Title: Adequacy of iodine status and associations with gut health: A prospective cohort study among infants in eight low- and middle-income countries

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Abstract *(word count=297)*

Background: Environmental enteric dysfunction increases the likelihood of micronutrient deficiencies among infants, but few studies have assessed the potential impact of gut health on urinary iodine concentration (UIC) among this vulnerable group.

Objective: We (1) describe trends of iodine status among infants from 6-24 months old and (2) examine associations between intestinal permeability, inflammation, and UIC from 6-15 months of age.

Methods: Data from 1557 children enrolled in this birth cohort study conducted in eight sites were included in these analyses. UIC was measured at 6, 15, and 24 months of age using the Sandell-Kolthoff technique. Gut inflammation and permeability were assessed via concentrations of fecal neopterin (NEO), myeloperoxidase (MPO) and alpha-1-anti-trypsin (AAT), and lactulose-mannitol (LM). A multinomial regression analysis was used to assess classified UIC (deficiency or excess). Linear mixed regression was used to test the effect of interactions among biomarkers on logUIC.

Results: All studied populations had adequate (≥100 µg/L) to excess (≥371 µg/L) median UIC at six months. Between 6–24 months, five sites displayed a significant decline in the infant's median UIC. However, median UIC remained within the optimal range. An increase of NEO and MPO concentrations by +1 unit in ln scale reduced the risk of low UIC by 0.87 [95% confidence interval (CI):0.78–0.97] and 0.86 [95% CI:0.77–0.95], respectively. AAT moderated the association between NEO and UIC ($P<0.0001$). The shape of this association appears to be asymmetric and in a reverse J-shape, with a higher UIC observed at both lower NEO and AAT concentrations.

Conclusions: Excess UIC was frequent at six months and tended to normalize at 24 months. Aspects of gut inflammation and increased permeability appear to reduce the prevalence of low UIC in children aged 6-15 months. Programs addressing iodine-related health should consider the role of gut permeability in vulnerable individuals.

Keywords: Infant; urinary iodine; iodine excess; gut inflammation; gut permeability

Introduction

Iodine is a crucial trace element for human health. Inadequate iodine intake may predispose to several pathologies. For example, during infancy, chronic iodine deficiency (ID)[1] may cause goiter, hypothyroidism, hyperthyroidism, mental retardation, and delayed growth and development (1). Preventing ID, especially during the first 1000 days of life, is a public health priority (2).

To prevent ID, *Jean-Baptiste Boussingault* proposed salt iodization in 1811 (3). More than a century later, universal salt iodization was adopted by the World Health Organization (WHO) as a global policy to tackle iodine deficiency disorders (IDD) (1). Now, universal salt iodization is considered to be the most successful food fortification program (4), illustrated by mandatory

[1] **Abbreviations**: AAT: alpha-1-anti-trypsin; BGD: Dhaka, Bangladesh site BRF: Fortaleza, Brazil site; CI: Confidence Interval at 0.95; CV: Coefficient of variation; EED: Environmental Enteric Dysfunction; EQUIP: Ensuring the Quality of Urinary Iodine Procedures; iccdr,b: International Centre for Diarrhoeal Disease Research, Bangladesh; ID: Iodine Deficiency; IDD: Iodine Deficiency Disorders; INV: Vellore, India site LMIC: Low and Middle Income Countries; LM: lactulose-mannitol ratio; LMZ: lactulose-mannitol ratio z-score; MAL-ED: Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health; MPO: Myeloperoxydase; NEB: Bhaktapur, Nepal site NEO: Neopterin; PEL: Loreto, Peru site; PKN: Naushero Feroze, Pakistan site; SAV: Venda, South Africa site; SES: composite socio-economic status; TZH: Haydom, Tanzania site; UIC: Urinary Iodine Concentration; UL: Upper limit; UNICEF: United Nations International Children's Emergency Fund; WHO: World Health Organization.

67 legislation for universal salt iodization in 108 countries (5) and the global coverage of iodized
68 salt consumption of any dose (≥0.1 ppm) reaching nearly 88% (6). However, even using a low
69 cut-off of ≥0.1 ppm of iodine content in salt, the coverage of iodized salt falls below 50% in
70 several low-and-middle income countries (LMIC) (6), indicating a gap between legislation and
71 effective implementation.
72 Limited access to iodized salt in some LMIC (7) exposes populations to an inadequate intake
73 of iodine (8,9). As a consequence, the iodine status in many countries remains dependent on
74 environmental factors, namely dietary patterns and availability in soil (10,11). Although ID is
75 most frequently reported in countries with weakly controlled universal salt iodization programs,
76 the occurrence of iodine excess is also a growing problem (12). The main causes of iodine excess
77 are high concentrations of iodine in ground or tap water, excessive iodine content or use of
78 iodized salt and high consumption of iodine-rich foods (e.g. dairy products and seafood) (13–17).
79 Studies conducted in Tanzania and Kenya, reported median urinary iodine concentrations (UIC)
80 among weaning infants that were three times higher than the current threshold (180 µg/L)
81 beyond which no health benefit is expected (18). This is concerning as it may cause dysthyroid
82 and subsequent possible impairments, especially in vulnerable groups (e.g., children under two
83 years) (19–21).
84 Absorption of dietary iodide in the small intestine is mediated by the sodium iodide
85 symporter (22) at the apical membrane of enterocytes. Several factors contribute to the regulation
86 of the sodium iodide symporter expression, with cytokines (e.g., tumor necrosis factor,
87 transforming growth factor β) downregulating its expression (23). Gut inflammation is common
88 among infants in LMIC, who often experience repeated exposure to enteropathogen infections

89 (24), leading to environmental enteric dysfunction (EED) (25). EED is partly characterized by an
90 increase in gut permeability (26,27) and altered inflammatory processes.
91 We hypothesized that both gut inflammation and increased permeability would have a
92 negative influence on iodine status (see Supplemental Figure 1). To our knowledge, no study has
93 been conducted to assess the association between intestinal permeability, inflammation and
94 iodine status even though the status of other nutrients has been shown to be disrupted under these
95 two physio-pathological conditions (26).
96 In this study, we measured urinary iodine excretion at multiple time points in children who
97 were participating in a birth cohort study in eight LMIC. We sought to 1) describe iodine
98 exposure across MAL-ED (Etiology, Risk Factors, and Interactions of Enteric Infections and
99 Malnutrition and the Consequences for Child Health) study sites via household salt use, 2)
100 describe trends in urinary iodine excretion during the first two years of life across MAL-ED sites
101 and in relation to regional information on iodine status, and 3) explore whether intestinal
102 permeability and inflammation are associated with iodine excretion.

103 ## Methodology

104 **Study context and sites**

105 The MAL-ED study is a birth cohort which began in November 2009 and was conducted in
106 eight sites in LMIC (29), namely Dhaka, Bangladesh; Fortaleza, Brazil; Vellore, India;
107 Bhaktapur, Nepal; Loreto, Peru; Naushero Feroze, Pakistan; Venda, South Africa; and Haydom,
108 Tanzania. The focus of the cohort study was to understand the relationships between
109 enteropathogen infection, dietary intake, nutritional status, gut physiology, growth, immune
110 function, vaccine response, and cognitive development (28).

111 **Subjects**

The study design has been described in detail elsewhere (28). Briefly, ~ 200 infants were enrolled per site within 17 days after birth with the goal of following them to 24 months of ag. The exclusion criteria were: the family planned to move outside the community in the next 6 months, maternal age < 16 years or the mother had another child in the MAL-ED study or was unable to provide informed consent, it was a multiple pregnancy, the neonate had severe or chronic disease requiring hospitalization and finally birth weight < 1500 g.

Iodine status

Child urinary iodine excretion was assessed at 6, 15 and 24 months during the administration of the Lactulose:Mannitol (LM) test of gut absorptive capacity and permeability. After a morning fast of two hours and provision of the dose of lactulose and mannitol, urine was collected using a specific collection bag (Fisher Scientific, Pittsburgh, PA, cat #22275347) (27) for up to five consecutive hours. In the Dhaka, Loreto and Naushero Feroze sites, an aliquot of the five-hour collection was separated for iodine determination whereas a spot urine sample was collected in Fortaleza, Vellore, Bhaktapur, Venda and Haydom. The volume of urine collected was measured in a graduated cylinder, and ~4 mL was immediately set aside for iodine assessment (27). At the Venda site, chlorhexidine (1-2 drops) was added to this sample. Samples at 24 months were collected and analyzed in 6 of 8 sites. Urine samples were stored at −80°C (27). Samples from Fortaleza, Bhaktapur, Loreto, Haydom and Venda were shipped on dry ice to Johns Hopkins University (USA) for analysis., Dhaka samples were assessed at International Centre for Diarrhoeal Disease Research, Bangladesh (iccdr,b), Vellore samples at Christian Medical College Hospital in Vellore, and Naushero Feroze samples at Aga Khan University. Seven laboratories used the Sandell-Kolthoff technique (1,29) adapted for 96 well plates to analyze urine samples for iodine concentration from children (30), and the EQUIP program (Ensuring the

Quality of Urinary Iodine Procedures; https://www.cdc.gov/labstandards/equip.html) led by the U.S. Centers for Disease Control and Prevention to ensure external validity. The lab in Vellore used an ion chromatography technique. All labs utilized internal quality control approaches (duplicate samples, inclusion of internal pools or spiked samples, according to their own practice) and adapted dilutions such that sample concentrations fell into the linearity range tested or calibration range. Reproducibility coefficient of variation (CV) for urinary iodine quantification varied from 1.6% to 15% for the Sandell-Kolthoff method and from 1.2% to 5.7% for the ion chromatography method.

According to the WHO, insufficient iodine intake corresponds to a median UIC at the population level below 100 µg/L (31). To approximate a tolerable upper limit of urinary iodine excretion that we could apply to define iodine excess in infants, we used the following logic:

- 92% of consumed iodine is excreted (32)
- 180 µg/d is the upper recommended daily intake (UL) for infants (less than 2 years) (33)
- Daily urine excretion is 2 ml/kg/hour (34)
- Infant weight at one year old will equal 9.6 kg for boys and 9 kg for girls (35) (*mean weight of 9.3 was used)*

$$UL\ of\ UIC = (UL \times 0.92) \div (wt\ (kg) \times 0.002 \times 24)$$

$$UL\ of\ UIC = (180 \times 0.92) \div (9.3 \times 0.002 \times 24) = 370.9\ \mu g/L \approx 371\ \mu g/L$$

Excess iodine status at the population level was therefore defined as a median UIC ≥371 µg/L.

A sensitivity to the classification based on the weight is displayed in Supplemental Table 1.

Gut biomarkers

Concentrations of neopterin (NEO) and myeloperoxidase (MPO) as indicators of inflammation and alpha-1-anti-trypsin (AAT) as an indicator of permeability were measured in non-diarrheal stool samples collected monthly in the first year and quarterly in the second (28). The mean fecal biomarker concentrations of all non-diarrheal samples available up to 94 days preceding the iodine quantification were used at 6 months. Only the concurrent measurement of each fecal biomarker was used at 15 months. The LM ratio was additionally used as an indicator of intestinal barrier permeability (27). The ratio was converted to a z-score (LMZ), standardized for age and sex using the Fortaleza site as reference. LMZ values used were contemporary to the iodine sampling (6 and 15 months).

Covariates

Use of iodized salt. Mothers were asked monthly about the use of iodized salt at home until infants were eight months old and we calculated the percentage of affirmative responses for each infant. UNICEF defines acceptable population use of iodized salt as >90% of households (36).

Socio-economic status. Mothers were surveyed biannually about household ownership of assets, average monthly income, access to improved water and sanitation facilities and maternal education. Answers were used to calculate a composite socio-economic (SES) index (range 0 [low] to 1 [high]) (37).

Data Analysis

Stata 16 (Stata Corporation, College Station, TX, USA) and R (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses. The alpha was 0.05. Results are presented as estimates and 95% confidence intervals (CI) or credibility intervals (ci) for Bayesian models. Excluded participants were compared to those retained in the analysis using

linear regression for continuous variables (iodized salt use and socio-economic score) and Pearson's chi-squared test for the categorical variable (sex).

UIC has a skewed distribution, and, therefore, the median UIC was used (1). The 95% CI was estimated by bootstrapping following the recommendations of UNICEF and the Global Iodine Network (38). For the comparison of the median UIC and mean biomarker concentrations within each site, we log-transformed biomarker values. Because of the longitudinal nature of the study, which involved repeated measures, we accounted for data clustering by including random effects for child.

A Bayesian multinomial logistic regression (brms package in R) was used to assess associations of the subject-level UIC in 3 categories (<100, ≥100 to <371, ≥371 µg/L) (39). The model, using non-informative priors, included a random intercept on the study site and the individual to account for unexplained variation in the model due to site variability and to account for repeated measures. The ≥100 to <371 µg/L class was used as the reference category and associations were quantified as crude or adjusted Relative Prevalence Ratios (RPR). Initially, variables were examined individually, adjusting only for age and the random intercept terms. In adjusted analyses, models were constructed to include additional covariates, namely: sex, household SES level, household frequency of iodized salt use (mean of four yes/no questions), urine sample collection protocol (spot urine *vs*. 5-hour collection), time difference between stool and urine collection as well as gut biomarkers for permeability (LMZ and AAT) and inflammation (MPO and NEO), respectively.

In order to assess the association of different indicators of gut permeability and inflammation with iodine status, six interaction terms were examined: LMZ (Independent variable (IV)) and AAT (moderator); LMZ (IV) and NEO (moderator); LMZ (IV) and MPO (moderator); AAT

(IV) and NEO (moderator); AAT (IV) and MPO (moderator); and NEO (IV) and MPO (moderator). For these models, we used linear mixed models to model the log-transformed UIC as a function of the biomarkers and again with random intercepts for the individual and site. We tested the improvement of the goodness of fit of the models with interaction terms in comparison with the model without introducing any interaction using the likelihood ratio test on models fitted by maximum likelihood method. Marginal effects were estimated, and results were plotted. To explain the interaction terms, we computed the simple slopes of the log UIC on the independent variable when the moderator variable is held constant at different combinations of low or high values. To assess the difference between simple slopes, we performed pairwise comparisons using the Dawson and Richter Method (40).

Results

From 2145 infants enrolled, 1557 (73%) had at least two UI measurements and complete covariate data and were therefore included in these analyses (**Figure 1**). Excluded participants' characteristics were compared to those included in the analyses at various data collection points. There was no difference in sex distribution across the three measurement points (p values were ≥0.15). Included infants were more likely to have a higher SES index at 6 (p=0.026) and 15 months (p<0.0001), while they had a lower SES index at 24 months (p<0.0001). Similarly, the household use of iodized salt was lower among included infants at 6 (p<0.0001).

Selected characteristics are presented in **Table 1**. Infants from Fortaleza displayed the highest anthropometric characteristics (all p values <0.005) with the exception of length in the Vellore sample (p=0.081). Sexes were equally represented across all study sites. The lowest socio-economic level was found in Haydomwith a SES score of 0.2 (0.1) and the highest level was reported in Fortaleza scoring 0.8 (0.1). The use of iodized salt (any iodine dose ≥0.1 ppm)

was low in Vellore and very low (less than 10%) in Naushero Feroze and Haydom. We also compared our findings regarding household iodized salt use to national or regional level data available for a similar timeframe and found dramatically lower use of iodized salt in Vellore, Naushero Feroze, Haydom, and Venda than would have been expected. The urine volume of infants ranged between 26.6 mL (TZH) to 78.5 mL (INV) at 6 months, and at 15 months, between 28.5 mL and 83.7 mL (Fortaleza and Dhaka sites, respectively).

Iodine status

The distribution of UIC by age and study site is reported in the supplemental material (**Supplemental Figure 2**). A non-normal distribution was found across all sites and ages (p<0.0001). In certain sites, the concentrations ranged from broadly normal (e.g., Naushero Feroze) to excess (e.g., Haydom) even though a very low proportion of households reported iodized salt use.

At six and 15 months, all studied populations had adequate to excess median UIC (**Table 2**). SAV had the lowest median UIC of 107 µg/L ;95% CI: 89, 125, whereas Bhaktapur had the highest median UIC (893 µg/L ;95% CI: 762, 1026]), nearly three times higher than our estimated upper limit of 371 µg/L. Comparing the first and the last available measurements, five sites displayed a significant decline in the infant's median UIC (e.g. Bhaktapur median UIC declined from 893 µg/L at 6 months to 396 µg/L at 24 months, p<0.0001) whereas in Fortaleza (p=0.53) and Dhaka (p=0.77) no change was observed in median UIC. The Loreto site displayed a significant quadratic UIC trend (p<0.0001) from 6, to 15, and to 24 months.

Gut biomarkers

The trends of the fecal biomarkers in the MAL-ED study have been reported elsewhere (41). Briefly, the concentrations of fecal biomarkers all decreased between six and 15 months (**Figure**

2). The LMZ score decreased significantly in Dhaka (p<0.0001) whereas a significant increase occurred in Vellore (p<0.0001) and Loreto (p<0.0001), with all other sites remaining relatively stable between six and 15 months. All values were calculated relative to the Fortaleza population, which was standardized by age, hence that remained at zero at both time points. For all the biomarkers, higher values indicate greater gut dysfunction (inflammation or greater permeability).

Covariate associations with urinary iodine concentration

In both crude and adjusted analyses, neither sex nor socioeconomic index were associated with UIC (**Table 3**). In analyses adjusted for covariates, an increase in iodized salt use by 1% was found to be associated with increased risk of high UIC (adjusted RPR= 2.58 ;95% CI: 1.29, 5.00], Bayesian p-value = 0.03)(41).

Association of iodine status with gut inflammation and permeability

Table 3 also shows the relative prevalence ratio of having low or high median UIC per unit increase in gut biomarker concentration, and the associations between these biomarkers with UIC expressed on a continuous scale are shown in **Figure 3**. For example, an MPO (overall sample, median =7.6 ln(ng/mL); interquartile range (IQR): 8.65, 9.5) increase of 1 unit in ln scale was found to reduce the prevalence of low UIC by 9% in crude and 14% in adjusted analyses (Table 3), consistent with a positive association of MPO with UIC in **Figure 3**. An increase of NEO (median =6.5 ln(ng/mL); interquartile range: 7.4, 8.1) concentrations by one log unit was associated with a 13% reduction in the prevalence of low UIC in the adjusted analysis. Point estimates for AAT were close to 1 for UICs both below and above the relevant cut-offs (all non-significant) but were exclusively above 1 (although NS) for the risk of having low UIC with

increasing LMZ. That directionality is consistent with lower UIC associated with higher LMZ in Figure 3.

Association between co-occurrence of gut permeability and inflammation and urinary iodine concentration

Only the interaction term between AAT and NEO had a statistically significant association with UIC ($P<0.0001$). For illustration, the highest UIC (coef.=10.8 log(µg/L), $p<0.0001$) is observed at low values of NEO (e.g. lowest observed value, -1 log(nmol/L)) and AAT (lowest observed value, -11 log(mg/g)) and the lowest UIC (coef.=2.5 log(µg/L), $p=0.007$) is observed at high NEO concentrations (e.g. highest value, 11 log(nmol/L)) and low AAT (-11 log(mg/g)) values. UIC variation across the different tested values of NEO and AAT is reported in **Figure 4**. Significant association was also found for LMZ (coef=-0.08, $p<0.0001$) with UIC.

Discussion

To our knowledge, this study is the first to assess iodine status with gut health among infants in low- and middle income settings. These results suggest that excess iodine (median UIC ≥371 µg/L) was common and especially occurred in the Bhaktapur (Nepal), Fortaleza (Brazil) and Haydom (Tanzania) sites. Secondly, our study indicates, for the first time, that UIC is associated with gut health biomarkers.

Infant iodine nutrition and current status of universal salt iodization programs

We found a general trend of declining UICs from 6 months to 24 months, but stable and high concentrations from 6 to 15 months in the Bhaktapur, Fortaleza and Haydom sites. Whether this reflects recent high iodine ingestion only or a continuous exposure over time is not known. Similar findings of excess iodine intake, with medians above 400 µg/L, have been reported previously among infants under two years in Nepal (42) and Tanzania(18) (Table 2 (18,42–55)).

Both in Bhaktapur and Fortaleza, the use of iodized salt exceeded 90% of households, which agreed with the coverage reported at the national level (Table 3). In Nepal, salt overiodization might explain the excess iodine intake (56). During the early 2000s, Brazil was classified as having an excessive iodine status (57), and ten years later (in 2013), the government reduced the level of salt iodization. Our study coincides with the pre-reduction period of iodine in salt, which could explain the reported excess of iodine nutrition among infants in our Brazil site (57). Surprisingly, in Haydom, iodine excess was identified despite low reported household use of iodized salt. Environmental factors (e.g., groundwater) may be the source of the excess as reported elsewhere (18). Irrespective of the cause of iodine excess, chronic exposure to high levels may cause the "escape" phenomenon from the protective cellular mechanism known as the *Wolff-Chaikoff* effect (58). Consequently, iodine excess may lead to dysthyroidism and the associated array of health impairments (19–21) which may be particularly detrimental for infants(59). That said, a multicenter cross-sectional study conducted in East Africa (n=808) found that chronic exposure to high iodine doses – confirmed by thyroglobulin measurements – had little impact on thyroid function (dysthyroidism affected less than 2% of infants) (18). The long-term effects of early exposure to excess iodine intake are not firmly established (18).

Iodine status was acceptable from 6 to 24 months at the Dhaka, Vellore, Naushero Feroze, Loreto, and Venda sites. Other studies in India and South Africa have also consistently shown acceptable iodine status among infants (0.6 – 6 months) (44,54). Low use of iodized salt might occur in India, where a wide range in use (18.2% to 91.9%) has been reported (60). In Pakistan, inconsistent legislation across provinces regarding the compulsory commercialization of iodized salt is believed to lead to low iodized salt use (61,62).

The WHO recommends assessing iodine status among school-age children as representative of the general population (1). In general, our determination of iodine status at 24 months was in agreement with the most recent national reports among school children (Table 2) (5,6,12). However, further assessments are needed to confirm the concordance of iodine status between school-age children and infants, given that the iodine nutrition status of school-age children is not necessarily a good indicator of iodine status among pregnant women (38,63).

Associations between gut health and urinary iodine concentration

In contrast to our expectation, we found that gut inflammation, defined by high concentrations of NEO, was protective against low UIC. Roughly, a 1 log unit is a shift of ~ 25% in the raw biomarker concentrations (compared to the IQR). Indeed, NEO is actively produced by macrophages and dendritic cells upon stimulation by interferon-gamma (64). As such, an overproduction of interferon in the context of inflammation could be accompanied by an elevated NEO. Pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha and beta, IL-6) have been found to downregulate sodium-iodide symporter expression in thyroid tissues (65). Our multinomial model results do not reflect this association, perhaps due to downregulation of the symporter (up to 80% has been reported) following exposure to high doses of iodine (at least 24 hours) (22). In major diseases or conditions, such as inflammatory bowel disease, there is an altered distribution of tight junction proteins (66) along with increased molecule passage through the paracellular route (67) and perhaps this is being exhibited in these children.

We found that gastrointestinal permeability –based on higher LMZ– showed a modest negative association with UIC (only in linear regression, **Supplemental Table 2**). Mechanistically, the LMZ biomarker measures intestinal integrity and, precisely, the permeability through the paracellular route (68). On the other hand, under normal conditions,

iodine is transported from the intestinal lumen to the blood circulation via active transport catalyzed by the sodium-iodide symporter (22). This absorption of iodide is against its electrochemical gradient. In increased uptake through the paracellular route, iodine could be taken in by passive diffusion favoring its concentration gradient. Stated differently, iodine is lost through a more permeable gut.

We also found that AAT moderated the association between NEO and UIC. The shape of this association appears to be asymmetric and in a reverse J-shape, with a higher UIC observed at both lower AAT and NEO values, bathyphase at lower AAT and higher NEO and a clear upturn of UIC at both high NEO and AAT concentrations. Fecal AAT concentration reflects the transition from blood to lumen, such that the moderation effect is probably due to iodine losses through the transcellular route. Accordingly, a negative association between AAT and UIC was found in both multinomial (RPR=0.95) and linear (coef=-0.22; p=0.06) regressions; however, it was not statistically significant.

Järnerot (1975) examined the 24-hour iodine excretion of patients affected by ulcerative colitis or Crohn's disease. Ten percent of patients affected by bowel disease had an iodine excretion of less than 40 µg per day than 5% of controls. However, overall group comparisons did not show a significant difference between controls and patients regarding the absorbed radioiodide (69). Navarro et al. (70) investigated the influence of intestinal malabsorption among adults on iodine in patients suffering from chronic pancreatitis or short bowel syndrome. No evidence of iodine malabsorption was found in patients vs. controls. In a more recent study, Thomassen et al. (71) investigated the intestinal failure effect on iodine status in pediatric patients (n=19, aged 2 to 18 years). Intestinal failure patients received most of their iodine from parental nutrition (63%). It was shown that despite the fact that intestinal failure patients

363 received 2.7 µg/day/kg of iodine (two folds higher than the recommended dose) they were iodine
364 deficient. Despite a very high intake, iodine deficiency prevailed even after correction for
365 hydration. These studies provide support for the hypothesis that intestinal function disturbance
366 could be associated with poor iodine status. However, their major limitations are that they used
367 small sample sizes, were cross-sectionally designed, and did not analyze intestinal permeability
368 or inflammation.

369 Beyond the provided explanations on the possible metabolic pathways, it is essential to
370 acknowledge that the studied populations face multiple nutritional problems. Nutrient adequacy
371 of the diet is generally inadequate, although there is variability across nutrients and across sites
372 (26). Anemia (varying from 40 % to 88 % across sites) and micronutrient deficiencies (e.g., zinc
373 and vitamin A deficiency up to 61 % and 73 %, respectively) are prevalent, and stunting
374 increased three-fold from enrolment to 24 months (26,72). Malnutrition has been reported to
375 affect intestinal absorption through a weakened immune system, mucosal damage, and frequent
376 enteric infections (also bacterial translocation). This phenomenon would lead to mucosal
377 inflammation (72). Iron deficiency has been reported to be associated with gut dysbiosis, leading
378 to severe inflammation state (73). Beyond the nutritional situation, a high frequency (56 %) of
379 antibiotic use has been reported over the first two years of life among the studied population
380 (74). According to Yoon and al. (75), antibiotic use induces high fecal serine protease activity,
381 which in turn has a detrimental effect on the intestinal barrier.

382 **Strengths and limitations**

383 The present study had several strengths, including the fact that this is the first large-scale
384 multicenter and longitudinal assessment of iodine status in LMIC where data are scarce among
385 infants (76). This study covers a crucial period of life (within the first 1000 days of life). Both

386 internal and external certified quality control samples were used for urinary iodine
387 quantification. A standard procedure of a morning fast of 2 hours before urine sampling was
388 followed which is important given the knowledge that recent intakes of iodine may introduce a
389 significant bias in terms of urinary iodine data interpretation (77).

390 Our study also has some limitations. First, we did not measure the iodine content in breast
391 milk or estimate intake from non-breast milk foods. Across the sites, 96% of infants were
392 breastfed at 6 months and 82% were breastfed at 15 months. We did not adjust for the use of
393 antibiotics, as available data rely only on the frequency of use, and the information on the
394 antibiotic class was missing for a substantial number of participants (74). The use of urine spot
395 collection for UIC assessment is recommended at the population level given the known within-
396 and between-day variation at the individual level (29,78). However, 24-hour urine collections to
397 assess the iodine status of infants would prove challenging in the framework of an
398 epidemiological survey. Had we measured thyroglobulin concentration as a biomarker of long-
399 term iodine intake, it would have partially overcome the limitation related to the urine spot. The
400 application of the population cut-offs to a single casual quantification of UIC may introduce a
401 misclassification of subjects, although this bias may have been reduced by the large sample size
402 (n> 500) of our study (79) as well as the longitudinal design. It is also known that the use of
403 casual urine samples tends to overestimate the 24-hour intake of iodine (29). In the absence of
404 defined clinical thresholds for excess iodine, we used an approximation based on theoretical
405 underpinning. That the excess limit that we defined coincides with the upturn of thyroglobulin
406 toward higher values predicted by a second-order polynomial according to Farebrother et al.,
407 (80) suggests that our estimated upper limit is reasonable. The same study showed that
408 thyroglobulin tends to be high at 100 µg/L, indicating that the lower limit should probably be

increased. Our objective was not to define the upper limit of UIC corresponding to an excess status (neither at the population nor at the individual level). Rather, we tried to approximate a certain limit based on a logical approach in order to assess the relationship between a low and high concentration of iodine with gut biomarkers. However, as published previously by Zimmermann et al. (81), a stringent approach is needed to define iodine excess based on median UIC (e.g., recruitment of healthy children, measurement of thyroid biomarkers such as thyroglobulin, UIC of recruited children should be evenly distributed over the range of intake from deficient to excessive).

Additionally, we did not determine the exact location of the inflammation and impaired permeability at the intestinal level (i.e. small or large intestine). This might be a limitation because the sodium iodide symporter is mainly expressed in the small intestine. Finally, iodine concentration may vary in urine spots based on the hydration status, and we did not have the data necessary to adjust for creatinine concentration. This may have contributed to findings of iodine excess in certain sites (i.e., Fortaleza, Haydom and Bhaktapur), where urine volume was ranging on average between 28 to 44 mL across all time points.

Conclusion

In line with the recent global estimates (76), we found that iodine status in eight LMIC varies from optimal to excess across the period of 6 to 24 months of age. Excess UIC was predominant at six months and tended to normalize at 24 months. Because the first 1000 days represent a unique nutritional opportunity for promoting optimal infant health and growth (73), tight monitoring of the iodine nutrition at this early phase of life should be recommended in these countries. EED is frequent in LMIC and has been associated with micronutrient deficiencies. The increase in gut permeability was found to be associated with a slightly higher iodine excretion,

and inflammation was found to reduce the prevalence of low UIC. The direction of association between inflammation and iodine excretion contradicted our expectation that inflammation would reduce UIC via down-regulation of the iodide transporter. UIC association with NEO and AAT follows a reversed J-shape curve with a reduction of UIC excretion observed at low AAT and very high NEO. However, as a future direction of research, molecular assessments (RNA sodium iodide symporter expression) at the gut level could shed light on the mechanistic nature of these associations. Our findings overall suggest that the role of gut health should further examined in relation to iodine status using UIC, particularly in populations with a high prevalence of gut inflammation.

Acknowledgment

BM, LEC, KS, and LEMK designed research; BM, LEC, KS, and LEMK conducted research; RD and BM analyzed data; and RD, BM, LEMK wrote the first draft of paper; All authors contributed to critical revision of the manuscript; All authors had primary responsibility for final content. All authors read and approved the final manuscript.

Data availability

The MAL-ED study data are publicly available (upon request) through the ClinEpiDB platform (https://clinepidb. org). The link to the specific record to explore study data and request a download of the data is https://clinepidb.org/ce/app/record/dataset/DS_5 c41b87221

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683 **Table 1**. Characteristics of the participants by study site

Site	**n**	**Weight at 6 months (kg)**	**Length at 6 months (cm)**	**Weight-for-length z-score**	**Girls %**	**SES index, mean (sd)**	**Iodized salt use % (in MALED study)**	**Use of iodized salt (>0.1 mg/kg) at the national level** (6) **(in the literature)**	
								Year	**%**
Bangladesh, Dhaka	164	6.87 ± 0.06	64.06 ± 0.16	-0.16 ± 0.08	44.9	0.5 (0.1)	99.0 (0.2)	2015	68.3
India, Vellore	219	6.55 ± 0.05	63.9 ± 0.14	-0.66 ± 0.07	46.6	0.5 (0.1)	19.2 (1.0)	2015-6	92.7
Nepal, Bhaktapur	229	7.38 ± 0.06	65.4 ± 0.14	0.11 ± 0.07	53.3	0.7 (0.1)	93.8 (0.6)	2016-7	94.2
Pakistan, Naushero Feroze	250	–	–	–	49.9	0.5 (0.2)	4.0 (0.5)	2011	69.1
Brazil, Fortaleza	162	8.42 ± 0.10	67.00 ± 0.19	1.00 ± 0.10	56.7	0.8 (0.1)	100	2006-7	98.4
Peru, Loreto	153	7.53 ± 0.07	63.84 ± 0.16	0.93 ± 0.08	53.9	0.5 (0.1)	97.6 (0.2)	2018	88.7
Tanzania, Haydom	189	7.16 ± 0.08	63.58 ± 0.17	0.47 ± 0.09	49.3	0.2 (0.1)	7.0 (0.8)	2015 - 6	76.0
South Africa, Venda	191	7.39 ± 0.08	64.4 ± 0.21	0.48 ± 0.10	52.1	0.8 (0.1)	17.8 (1.2)	2016	91.1

Table 2. Median urinary iodine concentration by age and site

Country	MAL-ED					Literature						
	Site	6 months	15 months	24 months	*P*-value[1]	Infant				School-age children		
		Median (95% confidence interval)				Median UIC [2] (µg/L)	Age (month)	Ref	Year	Median UIC [2] (µg/L)	Age (y.)	Ref
Bangladesh	Dhaka	243 (218 – 269)	233 (185– 282)	–	0.77	–	–	–	2011-12	146 (59 – 270) [3]	6 – 12	(43)
India	Vellore	204 (171 – 238)	114 (93 – 135)	106 (86 – 127)	<0.0001	168	0.6	(44)	2019	173	15 – 49	(45)
Nepal	Bhaktapur	893 (762 – 1026)	788 (627 – 949)	396 (336 – 457)	<0.0001	407	6.0-24.0	(42)	2016	314	6 – 9	(46)
Pakistan	Naushero Feroze	241 (215 – 279)	187 (167 – 208)	111 (98 – 123)	<0.0001	66	Neonates	(47)	2011	126	6 –12	(48)
Brazil	Fortaleza	590 (506 – 673)	588 (531 – 645)	–	0.53	56 (38 – 74) [3]	Neonates	(49)	2016	276	6 – 14	(52)
						293 (211 – 544) [3]	0 – 6.0	(50)				
Peru	Loreto	298 (250 – 346)	310 (251 – 370)	150 (116 – 184)	<0.0001	–	–	–	2013	259	5 – 17	(51)
Tanzania	Haydom	557 (434 – 680)	371 (228 – 513)	247 (191 – 303)	<0.0001	515 (279 – 886) [3]	2.4	(18)	2004	204	6 – 18	(53)
						528 (255 – 952) [3]	10.4					
South Africa	Venda	107 (89 – 125)	112 (95 – 130)	88 (43 – 132)	0.55	336 (214 – 604) [3]	6.0	(54)	2015	130	18 – 49	(55)

[1] Unadjusted P-value from the linear model of change in mean log(UIC) between first and last categories of age. [2-] Urinary Iodine Concentration. [3]Interquartile range (25^{th} – 75^{th}) where available.

Table 3. Subject level associations between urinary iodine concentration and socio-demographic factors, use of iodized salt4 in household and gut biomarkers

	Crude analysis				Adjusted analysis			
	UIC[1]<100 µg/L		UIC≥371 µg/L		UIC<100 µg/L		UIC≥371 µg/L	
	RPR[2]	95% CI[3]	RPR[4]	95% CI[3]	RPR[2]	95% CI[3]	RPR[4]	95% CI[3]
Time variable								
Age, months	1.05	1.03 – 1.06	0.94	0.93 – 0.95	1.04	1.01 – 1.07	1.00	0.97 – 1.02
Co-variates								
Sex								
Girls	1		1		1		1	
Boys	0.13	0.04 – 0.91	1.05	1.03 – 1.06	0.95	0.74 – 1.23	1.22	0.99 – 1.49
Socioeconomic level (score x 10)	0.98	0.91 – 1.05	1.04	0.98 – 1.11	1.01	0.92 – 1.11	1.01	0.93 – 1.09
Use of iodized salt	0.52	0.31 – 0.88	1.80	1.08 – 3.00	0.51	0.26 – 1.04	2.58	1.29 – 5.00
Gut biomarkers								
MPO[5] ln(ng/mL)	0.91	0.85 – 0.99	1.00	0.93 – 1.07	0.86	0.77 – 0.95	0.95	0.87 – 1.03
NEO[6] ln(nmol/L)	0.95	0.87 – 1.03	1.05	0.98 – 1.13	0.87	0.79 – 0.97	1.05	0.96 – 1.15
AAT[7] ln(mg/g)	0.93	0.86 – 1.01	0.95	0.88 – 1.03	0.95	0.84 – 1.07	0.98	0.87 – 1.09
LMZ[8]	1.09	0.97 – 1.24	0.93	0.83 – 1.03	1.09	0.97 – 1.24	0.93	0.83 – 1.03

[1-] UIC: Urinary iodine concentration; [2-]RPR: For category of cofactor vs. reference category (for which RPR = 1), crude or adjusted Relative Prevalence Ratio of having UIC < 100 vs. having UIC in the 100 < UIC < 371 category (base response category); [3-] C.I.: 0.95 credibility interval for crude or adjusted RPR. [4-] RPR: For category of cofactor vs. reference category (for which RPR = 1), crude or adjusted Relative Prevalence Ratio of having high iodine (UIC ≥ 371) vs. having UIC in the 100 < UIC < 371 category (base response category). [5-]Myeloperoxydase. [6-] Neopterin. [7-]Alpha-1-anti-trypsin; [8-]Lactulose Mannitol z score. Adjustment was made for all variables included in the table (age, sex, socioeconomic level score, use of iodized salt, MPO, NEO, AAT and LMZ) as well as for site as a random intercept.

Figure 1. Flowchart of study participants selection.

Figure 2. Trends in gut biomarker concentrations by age and site at 6 and 15 months old.

Figure caption: BGD: Dhaka, Bangladesh; INV: Vellore, India; NEB: Bhaktapur, Nepal; PKN: Naushero Feroze, Pakistan; BRF: Fortaleza, Brazil; PEL: Loreto, Peru; TZH: Haydom, Tanzania; SAV: Venda, South Africa; MPO: Myeloperoxydase; NEO: Neopterin; AAT: Alpha-1-antitrypsin; LM: Lactulose:Mannitol z-score.

Figure 3. Trends of UIC by concentration of gut biomarkers. The blue line gives the linear trend across the full range of the gut biomarker, the red line indicates the trend across the 10^{th} to 90^{th} percentile range of the biomarker. The horizontal dashed lines indicate 100, 180 and 371 UIC (µg/L). All observations across sampling times and sites are included.

Figure 4. Estimated effects of combined concentrations of AAT and NEO on lnUIC. Analyses are adjusted for sex, household socioeconomic level, household frequency of iodized salt use, urine sample collection protocol (spot urine vs. 5-hour collection), as well as gut biomarkers for permeability (LMZ) and inflammation (MPO). AAT: Alpha-1-anti-trypsin; NEO: Neopterin; LMZ: lactulose-mannitol ratio z score. The NEO concentrations are -1 (low, lowest observed value), 3 (moderate, at 42% of observed concentrations), 7 (high, at 95%), and 11 (very high, highest observed value) ln(nmol/L). The AAT concentrations are -11 (low, lowest observed value) and 1 (high, at 80% of observed values) ln(mg/g).