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Supplementary Materials for

Metabolic maturation in the first 2 years of life in resource-constrained settings and its association with postnatal growths

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Supplementary Materials

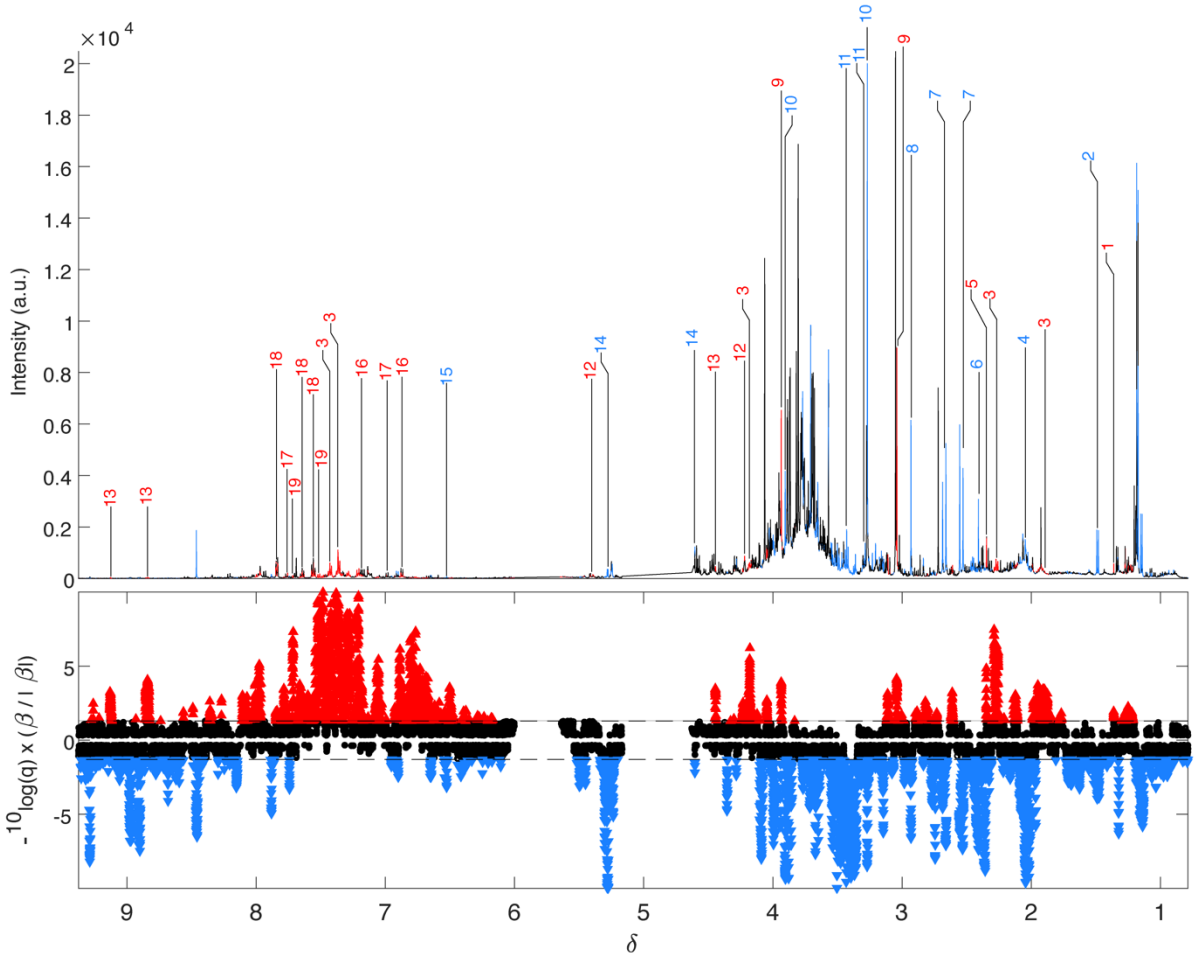

Fig. S1: Covariate-adjusted-projection to latent structures (CA-PLS) model built on the complete ^{1}H NMR spectral profiles from the urine of healthy infants across all ages identifying spectral features significantly associated with age. Model is adjusted for site. *Upper panel*. Mean ^{1}H NMR spectrum from all samples indicating metabolites that are excreted in greater amounts as the infant ages (red) and those excreted in lower amounts by the infant ages (blue). *Lower panel*. Manhattan plot showing −log10(q) × sign of regression coefficient (β) of the Monte Carlo cross-validation (MCCV) – PLS model for the 24,706 spectral variables. A *p*-value was calculated for each variable on the basis of 25 bootstrap re-samplings of the data in each of the 100 models to estimate the variance. Horizontal lines indicate cut-off values for the false discovery rate on the $\log_{10}$ scale. Blue points indicate metabolites significantly negatively associated with age and red points indicate those metabolites significantly positively associated with age. 1, 2-Hydroxyisobutyrate; 2, alanine; 3, phenylacetylglutamine; 4, *N*-acetylglycoprotein; 5, 4-cresyl

sulfate; 6, succinate; 7, citrate; 8, dimethylglycine; 9, creatine; 10, betaine; 11, taurine; 12, sucrose; 13, *N*-methylnicotinic acid (trigonelline); 14, galactose; 15, fumarate; 16, 4-hydroxyphenylacetate; 17, 4-hydroxyhippurate; 18, hippuric acid; 19, 3-indoxyl sulfate.

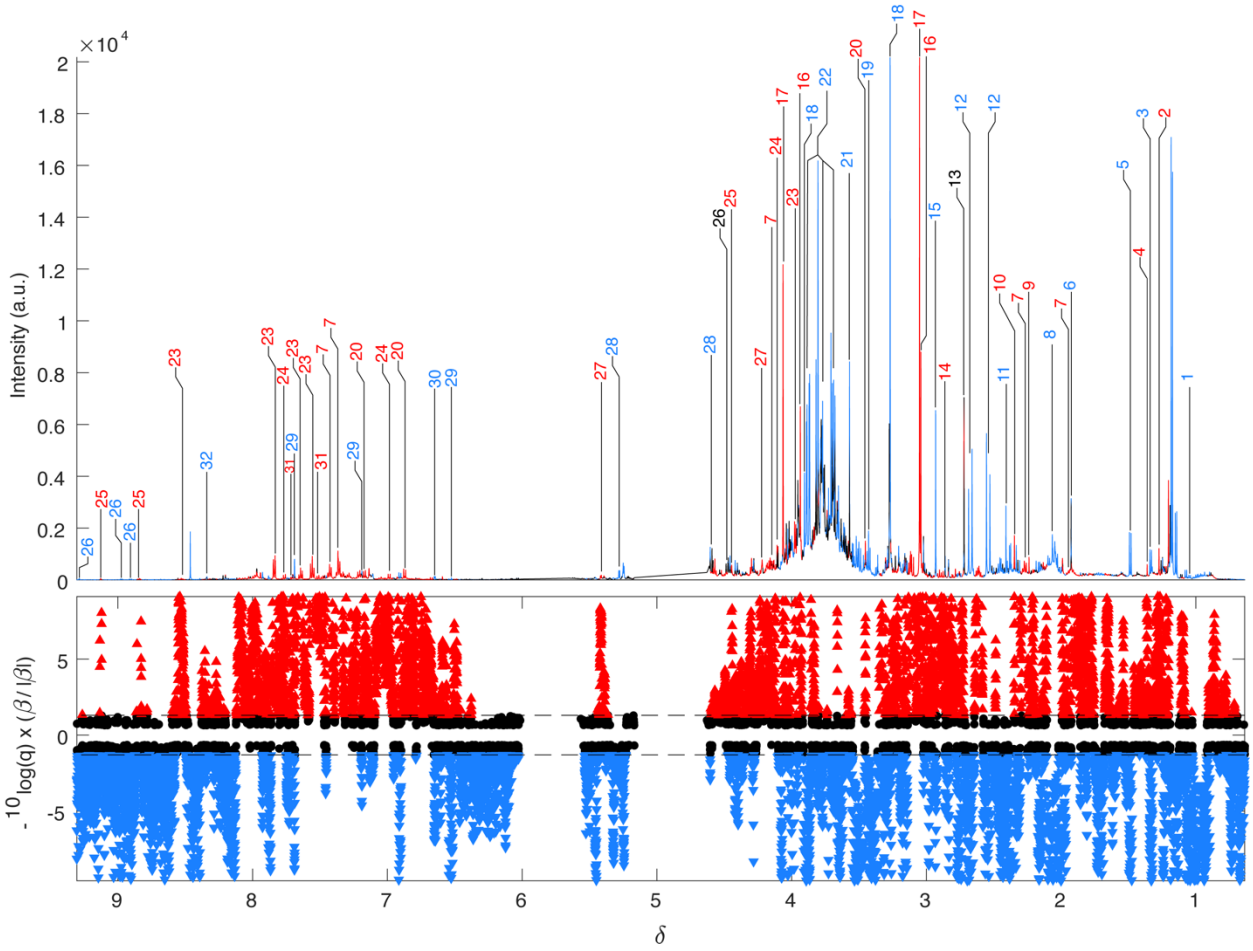

Fig. S2: Covariate-adjusted-projection to latent structures (CA-PLS) model built on the complete ^{1}H NMR spectral profiles from the urine of growth-constrained infants across all ages identifying spectral features significantly associated with age. Model is adjusted for site. *Upper panel*. Mean ^{1}H NMR spectrum from all samples indicating metabolites that are excreted in greater amounts as the infant ages (red) and those excreted in lower amounts by the infant ages (blue). *Lower panel*. Manhattan plot showing −log10(q) × sign of regression coefficient (β) of the Monte Carlo cross validation (MCCV) – PLS model for the 24,706 spectral variables. A *p*-value was calculated for each variable on the basis of 25 bootstrap re-samplings of the data in each of the 100 models to estimate the variance. Horizontal lines indicate cut-off values for the false

discovery rate on the $\log_{10}$ scale. Blue points indicate metabolites significantly negatively associated with age and red points indicate those metabolites significantly positively associated with age. 1, Valine; 2, beta-hydroxy-beta-methylbutyrate; 3, lactate; 4, 2-hydroxyisobutyrate; 5, alanine; 6, acetate; 7, phenylacetylgutamine; 8, *N*-acetylglycoprotein; 9, acetone; 10, 4-cresyl sulfate; 11, succinate; 12, citrate; 13, dimethylamine; 14, trimethylamine; 15, dimethylglycine; 16, creatine; 17, creatinine; 18, betaine; 19, taurine; 20, 4-hydroxyphenylacetate; 21, glycine; 22, mannitol; 23, hippuric acid; 24, 4-hydroxyhippurate; 25, *N*-methylnicotinic acid (trigonelline); 26, *N*-methylnicotinamide; 27, sucrose; 28, galactose; 29, fumarate; 30, 2-furoylglycine; 31, 3-indoxyl sulfate; 32, *N*-methyl-2-pyridone-5-carboxamide.

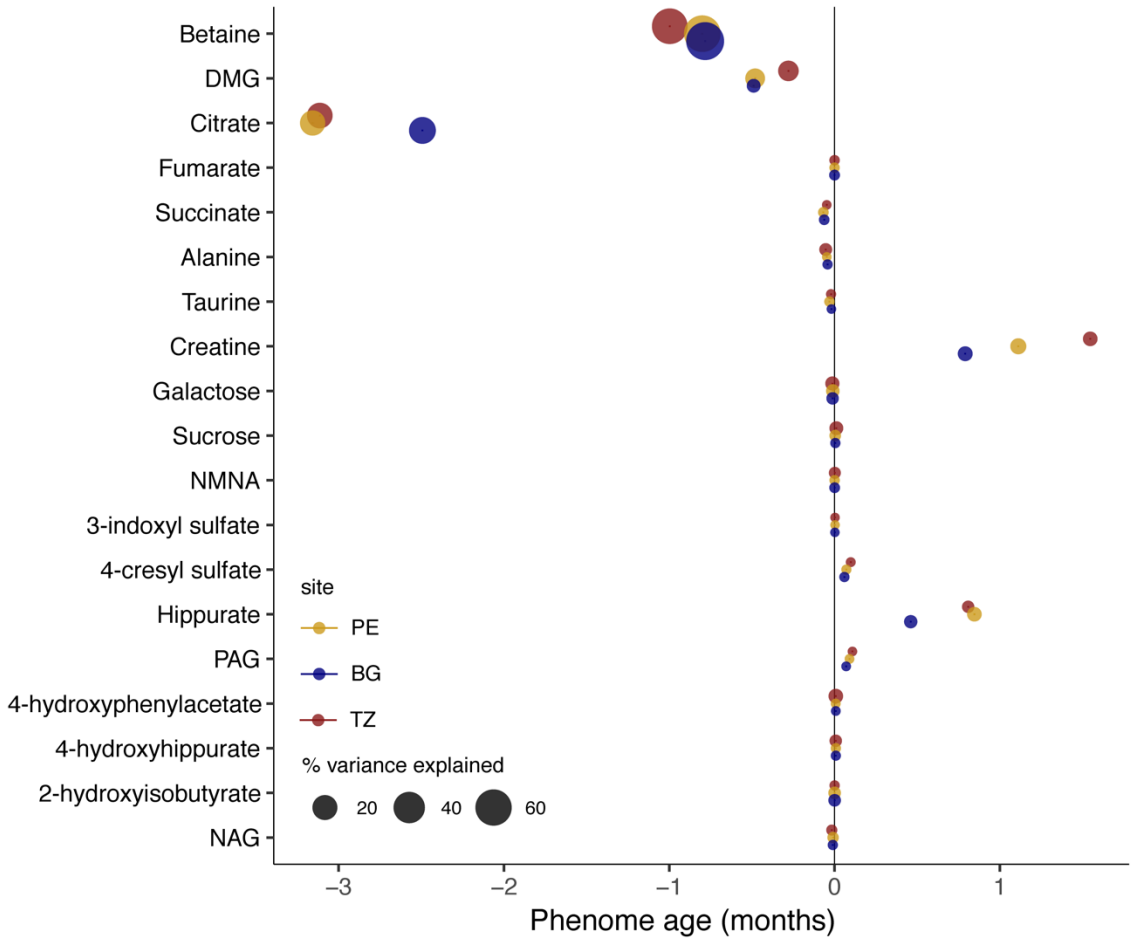

Fig. S3: Mean effect size of 19 age-discriminatory urinary metabolites on the phenome-for-age (PA) based on children with healthy growth trajectories, derived from PLS models. The effect size is depicted as the estimated change in PA for each standard deviation change a metabolite concentration. Color indicates cohort site and the size of the symbols indicate the percentage PA variance explained by each metabolite.

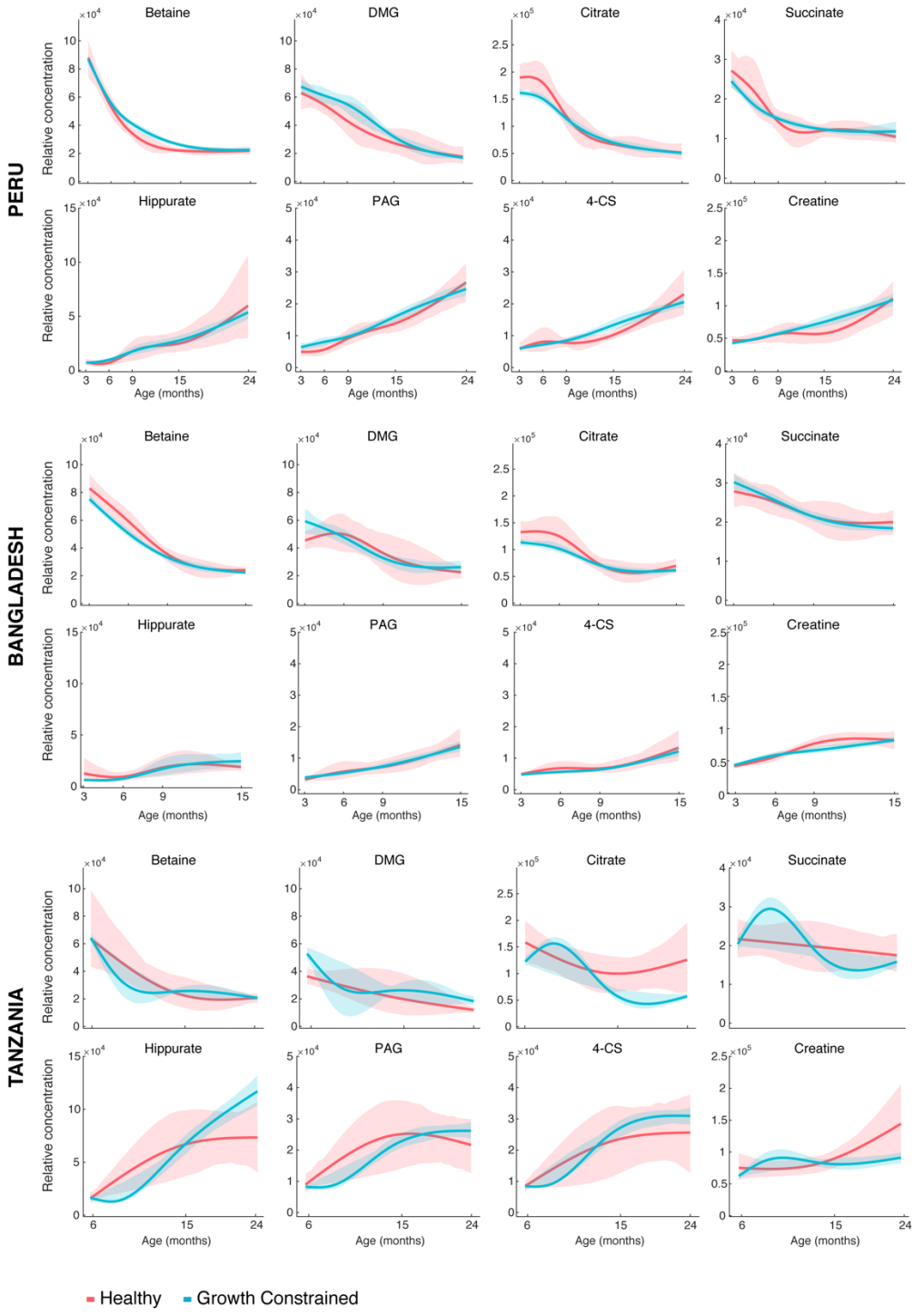

Fig. S4: Time-dependent variation in the eight urinary metabolites used to calculate the phenome age of the study children in Peru, Bangladesh, and Tanzania. Relative concentrations of metabolites were obtained by measuring the area under selected spectral regions corresponding to betaine, DMG, citrate, succinate, hippurate, PAG, 4-CS and creatine. Shaded area represents 95% confidence interval.

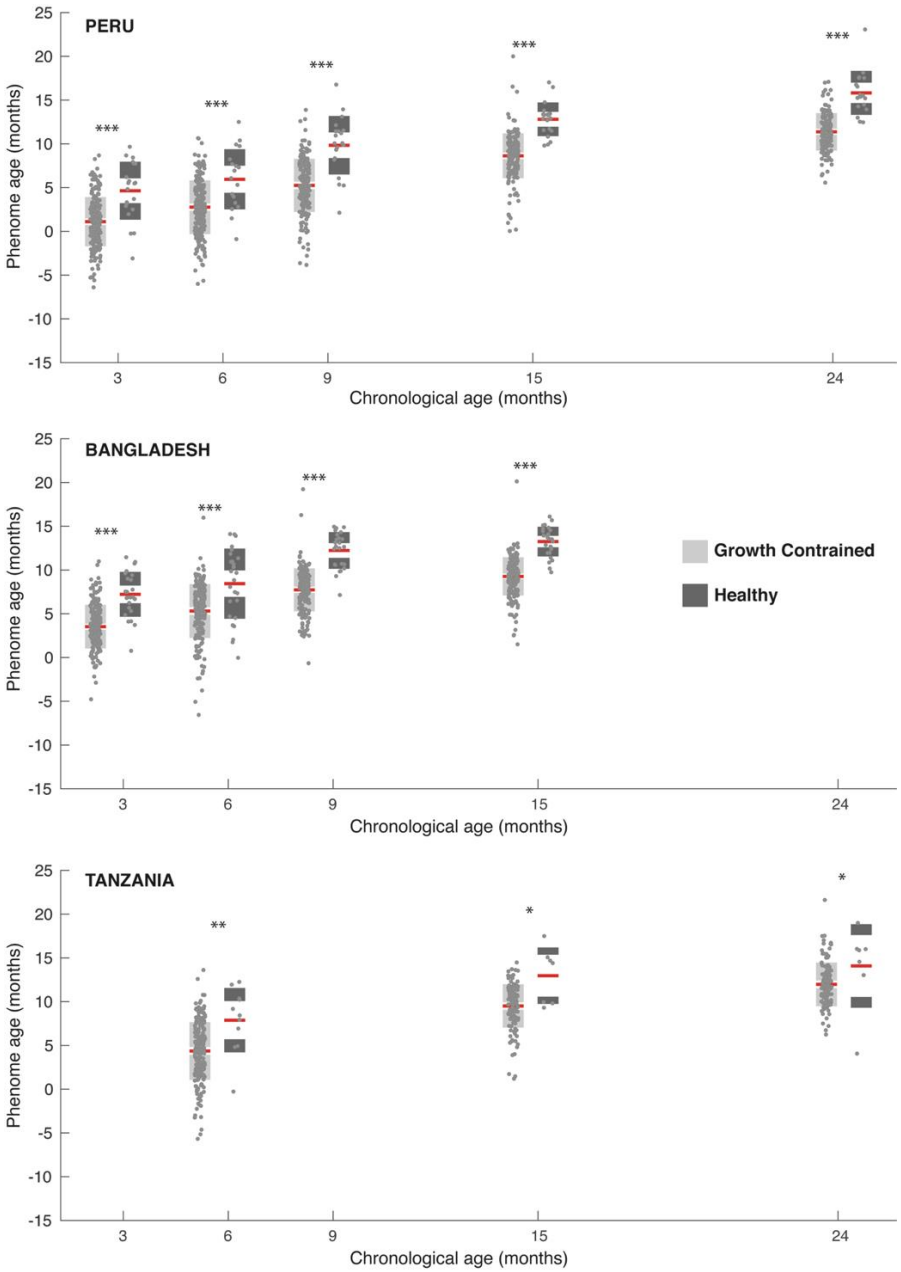

Fig. S5: Phenome age of growth constrained and healthy infants relative to their chronological age across the three sites. The PA score of healthy and growth constrained infants from each site was calculated from 8 age-discriminatory urinary metabolites. Significant differences were observed between healthy and growth constrained children at all sampling points in all cohorts. Mann-Whitney U test, * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$. (Healthy Peru: $N_{3\ months} = 21$, $N_{6\ months} = 19$, $N_{9\ months} = 20$, $N_{15\ months} = 20$, $N_{24\ months} = 18$; Growth constrained Peru: $N_{3\ months} = 220$, $N_{6\ months} = 214$, $N_{9\ months} = 197$, $N_{15\ months} = 183$, $N_{24\ months} = 141$; Healthy Bangladesh: $N_{3\ months} = 25$, $N_{6\ months} = 27$, $N_{9\ months} = 24$, $N_{15\ months} = 26$; Growth-

constrained Bangladesh: $N_{3\ months}$ = 196, $N_{6\ months}$ = 197, $N_{9\ months}$ = 181, $N_{15\ months}$ = 184; Healthy Tanzania: $N_{6\ months}$ = 11, $N_{15\ months}$ = 7, $N_{24\ months}$ = 7; Growth constrained Tanzania: $N_{6\ months}$ = 209, $N_{15\ months}$ = 129, $N_{24\ months}$ = 122).

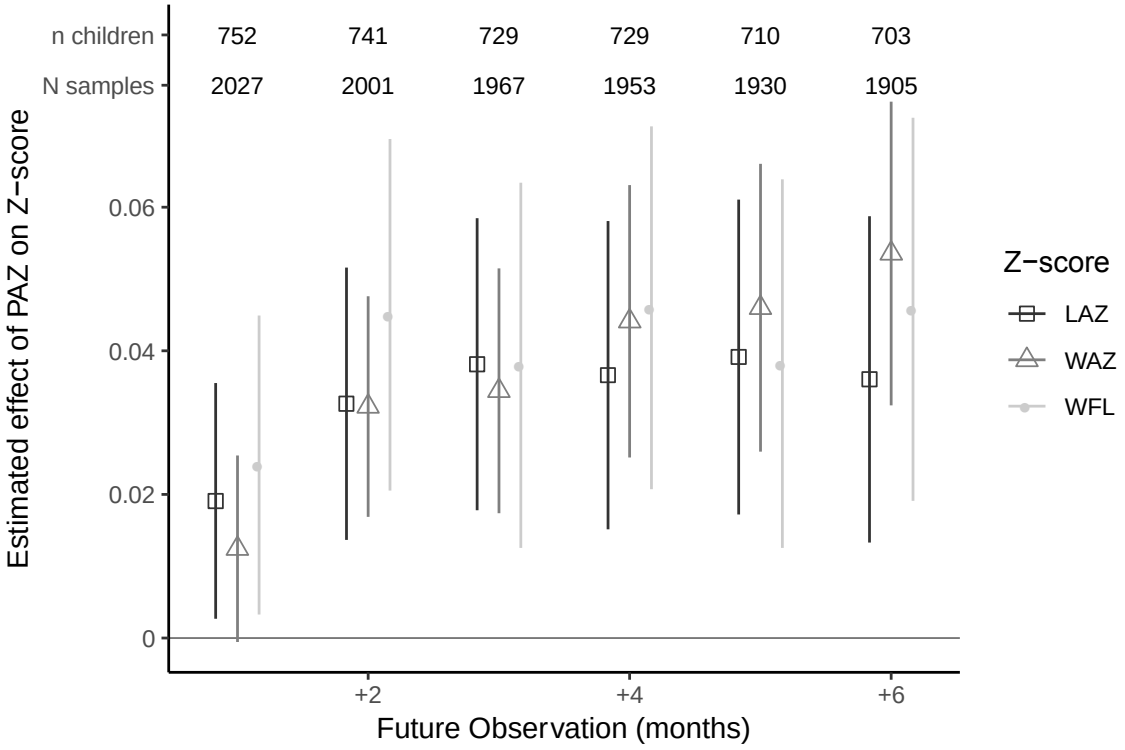

Fig. S6: The mean effect of each additional month of PAZ on LAZ and WAZ one to six months after the urine sampling time

Table S1. Characteristics of sampled population. Number of participants in each cohort site (N (%)), and their corresponding length-for-age (LAZ) Z-scores (median [IQR]).

	HEALTHY				GROWTH CONSTRAINED			
	Peru	**Bangladesh**	**Tanzania**	**Total**	**Peru**	**Bangladesh**	**Tanzania**	**Total**
Participants	22	28	13	63	259	221	236	716
Female	17 (77.3)	13 (46.3)	8 (61.5)	38 (60.3)	112 (43.2)	113 (51.1)	119 (50.4)	344 (48.0)
Enrolment length	49.8	50.5	50.0	50.4	48.6	48.0	49.0	48.3
(cm)	[48.1 - 50.9]	[50 - 51.1]	[49.8 - 52.0]	[49 - 51.1]	[47.0 - 49.8]	[46.5 - 49.4]	[47.5 - 50.0]	[47 - 49.8]
Enrolment weight	3.20	3.19	3.8	3.26	3.05	2.71	3.37	3.05
(kg)	[2.89 - 3.35]	[3.01 - 3.43]	[3.4 - 3.93]	[2.98 - 3.56]	[2.84 - 3.35]	[2.45 - 2.96]	[3.09 - 3.63]	[2.71 - 3.40]
LAZ								
Birth	-0.16	0.06	0.09	0.04	-1.02	-1.10	-0.97	-1.05
	[-0.88 - 0.62]	[-0.24 - 0.50]	[-0.31 - 0.45]	[-0.47 - 0.45]	[-1.63 - -0.41]	[-1.74 - -0.52]	[-1.65 - -0.31]	[-1.68 - -0.43]
3 months	-0.34	-0.01	0.07	-0.02	-1.49	-0.98	-1.22	-1.32
	[-0.41 - 0.34]	[-0.21 - 0.35]	[-0.15 - 0.22]	[-0.35 - 0.34]	[-2.08 - -0.84]	[-1.68 - -0.44]	[-1.87 - -0.64]	[-1.91 - -0.69]
6 months	-0.20	-0.01	-0.23	-0.04	-1.36	-1.16	-1.24	-1.24
	[-1 - 0.24]	[-0.15 - 0.18]	[-1.04 - 0.67]	[-0.53 - 0.26]	[-1.95 - -0.89]	[-1.88 - -0.75]	[-2.05 - -0.72]	[-1.94 - -0.75]
9 months	-0.29	-0.23	-0.38	-0.27	-1.57	-1.38	N/A	-1.66
	[-0.92 - 0.06]	[-0.43 - 0.12]	[-0.72 - 0.33]	[-0.71 - 0.27]	[-2.12 - -0.98]	[-2.04 - -0.89]		[-2.30 - -0.98]
15 months	-0.80	-0.73	-0.88	-0.76	-1.84	-1.93	-2.44	-2.00
	[-1.26 - -0.31]	[-0.91 - -0.46]	[-1.08 - -0.52]	[-1.07 - -0.44]	[-2.29 - -1.33]	[-2.5 - -1.39]	[-3.09 - -1.92]	[-2.67 - -1.45]
24 months	-0.67	-0.91	-0.98	-0.91	-1.95	-2.23	-2.74	-2.24
	[-1.05 - -0.46]	[-1.08 - -0.64]	[-1.05 - -0.67]	[-1.08 - -0.58]	[-2.51 - -1.66]	[-2.76 - -1.67]	[-3.53 - -1.99]	[-2.77 - -1.70]
Delta $LAZ_{3\text{-}24}$	-0.33	-0.90	-1.05	-0.89	-0.46	-1.25	-1.52	0.92

[1] Enrolment and Birth LAZ was ≤17 days after birth

Table S2. Summary of the PLS models returned for age associated metabolic variation in healthy and growth constrained infants. Models were built with one predictive component and no orthogonal components. R^2X: fraction of variation of the descriptive matrix explained by the model, $R^2\hat{Y}$: goodness of fit, $Q^2\hat{Y}$: predictive ability of the model, P: *p*-value following 1,000 permutations.

Model	*n*	R^2X	$R^2\hat{Y}$	$Q^2\hat{Y}$	P
Urine					
Healthy Combined	225	0.19	0.40	0.37	< 0.001
Healthy Peru	98	0.25	0.54	0.51	< 0.001
Healthy Bangladesh	102	0.23	0.43	0.32	< 0.001
Healthy Tanzania	25	0.32	0.56	0.35	< 0.001
Growth Constrained Combined	2198	0.14	0.44	0.47	< 0.001
Growth Constrained Peru	959	0.23	0.53	0.53	< 0.001
Growth Constrained Bangladesh	758	0.10	0.51	0.47	< 0.001
Growth Constrained Tanzania	481	0.22	0.46	0.45	< 0.001
Plasma					
Healthy Peru	36	0.29	0.18	0.12	< 0.001
Healthy Bangladesh	49	0.29	0.30	0.20	< 0.001
Growth Constrained Peru	375	0.05	0.23	0.18	< 0.001
Growth Constrained Bangladesh	355	0.36	0.24	0.23	< 0.001

Table S3. Projection to latent structures (PLS) models built on the urinary metabolic profiles of infants from Peru, Bangladesh and Tanzania at each sampling point investigating metabolic variation related to fever at the time of sampling, diarrhea, acute lower respiratory infections (ALRI), breast-feeding, sex and WAMI. The values shown relate to the predictive ability (Q^2Y) of the models and where appropriate the significance of the model (*p*-value based on 1,000 permutations).

Sampling age	Fever	Diarrhea	ALRI	Breast-fed	WAMI	Sex
Peru						
3 months	-0.112	0.005	0.008	-0.029	-0.013	-0.006
6 months	-0.015	*0.043 (0.004)	-0.069	-0.021	-0.072	*0.022 (0.011)
9 months	-0.093	*0.027 (0.001)	-0.103	-0.088	-0.070	-0.298
15 months	-0.011	-0.030	-0.032	-0.122	*0.037 (0.006)	-0.041
24 months	-0.072	-0.053	NA	-0.076	-0.176	-0.094
Bangladesh						
3 months	0.001	-0.040	-0.001	-0.295	-0.058	0.001
6 months	0.009	0.007	-0.057	-0.021	-0.034	-0.022
9 months	-0.010	-0.013	-0.025	-0.001	-0.007	-0.017
15 months	-0.003	0.010	-0.021	-0.029	-0.035	0.002
Tanzania						
6 months	-0.030	-0.003	-0.055	-0.048	-0.030	0.003
15 months	-0.044	-0.007	-0.026	*0.075 (0.004)	-0.030	0.022
24 months	-0.025	-0.131	NA	-0.101	*0.061 (0.008)	-0.039

Table S4. Summary of plasma amino acids measured.

Amino Acid
4-Hydroxyproline
Alanine
Arginine
Aspartic Acid
Asparagine
Ethanolamine
Glutamic Acid
Glutamine
Glycine
Histidine
Isoleucine
Leucine
Methionine
Phenylalanine
Proline
Serine
Tryptophan
Threonine
Tyrosine
Valine
Beta-Amino-iso-Butyric Acid
Citrulline
Sarcosine
Beta-Alanine

Table S5. Concentrations [median (IQR)] of the eight urinary metabolites used to calculate the phenome age in healthy infants expressed as μM/mM of creatinine.

Cohort	Age (months)	Betaine	DMG	Citrate	Succinate
Peru	**3**	879.68 (840.7-936.98)	186.02 (138.35-244.18)	1599.12 (1411.32-2010.15)	112.93 (77.34-160.12)
	6	446.2 (311.24-564.87)	146.36 (65.07-180.53)	1088.15 (637.38-1924.36)	66.47 (51-98.95)
	9	256.22 (213.16-360.56)	115.7 (88.79-144.72)	964.43 (408.16-1250.08)	54.52 (39.14-71.37)
	15	186.28 (164.43-206.87)	63.41 (45.45-69.47)	458.12 (295.96-747.74)	52.11 (40.26-62.44)
	24	160.96 (139.11-208.13)	41.93 (32.29-48.93)	413.49 (259.05-466.3)	35.2 (28.73-46.22)
Bangladesh	**3**	923.94 (783.89-1242.39)	174.56 (137.68-228.3)	1617.62 (1346.05-1979.09)	176.19 (152.55-235.17)
	6	590.92 (399.16-763)	154.35 (103.44-246.66)	1102.64 (628.34-1873.82)	135.82 (101.6-180.42)
	9	297.81 (253.8-394.93)	84.12 (66.9-150.92)	663.33 (448.6-1022.02)	97.27 (82.46-121.15)
	15	214.5 (172.28-261.53)	52.47 (36.71-78.22)	493.21 (353.05-885.36)	85.62 (73.26-100.39)
Tanzania	**6**	525.05 (386.09-759.45)	142.71 (98.53-217.83)	1004.96 (740-1419.72)	73.18 (57.09-99.08)
	15	185.75 (168.02-222.42)	75.13 (51.72-97.8)	361.86 (324.81-509.4)	79.98 (65.59-97.8)
	24	374.31 (191.18-793.1)	59.33 (38.22-122.31)	917.02 (420.93-1684.78)	178.11 (77.59-349.54)
		Hippurate	**PAG**	**4-CS**	**Creatine**
Peru	**3**	45.82 (33.16-113.3)	23.15 (20.37-32.69)	51.2 (43.29-61.06)	434.79 (317.94-538.18)
	6	54.36 (28.85-82.44)	27.37 (20.13-51.99)	50.2 (41.77-71.03)	351.61 (259.77-454.12)
	9	77.11 (48.09-200.21)	34.59 (26.17-62.49)	56.34 (46.16-76.94)	441.18 (315.56-616.67)
	15	123.62 (74.72-220.37)	60.14 (49.35-142.78)	76.08 (55.04-133.21)	539.29 (411.46-784.99)
	24	374.54 (244.3-422.7)	141.5 (121.57-192.22)	197.59 (157.68-297.33)	859.23 (509.13-1183.37)
Bangladesh	**3**	70.55 (46.36-104.59)	22.23 (20.88-25.69)	55.21 (51.12-66.48)	453.02 (386.54-623.61)
	6	61.92 (41.86-123.72)	27.32 (19.74-42.67)	57.86 (45.59-79.09)	583.94 (448.35-881.77)
	9	91.47 (54.07-168.63)	36.43 (23.84-71.81)	57.84 (44.85-83.79)	696.81 (600.8-838.2)
	15	149.05 (113.71-332.7)	76.8 (42.87-117.11)	91.25 (73.32-151)	775.5 (573.99-948.15)
Tanzania	**6**	81.88 (70.28-111.87)	31.47 (22.21-41.85)	69.72 (55.82-75.92)	487.92 (395.32-725.54)
	15	521.74 (341.82-857.08)	146.7 (90.14-270.1)	376.33 (208.22-476.35)	731.58 (513.38-851.32)
	24	1903.41 (1606.42-5408.27)	737.13 (299.23-1584.78)	1177.74 (447.92-2464.14)	803.94 (504.56-2080.85)

Table S6. Concentrations [median (IQR)] of the eight urinary metabolites used to calculate the phenome age in growth constrained infants expressed as µM/mM of creatinine.

Cohort	Age (months)	Betaine	DMG	Citrate	Succinate
Peru	**3**	847.76 (684.04-1051.31)	192.22 (142.59-260.46)	1632.13 (1252.43-2026.45)	111.15 (77.97-149.04)
	6	444.81 (316.6-576.49)	144.15 (101.64-202.43)	1190 (801.62-1619.5)	64.75 (49.94-88.84)
	9	299.5 (221.85-386.33)	116.92 (88.05-163.68)	878.54 (521.3-1266.58)	57.31 (45.15-72.27)
	15	188.63 (165.1-225.39)	75.17 (52.21-107)	451.86 (327.78-747.47)	44.49 (36.46-58.16)
	24	167.51 (137.6-187.98)	39.91 (27.64-50.91)	325.44 (256.95-480.2)	37.13 (30.33-48.58)
Bangladesh	**3**	964.81 (789.93-1155.61)	181.27 (128.29-274.06)	1424.86 (1048.16-1842.26)	167.71 (135.32-214.96)
	6	504.94 (392.83-638.67)	135.42 (96.2-192.82)	968.22 (674.28-1401.13)	128.18 (102.26-159.5)
	9	314.52 (253.26-411.94)	94.78 (65.29-139.86)	637.51 (420.73-997.52)	105.46 (82.38-135.55)
	15	216.02 (187.6-255.47)	66.32 (46.26-104.76)	527.68 (378.18-813.48)	86.86 (71.34-112.37)
Tanzania	**6**	499.31 (371.41-693.9)	125.9 (92.07-177.26)	1014.99 (626.77-1428.26)	76.87 (55.37-103.7)
	15	200.98 (175.12-281.58)	61.4 (43.87-106.67)	621.77 (395.62-1058.18)	77.52 (58.47-103.25)
	24	173.19 (147.28-212.64)	45.21 (31.27-72.2)	459.84 (344.14-791.44)	65.7 (52.57-88.22)
		Hippurate	**PAG**	**4-CS**	**Creatine**
Peru	**3**	40.58 (31.81-74.35)	26.61 (20.34-41.4)	52.23 (43.06-64.79)	388.27 (306.75-494.57)
	6	41.06 (28.05-78.57)	24.67 (18.21-48.81)	47.77 (39.4-63.76)	351.37 (264.83-479.49)
	9	69.07 (39.17-143.67)	42.08 (24.89-67.6)	56.38 (44.48-77.98)	430.7 (313.3-595.97)
	15	153.93 (76.89-234.73)	70.08 (42.26-105.73)	76.97 (55.82-105.55)	490.59 (326.56-704.28)
	24	309.37 (195.97-532.48)	122.06 (64.26-171.15)	145 (94.94-201.46)	798.28 (502.38-1095.71)
Bangladesh	**3**	60.89 (48.84-81.42)	23.21 (19.29-30.53)	58.35 (46.85-75.29)	500.94 (420.84-671.62)
	6	55.68 (43.85-87.15)	24.9 (20.03-35.51)	52.29 (43.5-65.97)	577.09 (396.11-765.21)
	9	85.84 (57.24-147.98)	34.33 (25.66-61.77)	62.19 (47.86-82.53)	658.98 (443.16-855.63)
	15	148.5 (92.99-239.09)	70.32 (36.49-124.24)	91.2 (61.52-132.93)	832.24 (554.3-1126.69)
Tanzania	**6**	101.53 (63.84-149.54)	37.37 (24.27-57.22)	63.56 (48.53-81.72)	471.9 (346.33-697.7)
	15	431.77 (209.67-821.72)	110.32 (66.43-183.6)	198.32 (118.07-305.82)	743.66 (496.94-1098.5)
	24	864.25 (607.32-1407.22)	137.95 (85.95-229.39)	227.11 (171.05-407.43)	882.6 (532.18-1269.26)