Nutrient intake and environmental enteric dysfunction among Nepalese children 9-24 months old– the MAL-ED birth cohort study

Running title: Nutrient intake and enteric dysfunction

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Abstract

Background: Nutrient deficiencies limit the growth and turnover of intestinal mucosa, but studies assessing whether specific nutrients protect against or improve environmental enteric dysfunction (EED) are scarce. We aimed to investigate associations between nutrient intake and EED assessed by lactulose:mannitol ratio, anti-1-antitrypsin, myeloperoxidase and neopterin among children 9-24 months in Bhaktapur, Nepal.

Methods: Among 231 included children, nutrient intake was assessed monthly by 24 hour recalls, and 3-month usual intake was estimated using Multiple Source Method. Associations between nutrient intake and L:M ratio (measured at 15 months) were assessed using multiple linear regression, while associations between nutrient intake and fecal markers (measured quarterly) were assessed using Generalized Estimating Equations (GEE) models.

Results: We found that associations between nutrient intake from complementary food and lactulose-mannitol (L:M) ratio, alpha-1-antitrypsin (AAT), myeloperoxidase (MPO) and neopterin (NEO) were generally negative but weak. The only significant associations between nutrient intake (Potassium, Magnesium, Phosphorous, Folate and Vitamin C) and markers for intestinal inflammation were found for MPO.

Conclusion: Negative but weak associations between nutrient intake and markers of intestinal inflammation were found. Significant associations between several nutrients and MPO might merit further investigation.

Introduction

Environmental enteric dysfunction (EED) refers to a highly prevalent condition affecting populations in low- and middle income countries (LMICs) with increased gut inflammation, increased intestinal permeability and reduced absorption of nutrients due to villous atrophy (1) and loss of enzymatic activity (2). EED is established during infancy and is associated with poor sanitation, gut infections, home births, micronutrient deficiencies and breastfeeding practices (3). Possible consequences include infectious disease, stunting, impaired cognitive development and reduced vaccine efficacy (4).

The biomarker most commonly used to diagnose EED in previous studies is the lactulose:mannitol (L:M) ratio. While mannitol is passively absorbed proportional to intestinal absorptive capacity, lactulose is a disaccharide which is not absorbed by the healthy intestine. Increased L:M ratio thus indicates intestinal damage demonstrated by reduced absorptive capacity and increased permeability (2). Among newer, less invasive biomarkers used to assess EED are the fecal markers alpha-1-antitrypsin (AAT) measuring intestinal permeability, myeloperoxidase (MPO) measuring neutrophil activity and neopterin (NEO) representing Th-1 immune stimulation (5).

Increased dietary diversity may enhance gut microbiota (1), which reduces the risk of intestinal inflammation (6). Further, generalized malnutrition, protein depletion and deficiencies of specific nutrients including essential fatty acids, folate, zinc, vitamin A, and vitamin B_{12} has been shown to inhibit the growth and turnover of the intestinal mucosa (7). Meanwhile, studies assessing improvements in EED with micronutrient supplementation either alone (8) or in combination with other interventions (9) show mixed results. For specific nutrients, zinc (10) and vitamin A (11) have been associated with reduced L:M ratio

in children and alanyl-glutamin intake improved trans-mucosal resistance in mice (12). However, studies assessing whether specific nutrients protect against or improve EED are scarce. Also, studies investigating associations between nutrient intake and fecal markers for EED are mainly lacking.

The population of Bhaktapur, Nepal, has high socioeconomic status compared to national averages (13). Meanwhile, micronutrient adequacy, especially for iron, zinc, vitamin A and niacin, among children in the MAL-ED Nepal cohort was very low (14), and the prevalence of anemia and zinc deficiency at 24 months was 29 and 23 % respectively (15). National governmental programs to improve micronutrient status are ready to use therapeutic food (RUTF) to children with severe malnutrition (16), a biannual vitamin A supplementation program for children 6-59 months and zinc supplementation to children with diarrhea (17). Finally, the main enteric pathogens causing diarrhea after 12 months age in the MAL-ED Nepal cohort were campylobacter and enterohaemorrhagic e-coli (EHEC), norovirus GII and rotavirus (18).

The aim of this study was to investigate by exploratory analysis associations between nutrient intake and environmental enteric dysfunction assessed by lactulose:mannitol ratio, anti-1-antitrypsin, myeloperoxidase and neopterin among children 9-24 months in Bhaktapur, Nepal.

Methods

Design and subjects

The MAL-ED Nepal site provided data for the analyses. The data collection took place in Bhaktapur, a peri-urban, agriculture-based community located 15 km east of Kathmandu. Children were enrolled within 17 days from birth and followed at least until 24 months. The data collection period for age 9-24 months was February 2011 to November 2012. Out of 240 enrolled children, 229 had complete nutrition data at 24 months, the number of urine samples (collected at 15 months) was 218, while the number of fecal samples varied throughout follow-up. Data was divided into five time slots (9-12, 12-15, 15-18, 18-21 and 21-24 months respectively). The study received ethical approval from Nepal Health Research Council (NHRC) and the Walter Reed Institute of Research (Silver Springs, Maryland) and all caregivers signed informed consent forms. Further details on design and methodology are reported elsewhere (4).

Dietary intake and socioeconomic status

Trained local fieldworkers conducted monthly 24-hour recall interviews to collect data on foods and amounts consumed the previous day. A separate form was used to collect details about recipes. Amounts were estimated using household utensils, portion size booklets and play dough. The FAO International Network of Food Data Systems (INFOODS) database for Asia (19) was the main food composition database, but supplementary nutrient values from other databases were also used.

The Multiple Source Method (20) was used to calculate individuals' usual intake of energy, animal source protein, fiber, poly-unsaturated fatty acids (PUFA), iron, zinc, calcium, sodium, potassium, magnesium, phosphorous, thiamin, riboflavin, niacin, pantothenic acid,

vitamin $B_{6,}$ folate, vitamin $B_{12,}$ A, C and E. Usual intake was calculated based on three 24h recalls in each time slot or four recalls in time slots with secondary recalls. Socioeconomic status was assessed by questionnaire at 12 months by a WAMI (**W**ater, **A**ssets, **M**aternal education and **I**ncome) index, with scores ranging from 0 to 1 (21). The 8 assets included were separate room for a kitchen, household bank account, mattress, refrigerator, TV, people per room (mean), table and chair or bench.

Nutrient density adequacy

The nutrient density (ND) was defined as the amount of nutrient consumed per 100 kcal of complementary food and calculated for 10 micronutrients: thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin C, vitamin A, calcium, iron and zinc. Context specific desired nutrient density (DND) and nutrient density adequacy (NDA) of complementary foods was calculated based on methodology by Dewey and Brown (22) for the same micronutrients. For each time slot and for each nutrient, context specific DNDs were calculated in the following way:

[Recommended nutrient intake (RNI) of nutrient χ – (concentration of nutrient χ in breastmilk x median breast milk intake in time slot)]/median energy intake from complementary food in time slot*100.

For iron, FAO/WHO micronutrient requirements corresponding to low absorption (5%), while for zinc low or middle absorption (23) (depending on the phytate:zinc ratio measured) was used. For nutrients where the levels in breast milk are negatively affected by maternal status (thiamin, riboflavin, vitamin B_6 and vitamin A), we used concentrations based on studies conducted among women in low income countries (24, 25). Otherwise, WHO values based on breast milk from western women (26) were used. Breast milk intake was not

assessed, but calculated the following way:

[Total energy requirements (body weight measured monthly * FAO energy requirement per kg body weight for the appropriate age) (27) - energy intake from complementary food] /energy density of breast milk (LMICs) (26)

For non-breastfed children, desired nutrient densities were calculated as FAO/WHO micronutrient requirements (23) divided by median energy intake in the non-breastfed group.

NDA was calculated for each nutrient and for each observation as the ND as percentage of the DND. Finally, mean nutrient density adequacy (MNDA) was calculated as the mean of individual NDAs for all ten micronutrients each capped at 100%. Mean MNDA based on three months measurements (i.e. measured at 9, 10 and 11 months for time slot 1) was used in the analysis. A more detailed description of calculations of MNDA are reported elsewhere (14).

L:M ratio and fecal markers for EED

Children were instructed to fast 2 hours prior to and 30 minutes after the L:M test and recommended to void before administration of the sugar dose. The L:M ratio was assessed in urine collected during voiding (5-hour follow-up period) following the administration of 250 mg/mL lactulose and 50 mg/mL mannitol at a dose of 2 mL/kg to a maximum administered dose of 20 mL at a concentration of 1002 mOsm/L. Aliquots were stored at -70°C until testing and concentrations of lactulose and mannitol measured by high-performance liquid chromatography (HPLC) and either pulsed amperometric detection or iron chromatography. Results were presented as molar ratio of lactulose to mannitol (5). During our follow-up

period L:M ratio was only measured at 15 months. Due to skewed values, the variable was log-transformed.

Stool samples were collected monthly for children < 12 months, then quarterly up to 36 months age (4). Samples collected at 12, 15, 18, 21 and 24 months were used in the analysis. The samples were stored for processing at -70°C without fixative (5). The concentrations of AAT, MPO and NEO were measured by ELISA tests at Walter Reed/AFRIMS Research Unit Nepal (WARUN) with initial dilutions of 1:500 ng/mL for MPO (ALPCO, Salem, NH), AAT (BioVendor, Candler, NC) and NEO (GenWay Biotech, San Diego, CA). Tests showing out of range values were run again at a 2-fold higher or lower (as appropriate) concentration (5). To avoid overly diluting the samples, stool samples collected either during or ≤ 7 days after a diarrheal episode (3 semi-liquid stools in a 24h period separated by ≥ 2 days without diarrhea) or at the same time as the urine sample for the lactulose:mannitol test of intestinal permeability inherent in MAL-ED protocol (4) were excluded. Due to skewed distributions, the variables were log transformed to obtain normality and ease interpretation of results.

Statistical analysis

Continuous data are presented as mean and standard deviation (SD) if normally distributed, and as median and interquartile range (IQR) if not normally distributed. Variables to be included in regression models were selected based on a theory-based approach. Models for associations between nutrient intake and MNDA and L:M ratio, AAT, MPO and NEO, respectively, are presented. Models for nutrient intake and L:M ratio and fecal markers were adjusted for energy intake from complementary food, WAMI, gender, season and age (only for fecal markers), while models with MNDA were not adjusted for energy intake. We also tried adjusting for stool consistency, but due to very little variation this made no changes to

the estimates and was excluded. Models describing associations between nutrient intake and L:M ratio were assessed with multiple linear regression analysis. All other analysis was performed using GEE with autoregressive (AR-1) covariance structure. Season was coded according to the date when the fecal sample was taken as pre-monsoon (March-May), monsoon (June-August), post-monsoon (September-November) and winter (December-January). Apart from season and gender, all variables were continuous. The statistical package for the social sciences (SPSS) version 24.0 was used for data analysis.

Results

The baseline characteristics of mother and child pairs are presented in Table 1. The mean (SD) age of mothers was 27 (4) years and 11% had 3 children or more. The median (IQR) number of assets (out of 8 assessed) was 6 (5, 7). Median (IQR) WAMI score was 0.7 (0.6, 0.8), where all participants had access to improved water and sanitation. The majority of participants (53%) were male.

L:M ratio, fecal markers for EED, nutrient intake, nutrient adequacy and information about breast feeding is presented in Table 2. All outcome variables were skewed with some very high values. The median (IQR) L:M ratio was 0.07 (0.05, 0.12), where 26% had values above the reference (0.12) (28). All fecal markers decreased gradually with age. The largest reductions between the first and the last time slot were seen for MPO (74%) and NEO (72%).

Associations between intake of individual nutrients and MNDA and L:M ratio, AAT, MPO and NEO are presented in Table 3. Associations were generally negative and weak with few significant findings in view of the number of models presented. Significant negative associations were found between intake of potassium (-0.33, C.I -0.61, -0.05), magnesium (-2.81, C.I -5.36, -0.26), phosphorous (-0.58, C.I -1.14, -0.02), folate (-2.08, C.I -3.90, -0.25), vitamin C (-0.01, C.I -0.001, 0) and MNDA (-0.01, C.I -0.01, 0) and log MPO. Weak but significant negative associations were also found between intake of zinc, calcium, potassium, magnesium, phosphorous and lactulose (data not shown), while for L:M ratio, AAT, NEO and mannitol no significant associations were found.

Discussion

We found that associations between nutrient intake from complementary food and L:M ratio, AAT, MPO and NEO were generally negative but weak and few reached statistical significance. The only significant associations between nutrient intake and markers for intestinal inflammation were found for MPO.

The weak associations found between nutrient intake and intestinal inflammation are comparable to a cross-sectional study among 18 months old children in Bangladesh (29), and likely has several explanations. Firstly, associations between nutrient intake and EED are hard to assess since they are bidirectional or cyclical in nature, with malnutrition being both a cause and a consequence of EED. The level of severity of EED in our population might also be questioned since most participants had L:M ratios below the reference standard. Intestinal permeability is mediated by inflammation (30), and effects of enteroaggressive pathogens on fecal markers are cumulative (31). Meanwhile, a murine study by Brown et al. (2017) showed that increased permeability due to enteropathogens was only present in mice who had consumed a malnourished (in energy and protein) diet (6). Although the severity of intestinal lesions associated with elevated MPO, to our knowledge, is unknown, our data suggest that in this population EED may be moderate and the demand (additional to daily requirements) for typical "repair nutrients", such as folate, zinc and vitamin B_{12} (7), relatively limited. Further, nutrient intake in our study was assessed only from complementary food, whereas estimates of breast milk intake performed in a previous study suggested that this population were high breast milk consumers (14). Adjusting for energy intake from complementary food in our analysis did not account for the favorable absorption of many nutrients (i.e zinc and iron) (32) from breast milk compared to complementary food with low bioavailability (14). As a result, associations between nutrient intake, vitamin status and EED may be distorted.

Other important aspects likely to weaken associations between nutrient intake and EED in our study is the length of follow-up and the age of the included children (9-24 months). The gut microbiota matures and becomes more stable during the first 3 years of life (33). This process is negatively influenced by malnutrition and frequent use of antibiotics (15) and likely positively influenced by increased dietary diversity (34). Improved microbiota maturity is in turn associated with increased resistance to pathogens (34). In addition, it is hypothesized that increased levels of the biomarkers assessed may be side-effects of self-limiting natural processes (intestinal immune maturation) up to a certain "turning-point" where after elevated levels indicate EED (30). If this turning point occurs within our period of follow-up but at different time points for each participant, it could further complicate interpretation of results. In the end, dividing the data into 3-month time slots may not be sufficiently refined to assess the complex temporal interplay between nutrient intake, pathogen exposure and markers of EED in our age group. Finally, correlations between the fecal markers assessed and between the fecal markers and L:M ratio are low (35), indicating that they reflect different biological processes. For this reason, more comprehensive scores may be needed to adequately describe EED and assess risk factors associated with EED (31).

Although the number of models performed in our study suggests that some significant associations likely are spurious, those found between several nutrients, MNDA and MPO may still be of importance. MPO is the fecal marker most strongly affected by the most prevalent enteroaggressive pathogen (Campylobacter) in this population and in MAL-ED overall (18). It was the only fecal marker out of the three assessed here which was significantly associated with length velocity among children in the Bhaktapur cohort (36) and may thus be the marker most indicative of EED in our population. The usefulness of MPO as a biomarker for

inflammatory bowel disease (IBD) is currently being investigated, and has been shown to increase both with onset and severity of the disease (37). Although studies assessing associations between nutrient intake and MPO are lacking, both magnesium, vitamin C, potassium and fruit- and vegetable intake have been associated with decreased risk of IBD (38). Although the pathways at present seem unclear, these foods and nutrients may protect against intestinal inflammation, which supports our findings. However, potential associations between intake of specific nutrients and MPO need to be corroborated by future studies.

The main strength of the study is the longitudinal design with monthly measurements of nutrient intake enabling calculations of within- and between subject variance and likely more valid assessment of nutrient intake than in a cross-sectional study. The level of detail of nutrient data collected was high and included estimation of amounts. Fecal markers were assessed from asymptomatic stool samples. Frequent assessment of diarrhea incidence (several times per week) was a major advantage which improved the quality of data for fecal marker concentrations. Both L:M ratio and fecal markers were assessed according to strict guidelines in laboratories undergoing regular quality checks and standardization of tests between MAL-ED sites (5). The sample was drawn from a relatively homogenous population. Finally, retention was favorable (85% in the final time slot) in the MAL-ED Nepal cohort.

The main limitation of our study was uncertainty about the reliability of the outcome variables assessed. The L:M ratio may be affected by mannitol believed to be present naturally in urine, and HPLC may lack sensitivity for determining low concentrations of lactulose (35) while both MPO and NEO are non-specific markers of intestinal immunity (2). Also, assessing only nutrient intake from complementary food in this group who are high breast milk consumers (14), weakens the strength of the inferences made from our study.

Further, since all participants had access to improved water and sanitation, it was impossible to assess differences between exposed and unexposed participants regarding water, sanitation and hygiene believed to be of major importance in the development of EED (31). The vast number of regression models increases the likelihood of spurious significant associations. Meanwhile, correction for multiple comparisons is not required in explorative studies (39). Finally, the lack of international reference standards for biomarkers for EED, complicates the interpretation of results.

Conclusions

We found that associations between nutrient intake from complementary food and L:M ratio, AAT, MPO and NEO were generally negative but weak in this group of children aged 9-24 months in Bhaktapur, Nepal. The only significant associations were found for intake of Potassium, Magnesium, Phosphorous, Folate, Vitamin C and MNDA and MPO. General approaches, such as improving dietary diversity, might have beneficial effects on microbiota and gut maturation and would likely be advantageous in reducing EED in our population and in similar settings.

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Disclosure

The authors declare no conflict of interest

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Table 1 Baseline characteristics, mother-child pairs, Bhaktapur, Nepal

Characteristic[a] (n=211)	
Mother's age in years, mean (SD)	27.4 (3.7)
Parity	
One child, %	47
Two children, %	42
Three or more children, %	11
Improved water and sanitation, %	100
Number of assets, median (IQR)	6 (5, 7)
Mother's education in years, median (IQR)	9 (6, 10)
Monthly household income (USD)[b], median (IQR)	157 (101, 248)
WAMI, median (IQR)	0.7 (0.6, 0.8)
Birth weight (kg), mean (SD)[c]	3.0 (0.4)
Child's gender, male (%)	53.1

Parts of the table has been presented previously.

[a]Measured at 12 months

[b] Exchange rates from Ouanda.com

[c] n=207

Table 2 Markers of environmental enteric dysfunction, nutrient intake from complementary food and prevalence of breastfeeding, children 9-24 months, Bhaktapur, Nepal*

	Recomm-ended	9-12 mo n=211[a]	12-15 mo n=144	15-18 mo n=205	18-21 mo n=207	21-24 mo n=192
L:M ratio, median (IQR)	<0.12[b]	NA	0.07 (0.05, 0.12)[c]	NA	NA	NA
AAT (mg/g), median (IQR)	<0.27[d]	0.40 (0.21, 0.70)	0.42 (0.23, 0.86)	0.35 (0.23, 0.60)	0.31 (0.15, 0.61)	0.27 (0.14, 0.53)
MPO (ng/mL), median (IQR)	<2000[e]	5935.2 (2524.5, 14267)	3883.8 (1880, 6660.3)	3162.5 (1184.5, 5693)	2001.7 (854.3, 3976)	1541.6 (592.9, 2983.5)
NEO (nmol/L), median (IQR)	<70[f]	2439.7 (1476.3, 3511.2)	1549.5 (822.5, 2438.8)	1378.1 (678.3, 2318.5)	957.1 (496.4, 1874.9)	683.1 (375.9, 1266.3)
Energy (kcal)	516[g]	235.8 (170, 323.8)	285.5 (214.8, 375)	370.4 (266.4, 467.9)	456.7 (355.3, 582.6)	596.4 (449.7, 729.3)
ASP (g)[h]	NA	4.1 (2.6, 5.8)	4.6 (3.2, 7)	6.2 (4.3, 9.2)	7 (5.1, 9.6)	8.9 (6.3, 12.4)
PUFA (g)	NA	1.2 (0.9, 1.7)	1.7 (1.3, 2.3)	2 (1.6, 2.9)	2.8 (1.9, 2.6)	3.3 (2.8, 4.2)
Fiber (g)	NA	1.6 (1.3, 2.2)	2 (1.5, 2.9)	2.5 (1.9, 3.3)	3.3 (2.7, 4.6)	4 (2.9, 5)
Iron (mg)	12[i]	0.81 (0.6, 1.07)	1.03 (0.75, 1.39)	1.28 (0.99, 1.67)	1.7 (1.28, 2.2)	1.91 (1.47, 2.56)
Zinc (mg)	5.6/2.7[j]	0.86 (0.57, 1.14)	1.01 (0.71, 1.33)	1.21 (0.93, 1.67)	1.55 (1.14, 2.1)	2.01 (1.44, 2.63)
Calcium (mg)	500	66.8 (35.8, 138.4)	81.7 (41.3, 157.3)	111.6 (54.6, 190.1)	134.4 (91.5, 227.6)	187.5 (114.5, 335.3)
Potassium (mg)	3000[k]	228.5 (148.9, 330.1)	284 (184.6, 384.3)	366.7 (254.9, 501.8)	458.9 (364.9, 598.8)	566.4 (395, 768.1)
Magnesium (mg)	60	29.9 (21, 40)	36.9 (25.2, 47.3)	44.3 (32.6, 57.3)	58.6 (42.7, 72.2)	69 (50.4, 90)
Phosphorous (mg)	460[k]	121.4 (78.6, 189.1)	144.5 (94.7, 203.5)	184.2 (124.5, 253.9)	227.5 (175.8, 305.7)	291.7 (202.5, 410.8)
Thiamin (mg)	0.5	0.09 (0.07, 0.15)	0.11 (0.08, 0.17)	0.16 (0.11, 0.22)	0.20 (0.14, 0.26)	0.24 (0.16, 0.33)
Riboflavin (mg)	0.5	0.15 (0.09, 0.24)	0.18 (0.12, 0.28)	0.24 (0.15, 0.37)	0.29 (0.19, 0.44)	0.38 (0.24, 0.55)
Niacin (mg)	6	0.92 (0.69, 1.17)	1.25 (0.94, 1.65)	1.52 (1.19, 2.01)	2.01 (1.58, 2.61)	2.51 (1.87, 3.15)
Pantothenic acid (mg)	2	0.68 (0.5, 0.96)	0.84 (0.59, 0.14)	1 (0.79, 1.43)	1.22 (0.98, 1.63)	1.63 (1.20, 2.13)
Vitamin B_6 (mg)	0.5	0.14 (0.10, 0.19)	0.17 (0.12, 0.24)	0.22 (0.16, 0.29)	0.28 (0.22, 0.37)	0.35 (0.25, 0.44)
Folate (mg)	160	26.4 (19.8, 34.8)	31 (24.9, 44.6)	39.2 (28.7, 52.3)	53.1 (38, 66.3)	62.8 (46.8, 82.4)
Vitamin B_{12} (µg)	0.9	0.31 (0.16, 0.58)	0.38 (0.23, 0.69)	0.52 (0.31, 0.83)	0.62 (0.38, 0.98)	0.85 (0.48, 1.37)
Vitamin A (µg)	400[l]	43.1 (26.2, 76.7)	56.7 (33.8, 86.8)	79.6 (50.7, 119.7)	99.1 (64.1, 146.2)	125.1 (84.3, 201.4)
Vitamin C (mg)	30	3.6 (2.6, 5.3)	4.9 (3.6, 7.5)	7.1 (4.6, 9.8)	9.9 (7.5, 12.6)	10.7 (7.8, 15.8)
Vitamin E (mg)	5[k]	0.65 (0.46, 0.90)	0.9 (0.65, 1.17)	1.09 (0.71, 1.44)	1.27 (0.98, 1.66)	1.56 (1.13, 1.93)
MNDA (%), median (IQR)	100	41.8 (33.3, 49)	39.2 (33.4, 44.7)	40.1 (33.5, 45.7)	42.1 (36.3, 49.6)	48.8 (40.4, 55.7)
Children being breastfed (%)[m]	NA	100	100	98	90	61

*Lactulose:mannitol (L:M) ratio, alpha-1-antitrypsin (AAT), myeloperoxidase (MPO) and neopterin (NEO). Nutrient intakes are median (IQR) usual intakes estimated by Multiple Source Method (19). Recommended nutrient intakes from WHO/FAO for children 1-3 years (22). MNDA: mean nutrient density adequacy. Parts of the table has been presented previously.

[a] Number of participants with both dietary data and fecal sample collected

[b] Lunn PG, Northrop-Clewes CA & Downes RM (1991) (26)

[c] n=218

[d] Beckmann (2000) (28)

[c] Saiki (1998) (38)

[f] Ledjeff (2001) (39)

[g] Energy requirement from complementary food for children with high breast milk intake (40)

[h] Only participants with intake of ASP included (n= 193, 136, 192, 197 and 183 respectively)

[i] Corresponding to low (5%) absorption

[j] Corresponding to low (5%) and medium (10%) absorption

[k] Adequate intake
[l] Recommended safe intake
[m] Measured at the end of the time slot (12, 15, 18, 21 and 24 months)

Table 3 Associations between nutrient intake from complementary foods and L:M ratio and fecal markers for environmental enteric dysfunction (EED), children 9-24 months, Bhaktapur, Nepal

	Lactulose:mannitol (L:M) ratio			**Alpha-1-antitrypsin (AAT)**			**Myeloperoxidase (MPO)**			**Neopterin (NEO)**		
	B	CI	ß	B	CI	ß	B	CI	ß	B	CI	ß
Nutrients[a]												
ASP (g)	0.013	-0.046, 0.072	0.035	0	-0.009, 0.009	0	-0.005	-0.015, 0.005	-0.036	-0.001	-0.011, 0.008	-0.009
PUFA (g)	-0.143	-0.373, 0.087	-0.110	-0.029	-0.064, 0.006	-0.088	0.009	-0.025, 0.043	0.022	-0.017	-0.048, 0.014	-0.055
Fiber (g)	-0.037	-0.163, 0.090	-0.045	-0.005	-0.024, 0.015	0.020	-0.019	-0.046, 0.007	0.060	-0.022	-0.045, 0	-0.092
Iron (mg)	-0.098	-0.435, 0.238	-0.051	-0.017	-0.066, 0.033	-0.032	-0.046	-0.107, 0.016	-0.069	-0.018	-0.079, 0.044	-0.036
Zinc (mg)	-0.224	-0.833, 0.386	-0.108	-0.013	-0.106, 0.081	-0.023	-0.094	-0.205, 0.016	-0.136	-0.004	-0.105, 0.097	-0.008
Calcium (g)	-0.944	-3.006, 1.119	-0.141	0.037	-0.269, 0.342	0.010	-0.354	-0.744, 0.036	-0.080	0.022	-0.285, 0.328	0.007
Potassium (g)	-0.956	-2.518, 0.607	-0.145	0.008	-0.199, 0.215	0.004	-0.329	-0.609, -0.048	-0.145*	-0.137	-0.358, 0.083	-0.080
Magnesium (g)	-5.763	-18.6, 7.075	-0.105	0.422	-1.583, 2.426	0.027	-2.814	-5.363, -0.264	-0.144*	-1.173	-3.619, 1.272	-0.079
Phosphorous (g)	-1.934	-4.976, 1.107	-0.168	0.060	-0.408, 0.528	0.018	-0.581	-1.144, -0.017	-0.141*	-0.023	-0.493, 0.448	-0.007
Thiamin (mg)	1.390	-1.193, 4.694	0.090	0.271	-0.197, 0.740	0.065	-0.251	-0.774, 0.272	-0.048	-0.149	-0.570, 0.272	-0.038
Riboflavin (mg)	-0.958	-2.408, 0.492	-0.126	0.069	-0.136, 0.274	0.030	-0.255	-0.544, 0.035	-0.091	0.068	-0.162, 0.298	0.032
Niacin (mg)	-0.006	-0.376, 0.363	-0.003	-0.016	-0.069, 0.036	-0.034	0.007	-0.054, 0.068	0.012	-0.048	-0.109, 0.012	-0.108
Pantothenic acid (mg)	-0.551	-1.235, 0.134	-0.225	0.007	-0.092, 0.105	0.010	-0.080	-0.176, 0.017	-0.096	0.061	-0.027, 0.148	0.097
Vitamin B_6 (mg)	-0.555	-2.756, 1.645	-0.049	0.150	-0.169, 0.470	0.048	-0.200	-0.597, 0.198	-0.051	-0.256	-0.539, 0.028	-0.087
Folate (g)	-5.767	-15.817, 4.282	-0.097	-0.479	-1.917, 0.960	-0.029	-2.077	-3.901, -0.253	-0.102*	-1.037	-2.850, 0.849	-0.065
Vitamin B_{12} (mg)	-0.116	-0.385, 0.153	-0.060	-0.007	-0.074, 0.059	-0.008	-0.027	-0.102, 0.048	-0.026	0.011	-0.054, 0.076	0.014
Vitamin A (mg)	-2.284	-6.273, 1.705	-0.093	-0.075	-0.659, 0.509	-0,011	-0.409	-1.124, 0.307	-0.048	0.080	-0.532, 0.693	0.013
Vitamin C (mg)	-0.023	-0.064, 0.019	-0.079	-0.002	-0.008, 0.004	-0.025	-0.008	-0.014, -0.002	-0.082*	-0.004	-0.010, 0.002	-0.054
Vitamin E (mg)	-0.295	-0.745, 0.154	-0.107	-0.010	-0.090, 0.069	-0.013	-0.036	-0.123, 0.051	-0.038	-0.004	-0.069, 0.060	-0.006
MNDA[b]	-0.005	-0.021, 0.010	-0.048	-0.001	-0.004, 0.002	-0.014	-0.004	-0.007, -0.001	-0.076*	0	-0.003, 0.002	0
N Samples		218			959			960			962	

Dependent variables were log- transformed, associations between nutrient intake and L:M ratio were assessed by multiple linear regression, other associations were assessed by GEE models with auto-regressive (AR-1) covariance structure, significant associations are marked by *

[a] Models adjusted for energy intake, WAMI, season, gender and age (in days) (only fecal markers)

[b] Models adjusted for WAMI, season, gender and age (only fecal markers)