsenic exposure had any effect on STAT5b expression at 9 years has not been examined. Further research is warranted to understand how food supplementation during pregnancy affects the growth pathway and their physiological relevance in growing children.

Earlier Mariko et al. have shown an inverse relation of cord blood 25(OH)D with weight-for-length Z-score and BAZ in MINIMat newborns (Doi et al., 2011). Consistent with this, our findings also indicated that plasma 25(OH)D was persistently higher in children with low BMI at both 4.5 and 9 years, with strong effects observed in early-food and MM groups at preadolescence. The data on association between vitamin D status and child growth, undernutrition and obesity are inconclusive (Giustina et al., 2019). The association between vitamin D and linear growth in children who have clinical manifestations of deficiency such as rickets is well known (Giustina et al., 2019). Although some investigators have reported a positive correlation between 25(OH)D status and height in infancy, childhood and young adulthood (Kremer et al., 2009; Roth et al., 2013; Zhu et al., 2017), many studies including ours found that vitamin D was not an important contributor to linear growth (Chowdhury et al., 2017; Gould et al., 2017; Sudfeld et al., 2017). Multiple studies have reported association of obesity or high BMI with poor vitamin D status in children and adolescents (Giustina et al., 2019). Again, others have shown that underweight children were more likely to have lower serum 25(OH)D compared to normal-weight children (Marasinghe et al., 2015; Mokhtar et al., 2018). Experimental studies have shown a role of active vitamin D and its metabolites in inhibiting the formation of adipocytes by inhibiting the expression of a key regulator of adipogenesis, Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) (Mutt et al., 2014). One study reported that vitamin D stimulates lipogenesis and inhibits lipolysis by interacting with membrane vitamin D receptor (Abbas, 2017). In support of these findings, data from the NHANES indicated an inverse relation between measures of body fat and serum 25(OH)D, with leaner individuals having higher 25(OH)D concentration than do heavier individuals (Kumar et al., 2009; Yetley, 2008), which is consistent with our findings.

The inverse relationship between 25(OH)D and PTH levels has been reported in various studies in children and adolescents (Abrams et al., 2005; Outila et al., 2001); we have further shown that the anthropometric z- scores in 9 years old MINIMat children were positively associated with PTH and inversely with 25(OH)D. Apparently in

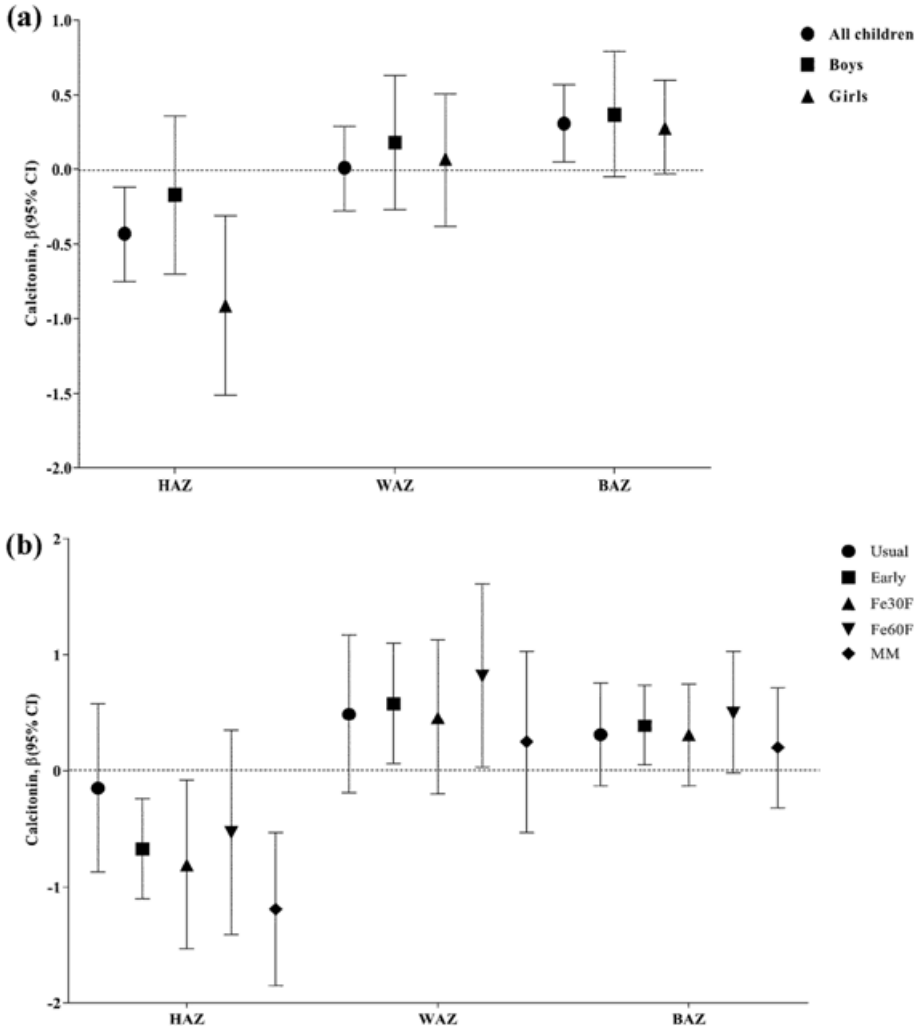


**FIGURE 4** Mean changes in calcitonin with anthropometric indices at 9 years of the children stratified by sex (a) and foods and micronutrient supplementation (b). Associations of plasma calcitonin concentrations with anthropometric-z scores exhibited inverse association with HAZ in all children which appeared to be stronger in girls. Positive association between calcitonin and BAZ was observed in the early-food group in all children. Calcitonin levels were inversely associated with HAZ in early-food, the Fe30F and MM groups. Positive associations between calcitonin and WAZ and BAZ were observed in the early-food group as well as in the Fe60F group. Fe30F = 30 mg iron and 400 μg of folic acid; Fe60F = 60 mg iron and 400 μg of folic acid; MM = multiple micronutrients, 15 micronutrients including 30 mg iron and 400 μg of folic acid

the presence of inverse association of vitamin D with growth indicators, PTH secretion increases to adapt to higher demand for growth in the pre-puberty age (Abrams et al., 2005). Population-based surveys support our findings of positive association of PTH with BAZ and WAZ scores in the children (Kamycheva et al., 2004; Reinehr et al., 2007). Moreover, we have shown that MM supplementation had stronger positive influence on association between BAZ/WAZ and circulating PTH levels at preadolescence. Our study demonstrates that calcitonin concentration in the early-food or Fe60F supplementation group is associated with higher body weight/body mass index. In support of our findings, a recent experimental study found that calcitonin in male mice may positively influence glucose and lipid metabolism, possibly via decreased adiponectin secretion from adipocytes (Nakamura et al., 2018). However, our data also exhibited inverse association of calcitonin with HAZ which appeared to be stronger in girls. In a Ca supplementation study among normal and GH deficient children, increase in serum calcitonin level was significantly lower in girls compared to boys among normal children (Saggese et al., 1993). Indeed, biomarker levels in children might vary greatly based on their age, sex and puberty stage—since hormones are important regulators of body growth. When stratified by sex, we found differences in associations between multiple growth biomarkers and anthropometric z scores. Further longitudinal studies are necessary to understand how biomarkers levels changes with height/weight among boys compared to girls.

For optimal skeletal health, vitamin D is essential for facilitating absorption of Ca. Inadequate Ca status among MINIMat children with lower 25(OH)D status may suggest inefficient utilization of Ca for calcification. Many epidemiological studies have shown relationships between high Ca intake and decreased body weight in children (Carruth & Skinner, 2001; Shapses et al., 2001) which substantiates the finding of inverse association between plasma Ca and BAZ in the MINIMat children. Increased intracellular Ca concentrations promote lipogenesis and suppress lipolytic activities in adipocytes in a coordinated fashion (Xue et al., 2001; Zemel et al., 2000). Recent evidence suggests that for efficient metabolization of vitamin D, adequate levels of Mg are also essential. Magnesium plays a key role in regulating the enzymatic processes and glucose metabolism, eventually determining the weight of an individual. One study in school children aged 10–12 years reported that children exposed to lower-than-recommended levels of Mg (10 mg/L) and Ca (20 mg/L) in drinking water exhibited reduced height and reduced rate of growth

compared to those with higher mineral exposure (Huang et al., 2018). Our finding of positive association between Mg and BAZ/WAZ in the standard iron supplementation group (Fe60F) may reflect an additional influence of iron on child growth.

In our cohort, IGF-1 levels increased with increasing age and were associated with physical growth indicators (HAZ, WAZ) as seen in growing children in other populations (Xu et al., 2010). However, concentrations of IGF-1 and IGF-BP3 in MINIMat children were about twofold lower than those found in Chinese preadolescents. This may be related to higher rate of undernutrition prevalent in our cohort. Furthermore, girls had significantly higher IGF-1 levels than boys at both 4.5 and 9 years of age, whereas higher IGF-1 levels in Chinese girls were found at the age of 9–12 years, most likely due to occurrence of early puberty. Our finding of high prevalence of stunting among girls at 4.5 and 10 years follows the scenario in the larger MINIMat cohort (Svefors et al., 2016); higher concentration of IGF-1 in girls thus might indicate increased requirement of IGF-1 for growth; IGFBP-3 levels were also higher in 9-year-old girls. However, we did not find any effect of timing of introduction of prenatal food or types of micronutrient supplementation on IGF-1/IGFBP-3 related changes.

The study has a number of limitations. When analysing the effect of maternal supplementation on child outcome, we need to consider the trade-offs between the mother and the child, for example, a child from a mother with an inadequate Ca status cannot receive a full benefit of the supplementation. Even though Ca was not contained in the MM supplement for the MINIMat mothers, vitamin D was. Since maternal growth/bone biomarker status was not measured, drawing inference regarding effects of prenatal supplementation cannot be generalized to all adolescents. One limitation was that GH concentration was measured in fasting blood without any stimulation; the levels obtained were thus basal levels and hence may not have truly reflected any influence of prenatal intervention. Another drawback was the lack of collection of dietary intake data at either time points, consequently missing one important covariate. Furthermore, being the very first study in the field, we could not refer to any earlier studies for STAT5b/SOCS2 assays, to evaluate the impact of prenatal supplementation on growth-associated gene expression in children. The strength of our study lies in the fact that we have determined important biochemical and molecular growth biomarkers in relation to physical growth parameters in a prospective design in more than 500 preadolescent children and our data was adjusted for several potential confounders.

## 5 | CONCLUSION

In the present study, we sought to obtain a comprehensive picture of the long-term effects of prenatal nutritional interventions on growth in relation with various growth biomarkers as well as their association with anthropometric indices among children in a randomized control trial in an impoverished setting. Although prenatal supplementations had limited effect on child growth indicators, the associations of growth biomarkers with anthropometric indices were predominantly driven by early introduction of food or multiple micronutrient supplementations during pregnancy. Further studies are needed to investigate the interplay between growth markers and anthropometric indices, which may be important in designing global strategies for appropriate interventions to combat impaired child growth effectively.

Malnutrition is frequently more pronounced among women in countries such as Bangladesh. The prenatal period is vulnerable and provision of maternal nutrition supplementation during this stage may beneficially affect various biomarkers involved in both short- and long-term growth. Further research is necessary to understand the independent role of maternal nutrition interventions in sequential measures of child growth and their association with other biomarkers involved in bone growth/hormonal maturation pathway. Thus a clear priority is to support the discovery, and assay validation of biomarkers that more evidently reflect the consequences of impaired growth as well as indicate specific prenatal interventions necessary to address them. Such assessment will identify best biochemical and molecular signature indicators of child growth trajectory/growth faltering that may be adopted for growth assessment by public health experts to detect deficits in early growth and combat its adverse consequences in children.

## ACKNOWLEDGMENTS

icddr,b acknowledges the following core donors which provide unrestricted support to icddr,b for its operations and research. Current donors providing unrestricted support include: Government of the Peoples Republic of Bangladesh; Global Affairs Canada (GAC); Swedish International Development Cooperation Agency (Sida) and the Department for International Development (UK Aid). We gratefully acknowledge these donors for their support and commitment to icddr,bs research efforts.

This work was supported by the Japan Society for the Promotion of Science (JSPS; Grant No. 18256005), icddrb (Grant No. 384, SWE-2008-065) and Swedish International Development Cooperation Agency (Grant No. 54100089). The funders had no role in the design, analysis or writing of this article.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

## CONTRIBUTIONS

RR, TJS, YW, E-CE, and SEA conceptualized and designed the study. AKR, EA, and MNAA were responsible for material preparation, data collection and laboratory analysis.

MAH and TJS analysed data and performed statistical analysis. MIH and TA provided technical expertise. TJS wrote the manuscript and RR critically reviewed it. All authors contributed to the development of this manuscript and read and approved the final version.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## ORCID

*Rubhana Raqib* https://orcid.org/0000-0002-8116-4511


## REFERENCES


Abbas, M. A. (2017). Physiological functions of vitamin D in adipose tissue. *The Journal of Steroid Biochemistry and Molecular Biology*, *165*(Pt B), 369–381.

Abrams, S. A., Griffin, I. J., Hawthorne, K. M., Gunn, S. K., Gundberg, C. M., & Carpenter, T. O. (2005). Relationships among vitamin D levels, parathyroid hormone, and calcium absorption in young adolescents. *The Journal of Clinical Endocrinology and Metabolism*, *90*(10), 5576–5581. https://doi.org/10.1210/jc.2005-1021

Ahmed, S., Rekha, R. S., Ahsan, K. B., Doi, M., Grandér, M., Roy, A. K., Ekström, E. C., Wagatsuma, Y., Vahter, M., & Raqib, R. (2013). Arsenic exposure affects plasma insulin-like growth factor 1 (IGF-1) in children in rural Bangladesh. *PLoS ONE*, *8*(11), e81530. https://doi.org/10.1371/journal.pone.0081530

Arifeen, S. E., Ekström, E. C., Frongillo, E. A., Hamadani, J., Khan, A. I., Naved, R. T., Rahman, A., Raqib, R., Rasmussen, K. M., Selling, K. E., & Wagatsuma, Y. (2018). Cohort profile: The Maternal and Infant Nutrition Interventions in Matlab (MINIMat) cohort in Bangladesh. *International Journal of Epidemiology*, *47*(6), 1737–1738e.

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R., & Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet*, *382*(9890), 427–451. https://doi.org/10.1016/S0140-6736(13)60937-X

Carruth, B. R., & Skinner, J. D. (2001). The role of dietary calcium and other nutrients in moderating body fat in preschool children. *International Journal of Obesity and Related Metabolic Disorders*, *25*(4), 559–566. https://doi.org/10.1038/sj.ijo.0801562

Carter, P. H., & Schipani, E. (2006). The roles of parathyroid hormone and calcitonin in bone remodeling: Prospects for novel therapeutics. *Endocrine, Metabolic & Immune Disorders Drug Targets*, *6*(1), 59–76. https://doi.org/10.2174/187153006776056666

Chowdhury, R., Taneja, S., Bhandari, N., Kvestad, I., Strand, T. A., & Bhan, M. K. (2017). Vitamin-D status and neurodevelopment and growth in young north Indian children: A secondary data analysis. *Nutrition Journal*, *16*(1), 59. https://doi.org/10.1186/s12937-017-0285-y

Christian, P., Kim, J., Mehra, S., Shaikh, S., Ali, H., Shamim, A. A., Wu, L., Klemm, R., Labrique, A. B., & West, K. P. Jr. (2016). Effects of prenatal multiple micronutrient supplementation on growth and cognition through 2 y of age in rural Bangladesh: The JiVitA-3 Trial. *The American Journal of Clinical Nutrition*, *104*(4), 1175–1182. https://doi.org/10.3945/ajcn.116.135178

Devakumar, D., Fall, C. H. D., Sachdev, H. S., Margetts, B. M., Osmond, C., Wells, J. C. K., Costello, A., & Osrin, D. (2016). Maternal antenatal multiple micronutrient supplementation for long-term health benefits in children: A systematic review and meta-analysis. *BMC Medicine*, *14*, 90. https://doi.org/10.1186/s12916-016-0633-3

Doi, M., Sultana Rekha, R., Ahmed, S., Okada, M., Kumar Roy, A., el Arifeen, S., Ekström, E. C., Raqib, R., & Wagatsuma, Y. (2011). Association between calcium in cord blood and newborn size in Bangladesh. *The British Journal of Nutrition*, *106*(9), 1398–1407. https://doi.org/10.1017/S0007114511001747

Ekstrom, E. C., Lindström, E., Raqib, R., El Arifeen, S., Basu, S., Brismar, K., Selling, K., & Persson, L. (2016). Effects of prenatal micronutrient and early food supplementation on metabolic status of the offspring at 4.5 years of age. The MINIMat randomized trial in rural Bangladesh. *International Journal of Epidemiology*, *45*(5), 1656–1667. https://doi.org/10.1093/ije/dyw199

Eugster, E. A., & Pescovitz, O. H. (2003). New revelations about the role of STATs in stature. *New England Journal of Medicine*, *349*(12), 1110–1112. https://doi.org/10.1056/NEJMp038134

Flores-Morales, A., Greenhalgh, C. J., Norstedt, G., & Rico-Bautista, E. (2006). Negative regulation of growth hormone receptor signaling. *Molecular Endocrinology*, *20*(2), 241–253. https://doi.org/10.1210/me.2005-0170

Giustina, A., Adler, R. A., Binkley, N., Bouillon, R., Ebeling, P. R., Lazaretti-Castro, M., Marcocci, C., Rizzoli, R., Sempos, C. T., & Bilezikian, J. P. (2019). Controversies in vitamin D: Summary statement from an international conference. *The Journal of Clinical Endocrinology and Metabolism*, *104*(2), 234–240. https://doi.org/10.1210/jc.2018-01414

Gliga, A. R., Engström, K., Kippler, M., Skröder, H., Ahmed, S., Vahter, M., Raqib, R., & Broberg, K. (2018). Prenatal arsenic exposure is associated with increased plasma IGFBP3 concentrations in 9-year-old children partly via changes in DNA methylation. *Archives of Toxicology*, *92*(8), 2487–2500. https://doi.org/10.1007/s00204-018-2239-3

Gould, J. F., Anderson, A. J., Yelland, L. N., Smithers, L. G., Skeaff, C. M., Zhou, S. J., Gibson, R. A., & Makrides, M. (2017). Association of cord blood vitamin D with early childhood growth and neurodevelopment. *Journal of Paediatrics and Child Health*, *53*(1), 75–83. https://doi.org/10.1111/jpc.13308

Greenhalgh, C. J., Rico-Bautista, E., Lorentzon, M., Thaus, A. L., Morgan, P. O., Willson, T. A., Zervoudakis, P., Metcalf, D., Street, I., Nicola, N. A., Nash, A. D., Fabri, L. J., Norstedt, G., Ohlsson, C., Flores-Morales, A., Alexander, W. S., & Hilton, D. J. (2005). SOCS2 negatively regulates growth hormone action in vitro and in vivo. *The Journal of Clinical Investigation*, *115*(2), 397–406. https://doi.org/10.1172/JCI200522710

Harkare, H. V., Corsi, D. J., Kim, R., Vollmer, S., & Subramanian, S. V. (2021). The impact of improved data quality on the prevalence estimates of anthropometric measures using DHS datasets in India. *Scientific Reports*, *11*(1), 1–13.

Huang, Y., Wang, J., Tan, Y., Wang, L., Lin, H., Lan, L., Xiong, Y., Huang, W., & Shu, W. (2018). Low-mineral direct drinking water in school may retard height growth and increase dental caries in schoolchildren in China. *Environment International*, *115*, 104–109. https://doi.org/10.1016/j.envint.2018.02.021

Islam Khan, A. (2013). Effects of pre- and postnatal nutrition interventions on child growth and body composition: The MINIMat trial in rural Bangladesh. *Global Health Action*, *6*, 22476. https://doi.org/10.3402/gha.v6i0.22476

Kamycheva, E., Sundsfjord, J., & Jorde, R. (2004). Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *European Journal of Endocrinology*, *151*(2), 167–172. https://doi.org/10.1530/eje.0.1510167

Khan, A. I., Kabir, I., Ekström, E. C., Åsling-Monemi, K., Alam, D. S., Frongillo, E. A., Yunus, M., Arifeen, S., & Persson, L. Å. (2011). Effects of prenatal food and micronutrient supplementation on child growth from birth to 54 months of age: A randomized trial in Bangladesh. *Nutrition Journal*, *10*, 134. https://doi.org/10.1186/1475-2891-10-134

Kremer, R., Campbell, P. P., Reinhardt, T., & Gilsanz, V. (2009). Vitamin D status and its relationship to body fat, final height, and peak bone mass in young women. *The Journal of Clinical Endocrinology and Metabolism*, *94*(1), 67–73. https://doi.org/10.1210/jc.2008-1575

Kumar, J., Muntner, P., Kaskel, F. J., Hailpern, S. M., & Melamed, M. L. (2009). Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. *Pediatrics*, *124*(3), e362–e370. https://doi.org/10.1542/peds.2009-0051

Lanou, H., Huybregts, L., Roberfroid, D., Nikiema, L., Kouanda, S., van Camp, J., & Kolsteren, P. (2014). Prenatal nutrient supplementation and postnatal growth in a developing nation: An RCT. *Pediatrics*, *133*(4), e1001–e1008. https://doi.org/10.1542/peds.2013-2850

Lu, W. P., Lu, M. S., Li, Z. H., & Zhang, C. X. (2014). Effects of multimicronutrient supplementation during pregnancy on postnatal growth of children under 5 years of age: A meta-analysis of randomized controlled trials. *PLoS ONE*, *9*(2), e88496. https://doi.org/10.1371/journal.pone.0088496

Mannan, T., Ahmed, S., Akhtar, E., Roy, A. K., Haq, M. A., Roy, A., Kippler, M., Ekström, E. C., Wagatsuma, Y., & Raqib, R. (2016). Maternal micronutrient supplementation and long term health impact in children in rural Bangladesh. *PLoS ONE*, *11*(8), e0161294. https://doi.org/10.1371/journal.pone.0161294

Marasinghe, E., Chackrewarthy, S., Abeysena, C., & Rajindrajith, S. (2015). Micronutrient status and its relationship with nutritional status in preschool children in urban Sri Lanka. *Asia Pacific Journal of Clinical Nutrition*, *24*(1), 144–151. https://doi.org/10.6133/apjcn.2015.24.1.17

Mokhtar, R. R., Holick, M. F., Sempértegui, F., Griffiths, J. K., Estrella, B., Moore, L. L., Fox, M. P., & Hamer, D. H. (2018). Vitamin D status is associated with underweight and stunting in children aged 6-36 months residing in the Ecuadorian Andes. *Public Health Nutrition*, *21*(11), 1974–1985. https://doi.org/10.1017/S1368980017002816

Moller, N., & Jorgensen, J. O. (2009). Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocrine Reviews*, *30*(2), 152–177. https://doi.org/10.1210/er.2008-0027

Mridha, M. K., Matias, S. L., Chaparro, C. M., Paul, R. R., Hussain, S., Vosti, S. A., Harding, K. L., Cummins, J. R., Day, L. T., Saha, S. L., Peerson, J. M., & Dewey, K. G. (2016). Lipid-based nutrient supplements for pregnant women reduce newborn stunting in a cluster-randomized controlled effectiveness trial in Bangladesh. *The American Journal of Clinical Nutrition*, *103*(1), 236–249. https://doi.org/10.3945/ajcn.115.111336

Mutt, S. J., Hyppönen, E., Saarnio, J., Järvelin, M. R., & Herzig, K. H. (2014). Vitamin D and adipose tissue-more than storage. *Frontiers in Physiology*, *5*, 228.

Nakamura, M., Nomura, S., Yamakawa, T., Kono, R., Maeno, A., Ozaki, T., Ito, A., Uzawa, T., Utsunomiya, H., & Kakudo, K. (2018). Endogenous calcitonin regulates lipid and glucose metabolism in diet-induced obesity mice. *Scientific Reports*, *8*(1), 17001. https://doi.org/10.1038/s41598-018-35369-5

Osmani, S., & Sen, A. (2003). The hidden penalties of gender inequality: Fetal origins of ill-health. *Economics and Human Biology*, *1*(1), 105–121. https://doi.org/10.1016/S1570-677X(02)00006-0

Outila, T. A., Karkkainen, M. U., & Lamberg-Allardt, C. J. (2001). Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: Associations with forearm bone mineral density. *The American Journal of Clinical Nutrition*, *74*(2), 206–210. https://doi.org/10.1093/ajcn/74.2.206

Pekkinen, M., Viljakainen, H., Saarnio, E., Lamberg-Allardt, C., & Mäkitie, O. (2012). Vitamin D is a major determinant of bone mineral density at school age. *PLoS ONE*, *7*(7), e40090. https://doi.org/10.1371/journal.pone.0040090

Persson, L. A., Arifeen, S., Ekström, E. C., Rasmussen, K. M., Frongillo, E. A., Yunus, M., & MINIMat Study Team. (2012). Effects of prenatal micronutrient and early food supplementation on maternal hemoglobin, birth weight, and infant mortality among children in Bangladesh: The MINIMat randomized trial. *JAMA*, *307*(19), 2050–2059. https://doi.org/10.1001/jama.2012.4061

Raiten, D. J., Raghavan, R., & Kraemer, K. (2013). Biomarkers in growth. *Annals of Nutrition and Metabolism*, *63*(4), 293–297. https://doi.org/10.1159/000357572

Ramakrishnan, U., Grant, F. K., Goldenberg, T., Bui, V., Imdad, A., & Bhutta, Z. A. (2012). Effect of multiple micronutrient supplementation on pregnancy and infant outcomes: A systematic review. *Paediatric and Perinatal Epidemiology*, *26*(Suppl 1), 153–167. https://doi.org/10.1111/j.1365-3016.2012.01276.x

Raqib, R., Ahmed, S., Ahsan, K. B., Kippler, M., Akhtar, E., Roy, A. K., Lu, Y., Arifeen, S. E., Wagatsuma, Y., & Vahter, M. (2017). Humoral Immunity in arsenic-exposed children in rural Bangladesh: Total immunoglobulins and vaccine-specific antibodies. *Environmental Health Perspectives*, *125*(6), 067006. https://doi.org/10.1289/EHP318

Raqib, R., Ahmed, S., Sultana, R., Wagatsuma, Y., Mondal, D., Hoque, A. M. W., Nermell, B., Yunus, M., Roy, S., Persson, L. A., Arifeen, S. E., Moore, S., & Vahter, M. (2009). Effects of in utero arsenic exposure on child immunity and morbidity in rural Bangladesh. *Toxicology Letters*, *185*(3), 197–202. https://doi.org/10.1016/j.toxlet.2009.01.001

Reinehr, T., de Sousa, G., Alexy, U., Kersting, M., & Andler, W. (2007). Vitamin D status and parathyroid hormone in obese children before and after weight loss. *European Journal of Endocrinology*, *157*(2), 225–232. https://doi.org/10.1530/EJE-07-0188

Roth, D. E., al Mahmud, A., Raqib, R., Akhtar, E., Perumal, N., Pezzack, B., & Baqui, A. H. (2013). Randomized placebo-controlled trial of high-dose prenatal third-trimester vitamin D3 supplementation in Bangladesh: The AViDD trial. *Nutrition Journal*, *12*, 47. https://doi.org/10.1186/1475-2891-12-47

Rotwein, P., & Chia, D. J. (2010). Gene regulation by growth hormone. *Pediatric Nephrology*, *25*(4), 651–658. https://doi.org/10.1007/s00467-009-1258-y

Saggese, G., Baroncelli, G. I., Bertelloni, S., Cinquanta, L., & di Nero, G. (1993). Effects of long-term treatment with growth hormone on bone and mineral metabolism in children with growth hormone deficiency. *The Journal of Pediatrics*, *122*(1), 37–45. https://doi.org/10.1016/S0022-3476(05)83484-5

Shapses, S. A., von Thun, N. L., Heymsfield, S. B., Ricci, T. A., Ospina, M., Pierson, R. N. JR., & Stahl, T. (2001). Bone turnover and density in obese premenopausal women during moderate weight loss and calcium supplementation. *Journal of Bone and Mineral Research*, *16*(7), 1329–1336. https://doi.org/10.1359/jbmr.2001.16.7.1329

Shrimpton, R., Victora, C. G., de Onis, M., Lima, R. C., Blossner, M., & Clugston, G. (2001). Worldwide timing of growth faltering: Implications for nutritional interventions. *Pediatrics*, *107*(5), E75. https://doi.org/10.1542/peds.107.5.e75

Stagi, S., Cavalli, L., Iurato, C., Seminara, S., Brandi, M. L., & de Martino, M. (2013). Bone metabolism in children and adolescents: Main characteristics of the determinants of peak bone mass. *Clinical Cases in Mineral and Bone Metabolism*, *10*(3), 172–179.

Sudfeld, C. R., Mugusi, F., Aboud, S., Nagu, T. J., Wang, M., & Fawzi, W. W. (2017). Efficacy of vitamin D3 supplementation in reducing incidence of pulmonary tuberculosis and mortality among HIV-infected Tanzanian adults initiating antiretroviral therapy: Study protocol for a randomized controlled trial. *Trials*, *18*(1), 66. https://doi.org/10.1186/s13063-017-1819-5

Svefors, P., Rahman, A., Ekström, E. C., Khan, A. I., Lindström, E., Persson, L. Å., & Ekholm Selling, K. (2016). Stunted at 10 years. Linear growth trajectories and stunting from birth to pre-adolescence in a rural Bangladeshi cohort. *PLoS ONE*, *11*(3), e0149700.

Uauy, R., Kain, J., & Corvalan, C. (2011). How can the Developmental Origins of Health and Disease (DOHaD) hypothesis contribute to improving health in developing countries? *The American Journal of Clinical Nutrition*, *94*(6 Suppl), 1759s–1764s.

Vance, M. L. (1990). Growth-hormone-releasing hormone. *Clinical Chemistry*, *36*(3), 415–420. https://doi.org/10.1093/clinchem/36.3.415

Victora, C. G., de Onis, M., Hallal, P. C., Blossner, M., & Shrimpton, R. (2010). Worldwide timing of growth faltering: Revisiting implications for interventions. *Pediatrics*, *125*(3), e473–e480. https://doi.org/10.1542/peds.2009-1519

Woelfle, J., Harz, K., & Roth, C. (2007). Modulation of circulating IGF-I and IGFBP-3 levels by hormonal regulators of energy homeostasis in obese children. *Experimental and Clinical Endocrinology & Diabetes*, *115*(01), 17–23.

Xu, S., Gu, X., Pan, H., Zhu, H., Gong, F., Li, Y., & Xing, Y. (2010). Reference ranges for serum IGF-1 and IGFBP-3 levels in Chinese children during childhood and adolescence. *Endocrine Journal*, *57*(3), 221–228. https://doi.org/10.1507/endocrj.K09E-200

Xue, B., Greenberg, A. G., Kraemer, F. B., & Zemel, M. B. (2001). Mechanism of intracellular calcium ([Ca2+]i) inhibition of lipolysis in human adipocytes. *The FASEB Journal*, *15*(13), 2527–2529. https://doi.org/10.1096/fj.01-0278fje

Yetley, E. A. (2008). Assessing the vitamin D status of the US population. *The American Journal of Clinical Nutrition*, *88*(2), 558s–564s.

Zemel, M. B., Shi, H., Greer, B., Dirienzo, D., & Zemel, P. C. (2000). Regulation of adiposity by dietary calcium. *The FASEB Journal*, *14*(9), 1132–1138. https://doi.org/10.1096/fasebj.14.9.1132

Zhu, K., Oddy, W. H., Holt, P., Ping-Delfos, W. C. S., Mountain, J., Lye, S., Pennell, C., Hart, P. H., & Walsh, J. P. (2017). Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass. *The American Journal of Clinical Nutrition*, *106*(1), 276–283. https://doi.org/10.3945/ajcn.116.150524

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Siddiqua, T. J., Roy, A. K., Akhtar, E., Haq, M. A., Wagatsuma, Y., Ekström, E.-C., Afsar, M. N. A., Hossain, M. I., Ahmed, T., El Arifeen, S., & Raqib, R. (2022). Prenatal nutrition supplementation and growth biomarkers in preadolescent Bangladeshi children: A birth cohort study. *Maternal & Child Nutrition*, 18:e13266. https://doi.org/10.1111/mcn.13266