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Lower intakes of protein, carbohydrate, and energy are associated with increased global DNA methylation in 2- to 3-year-old urban slum children in Bangladesh

Mohd S. Iqbal[1] | Sabuktagin Rahman[2] | Md Ahshanul Haque[1] | Mohammad Junayed Bhuyan[1] | Abu Syed Golam Faruque[1] | Tahmeed Ahmed[1]

[1] Nutrition and Clinical Services Division, International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), Dhaka, Bangladesh

[2] Department of Public Health, School of Medicine, Griffith University, Southport, Queensland, Australia

Correspondence
Mohd Shamim Iqbal, Nutrition and Clinical Services Division, International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), Dhaka 1212, Bangladesh.
Email: msiqbal@icddrb.org

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Abstract

Stunting in children is a global public health concern. We investigated how global DNA methylation relates to food intakes, dietary diversity, and development of stunting among 324 children aged 24–36 months in a slum community in Dhaka, Bangladesh. Stunted children (height-for-age *z* score <−2; *n* = 162) and their age- and sex-matched nonstunted counterparts (height-for-age *z* score >−1; *n* = 162) were selected by active community surveillance. We studied global DNA methylation, measured as 5-mC% content in whole blood. Dietary intake, anthropometric measurement, and sociodemographic information were obtained. In the multiple linear regression model, increased global DNA methylation level in children was significantly associated with consumption of lower amount of energy, coef: .034 (95% CI [.014, .053]); *P* = .001, protein, coef: .038 (95% CI [.019, .057]); *P* = .000, carbohydrate, coef: .027 (95% CI [.008, .047]); *P* = .006, zinc, coef: .020 (95% CI [.001, .039]); *P* = .043, total dietary intakes, coef: .020 (95% CI [.001, .039]); *P* = .043, and intake from plant sources, coef: .028 (95% CI [.009, .047]); *P* = .005, after adjusting for other covariates. Moreover, higher fruits and vegetables consumption was significantly associated with lower 5-mC% level, coef: −.022 (95% CI [−.041, −.002]); *P* = .028. Our findings suggest a significant association between low dietary intakes and increased global DNA methylation. We also found increased global DNA methylation in stunted children. To establish the relationship among the macronutrient intakes, global DNA methylation, and stunting, future prospective studies are warranted in resource-poor settings.

KEYWORDS

Bangladeshi children, dietary diversity, dietary intake, epigenetics, global DNA methylation, stunting

1 | INTRODUCTION

Stunted growth or low height-for-age *z* score (HAZ) is the most common manifestation of undernutrition inflicting 155 million children under 5 years of age in the low- and middle-income countries (Development Initiatives, 2017). Despite concerted efforts at improving childhood nutrition and health status during the Millennium Development Goal era, stunting remains an intractable problem (Bhutta et al., 2013). Stunting in early life is associated with various adverse health consequences including increased child mortality, decreased

cognitive function, behavioural abnormalities, impaired immunity, and shorter adult stature and low productivity (Huey & Mehta, 2016; Victora et al., 2008; World Health Organization [WHO], 2012).

Certain risk factors for stunting are known, but there remains a lack of clarity about specific aetiology of this very common childhood condition. In developing countries, growth faltering such as stunting initiates as early as in the developmental stages. Data from 54 resource-poor countries in Africa and Southeast Asia demonstrated a reduction in the HAZ during the first 2 years of life (Prentice et al., 2013). After the first 1,000 days, stunting generally does not worsen but is commonly irreversible, leading to adults with shorter stature. In Bangladesh, stunting among children under 5 has declined from 51% in 2004 to 36% in 2014, but it remained as 41.4% for children aged 2–3 years (NIPORT, Mitra and Associates & ICF International, 2016). According to the National Micronutrient Survey, the prevalence of stunting is higher, 51.1% in the slum for preschool-aged children (icddr'b, UNICEF/Bangladesh, GAIN, & IPHN, 2013). Adverse environmental conditions and poverty are more pronounced for the slum dwellers, with women and children under 5 being particularly vulnerable.

Nutritional factors play a crucial role in health and diseases. Over the last two decades, epigenetics evolved as one of the most important mechanisms through which nutrients and bioactive compounds affect metabolic traits. Epigenetic processes, such as DNA methylation, induce heritable changes in the gene expression and function without a change in nucleotide sequences. Numerous studies have demonstrated effects on DNA methylation of alcohol, the B vitamins, protein, micronutrients, functional food components, and general nutritional status (Haggarty, 2013). We have recently shown that DNA methylation differences in young Bangladeshi adults exposed and unexposed to famine in the postnatal period have methylation differences implicating in the regulation of growth factors signalling (Finer et al., 2016). We posit that in addition to fetal exposure during gestation, foods and environment might play important role in the aetiopathology of stunting through epigenetic mechanisms. In the present study, we investigated how the epigenetic state (global DNA methylation) relates to food intakes and development of stunting in young children in an urban slum community in Bangladesh. In addition, we examined the relationships among global DNA methylation, plasma micronutrient status, and dietary diversity. We also identified child, maternal, and household characteristics associated with the global DNA methylation among these children.

Key messages

- Higher global DNA methylation level was significantly associated with lower dietary protein, carbohydrate, and energy intakes in 2- to 3-year-old children in low-resource settings.
- Consumption of fruits and vegetables was significantly associated with lower global DNA methylation level in this setting.
- Stunted children had increased global DNA methylation level, which urges future prospective studies to explore further relationship among the dietary intakes, epigenetics, and stunting in resource-poor settings.

2 | MATERIALS AND METHODS

2.1 | Study population and design

This was a cross-sectional exploratory study, and the study population composed of 2- to 3-year-old children of an urban slum community in Dhaka, Bangladesh. For inclusion in the study, children had to be aged 24–36 months and had to have lived in the study area for at least 6 months. Children with a HAZ of <−2 were considered as stunted and HAZ of >−1 as nonstunted control. We did not find any previous study to estimate our sample size in the stunted population regarding epigenetics status (global DNA methylation). Considering the budget and timeline, a total of 324 children were purposively selected for the study out of a total of 1,196 children through community-based screening and considering the inclusion and exclusion criteria. Age of the nonstunted children was matched to the stunted children with a maximum allowance of ±1.2 months as based on the standard deviation of the mean age of the children under 5 as observed in the National Micronutrient Survey 2011–2012 (icddr'b et al., 2013). Data collection was performed at the participant's home and took place between November 2015 and March 2017. After a thorough explanation of the study, written informed consent was obtained from the mother of each child. The study was ethically approved by the institutional review board of icddr,b.

2.2 | Sociodemographic information

Data on socio-economic status, household possession of assets, household construction material, household food insecurity, morbidity of children, and food consumption were analysed. The asset index used in this study is a composite indicator of the household asset calculated using principal component analysis, following a method similar to that used in the Bangladesh Demographic and Health Survey (Gwatkin et al., 2000; NIPORT, Mitra and Associates & ICF International, 2016). Household food insecurity was assessed by the Household Food Insecurity Access Scale for measurement of food access (Coates, Swindale, & Bilinsky, 2007). Water, sanitation, and hygiene indicators were measured according to published studies and other relevant studies by icddr,b (Policy Support Unit & Local Government Division, 2014).

2.3 | Anthropometric status

Height was measured to the nearest 0.1 cm in a standing position, with the head in the Frankfurt plane the feet together and knees

straight, using a portable SECA 217 stadiometer for mobile height measurement (Seca GmbH & Co., Hamburg, Germany). Weight was measured to the nearest 0.05 kg using an electronic load cell scale (HD-661 TANITA Corporation, Tokyo, Japan). The scale was placed on a flat, hard surface and calibrated daily with an object of known weight. All measurements were taken in light clothing and without shoes or cap. HAZ for children was calculated using WHO (2006) growth standards.

2.4 | Dietary intake

A Semiquantitative Food Frequency Questionnaire was used taking into consideration commonly consumed Bangladeshi foods (46 food items) with special attention to vitamin A, iron, and zinc-rich foods (Buch-Andersen, Perez-Cueto, & Toft, 2016; Cheng et al., 2008; Rahman et al., 2017; Tang et al., 2015; Zhang et al., 2015). The tool was administered by the field data collectors and mother or the primary caretakers of the children responded on children's consumption. The respondent was asked to tell about the number of portions (servings) of particular foods that she and/or her child had consumed over the past 7 days. She was required to indicate the portion size (grams and millilitres) of the consumption from the standardized food photographs or commonly used household utensils, which were displayed and explained at the time of the interview. The number of portions consumed over the past 7 days was multiplied by the average size of the portions, to yield the total weekly consumption of a particular food (grams and millilitres). Raw food weight was calculated by using appropriate conversion factors (Ali & Pramanik, 1991). Nutrient values for macronutrients (energy, protein, carbohydrate, lipid, etc.) and micronutrients (iron, zinc, vitamin A, etc.) were calculated per 100 g of raw food consumed through use of an updated food composition table on Bangladeshi foods (Islam, Khan, & Akhtaruzzaman, 2010). All dietary intake variables were transformed to binary variables using cut-off value median, that is, 1 means below median and 0 means median or above.

2.5 | Dietary diversity

We grouped the different food items into 12 food groups consumed by the children in the last 7 days prior to the interview based on 7-day mother-reported food recall. The food groups were starchy staples (grains, roots, and tubers); legumes and nuts; dairy products (milk yogurt and cheese); meats (meat, poultry, and liver/organ meat); eggs; vitamin A-rich fruits and vegetables; other fruits and vegetables; fish; candies and desserts; snacks; sweetened beverages; and fast foods. Dietary diversity was estimated by the total of food groups that were consumed at least 10 g in the previous 7 days (Gómez-Acosta et al., 2017).

2.6 | Biochemical analyses

Haemoglobin was measured by the cyanmethaemoglobin method using Drabkin's reagent from Sigma-Aldrich Co. (3050 Spruce Street, St. Louis, MO, USA) on ultraviolet–visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Serum retinol was measured by high-performance liquid chromatography (Shimadzu Corporation) with photodiode array detection. Ferritin, vitamin D, folate, and vitamin B12 were measured by electrochemiluminescence immunoassay using Roche commercial kit on the Roche automated immunoassay analyser (Cobas e601). As the micronutrient levels, such as ferritin, retinol, and serum zinc, are influenced by infection, their values were adjusted by the infection biomarkers, for example, C-reactive protein (CRP) and 1-α-acetylated glycoprotein (AGP). CRP and AGP were measured by immunoturbidimetric assay using commercial kits from Roche diagnostics on Roche automated clinical chemistry analyser (Cobas C311; Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Serum zinc was measured by flame atomic absorption spectrophotometry (Shimadzu AA-6501S, Kyoto, Japan).

2.7 | Epigenetics analysis

Global methylation was detected in DNA isolated from whole blood samples using 5-mC DNA ELISA Kit (Zymo Research) according to the manufacturer's instructions. Levels of 5-mC in DNA of all biological samples are reported as the amount of methylated cytosine relative to the cytosine genomic content (per cent). For the global DNA methylation assay, the input DNA concentration was 100 ng. All samples (or repeats) were loaded using the same amount of DNA in the assay plate. All samples and methylated standards were measured in duplicate, and the average is reported. High methylation was defined using median cut-off point.

2.8 | Adjusting for infection

Serum ferritin, zinc, and retinol were adjusted for infection by estimating biomarkers of infection: CRP and AGP. The adjustment of serum ferritin, retinol, and zinc levels for elevated CRP (>10·0 mg/L)(ref) and AGP (>1·0 g/L) was done by calculating the correction factors following the methods described by Thurnham et al. (2010).

2.9 | Statistical analysis

Descriptive statistics such as mean, median, and proportion were used to examine the distribution and summarize the data. We performed bivariate analyses to explore the relationships using chi-square tests, Kruskal–Wallis rank test for asymmetric data, Mann–Whitney test for ordinal measurement, *t* test, and simple linear regression. To estimate the inferential statistic, 95% CI was used. All dietary intake variables were transformed to binary variables using cut-off value of median, that is, 1 represents below median and 0 represents median or above. As the data were not symmetric, median and interquartile range (IQR) were used to summarize the dietary intake data. Methylation data were continuous and asymmetric with a few extreme values. Moreover, log transformation did not result in symmetric data, and thus, we kept the original values

without any transformation. Simple linear regression was used to determine crude relationship between dietary intake data and methylation. But dietary intake data were highly correlated to each other. Therefore, multiple linear regression models were constructed for each variables to test the association between dietary intakes and methylation where the dietary components were categorized using the cut-off value of median after adjusting household food insecurity, at least 1-year formal education of household head, affected by at least one disease, hand washing using soap in the five critical times, and sex of child. Similarly, association between dietary diversity and methylation was carried out. All analyses were performed using the statistical software STATA 13.0 SE (StataCorp, College Station, TX, USA).

3 | RESULTS

Household general characteristics are given in Table 1. Only 12.65% household heads, mostly mothers, had completed high school or higher, and the proportion is lower ($P < .033$) in the stunted children group (8.02%) than in the nonstunted group (17.28%), and asset index ($P < .001$) was significantly associated with stunting. About 59% of the household were food secure, and this proportion in stunted and nonstunted groups was almost similar. Mildly, moderately, and severely food insecure households were 16.36%, 20.68%, and 4.32%, respectively. Household food insecurity was not significantly related with stunting. Mean age of the children was 28.52 months and comparable in the stunted and nonstunted groups. The proportion of children

TABLE 1 Household general characteristics

Indicator/variable	Stunted *n* (%)	Nonstunted *n* (%)	Total *n* (%)	*P* value
Level of formal education of the household head				
No education	31 (19.14)	19 (11.73)	50 (15.43)	.005
Primary incomplete	50 (30.86)	39 (24.07)	89 (27.47)	
Primary complete	27 (16.67)	32 (19.75)	59 (18.21)	
Secondary incomplete	41 (25.31)	42 (25.93)	83 (25.62)	
Secondary complete or higher	13 (8.02)	28 (17.28)	41 (12.65)	
Asset index				
1st quintile	42 (25.93)	24 (14.81)	66 (20.37)	.000
2nd quintile	41 (25.31)	23 (14.2)	64 (19.75)	
3rd quintile	31 (19.14)	35 (21.6)	66 (20.37)	
4th quintile	28 (17.28)	36 (22.22)	64 (19.75)	
5th quintile	20 (12.35)	44 (27.16)	64 (19.75)	
Household food insecurity				
Food secure	91 (56.17)	99 (61.11)	190 (58.64)	.337
Mildly food insecure	28 (17.28)	25 (15.43)	53 (16.36)	
Moderately food insecure	34 (20.99)	33 (20.37)	67 (20.68)	
Severely food insecure	9 (5.56)	5 (3.09)	14 (4.32)	
Child morbidity				
Child's age (mean)	28.50 ± 3.54	28.53 ± 3.58	28.52 ± 3.55	
Child's sex				
Male	81 (50.0)	81 (50.0)	162 (50.0)	1.000
Female	81 (50.0)	81 (50.0)	162 (50.0)	
Morbidity status of the children (2 weeks prior to the interview)				
Diarrhoea	15 (9.26)	21 (12.96)	36 (11.11)	.289
Fever	76 (46.91)	71 (43.83)	147 (45.37)	.577
Cough and fast breathing/difficulty breathing	57 (35.19)	57 (35.19)	114 (35.19)	1.000
Measles in the last 6 months prior to the interview	4 (2.47)	8 (4.94)	12 (3.7)	.244
	105 (64.81)	100 (61.73)	205 (63.27)	.564
Affected by at least one disease				
Receipt of antihelminthic tablet/syrup in the last 6 months prior to the interview	91 (56.17)	83 (51.23)	174 (53.7)	.373
Children who have been breastfed	159 (98.15)	156 (96.3)	315 (97.22)	.310

having diarrhoea, fever, and cough/difficulty breathing was 11.11%, 45.37%, and 35.19%, respectively. In the last 6 months prior to the interview, 3.7% of children were affected by measles; 63.27% of the children were affected by at least one of these diseases. Almost all (97.22%) children have been breastfed. These variables were not significantly different over the stunted and nonstunted groups. Table 2 showed that the average daily dietary intake of energy was 750.9 g (IQR: 561, 952), protein was 28.2 g (IQR: 20.7, 37.2), carbohydrate was 123.9 g (IQR: 89, 162.6), lipid was 15 g (IQR: 10.2, 21.8), iron was 3.8 mg (IQR: 2.7, 5.5), zinc was 2,802.8 μg (IQR: 1,864.4, 3,876.2), vitamin A was 472.5 RAE (IQR: 273.9, 781.6), folate was 82 μg (IQR: 53, 118.5), vitamin B12 was 3.2 mg (IQR: 1.4, 7.5), phytate was 194 mg (IQR: 135.9, 269.2), vitamin D was 2.2 mg (IQR: 1.1, 4), calcium was 158.9 mg (IQR: 89.9, 299), dietary fibre was 3.8 g (IQR: 2.5, 5.6), total intake was 438.1 g (IQR: 306.3, 584.5), intake from animal sources was 154.4 g (IQR: 78.1, 275.9), and intake from plant sources was 250.5 g (IQR: 175.5, 346). Association between dietary intake and global DNA methylation is shown in Table 3. In the multiple linear regression model, higher global DNA methylation level in children was significantly associated with consuming low levels of energy, coef: .034 (95% CI [.014, .053]); P = .001, protein, coef: .038 (95% CI [.019, .057]); P = .000, carbohydrate, coef: .027 (95% CI [.008, .047]); P = .006, zinc, coef: .020 (95% CI [.001, .039]); P = .043, total dietary intakes, coef: .020 (95% CI [.001, .039]); P = .043, and intake from plant sources, coef: .028 (95% CI [.009, .047]); P = .005, after adjusting for household food insecurity, at least 1-year formal education of household head, suffered from at least one disease, hand washing using soap in the five critical times, and sex of child. The relationship between consuming different types of food groups and global DNA methylation level in children is shown in Table 4. Among the food groups, fruits and vegetables consumption was significantly associated with lower 5-mC% level, coef: −.022 (95% CI [−.041, −.002]); P = .028. Table 5 showed the mean global DNA methylation level as 5-mC%. The mean global DNA methylation level was .166 for all children, which was .170 for stunted children and .162 for nonstunted children. No difference was observed in between the plasma micronutrients and global DNA methylation level (Table S1).

4 | DISCUSSION

Stunting, inflicting one quarter of the world's young children, remains as an unsolved problem. Even if interventions are applied at 90% coverage, only a 20% reduction in stunting would be achieved (Bhutta et al., 2013). It is important to know the aetiology of stunting, especially the role of epigenetics and dietary habits. In the present study, we observed that global DNA methylation level (5-mC%) was higher in 2- to 3-year-old stunted children and lower intakes of protein, carbohydrate, and energy were significantly associated with increased global DNA methylation after controlling for other factors such as household food insecurity, literacy of household head, hand washing using soap in the five critical times, child morbidity, and sex.

A large number of animal-based studies have demonstrated epigenetic status, and both biology and physiology are influenced by nutritional exposure at particular life stages (Haggarty, 2013). Recent evidence from studies involving human subjects has also emerged where nutritional exposure has a direct relation with malnutrition through epigenetic mechanisms (Bollati et al., 2014; Huang et al., 2015; Prentice et al., 2013). This study would be the first to show any association with global DNA methylation status for children with

TABLE 2 Distribution of dietary intakes by linear growth in 2- to 3-year-old children in an urban slum community

Dietary intake, median (IQR)	Nonstunted	Stunted	Total	*P* value
Energy (kcal)	798.5 (645.4, 1,018.2)	668.1 (506.5, 873.4)	750.9 (561.3, 952)	.000
Protein (g)	31.8 (23.4, 39.7)	25.8 (17.6, 34.2)	28.2 (20.7, 37.2)	.000
Carbohydrate (g)	134.6 (103.3, 167.7)	112.2 (79.3, 145.9)	123.9 (89, 162.6)	.000
Lipid (g)	16.8 (12.1, 23.8)	13.9 (8.8, 19.4)	15 (10.2, 21.8)	.000
Iron (mg)	4 (2.9, 5.9)	3.5 (2.6, 5)	3.8 (2.7, 5.5)	.012
Zinc (μg)	3,048.3 (2,274, 4,269.5)	2,380.6 (1,716.8, 3,386.9)	2,802.8 (1,864.4, 3,876.2)	.000
Vitamin A (RAE)	512.5 (304.8, 884.3)	443.9 (254.1, 734.6)	472.5 (273.9, 781.6)	.018
Folate (μg)	85.8 (57.8, 123.1)	76.9 (48.8, 112.9)	82 (53, 118.5)	.034
Vitamin B12 (mg)	3.4 (1.5, 8.3)	2.7 (1.3, 7.2)	3.2 (1.4, 7.5)	.144
Phytate (mg)	209.2 (148.9, 297.6)	181.6 (124, 248.9)	194 (135.9, 269.2)	.004
Vitamin D (mg)	2.3 (1.1, 4.2)	2.1 (1, 3.8)	2.2 (1.1, 4)	.242
Calcium (mg)	187.1 (96.7, 332.8)	134.6 (85.4, 237.9)	158.9 (89.9, 299)	.001
Dietary fibre (g)	3.8 (2.5, 6.2)	3.6 (2.3, 5.1)	3.8 (2.5, 5.6)	.022
Total intake (g)	490.4 (364.7, 633.5)	383.5 (275, 497.1)	438.1 (306.3, 584.5)	.000
Intake from animal sources (g)	177.5 (87.4, 311.8)	122 (67.2, 225.1)	154.4 (78.1, 275.9)	.001
Intake from plant sources (g)	268.1 (190.5, 377.5)	222.1 (161.6, 309)	250.5 (175.5, 346)	.004

Abbreviation: IQR, interquartile range (difference between the first and third quartile).

TABLE 3 Relationship between lower global DNA methylation and dietary intakes

Dietary intake[a]	Unadjusted coef. (95% CI)	*P* value	Adjusted coef. (95% CI)	*P* value
Energy (kcal)	.029 [.010, .048]	.003	.034 [.014, .053]	.001
Protein (g)	.037 [.018, .056]	.000	.038 [.019, .057]	.000
Carbohydrate (g)	.025 [.005, .044]	.013	.027 [.008, .047]	.006
Lipid (g)	.012 [−.007, .032]	.216	.011 [−.008, .031]	.255
Iron (mg)	.013 [−.007, .032]	.200	.015 [−.004, .035]	.126
Zinc (μg)	.020 [.000, .039]	.047	.020 [.001, .039]	.043
Vitamin A (RAE)	.011 [−.008, .031]	.265	.011 [−.008, .031]	.262
Folate (μg)	.015 [−.004, .034]	.130	.015 [−.004, .035]	.124
Vitamin B12 (mg)	.002 [−.018, .021]	.842	.003 [−.016, .023]	.727
Phytate (mg)	.013 [−.006, .033]	.186	.016 [−.003, .036]	.105
Vitamin D (mg)	−.001 [−.020, .019]	.927	.000 [−.020, .020]	.990
Calcium (mg)	.000 [−.020, .019]	.968	−.001 [−.020, .019]	.937
Dietary fibre (g)	.010 [−.010, .029]	.325	.010 [−.009, .030]	.310
Total intake (g)	.020 [.001, .040]	.041	.020 [.001, .039]	.043
Intake from animal sources (g)	.007 [−.012, .027]	.478	.006 [−.013, .026]	.536
Intake from plant sources (g)	.025 [.006, .044]	.012	.028 [.009, .047]	.005

Note. Adjusted: Household food insecurity, at least 1-year formal education of household head, affected by at least one disease, hand washing using soap in the five critical times, child's age, and sex of child.

[a]Variables are transformed to binary variables using cut-off value median, that is, 1 means below median and 0 means median or above.

TABLE 4 Association between consumed food groups and lower global DNA methylation

Food groups	Unadjusted coef. (95% CI)	*P* value	Adjusted* coef. (95% CI)	*P* value
Starchy staples (grains, roots, and tubers)				
Legumes and nuts	.014 [−.008, .036]	.213	.013 [−.009, .035]	.234
Dairy products (milk yogurt and cheese)	.004 [−.018, .027]	.701	.005 [−.017, .028]	.640
Meats (meat, poultry, and liver/organ meat)	−.019 [−.043, .006]	.130	−.020 [−.044, .004]	.098
Eggs	.000 [−.027, .026]	.972	.004 [−.023, .031]	.787
Vitamin A-rich fruits and vegetables	−.039 [−.117, .038]	.318	−.033 [−.111, .044]	.399
Other fruits and vegetables	−.021 [−.04, −.001]	.036	−.022 [−.041, −.002]	.028
Fish	−.018 [−.05, .015]	.282	−.019 [−.051, .013]	.251
Candies and desserts	−.016 [−.063, .031]	.504	−.015 [−.062, .033]	.548
Snacks	.002 [−.017, .022]	.826	.000 [−.020, .020]	.998
Sweetened beverages	.008 [−.011, .028]	.404	.009 [−.011, .029]	.367
Fast food	−.019 [−.048, .010]	.209	−.022 [−.051, .007]	.130

Note. Reference value: consume ≥ 10 mg in previous 7 days.

*Household food insecurity, at least 1-year formal education of household head, affected by at least one disease, hand washing using soap in the five critical times, and sex of child.

dietary intakes and chronic malnutrition, that is, stunting. Dietary habits may have direct consequences on health and diseases through epigenetic processes. It may be also responsible for future metabolic programming (Haggarty, 2013). Consistent with this, the present study demonstrated that lower intakes of energy, protein, and carbohydrate are significantly associated with increased global DNA methylation in children. Several studies have shown that inadequate energy intake is common, particularly in developing communities, and in some circumstances, this energy deficiency is associated with stunting (Millward, 2017). A systematic review of protein intakes and growth in children in the Nordic countries revealed that higher protein intakes in infancy and early childhood are associated with increased growth and higher BMI in childhood (Hörnell, Lagström, Lande, & Thorsdottir, 2013; Millward, 2017). Also in healthy Danish preschool children,

TABLE 5 Global DNA methylation status in urban slum children

Indicator	Stunted *n* = 157 Mean ± *SD*	Nonstunted *n* = 157 Mean ± *SD*	*P* value	OR (95% CI)	*P* value	AOR[a] (95% CI)	*P* value
Global methylation level (mean 5-mC% content)	0.170 ± 0.096	0.162 ± 0.078	.391	3.05 [0.24, 38.7]	.390	2.90 [0.22, 39.07]	.422

Abbreviation: OR, odds ratio.

[a]Household food insecurity, at least 1-year formal education of household head, affected by at least one disease, hand washing using soap in the five critical times, and sex of child.

height was positively associated with protein intakes from milk but not from meat. Recently, Semba et al. (2016) have shown that serum concentration of most amino acids was low in stunted preschool children from rural Malawi compared with nonstunted children.

We observed that among the food groups, higher fruits and vegetables consumption was significantly associated with lower global DNA methylation level. This may be due to the polyphenols contents in the fruits and vegetables with antioxidant activities, which have shown to have multiple beneficial effects on health and diseases. A recent report by Huang et al. (2018) has shown that some of these polyphenols, flavonoid compounds, and cruciferous vegetables can also change DNA methylation, histone modifications, and expression of microRNA (Lima, Vianello, Corrêa, Campos, & Borguini, 2014). However, no difference was observed between the plasma micronutrients and global DNA methylation in these children. It is difficult to explain the reason for a nonassociation of the serum micronutrient levels and global DNA methylation. However, the serum levels of micronutrients are dependent on several factors, such as the intakes of the macronutrients (e.g., proteins), level of body stores, infection status, and bioavailability. Therefore, it is easier to elicit an association of macronutrient intakes and DNA methylation than that of the micronutrient levels and the methylation status. Despite that, we observed an apparently higher but statistically non-significant level of the micronutrients (e.g., iron, zinc, vitamin A, and folate) in the low DNA methylation groups. Perhaps, a larger study might have higher power to observe the association of micronutrient levels and DNA methylation. We observed higher global DNA methylation level (5-mC%) in 2- to 3-year-old stunted children, but the association was not statistically significant.

The strength of our study was that it was conducted in an urban slum area with a high prevalence of stunting, with concomitant insufficient quality of diet. This makes this an ideal setting for studying the possible links among epigenetics, dietary intakes, dietary diversity, and childhood stunting. Another strength is that all measurements and interviews were done by the expert field research assistants and health workers who live in the area and are familiar with the cultural background and habits of the study population, thus optimizing data quality.

A limitation of our study is that the study population was aged 2–3 years, and therefore, we did not have data pertaining to the effect of the first 1,000 days, whereas most of the stunting is believed to occur between 6 and 36 months, the complementary feeding period (Black et al., 2013; Huey & Mehta, 2016; Prentice et al., 2013). We did not have data on dietary intake during 6–23 months of age, other than on breastfeeding. Hence, some important data were lacking, which could have further strengthened the findings. Moreover, being the very first study in the field, we failed to find or refer to any previous studies to decide on the appropriate sample size and design. After doing power analysis of dietary intake data, only energy (kcal) and protein (grams) intake were adequately powered (Table S2), which urges further studies with adequate sample size to test effect of the methylation on child growth in resource-poor settings.

In conclusion, the findings suggest significant association between lower dietary intakes and increased global DNA methylation in an impoverished setting in Bangladesh. The global DNA methylation and stunting in children showed an association. To establish the relationship among the dietary intakes, global DNA methylation, and stunting, future prospective studies are warranted, which may provide further understanding of the aetiology of stunting in resource-poor settings.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

MSI and TA designed this study. MSI, SR, MAH, MJB, and ASGF were responsible for the data analyses. MSI was responsible for drafting the manuscript. MSI, JB, and SR were responsible for the

study protocol and fieldwork. All authors contributed to the interpretation of the results. All authors read and approved the final manuscript.

ORCID

Mohd S. Iqbal https://orcid.org/0000-0002-1831-9939

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