Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor

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Science ***339, 548 (2013)***

Dept Meeting, 28 May 2013, M. UMEZAKI

ABSTRACT. Kwashiorkor, an enigmatic form of severe acute malnutrition, is the consequence of inadequate nutrient intake plus additional environmental insults. To investigate the role of the gut microbiome, we studied 317 Malawian twin pairs during the first 3 years of life. During this time, half of the twin pairs remained well nourished, whereas 43% became discordant, and 7% manifested concordance for acute malnutrition. Both children in twin pairs discordant for kwashiorkor were treated with a peanut-based, ready-to-use therapeutic food (RUTF). Time-series metagenomic studies revealed that RUTF produced a transient maturation of metabolic functions in kwashiorkor gut microbiomes that regressed when administration of RUTF was stopped. Previously frozen fecal communities from several discordant pairs were each transplanted into gnotobiotic mice. The combination of Malawian diet and kwashiorkor microbiome produced marked weight loss in recipient mice, accompanied by perturbations in amino acid, carbohydrate, and intermediary metabolism that were only transiently ameliorated with RUTF. These findings implicate the gut microbiome as a causal factor in kwashiorkor.

Moderate Acute Malnutrition (MAM)

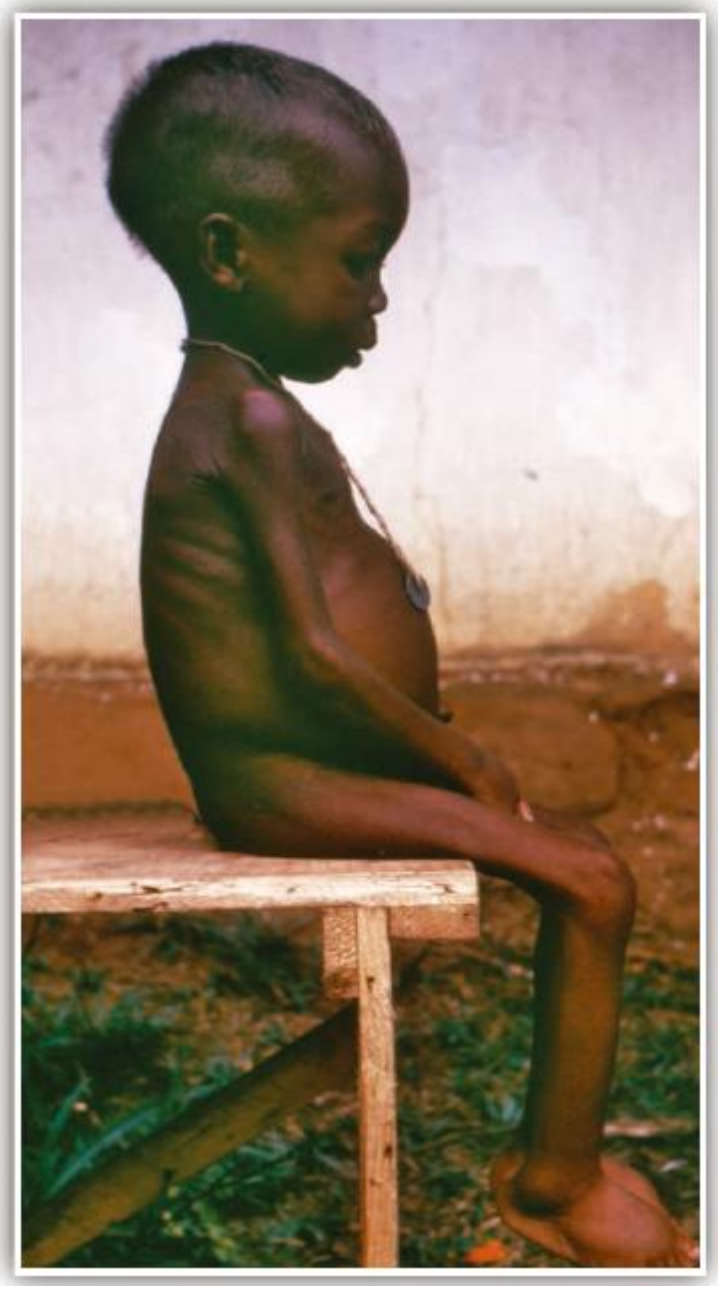
-3SD < WHZ < -2 SD

Severe Acute Malnutrition (SAM)

Marasmus: WHZ < -3 SD

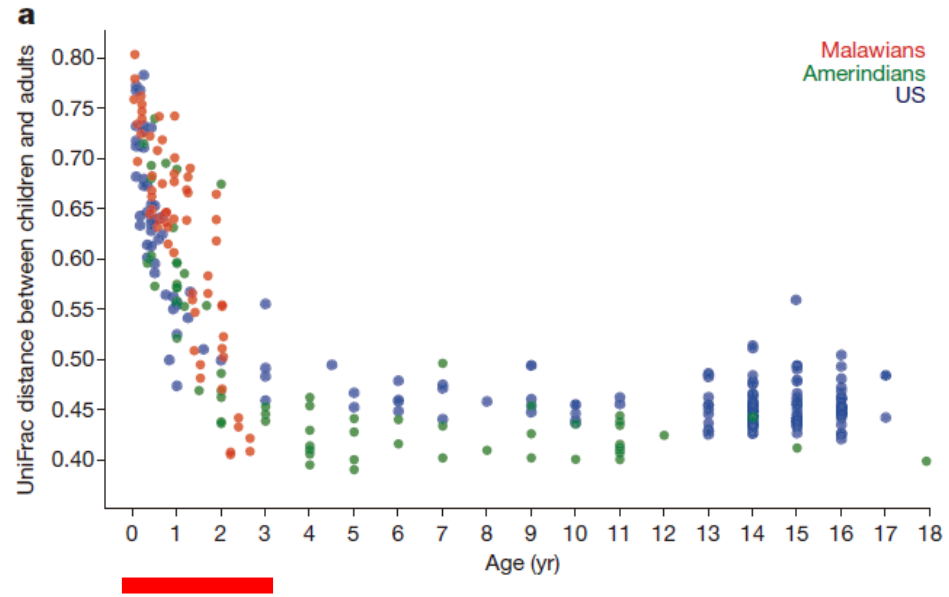
Kwashiorkor: edema,
hepatic steatosis
skin rashes
ulcerations

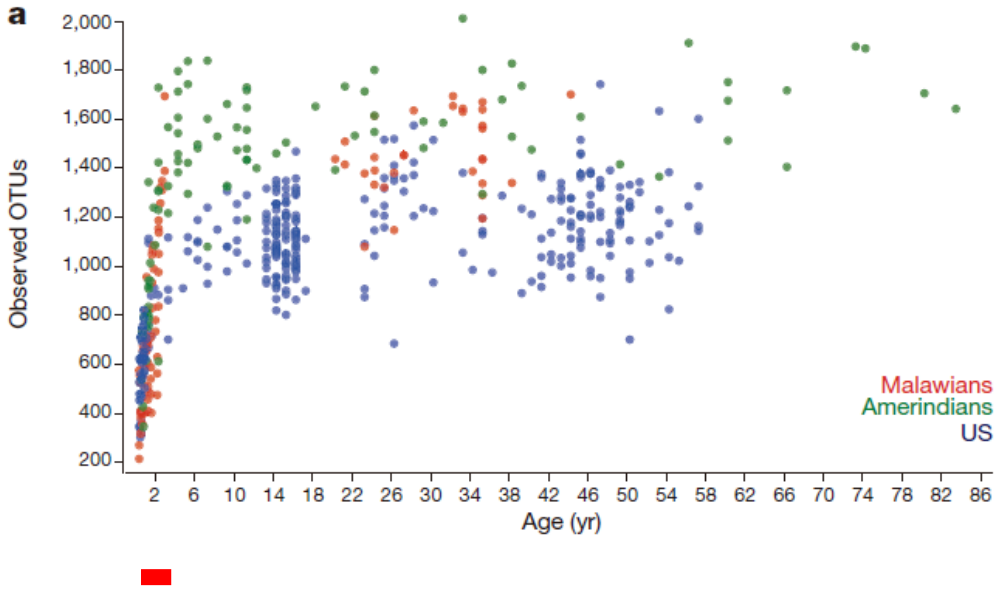
Inadequate protein intake?
Excessive oxidative stress?



Postnatal period
(0-3 years)

Genes encoding functions related to micro- and macro-nutrient biosysthesis and metabolism ↑





Yasunenko et al. (2012) Nature

Hypothesis

1) Gut microbiome provides essential functions for healthy development and growth
2) Disturbance in microbiome functions affect the risk of Kwashiorkor

Study population

317 twin pairs in Malawi, monthly visits
50% both well-nourished
43% one was acute malnutrition
7% both acute malnutrition

Monthly visit

7.4%/634 children: Kwashiorkor

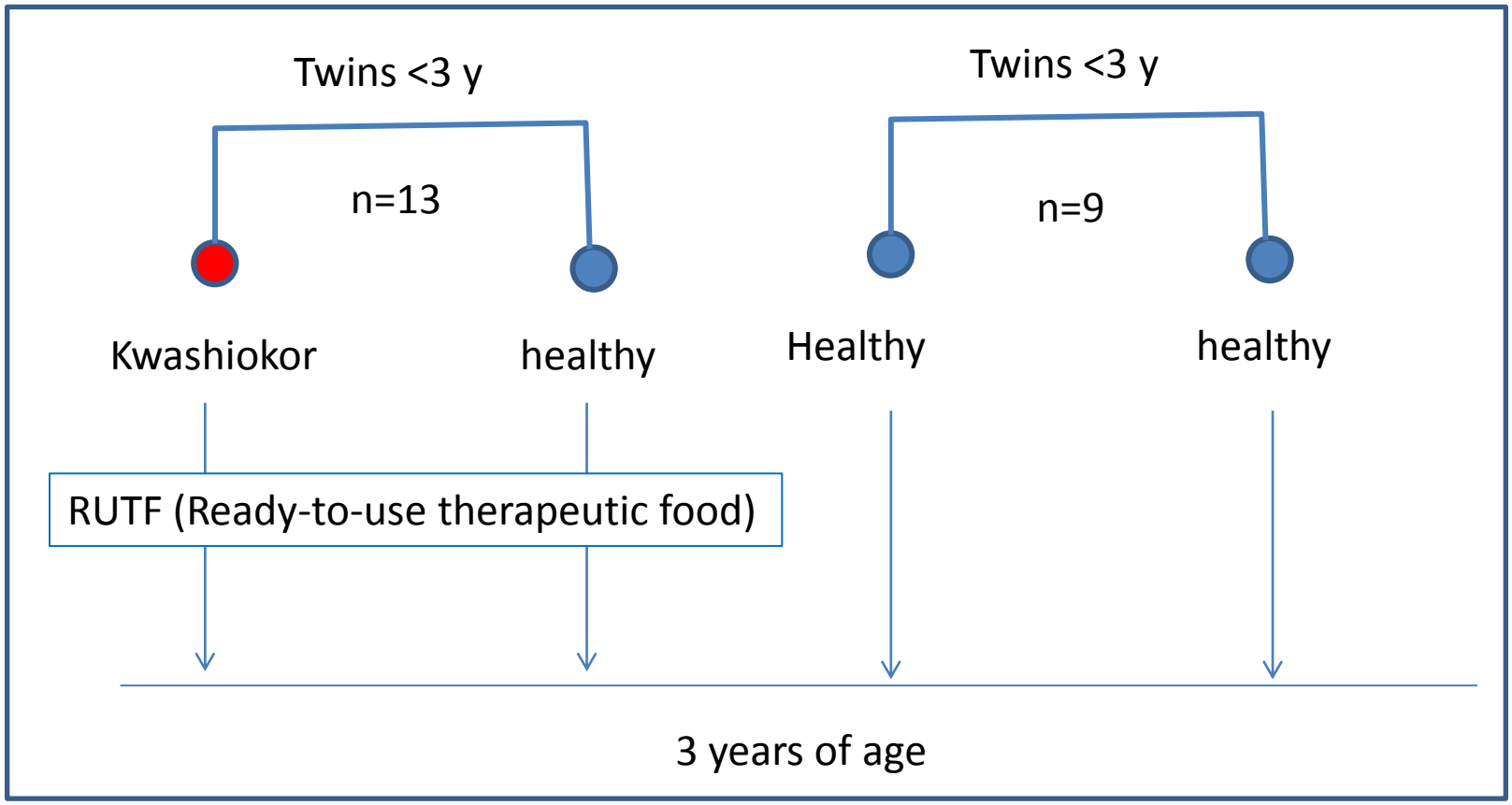
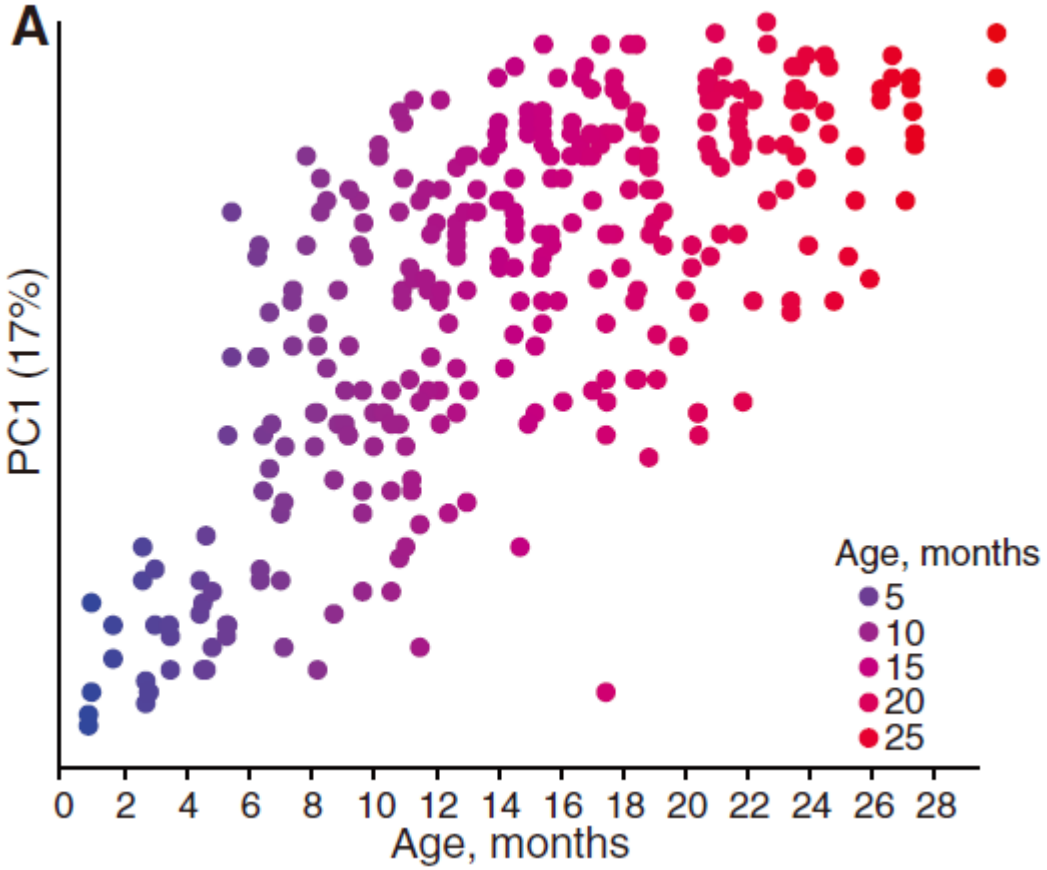
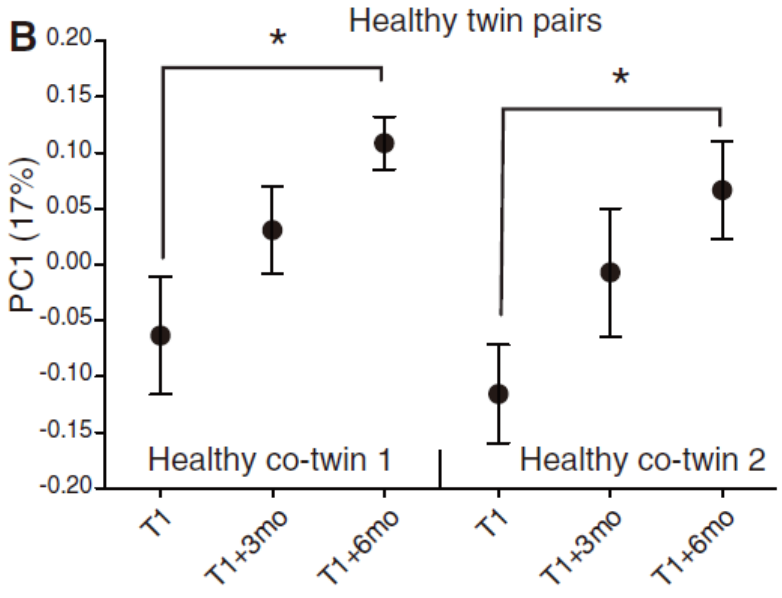


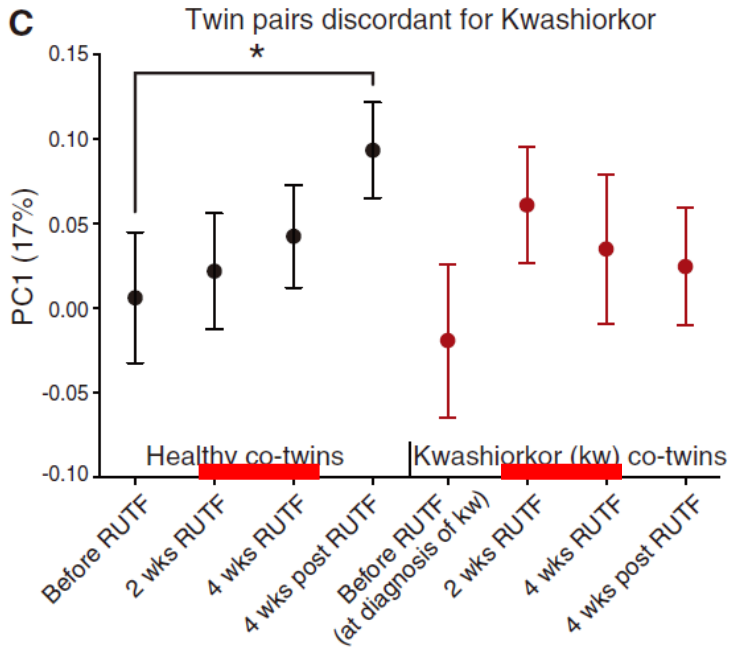
Fig. 1. Functional development of the gut microbiomes of Malawian twin pairs concordant for healthy status and twin pairs who became discordant for kwashiorkor. (A) Principal coordinates analysis of Hellinger distances between KEGG EC profiles. The position of each fecal microbiome along PC1, which describes the largest amount of variation (17%) in this data set of 308 sequenced twin fecal microbiomes, is plotted against age. Each circle represents a microbiome colored by the age of the human donor. PC1 is strongly associated with age, as well as with family membership (linear mixed-effects model, table S4). We did not find significant associations between the positions of samples along other principal coordinates and the other host parameters presented in table S2A. On average, the degree of intrapersonal variation in a co-twin was not smaller than the variation between co-twins (fig. S2). Similar to twins who remained healthy, the temporal variation within a co-twin member of a discordant twin pair was equal to the variation between co-twins, but still smaller compared with unrelated children (fig. S2).



Functional development of microbiome with age

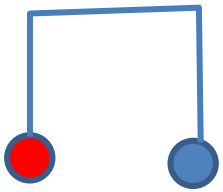
Fig 1. (B) Average T SEM (error bars) PC1 coordinate obtained from the data shown in (A) for microbiomes sampled at three consecutive time points from nine twin pairs who remained well nourished (healthy) during the study (participants surveyed between 3 weeks and 24.5 months of age). (C) Average T SEM (error bars) PC1 coordinate obtained from (A) for microbiomes sampled before, during, and after RUTF treatment from co-twins discordant for kwashiorkor. *P < 0.05, Friedman test with Dunn's post-hoc test applied to data shown in (B) and (C). Similar results were obtained using other distance metrics [Bray Curtis, Euclidian, and Kulzyncki (fig. S3)]. (See also fig. S4, which shows how changes in the relative proportion of Actinobacteria parallel the patterns observed with the changes along PC1. Children with kwashiorkor manifested a statistically significant decrease in Actinobacteria with the introduction of RUTF, unlike their healthy co-twins).





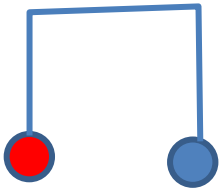
No vomiting, no diarrhea,
no antibiotics, similar age,

Family 196



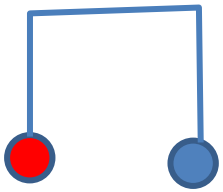
Kwashiokor healthy

Family 56



Kwashiokor healthy

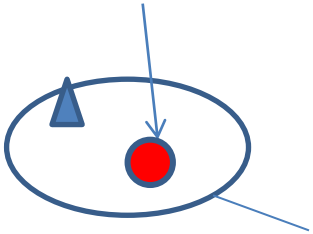
Family 57



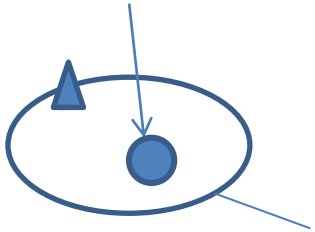
Kwashiokor healthy

Pre-treatment fecal samples

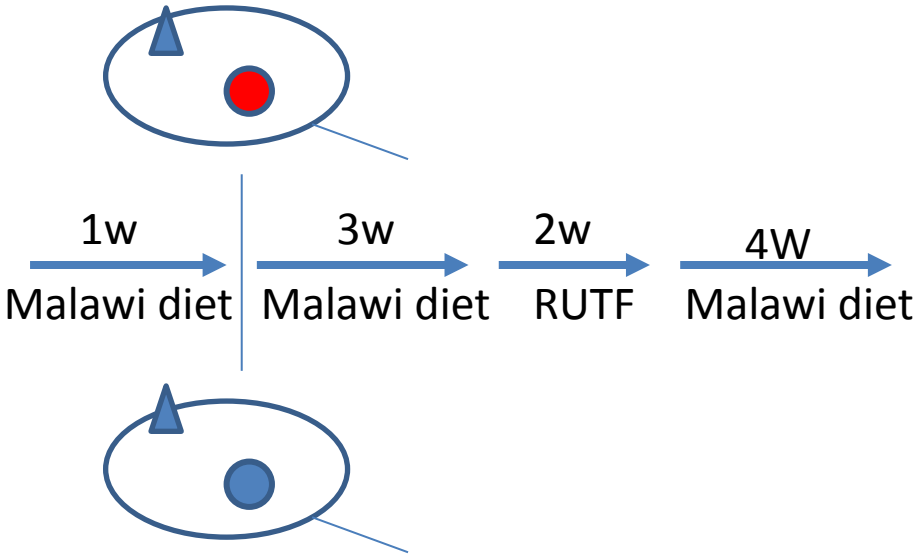
Kwashiorkor feces



Healthy feces



8 wks male C57BL/6J germ-free mice



Malawi diet: Corn + onion

RUTF: peanuts base

	NPAL tested Malawian Diet (per 1000g)	Calculated RUTF Diet (per 1000g)
Water, g	760	
Protein, g	26	136
Carbohydrate, g		438
Fat, g	10	354
Linolelic acid, g		
Fiber, g	27	
Lysine, g		8.3
Calcium, mg	205.7	3000
Phosphorus, mg	784.6	3500
Magnesium, mg	280.7	800
Potassium, mg	1000.4	11000
Iron, mg	6.9	120
Sodium, mg	66.7	1300
Copper, mg	<1	16.6
Zinc, mg	4.9	135.6
Manganese, mg	1.6	5.3
Thiamin, mg		6
Riboflavin, mg		18
Niacin, mg		53
Pantothenic acid, mg		31
Vitamin B6, mg		6
Folate, mg		2.08
Choline, mg		138.3
Vitamin A, IU	<1.8	32464
Vitamin E, mg		217

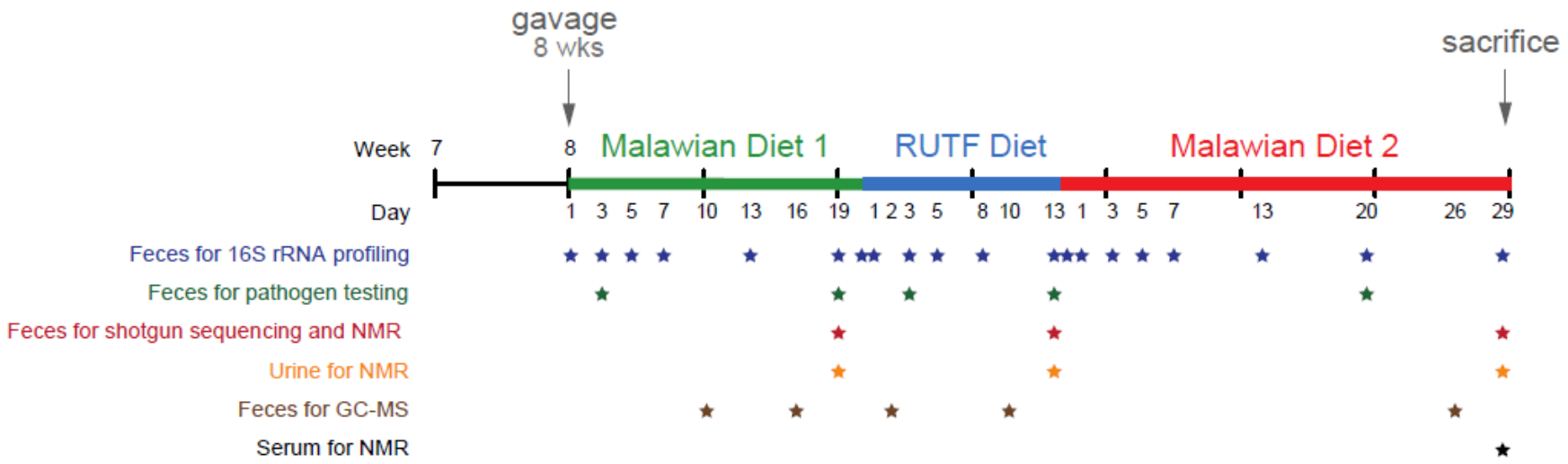


fig. S6. Sampling scheme for gnotobiotic mouse recipients of fecal microbiota transplants from discordant twin pair 196. A star designates the time point at which a sample was used for the indicated analysis. Amplicons were generated from the V4 region of bacterial 16S rRNA genes present in the input human samples, as well as in the fecal microbiota of all mouse recipients, and sequenced (204,585±181,417 reads/sample; 10 recipients/microbiota; up to 8 time points surveyed/diet/mouse; n=379 samples; table S2B). DNA isolated from fecal samples collected at the end of each diet period was also subjected to shotgun pyrosequencing (n=54 samples; table S2C).

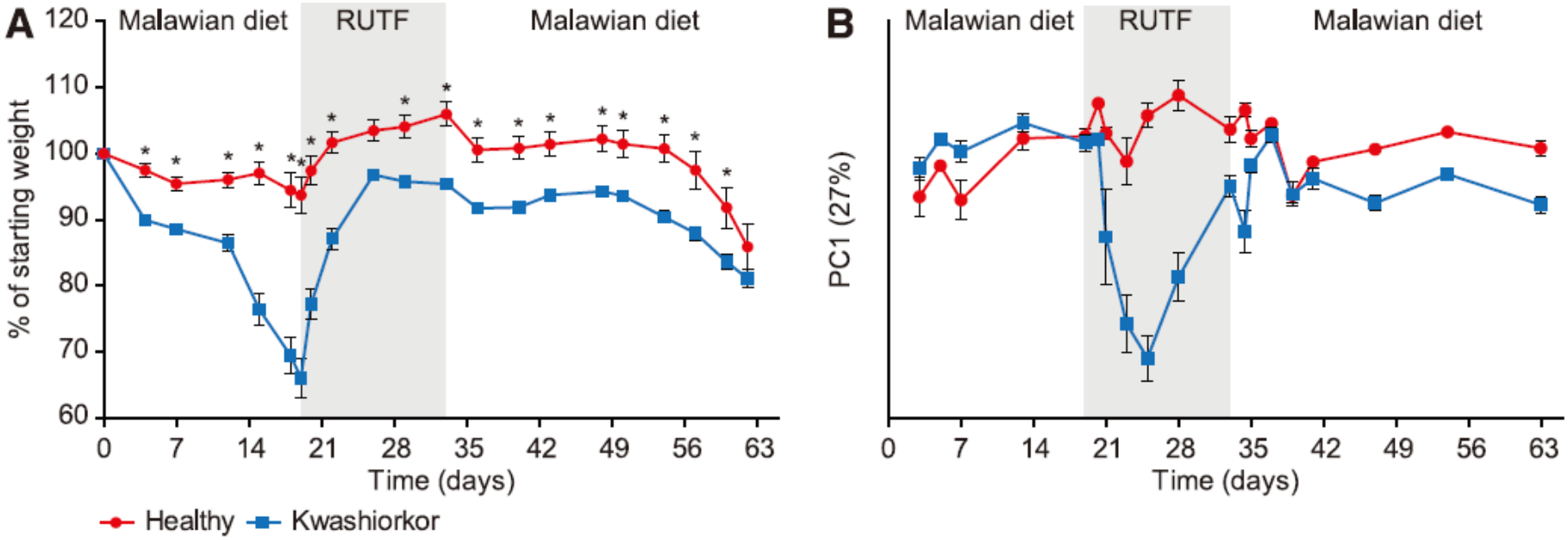
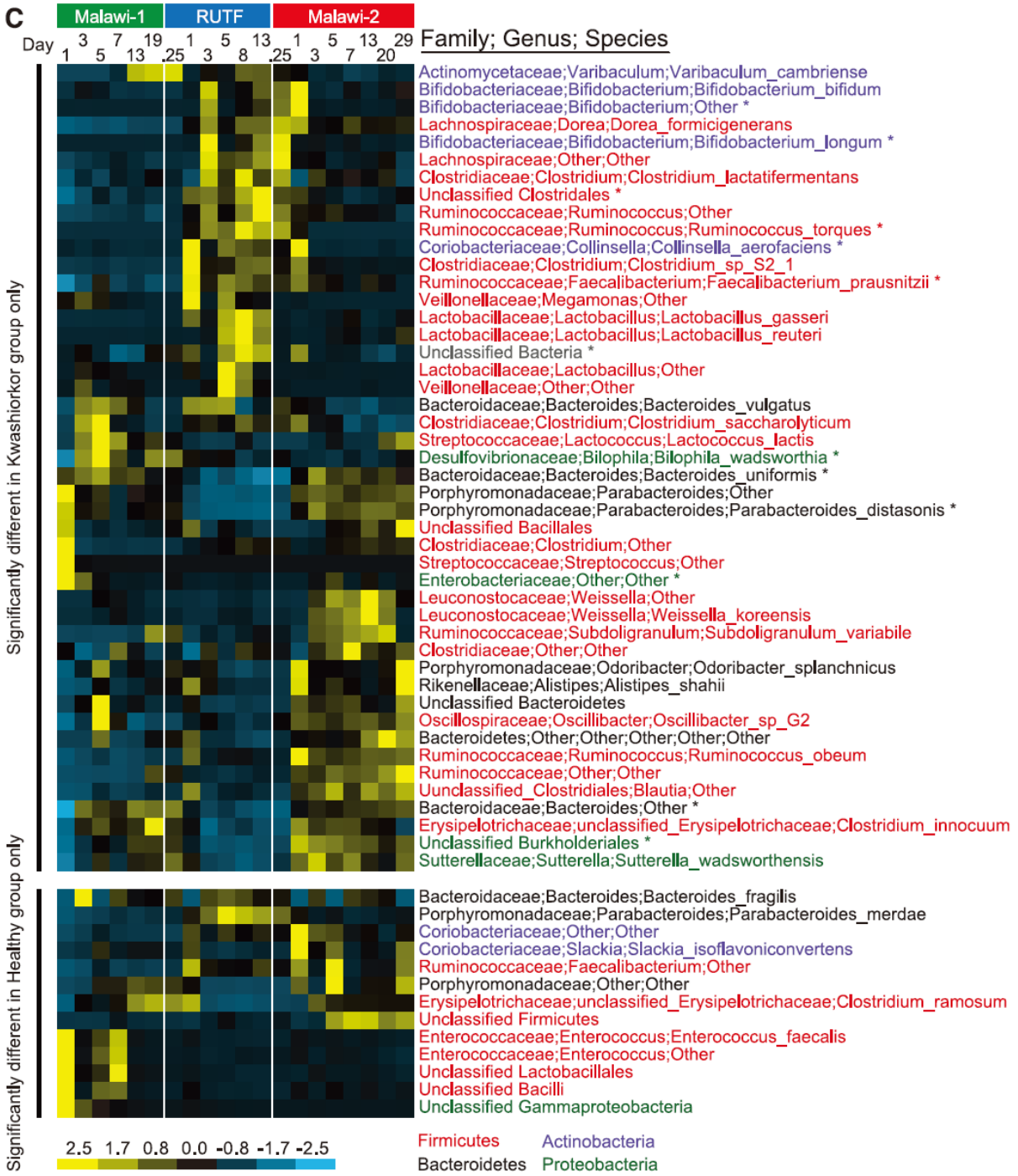


Fig. 2. Transplantation of fecal microbiota from kwashiorkor and healthy co-twins from family 196 into gnotobiotic mice fed Malawian and RUTF diets. (A) Discordant weight loss in recipient mice (n = 10 mice per group, *P < 0.05, Student's t test). Data points are colored by recipient group: blue, kwashiorkor co-twin fecal microbiota recipients; red, healthy co-twin fecal microbiota recipients. Error bars indicate SEM. (B) Average T SEM (error bars) PC1 coordinate obtained from the weighted UniFrac distances shown in fig. S9, A and B, for fecal microbiota sampled from mice over time. Same color key as in (A).

Fig 2. (C) Heatmap of phylotypes assigned to species-level taxa whose representation in the fecal microbiota of gnotobiotic mice changed significantly ($P < 0.05$, Student's t test with Bonferroni correction) as a function of donor microbiota and Malawian versus RUTF diets. Asterisks indicate taxa that changed significantly in both healthy and kwashiorkor microbiota transplant recipients. Species level taxa are colored by phylum: red, Firmicutes; blue, Actinobacteria; black, Bacteroidetes; and green, Proteobacteria.



Kwashiorkor mice: Malawi diet to RUTF

↑

Bifidobacteria (*B. longum, B. bifidum*, plus another unclassified taxon),

Lactobacilli [*L. reuteri and L. gasseri]:*
eliminate various enteropathogens

Ruminococcus [*R. torques, Faecalibacterium prausnitzii*]:
anti-inflammatory activity

↓

Bacteroidales (B. uniformis, Parabacteroides distasonis)

GC-MS analysis of

1. short-chain fatty acids and
2. 69 other products of carbohydrate, amino acid, nucleotide, and lipid metabolism in fecal and cecal samples

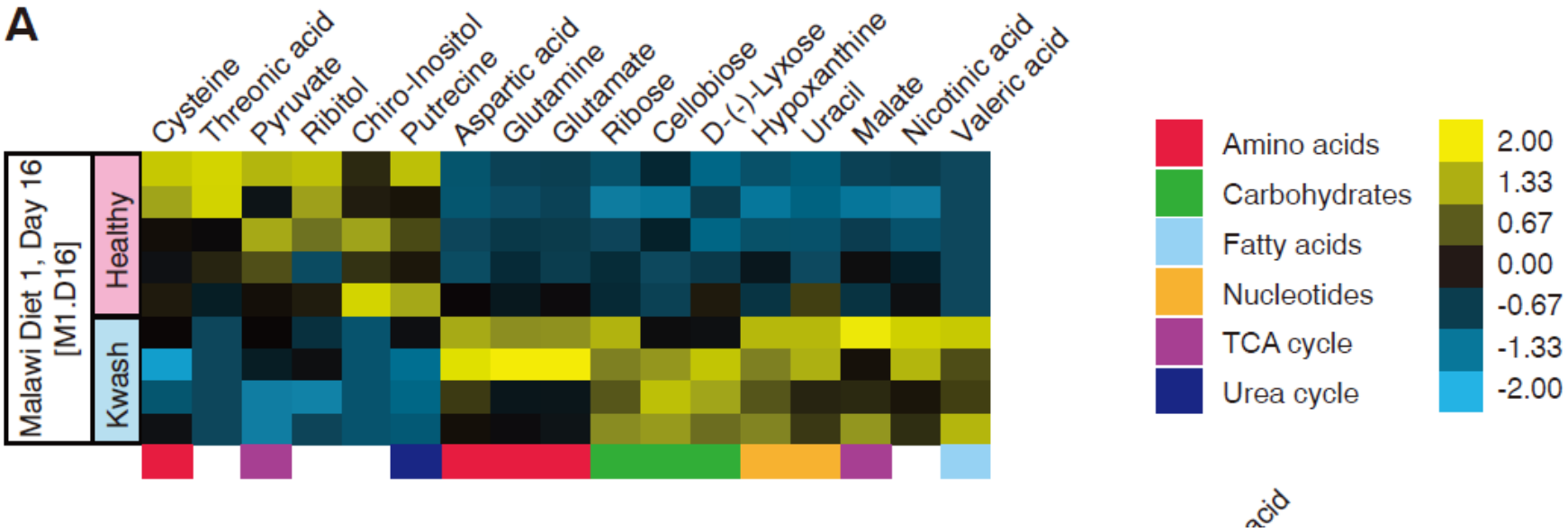


Fig. 3. Metabolites with significant differences in their fecal levels in gnotobiotic mice colonized with microbiota from discordant twin pair 196 as a function of diet. Data are from fecal samples collected 3 days before the end of (A) the first period of consumption of theMalawian diet (M1, day 16; abbreviated M1.D16),

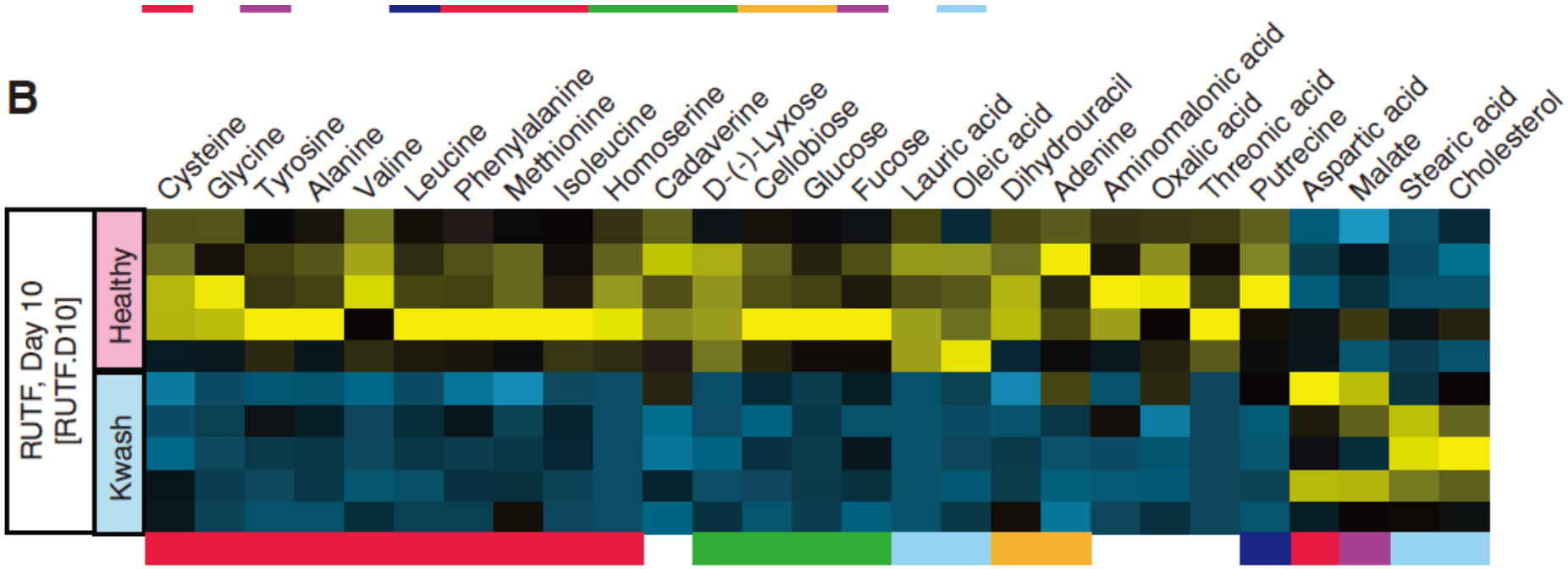


Fig 3. (B) RUTF treatment (RUTF.D10)

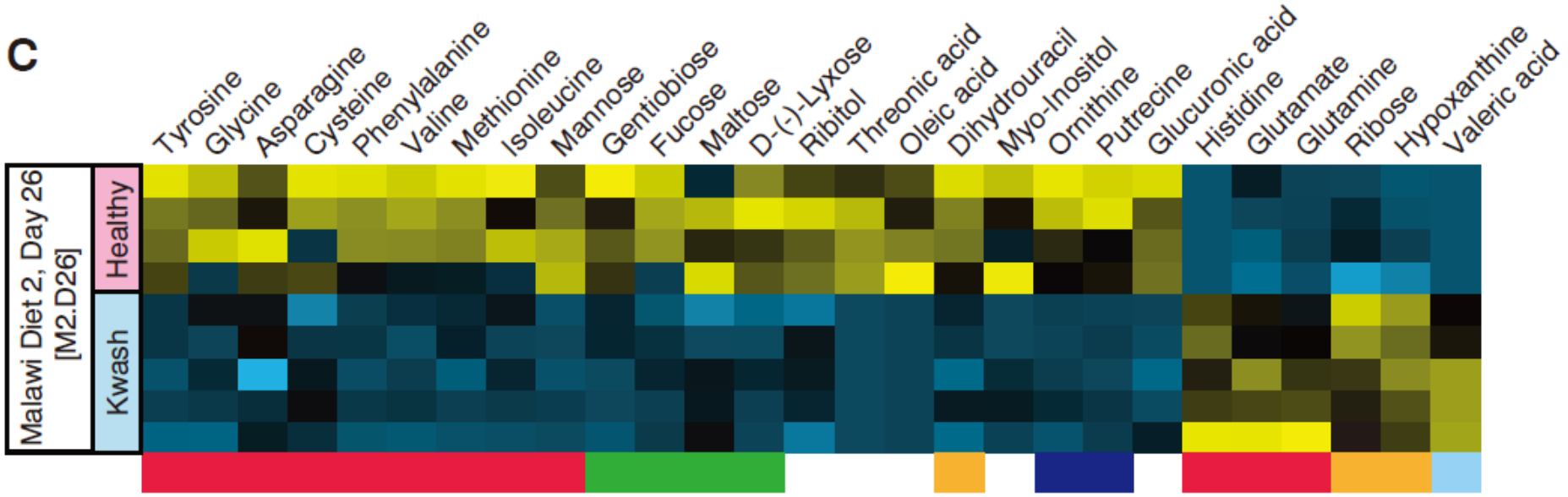
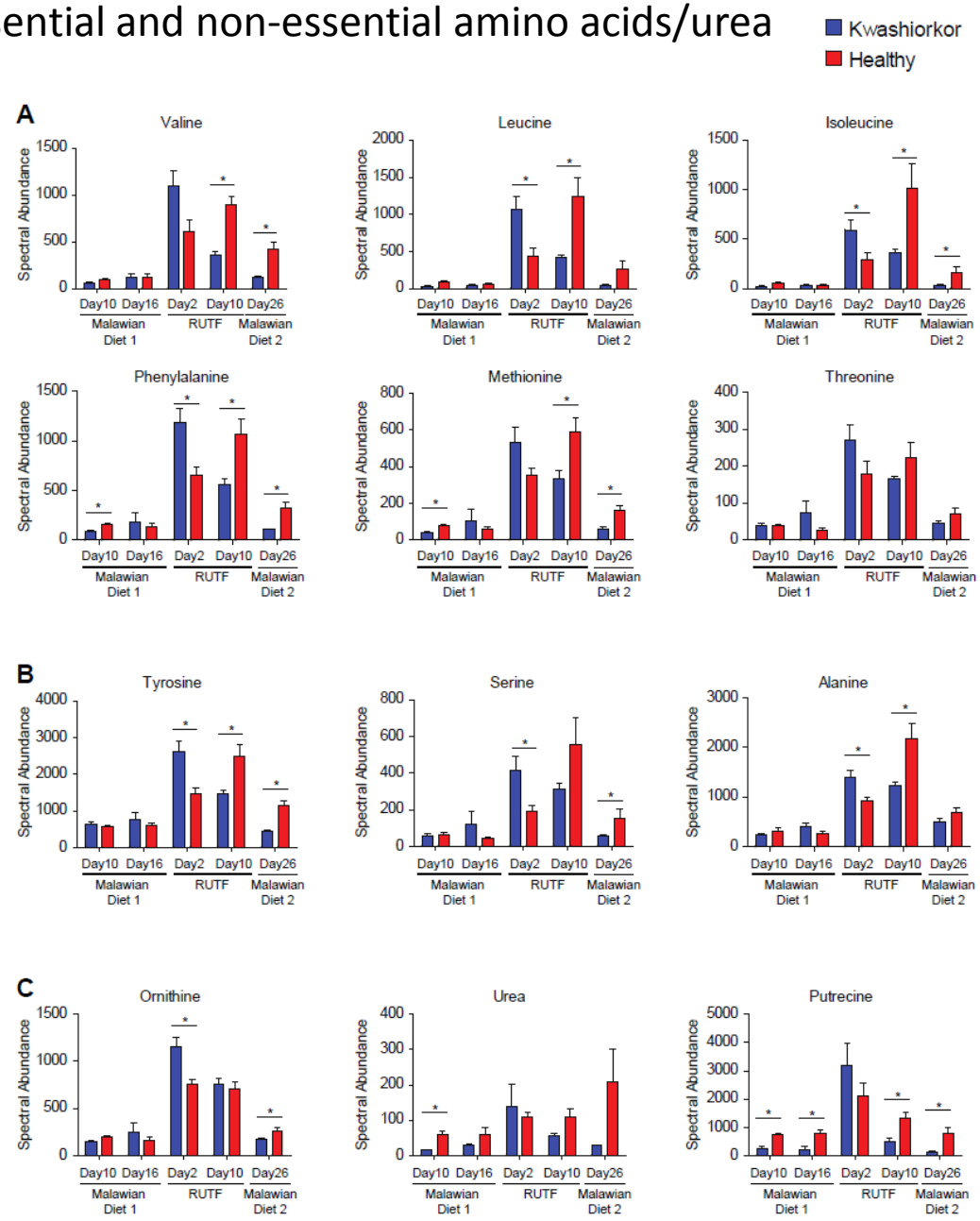


Fig 3. (C) the second period of Malawian diet consumption (M2. D26). Significant differences are defined as $P < 0.05$, according to Student's t test. Procrustes analysis of data obtained from the transplantedmicrobiota from discordant co-twins in family 196 (fig. S13) revealed a significant correlation between metabolic and taxonomical profiles on each diet with an overall goodness of fit (M2 value) of 0.380 ($P < 0.0001$; 1000 Monte Carlo label permutations) for all diets and microbiota.

Essential and non-essential amino acids/urea



Kw: response was initially greater; healthy: change persisted after they returned to Malawi diet

NMR spectrometry to generate urine metabolite profiles

Urinary concentration

KW<H

H>KW

A

	M1	RUTF	M2
R^2X	0.38	0.49	0.36
Q^2Y	0.62	0.8	0.78
2-oxoadipate	0.8747	0.9069	0.6603
taurine		0.8578	0.6236
lactate			0.4618
creatine	0.9303		
creatinine	0.7692		
methylamine	0.6755	0.8338	0.7646
dimethylamine	0.7732		
trimethylamine	0.8469		
trimethylamine *N*-oxide			
phenylacetylglycine	0.8522	0.8816	
indoxyl sulfate	0.6591	0.8138	
hippurate	0.6301	0.9485	0.9786
allantoin	0.7662		

Higher metabolite concentration in recipients of kwashiorkor co-twin microbiota

Higher metabolite concentration in recipients of healthy co-twin donor microbiota

Table 1. Metabolite analysis of urine samples obtained from mice with transplanted healthy or kwashiorkor co-twin microbiota from family 196 at each diet phase. (A) Urinary metabolites with differences in their levels inmice transplanted with the healthy co-twin versus the kwashiorkor co-twin microbiota within a given diet. Color code: blue, higher in kwashiorkor co-twin microbiota recipients; red, higher in healthy co-twin microbiota recipients; white, no significant difference between kwashiorkor and healthy.

Table 1. (B) Urinary metabolites with differences in their representation of mice transplanted with healthy or kwashiorkor co-twin microbiota between diets. Color code: red, higher during the M1 diet phase relative to RUTF or relative toM2; orange, higher on RUTF relative toM1 orM2; blue, higher on M2 compared to RUTF or M1; white, no significant differences in the indicated diet comparison.

B

	Recipients of healthy co-twin donor microbiota			Recipients of kwashiorkor co-twin donor microbiota		
	M1 vs RUTF	RUTF vs M2	M1 vs M2	M1 vs RUTF	RUTF vs M2	M1 vs M2
R^2X	0.66	0.58	0.61	0.74	0.52	0.67
Q^2Y	0.57	0.94	0.31	0.87	0.93	0.68
2-oxoglutarate	0.7448	0.8538		0.9062	0.8761	
citrate	0.7065	0.7921		0.827	0.7137	
succinate	0.6794	0.6841		0.8348	0.846	
fumarate	0.7439	0.7553				
acetate				0.7		
2-oxoadipate	0.8382	0.6198				
taurine	0.632	0.7469			0.5998	
lactate	0.5558					0.4733
creatine		0.7432		0.9474	0.759	0.8781
creatinine				0.8297	0.7051	0.6838
methylamine					0.7562	
dimethylamine			0.7469			
trimethylamine	0.7149	0.6639			0.8534	0.878
trimethylamine *N*-oxide					0.799	
phenylacetylglycine		0.8447		0.5716		0.6616
indoxyl sulfate	0.8021	0.8699		0.9593		
hippurate		0.8602				
allantoin		0.8203		0.9133	0.93	0.769
1-methylnicotinamide						0.7554

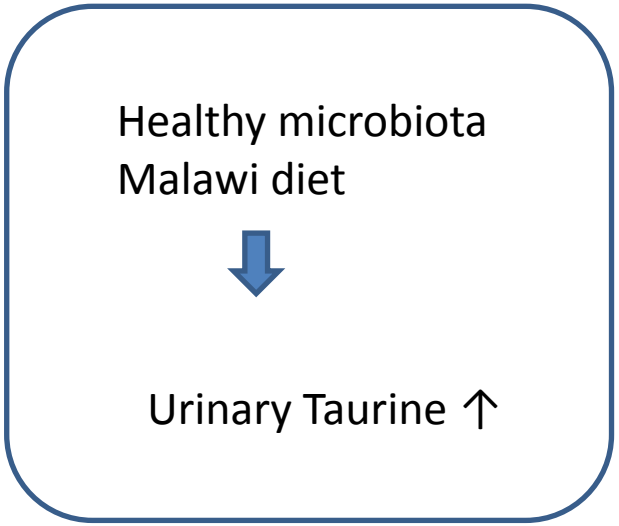
Higher metabolite concentration during Malawian diet phase 1

Higher metabolite concentration during RUTF

Higher metabolite concentration during Malawian diet phase 2

Urinary Taurine: RUTF, Healthy>KW
ML, Healthy>KW

KW: Mal>RUTF
Healthy: Mal>RUTF



Kwashiokor → B. Wadsworthia ↑,
which convert taurine to ammonia, acetate, or sulfide

Malawi diet: sulfa deficiency

Abnormal sulfur metabolism

Selective inhibition of TCA cycle intermediates?

TCA cycle intermediates

RUTF>Malawi

Disruption of TCA cycles among KW mice

succinate 3 × in cecal samples

Succunate-to-fumarate ratio ↑

KW microbiota generate chemical products that inhibit TCA cycle?

Summary

Mice to which Kwashiokor feces were transplanted:

(1) decreased body weight with Malawi diet

(2) showed different pattern of microbiome changes when fed RUTF

(3) showed different gut metabolism/synthesis when fed RUTF and Malawi diet

Abnormal sulfa metabolism?

Disruption of TCA cycle?