

No associations between microbiota signalling substances and cognitive, language and motor development among three-year-old rural Ugandan children

Child undernutrition remains a considerable problem in poor regions, due to unhygienic living conditions and chronically infected guts that make it hard to absorb nutrients. Undernutrition may negatively affect child development,¹ and findings suggest that impaired gut microbiota may be central to poor brain development.² However, clinical data, particularly randomised controlled trials (RCT), are needed to confirm this. Our previous RCT showed that maternal education in nutrition, hygiene and stimulation markedly improved children's cognitive, language and motor skills.³ The number of bacteria and species did not differ between the intervention and control groups. However, bacteria-derived signalling mediators could play a causative role in mediating these positive developmental findings, such as short-chain fatty acids (SCFA) and/or other elements of the gut-brain axis, such as the kynurenine pathway.^{4,5} This study examined possible links between the urine concentrations of SCFAs and metabolites in the kynurenine pathway and cognitive, language and motor development.

This was a secondary analysis of our open cluster RCT among poor mothers of children aged 6–8 months in south-western Uganda.³ It was approved by the Uganda National Council for Science and the Norwegian Regional Committee for Medical and Health Research Ethics. The mothers provided written or thumb-printed consent.

We randomised 511 mother-child pairs from the original study: 263 in the intervention received a 6-month education intervention that emphasised nutrition, hygiene and child stimulation and 248 controls did not.³ Booster education sessions were conducted every 3 months until the children were 36 months. The child had to be 20–24 months to be included in the follow-up study since developmental milestones at this age may predict intelligence quotient when children are about to start school. At 36 months, we collected 74 spot urine samples from the 155 children who participated in the follow-up study: 40 in the intervention group and 34 controls.

Child development was assessed with the Bayley Scales of Infant and Toddler Development—Third Edition (BSID-III), which was adapted for rural Uganda³ and conducted in hired rooms to minimise distractions.

The analyses of the urine metabolite concentrations and the statistical methods for handling the data are given in the Supporting Information.

As previously reported, the follow-up sample did not differ significantly from the original sample at baseline in terms of socio-demographic factors, anthropometry or BSID-III composite scores.³ We measured the concentrations of the three SCFAs, plus tryptophan and kynurenine, and there were no significant differences between the two study groups when these were reported as absolute concentrations or corrected normalised ratios (Table 1). Similarly, there was no significant difference in the ratio of kynurenine and tryptophan concentration, a marker of indoleamine 2,3-dioxygenase expression, which is the first and rate-limiting step of tryptophan metabolism along the kynurenine pathway. Since there were no significant differences in the urine concentrations between the two groups, we pooled the data from them. We then correlated the urine concentrations of the five tested mediators with the BSID-III composite scores for cognitive, language and motor development outcomes. No significant correlations were detected (Table S1).

Despite the suggested links between gut microbiota and child development, the intervention did not appear to have any effect on the urine concentrations of various metabolites known to mediate signalling in the gut microbiota-brain axis. In addition, there were no associations between the three SCFAs, tryptophan or kynurenine and the developmental outcomes.

Mechanistic data have suggested that dysregulated gut microbiota caused impaired child development in low-resource settings.² Since both diet and hygiene determine gut microbiota composition, we hypothesised that the positive effect on child cognition, language and motor skills observed in our education RCT³ could have been mediated by a change in the metabolites mediating signalling between the gut and the brain.

There could be several explanations for our negative findings. The sample size was low and the intervention was not primarily designed to study the impact on gut microbiota. In addition, we cannot exclude any significant role of microbiota sampled from

Compound	Intervention group	Control group	P-value
	(n = 40)	(n = 34)	
Creatinine (mmol/L)	1.9 (1.1-3.1)	1.9 (1.2-2.9)	.91
Tryptophan (μmol/L)	24 (9-47)	26 (13-48)	.64
Corrected ratio tryptophan	12 (8-16)	13 (8-19)	.68
Kynurenine (μmol/L)	1.3 (0.5-2.4)	1.2 (0.6-2.7)	.76
Corrected ratio kynurenine	0.7 (0.4-1.1)	0.8 (0.5-1.0)	.77
Ratio kynurenine/tryptophan	0.055 (0.040-0.080)	0.055 (0.038-0.10)	.96
Acetic acid (μmol/L)	151 (82-255)	131 (86-219)	.52
Corrected ratio acetic acid	79 (52-123)	77 (54-115)	.84
Propionic acid (μmol/L)	4.4 (3.1-8.2)	3.2 (2.7-5.9)	.15
Corrected ratio propionic acid	2.3 (1.3-4.4)	2.4 (1.4-4.0)	.87
Butyric acid (μmol/L)	1.3 (0.8-2.8)	1.4 (0.6-2.6)	.81
Corrected ratio butyric acid	0.7 (0.4-1.6)	0.8 (0.5-1.1)	.66

Note: Urine concentrations of the signalling mediators: tryptophan, kynurenine and short-chain fatty acids.

Values are medians and interquartile values. There are no available childhood reference ranges reported for any of these metabolites or their corrected ratios, probably due to methodological issues.

other anatomical locations, such as the oral cavity. Furthermore, we measured urine, not blood, concentrations of the selected metabolites, for pragmatic and logistic reasons as rural Uganda is a challenging setting. Major strengths included collecting data from a robust, pragmatic RCT, including several possible mediators of gut-brain signalling and using a validated tool, namely BSID-III, to assay developmental outcomes in this setting and age group.

In conclusion, the urine concentration of the gut microbiota-brain signalling mediators tryptophan, kynurenine and the SCFAs, such as acetic acid, propionic acid and butyric acid, did not significantly correlate with developmental outcomes among small children living in low-resourced rural Uganda.

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CONFLICT OF INTEREST

None declared.

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TABLE 1 Main study results

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.