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Authors

May, Thaddaeus
de la Haye, Bethany
Nord, Gabrielle
et al.

Publication Date

2022

DOI

10.1016/j.ebiom.2021.103791

Peer reviewed

One-carbon metabolism in children with marasmus and kwashiorkor



Thaddaeus May,^{a,*} Bethany de la Haye,^g Gabrielle Nord,^c Kevin Klatt,^{a,h} Kevin Stephenson,^g Sara Adams,^d Lucy Bollinger,^g Neil Hanchard,^{e,i} Erland Arning,^f Teodoro Bottiglieri,^f Kenneth Maleta,^b Mark Manary,^{a,b,g} and Farook Jahoor,^a

^aChildren's Nutrition Research Center, Baylor College of Medicine, One Baylor Plaza, Houston TX, USA

^bThe University of Malawi College of Medicine, Malawi

^cStanford University School of Medicine, USA

^dPATH, USA

^eNational Institutes of Health, USA

^fCenter of Metabolomics, Institute of Metabolic Disease, Baylor Scott and White Research Institute

^gWashington University in St. Louis School of Medicine, USA

^hCenter for Precision Environmental Health, Baylor College of Medicine

ⁱNational Human Genome Research Institute, National Institutes of Health

Summary

Background Kwashiorkor is a childhood syndrome of edematous malnutrition. Its precise nutritional precipitants remain uncertain despite nine decades of study. Remarkably, kwashiorkor's disturbances resemble the effects of experimental diets that are deficient in one-carbon nutrients. This similarity suggests that kwashiorkor may represent a nutritionally mediated syndrome of acute one-carbon metabolism dysfunction. Here we report findings from a cross-sectional exploration of serum one-carbon metabolites in Malawian children.

Methods Blood was collected from children aged 12–60 months before nutritional rehabilitation: kwashiorkor ($N = 94$), marasmic-kwashiorkor ($N = 43$), marasmus ($N = 118$), moderate acute malnutrition ($N = 56$) and controls ($N = 46$). Serum concentrations of 16 one-carbon metabolites were quantified using LC/MS techniques, and then compared across participant groups.

Findings Twelve of 16 measured one-carbon metabolites differed significantly between participant groups. Measured outputs of one-carbon metabolism, asymmetric dimethylarginine (ADMA) and cysteine, were lower in marasmic-kwashiorkor (median $\mu\text{mol/L}$ (\pm SD): 0.549 (± 0.217) $P = 0.00045$ & 90 (± 40) $P < 0.0001$, respectively) and kwashiorkor (0.557 (± 0.195) $P < 0.0001$ & 115 (± 50) $P < 0.0001$), relative to marasmus (0.698 (± 0.212) & 153 (± 42)). ADMA and cysteine were well correlated with methionine in both kwashiorkor and marasmic-kwashiorkor.

Interpretation Kwashiorkor and marasmic-kwashiorkor were distinguished by evidence of one-carbon metabolism dysfunction. Correlative observations suggest that methionine deficiency drives this dysfunction, which is implicated in the syndrome's pathogenesis. The hypothesis that kwashiorkor can be prevented by fortifying low quality diets with methionine, along with nutrients that support efficient methionine use, such as choline, requires further investigation.

Funding The Hickey Family Foundation, the American College of Gastroenterology, the NICHD, and the USDA/ARS.

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Keywords: Severe acute malnutrition; Methionine; Choline; Methyl donors; Nutritional edema; Edematous malnutrition

Abbreviations: ADMA, Asymmetric dimethylarginine; DMG, Dimethylglycine; HAZ, Height for age Z; Hcy, Homocysteine; LC–ESI/MS–MS, Liquid chromatography–electrospray ionization tandem mass spectrometry; MUAC, Mean upper arm circumference; MTHF, 5-Methyl tetrahydrofolate; PLP, Pyridoxal phosphate; RUTF, Ready-to-use therapeutic food; SAH, S-adenosyl homocysteine; SAME, S-adenosyl methionine; SHMT, Serine hydroxyl methyl transferase; SDMA, Symmetric dimethylarginine; WHO, World Health Organization; WHZ, Weight for height Z

*Corresponding author.

E-mail address: tdmay@bcm.edu (T. May).

Research in context

Evidence before this study

Kwashiorkor is an often-lethal syndrome of childhood malnutrition. Unlike marasmus, kwashiorkor is defined by nutritional edema rather than severe weight loss. Although kwashiorkor was formally described in 1933 its pathogenesis remains uncertain. The current piecemeal understanding of kwashiorkor is inadequate. *Why do some children develop marasmus while others develop kwashiorkor?* Discovery of the nutrient deficiencies that precipitate kwashiorkor will allow the development of better strategies for its alleviation. In addition to edema, kwashiorkor is distinguished by a consistent pattern of molecular and organ-level disturbances. These disturbances resemble those that occur in animals subjected to diets deficient in essential one-carbon nutrients, especially methionine and choline. This resemblance offers support for the hypothesis that kwashiorkor is a nutritionally mediated syndrome of one-carbon metabolism dysfunction that is precipitated by inadequate intake of particular one-carbon nutrients. However, the current understanding of one-carbon metabolism in kwashiorkor and marasmus remains limited. This knowledge gap hinders efforts to develop better strategies for the treatment and prevention of kwashiorkor.

Added value of this study

Kwashiorkor's unique risk factors and lesions have not been integrated into a gathered syndrome of malnutrition. The purpose of this study was to explore the hypothesis that kwashiorkor is a nutritionally mediated syndrome of one-carbon metabolism dysfunction. To do so, we characterized one-carbon metabolites in Malawian children who differed by nutritional status. This study is the largest published comparison of one-carbon metabolites in kwashiorkor and marasmus to date. We observed that kwashiorkor (including marasmic-kwashiorkor) was distinguished by evidence of greater one-carbon metabolism dysfunction relative to other groups of acutely malnourished children and controls. These observations suggest that one-carbon metabolism offers a molecular grammar for narrating the pathogenesis of kwashiorkor, from its preceding risk factors to its end-stage lesions.

Implications of all the available evidence

The findings of this study are consistent with the concept that kwashiorkor is nutritional syndrome of systemic one-carbon metabolism dysfunction. Correlative findings presented here suggest that methionine deficiency is necessary for the pathogenesis of this dysfunction. We also observed that methionine was well correlated with methyl donors. Methyl donors sustain efficient methionine recycling. These observations suggest that methyl donors support methionine status in this population of children. Together these findings

support the hypothesis that kwashiorkor can be prevented by fortifying meager diets with methionine and methyl donors, such as choline. Clinical trials are needed to test this hypothesis.

Introduction

Kwashiorkor and marasmus are separate conditions of severe acute malnutrition. Both contribute to the global burden of childhood undernutrition,¹ which is associated with 45% of deaths occurring before the age of five.² The cause of marasmus is not mysterious; a negative energy balance that results in severe wasting. Kwashiorkor is different. Most children with kwashiorkor are not wasted.³ Instead of wasting, kwashiorkor is characterized by a constellation of disturbances. This syndrome includes fatty liver disease, skin disturbances, glutathione depletion, as well as kwashiorkor's defining disturbance, edema.¹ Although the cause of kwashiorkor remains uncertain^{4,5} it is established that this distinctive syndrome only occurs in children who have been subjected to monotonous low quality diets.^{6,7} Kwashiorkor's association with meager diets transcends economic, sanitary, and geographical differences.^{8–11} The consistency of this pattern indicates that kwashiorkor is fundamentally a problem of poor nutrition. The first formal description of kwashiorkor¹² sparked debates about its etiology.¹³ Later, by the middle of the 20th century, the belief that kwashiorkor is simply due to protein deficiency became popular.¹⁴ This reasonable theory was supported by the observations that children who consume ample quantities of animal protein do not develop kwashiorkor and that skim milk powder is an effective therapeutic regimen.^{15,16} However, subsequent epidemiologic studies demonstrated that children with kwashiorkor do not necessarily consume less protein than those who develop marasmus.^{6,7} Nor is edema in kwashiorkor consistently correlated with plasma proteins, such as albumin.^{17–20} Likewise, the incidence of kwashiorkor among children who consume low-protein cereal-based diets is perplexingly sporadic. Sometimes kwashiorkor even varies between identical twins eating the same food in the same home.²¹ Low-protein diets are the syndrome's etiologic context, not its precise cause; where kwashiorkor happens, not why. Additional hypotheses need testing. Kwashiorkor's distinctive metabolic and organ lesions bear a striking resemblance to the effects of experimental diets that are deficient in nutrients that support one-carbon metabolism. This category of biochemical processes sustains the movement of methyl groups and the transsulfuration pathway (Figure 1).^{22–24} Kwashiorkor's phenotypic overlap with the pathologic effects of one-carbon nutrient deficient diets suggests that it may be a syndrome of one-carbon metabolism dysfunction, which is precipitated by one-

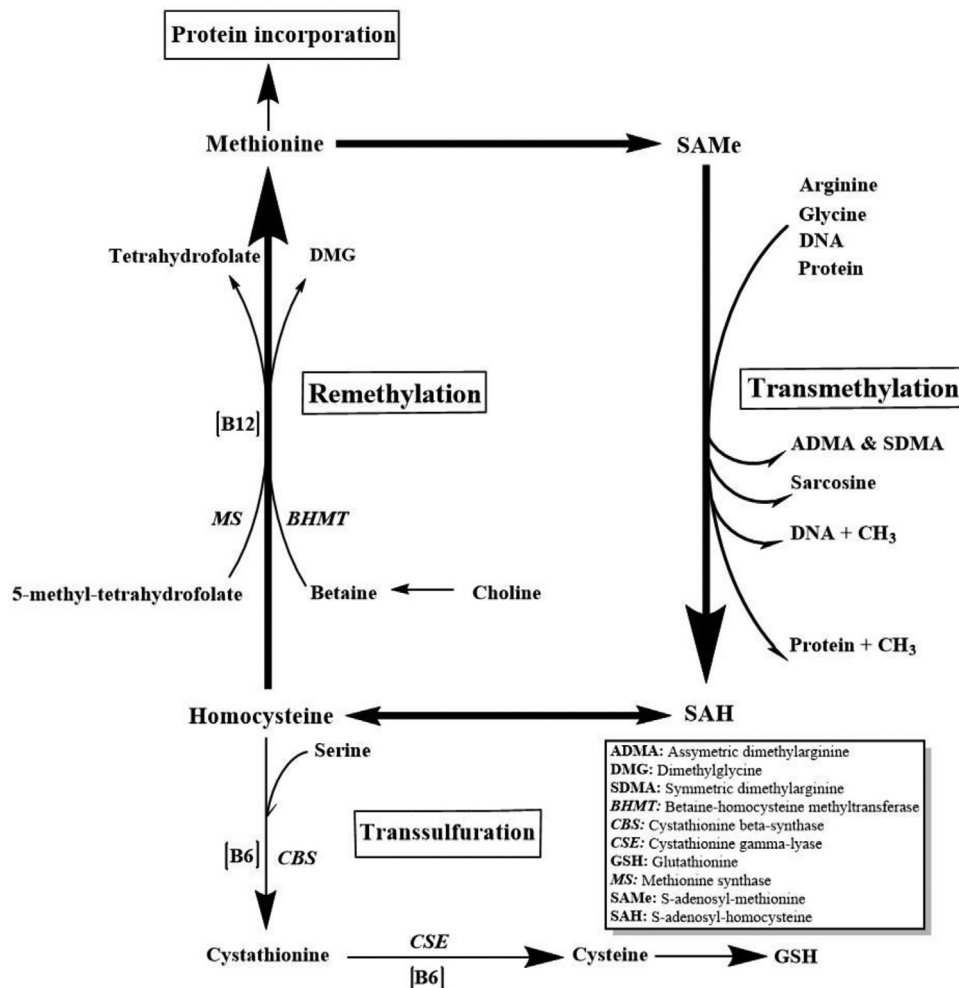


Figure 1. One-carbon metabolism schematic, adapted with permission.¹⁷⁰

carbon nutrient deficiencies. This concept may be useful for defining the underlying molecular pathways that link kwashiorkor's environmental determinants with its hallmark organ level lesions and serum biochemical differences. However, one-carbon metabolism in malnutrition remains poorly characterized. The purpose of this cross-sectional study was to compare circulating concentrations of one-carbon metabolites in groups of Malawian children who differed by nutritional status: kwashiorkor, marasmic-kwashiorkor, marasmus, moderate acute malnutrition (MAM), and controls.

Methods

Study design

This cross-sectional study was undertaken among participants who were recruited from a network of 25 rural community-based malnutrition surveillance clinics in southern Malawi. These clinics are operated by the St.

Louis Nutrition Project, a non-governmental research organization affiliated with Washington University in St. Louis School of Medicine and the University of Malawi College of Medicine.

Ethics

This study was approved and supervised by the University of Malawi College of Medicine Research and Ethics Committee (P.07/15/1766), as well as the Institutional Review Boards of Washington University in St. Louis (201,512,104), and Baylor College of Medicine (H-37,400). The local safety monitoring board of the University of Malawi College of Medicine supervised the portions of this study conducted in Malawi. The institutional review boards at Baylor College of Medicine and Washington University in St. Louis supervised the portions of this investigation conducted in the USA. Written and verbal informed consents were obtained from each participant's parent or guardian in their preferred

language. Ineligible children, as well as those whose guardians declined to participate, received the same cost-free care that was provided to study participants.

Participants

Participants were recruited during a 20-week period spanning January to May of 2016. Children were brought to the aforementioned network of clinics for a variety of reasons. These ranged from referrals by local clinicians who were concerned about a child's nutritional status to routine nutritional surveillance visits for children without any apparent malnutrition. Eligible participants were between the ages of 12 and 60 months at the time of enrollment, without any prior treatment for malnutrition in the preceding 28 days. Aside from undernutrition, participants did not have chronic medical conditions, such as cerebral palsy, congenital heart disease, tuberculosis, or HIV. Caregivers were questioned as to whether their child had experienced cough, diarrhea, and fever during the preceding seven days. Reports of such symptoms are common among children who present to this network of malnutrition surveillance clinics, and were not used as exclusionary criteria for malnourished participants or controls. Participants who met criteria for acute malnutrition were categorized according to their specific condition: kwashiorkor, marasmic-kwashiorkor, marasmus, or moderate acute malnutrition (MAM). These diagnoses were based on the detection of edema and anthropometric measurements on the day of enrollment, using cutoffs established by the World Health Organization (WHO).¹ Thus, in the context of this study *Marasmus* (i.e. 'non-edematous severe acute malnutrition') means that a participant had severe wasting (i.e. weight for height Z score (WHZ) < -3 SD or mean upper arm circumference (MUAC) < 11.5 cm). *Kwashiorkor* (i.e. 'edematous severe acute malnutrition' or 'nutritional edema') means that a child had bilateral pitting pedal edema (+, ++, or +++) without severe wasting. *Marasmic-kwashiorkor* means that a participant had bilateral pitting edema and severe wasting. MAM was defined by the presence of moderate wasting (i.e. WHZ < -2 or MUAC < 12.5 cm). Anthropometric values, WHZ, and height for age Z score (HAZ), were calculated using Anthro (version 3.2.2), an anthropometric Z-score calculator developed by the WHO. Controls were recruited as a convenience sample from the same network of malnutrition surveillance clinics. Controls were distinguished by the absence of acute malnutrition, as evidenced by edema, or wasting, whether severe or moderate. Children with stunting, a condition of chronic undernutrition, and those who reported acute health complaints (i.e. diarrhea or fever), were not excluded from participating as controls. This approach ensured that controls were distinguished primarily by the absence of acute malnutrition rather than generally superior health. In

the context of this study *control* does not mean that the child was entirely well-nourished and free of all health complaints. Rather, *control* means that the child did not meet WHO diagnostic criteria for acute malnutrition.

Participation

After obtaining informed consent, health histories were collected by Malawian research staff in the caregiver's preferred language. Malawian nurses then collected one mL of venous blood into a vacuum-sealed collection tube containing an inert silica-based pro-coagulant. Whole blood specimens were stored at 2 °C during transport to laboratories at the University of Malawi College of Medicine in Blantyre, (~ transport time 6 h). There, the serum component was separated for storage at -80 °C before transport to Baylor College of Medicine in Houston Texas. After venipuncture, participants received a 30 g test dose of ready-to-use therapeutic food (RUTF) under the supervision of a study nurse. After demonstrating appropriate feeding technique to the child's caregiver a study nurse confirmed that the participant had sufficient appetite for outpatient nutritional rehabilitation. RUTF was provided by Project Peanut Butter, a non-governmental organization based in Malawi. RUTF was administered for up to 12 weeks, in accord with the local standard of care. Participants whose condition deteriorated and those who failed to improve after 12 weeks of outpatient treatment, were considered to have failed outpatient treatment. These children were referred for inpatient care. Adequate serum from 357 participants was available for analysis (Patient flow-chart: Supplemental Figure 1).

Metabolic parameters

A panel of sixteen circulating one-carbon metabolites and functional outputs was quantified in order to assess one-carbon metabolism in different conditions of malnutrition, and in controls. These included choline, betaine, dimethylglycine (DMG), glycine, sarcosine, 5-methyltetrahydrofolate (MTHF), serine, methionine, S-adenosylmethionine (SAME), S-adenosylhomocysteine (SAH), homocysteine, cysteine, cystathionine, pyridoxal phosphate (PLP) and asymmetric dimethylglycine (ADMA). Individual un-pooled serum samples were analyzed in batches. All metabolic analyses were conducted at the Center of Metabolomics, Baylor Scott & White Research Institute, Dallas Texas. Serum homocysteine was quantified using a liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS-MS) approach,²⁵ with additional modifications for the measurement of total cysteine. Serum concentrations of betaine, choline, methionine, cystathionine, PLP, SAME, and SAH were measured using previously described LC-ESI/MS/MS methods,^{26,27} which were modified to include glycine, DMG, sarcosine, and ADMA.²⁵ Serum MTHF was

	Kwashiorkor (N = 94)	Marasmic-Kwashiorkor (N = 43)	Marasmus (N = 118)	Moderate Acute Malnutrition (N = 56)	Controls (N = 46)
Demographics & anthropometry¹					
Number of females (total /%)	47 (50%) ^a	19 (44%) ^a	68 (58%) ^a	44 (79%) ^b	28 (61%) ^{a, b}
Age mo. (± SD)	29 (11) ^a	25 (9) ^a	26 (11) ^a	28 (11) ^a	28 (11) ^a
MUAC cm. (± SD)	13.04 (0.89) ^a	10.9 (0.79) ^b	11.21 (0.78) ^b	12.19 (0.36) ^c	13.99 (1.03) ^d
Weight for Height Z score (± SD)	−1.4 (0.9) ^a	−3.29 (0.9) ^b	−3.01 (0.90) ^b	−2.11 (0.63) ^c	−0.30 (0.74) ^d
Height for Age Z score (± SD)	−2.66 (2.43) ^a	−3.56 (1.54) ^a	−3.15 (1.58) ^a	−2.60 (1.67) ^a	−2.67 (1.42) ^a
Nutritional characteristics					
Breastfeeding ² (no. /%)	17 / 19 ^a	7 / 17 ^{a, b}	46 / 40 ^b	18 / 33 ^{a, b}	18 / 43 ^b
Age solids introduced (mo. / ± SD)	9 (6) ^a	9 (6) ^a	8 (4) ^a	8 (5) ^a	8 (5) ^a
Cassava consumption ³ (no. /%)	5 (5.3%) ^a	1 (2.3%) ^a	5 (4.2%) ^a	4 (7.1%) ^a	4 (8.7%) ^a
Egg consumption ³ (no. /%)	17 (18%) ^a	9 (21%) ^a	19 (16%) ^a	13 (23%) ^a	10 (22%) ^a
Vitamin A use ⁴ (no. /%)	16 (17%) ^a	3 (7%) ^a	29 (25%) ^a	16 (29%) ^a	12 (26%) ^a
Health history					
Diarrhea ⁵ no. (%)	53 (58%) ^a	30 (71%) ^a	64 (56%) ^a	15 (28%) ^b	13 (30%) ^b
Bloody diarrhea ⁵ no. (%)	8 (15%) ^a	3 (12%) ^a	7 (11%) ^a	0 (0%) ^a	2 (13%) ^a
Fever ⁵ no. (%)	71 (79%) ^a	29 (66%) ^{a, b}	87 (75%) ^a	22 (39%) ^b	19 (45%) ^b
Rash ⁵ no. (%)	21 (23%) ^a	10 (23%) ^a	23 (20%) ^a	6 (11%) ^a	3 (7.0%) ^a
Vomiting ⁵ no. (%)	26 (28%) ^a	12 (29%) ^a	31 (27%) ^a	12 (22%) ^a	9 (21%) ^a
Cough ⁵ no. (%)	41 (44%) ^a	23 (53%) ^a	51 (44%) ^a	28 (50%) ^a	26 (60%) ^a
Use of deworming medicine ⁶ no. (%)	26 (29%) ^a	11 (26%) ^a	48 (42%) ^{a, c}	39 (72%) ^b	23 (53%) ^{b, c}
Treatment outcome⁷					
Completed treatment	86 (94%)	34 (75%)	107 (91%)	54 (96%)	
Lost to follow-up	7 (7%)	4 (9%)	11 (9%)	2 (4%)	
Death	1 (1%)	5 (12%)	0 (0%)	0 (0%)	

Table 1: Demographic, nutritional, and health history characteristics of subjects.

¹ Shared letters indicate insignificant that pairwise differences were insignificant (i.e. $P > 0.05$).

² Any reported consumption of breastmilk at enrollment.

³ Consumption reported during the preceding two weeks.

⁴ Vitamin A supplementation in the preceding 6 months.

⁵ Symptoms reported in the 7 days preceding enrollment.

⁶ Any reported use of deworming medicine.

⁷ Low event frequency precluded formal statistical comparison.

quantified using previously described LC–ESI/MS–MS techniques.²⁸ Inter-assay coefficients of variation for all analytes were less than 15%. Analyses were performed on a 4000 QTrap and 5500 QTrap mass spectrometry instruments (Sciex, Framingham, MA) coupled to LC systems (Shimadzu, Columbia, MD) with data collected and processed using Analyst Software Version 1.6.2 (Sciex, Framingham, MA). Specimens were allocated to separate batched groups in a randomized fashion. A system of randomly generated participant identifiers was used to keep laboratory personnel blinded to each specimen's diagnosis group. Two quality control measurements were made for each batch of serum specimens by using internal standards to assess within and between assay variations, which was < 10% for all metabolites. Relevant calculated metabolite ratios were used to approximate the activity of certain metabolic reactions within one-carbon metabolism.

Statistical analyses

Prior to this study most of the metabolites that were targeted for quantification had not been characterized in malnourished children before treatment. Hence, the precise calculation of sample sizes for detecting intergroup differences between measured metabolites was not possible. A target sample size of 350–425 participants was estimated using previous reports of similar serum metabolites in this population.^{29,30} The normality (i.e. Gaussian distribution) of each parameter was first established visually, and then confirmed using a Kolmogorov-Smirnov (K-S) test. Parameters with missing data points (i.e. cysteine, MTHF, and PLP) were also normally distributed. Hence, these were analyzed using the same statistical procedures. Missing data points were not imputed or inferred. Reported medians and standard deviations were calculated using raw data. Kernel probability density plots for one-carbon metabolites and relevant one-carbon metabolite ratios were

Metabolic parameter ¹ (Median (±SD))	Marasmic kwashiorkor (N = 43)	Kwashiorkor (N = 94)	Marasmus (N = 118)	Moderate acute malnutrition (N = 56)	Controls (N = 46)
Methionine (µmol/L)	10.9 (5.0) ^a	12.4 (4.7) ^a	16.5 (7.1) ^b	16.1 (5.6) ^b	16.3 (6.0) ^b
SAMe ² (nmol/L)	125 (80) ^a	98 (35) ^b	104 (40) ^b	92 (37) ^b	85 (19) ^b
SAH ³ (nmol/L)	58 (57) ^a	46 (42) ^a	46 (45) ^a	22 (18) ^b	23 (18) ^b
Homocysteine (µmol/L)	4.3 (2.5) ^a	6.3 (4.2) ^{a,c}	8.0 (4.8) ^b	8.0 (3.6) ^{b,c}	8.5 (4.5) ^b
Glycine (µmol/L)	287 (99) ^a	271 (96) ^a	274 (96) ^a	241 (84) ^{a,b}	215 (64) ^b
Serine (µmol/L)	180 (63) ^a	133 (50) ^b	173 (69) ^a	136 (49) ^b	122 (34) ^b
Choline (µmol/L)	9.1 (4.0) ^a	9.2 (3.6) ^a	10.7 (4.7) ^a	9.8 (5.3) ^a	10.0 (2.8) ^a
Betaine (µmol/L)	230 (213) ^a	128 (80) ^b	107 (81) ^{b,c}	80 (31) ^c	79 (28) ^c
DMG ⁴ (µmol/L)	6.0 (4.1) ^a	5.9 (4.1) ^a	7.7 (7.9) ^a	6.3 (5.9) ^a	5.5 (3.2) ^a
Sarcosine (µmol/L)	2.34 (0.97) ^{a,b}	2.02 (1.16) ^a	2.89 (1.86) ^b	2.27 (1.52) ^{a,b}	2.16 (1.03) ^a
ADMA ⁵ (nmol/L)	549 (217) ^a	557 (195) ^a	698 (212) ^b	647 (208) ^{a,b}	648 (125) ^{a,b}
SDMA ⁶ (nmol/L)	910 (692) ^a	640 (163) ^{b,c}	678 (279) ^b	555 (167) ^{b,c}	517 (82) ^c
PLP ⁷ (nmol/L)	10 (4) ^a	20 (56) ^a	19 (14) ^a	23 (16) ^a	21 (11) ^a
MTHF ⁸ (nmol/L)	28 (21) ^a	38 (34) ^a	41 (28) ^a	46 (24) ^a	47 (29) ^a
Cysteine ⁹ (µmol/L)	90 (40) ^a	115 (50) ^a	153 (42) ^b	178 (38) ^c	178 (26) ^c
Cystathionine (µmol/L)	0.79 (0.44) ^a	0.59 (0.42) ^b	0.41 (0.34) ^c	0.28 (0.17) ^c	0.25 (0.18) ^c
Methionine/SAMe	0.11 (0.06) ^a	0.14 (0.07) ^a	0.18 (0.09) ^b	0.19 (0.08) ^b	0.20 (0.08) ^b
SAMe/SAH	2.84 (1.25) ^a	3.07 (1.59) ^a	3.35 (1.79) ^a	5.74 (3.66) ^b	5.15 (2.85) ^b
SAH/Homocysteine	17 (20) ^a	11 (15) ^b	7 (7) ^{b,c}	3 (2) ^c	3 (2) ^c
Homocysteine/Cysteine	0.055 (0.022) ^{a,b}	0.058 (0.028) ^a	0.057 (0.033) ^{a,b}	0.045 (0.018) ^b	0.047 (0.022) ^{a,b}
Homocysteine/Methionine	0.46 (0.33) ^a	0.55 (0.37) ^a	0.57 (0.46) ^a	0.55 (0.30) ^a	0.58 (0.34) ^a
Betaine/DMG	52 (75) ^a	28 (25) ^b	18 (11) ^b	17 (8) ^b	17 (6) ^b
Choline/Betaine	0.06 (0.05) ^a	0.10 (0.06) ^b	0.12 (0.05) ^c	0.13 (0.05) ^c	0.13 (0.04) ^c
Glycine/Sarcosine	143 (89) ^{a,b}	165 (101) ^a	122 (66) ^b	125 (54) ^b	114 (42) ^b
SDMA/ADMA	1.72 (1.20) ^a	1.24 (0.41) ^b	1.01 (0.40) ^c	0.88 (0.23) ^c	0.82 (0.18) ^c

Table 2: Metabolic parameters in kwashiorkor, marasmic-kwashiorkor, moderate acute malnutrition, and controls[†].

1: Shared letters indicate insignificant pairwise comparisons (Tukey post-hoc analysis, $P > 0.05$) after adjusting for multiple comparisons.
2: S-adenosyl methionine.
3: S-adenosyl homocysteine.
4: Dimethylglycine.
5: Asymmetric dimethylarginine.
6: Symmetric dimethylarginine.
7: Pyridoxyl phosphate, N=304 (Kwashiorkor: 89, Marasmic-kwashiorkor: 21, Marasmus: 93, MAM: 55, Controls: 46).
8: Methyl tetrahydrofolate, N=298 (Kwashiorkor: 87, Marasmic-kwashiorkor: 19, Marasmus: 91, MAM: 55, Controls: 46)
9: Cysteine, N=306, (Kwashiorkor: 89, Marasmickwashiorkor: 22, Marasmus: 94, MAM: 55, Controls: 46).

estimated using an existing software package,³¹ (Supplemental Figs. 2–4). Intergroup differences were detected using a one-way ANOVA on ranks (i.e. Kruskal-Wallis test).³² Pairwise comparisons for continuous variables were made using Tukey's post-hoc test, which was adjusted for multiple comparisons. Categorical variables were assessed using Pearson's chi-square procedure. P values reported in Tables 1, 2, and Figure 2 were adjusted for multiple comparisons. Shared superscripted letters in Tables 1, 2, and Figure 2 indicate that P values were not significantly different (i.e. $P > 0.05$), after adjusting for multiple comparisons. Logistic regression was used to characterize associations between one-carbon metabolites and the presence of edematous malnutrition (i.e. kwashiorkor or marasmic-kwashiorkor), after adjusting for sex, study visit WHZ, MUAC, and HAZ, as well as reports of fever or diarrhea during the preceding seven days. Metabolite

interquartile range effects were summarized as odds ratios with 95% confidence intervals (CIs). ANOVA plots were created using an existing software package for the Wald chi-square test,³³ (Figure 3 and Supplemental Figs. 6–8). Additionally, we calculated Pearson's correlation coefficients between metabolic parameters and individual measures of nutritional status: MUAC, WHZ, and HAZ.³⁴ These univariate correlation coefficient values are depicted in Supplemental Figs. 9–16. Statistical analyses were performed using SPSS[™] Version 25 and R Statistical Software, Version 4.0.2.

Role of funding sources

Funders did not contribute to the conceptualization, study design, data collection, analysis, data interpretation, manuscript preparation, or journal selection for this research.

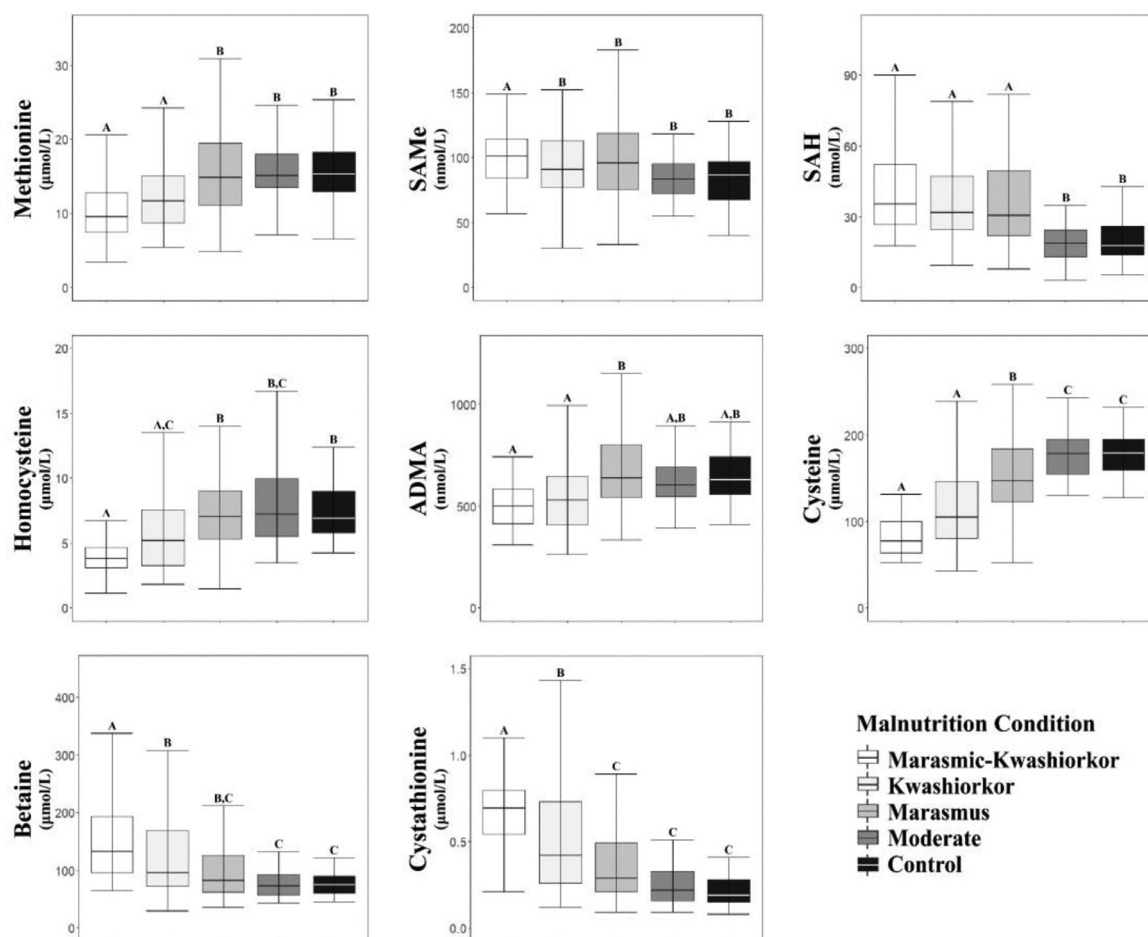


Figure 2. One-carbon metabolites in serum: Subject groups are reflected by gray-scale differences according to the legend: kwashiorkor ($N = 94$), marasmic-kwashiorkor (43), marasmus ($N = 118$), moderate acute malnutrition ($N = 56$) controls ($N = 46$). Box plots depict serum concentrations (y axis) of methionine, S-adenosyl methionine (SAME), S-adenosyl homocysteine (SAH), homocysteine, asymmetric dimethylarginine (ADMA), cysteine, cystathionine, and betaine. Lower, middle, and upper boundaries of bars represent the 25th, 50th, and 75th percentiles respectively. Lower and upper whiskers represent the 5th and 95th percentiles respectively. Shared letters indicate that pairwise comparison of means was insignificant (i.e. Tukey post-hoc test $P > 0.05$), after adjusting for multiple comparisons.

Results

Participants

Serum was collected from 422 children. Of these, sufficient quantities of non-hemolyzed serum for metabolic analyses were available from 357, (43 marasmic-kwashiorkor, 94 kwashiorkor, 118 marasmus, 56 MAM, and 46 controls; Supplemental Figure 1). All participants lived in rural communities where household food security is linked to subsistence patterns of agriculture. In this respect participants' economic and living conditions resembled those of other children in rural areas of Sub-Saharan Africa, where risk for malnutrition is high. Overall, there were slightly more female participants (58%) than male. Among the 137 participants with

edema (i.e. edematous malnutrition) there were 43 (33%) who also had severe wasting ($WHZ < -3$ or $MUAC < 11.5$ cm) at the time of enrollment. These participants were grouped together for separate consideration as marasmic-kwashiorkor. Enrollment age was similar across participant groups (Table 1). Stunting is widespread in Malawi.²⁹ Stunting (i.e. $HAZ < 2$), which was present in 267 of 357 participants (75%), was distributed similarly in each participant group (Pearson's chi square ≥ 0.2 for all pairwise comparisons). Reports of rash, vomiting, and cough, were similar in all three groups (Table 1). In contrast, diarrhea and fever were more common in children with marasmus, kwashiorkor, or marasmic-kwashiorkor (Table 1). Like malnourished participants, caregivers for controls reported

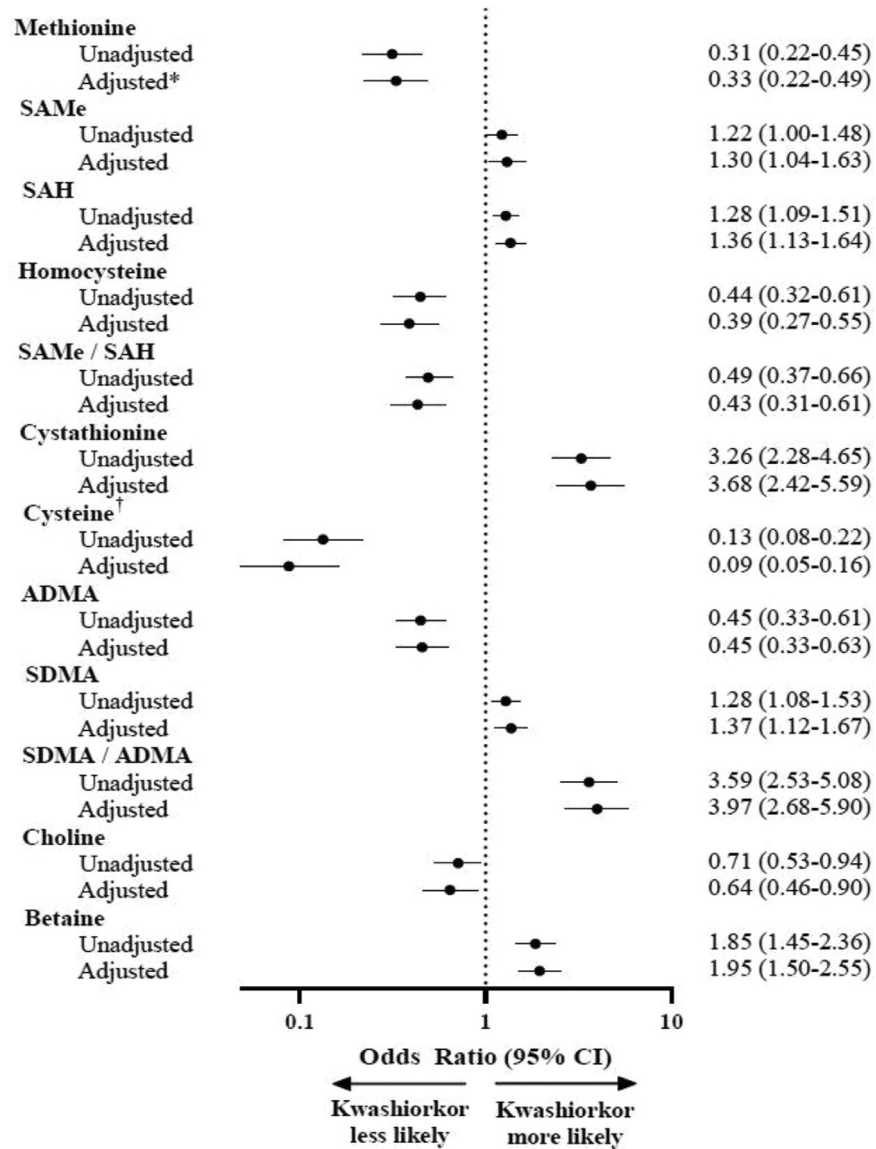


Figure 3. Covariate regression analysis: Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression. Depicted ORs and CIs reflect interquartile range effects for the prediction of kwashiorkor (including marasmic-kwashiorkor), before and after adjusting for mean upper arm circumference (MUAC), height for age Z score (HAZ), weight-for-height Z score (WHZ), age, sex, diarrhea, and fever. *N = 345: Kwashiorkor: 89, Marasmic-kwashiorkor: 22, Marasmus: 94, MAM: 55, Controls: 46. S-adenosyl-methionine (SAMe), S-adenosyl-homocysteine (SAH), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). † Cysteine, N = 306. Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA).

frequent acute health complaints. Specifically, the total number of health complaints in controls was not lower relative to other participant groups: i.e. controls (3.3/ ± 1.3), marasmic-kwashiorkor (2.6 SD ± 1.2), kwashiorkor (2.7/ ± 1.3), marasmus (2.8/ ± 1.3), and MAM (3.5/ ± 1.4). Controls were distinguished by the absence of acute malnutrition rather than perfect health. Empiric use of antibiotics to treat routine childhood illnesses is

common in Malawi. There were 33 participants whose caregivers reported use of one or more antibiotics during the preceding two weeks (sulfamethoxazole-trimethoprim N = 23, artemether-lumefantrine N = 12, and amoxicillin N = 3). Of these antibiotics, only sulfamethoxazole-trimethoprim targets one-carbon metabolism. Reports of sulfamethoxazole-trimethoprim use were distributed asymmetrically across participant groups

Feature	1CNDDs	Kwashiorkor
Organ changes		
Liver steatosis	↑ ²⁴	↑ ⁸⁸
Pancreatic atrophy	↑ ⁸⁹	↑ ⁸⁸
Exocrine pancreas <i>fxn.</i>	↓ ⁹⁰	↓ ⁹¹
Intestinal thickness	↓ ^{48,92}	↓ ⁹³
Intestinal permeability	↑ ⁹⁴	↑ ⁹⁵
Intestinal inflammation	↑ ⁹⁶	↑ ⁹⁷
Skin disturbances	↑ ^{98,99}	↑ ¹⁰⁰
Cellular immune <i>fxn.</i>	↓ ¹⁰¹	↓ ¹⁰²
Edema†	↑ ^{103,‡}	↑ ¹⁴
Molecular changes		
Transmethylation	↓ ¹⁰⁴	↓ ⁵⁷
DNA methylation	↓ ¹⁰⁵	↓ ⁸⁰
Plasma carnitine	↓ ¹⁰⁶	↓ ¹⁰⁷
Plasma cysteine	↓ ¹²³	↓ ⁵⁷
Plasma glutathione	↓ ¹⁰⁸	↓ ⁷¹
Sulfated GAGs‡	↓ ¹⁰⁹	↓ ¹¹⁰
Plasma albumin	↓ ¹¹¹	↓ ¹¹²
Hepatic PPARα§	↓ ¹¹³	↓ ¹¹⁴
Plasma triglycerides¶	↓ ¹¹⁵	↓ ¹¹⁶
Fatty acid oxidation	↓ ¹¹⁷	↓ ¹¹⁸
Lipid peroxidation	↑ ¹¹⁹	↑ ¹²⁰
'Oxidative stress'	↑ ²⁴	↑ ^{82,83}
Metalloproteinase-2	↑ ¹²¹	↑ ¹⁹⁹
Plasma TNF-α	↑ ¹²²	↑ ¹²³

Table 3: Disturbances in kwashiorkor and experimental one-carbon nutrient deficient diets* (1CNDDs).

* Most experimental diets referenced here are deficient in methionine and choline.

† Nutritional edema in rats is prevented completely by supplementation with choline, and prevented partially with cobalamin.

‡ Glycosaminoglycans.

§ Hepatic PPARα signaling in kwashiorkor has not been directly characterized. Hepatic peroxisomes are reduced in kwashiorkor, suggesting that PPARα signaling is suppressed.

¶ In kwashiorkor plasma triglycerides are lower at diagnosis, then rise during treatment.

(marasmic-kwashiorkor $N = 1$, kwashiorkor $N = 5$, marasmus $N = 14$, and controls $N = 1$), and were not associated with evidence of greater one-carbon disturbances.

Methionine cycle

The four intermediates of the methionine cycle differed among participant groups. Methionine and homocysteine were lower in kwashiorkor ($P \leq 0.0025$) and marasmic-kwashiorkor ($P < 0.0001$), relative to marasmus and controls (Figure 2, Table 2). We did not observe a consistent pattern of SAME and SAH differences in kwashiorkor and marasmic-kwashiorkor. SAME, the universal methyl donor, was significantly higher in marasmic-kwashiorkor, relative to the other four participant groups. In contrast, SAH, the demethylated analogue of SAME, was not significantly different in

kwashiorkor or marasmic-kwashiorkor, relative to marasmus. However, SAH was significantly higher in kwashiorkor, marasmic-kwashiorkor, and marasmus when these three participant groups were compared individually with MAM or controls. SAME to SAH ratios were similar in kwashiorkor, marasmic-kwashiorkor, and marasmus, but lower ($P < 0.0001$) when these three conditions of severe acute malnutrition were compared individually with MAM and controls (Table 2). SAME to SAH ratios fall when transmethylation capacity is limited.³⁵ The observation of lower SAME to SAH ratios in kwashiorkor, marasmic-kwashiorkor, and marasmus suggests that reduced transmethylation potential is common in each of these three conditions of malnutrition. Although SAME to SAH ratios were not significantly lower in kwashiorkor and marasmic-kwashiorkor when compared directly with marasmus, this indicator of transmethylation capacity was significantly associated with edema in an adjusted multivariate model (Figure 3). This observation suggests that decreased methylation potential is a predictor of which children develop kwashiorkor (including marasmic-kwashiorkor), as opposed to marasmus. Ratios of glycine to sarcosine in kwashiorkor and marasmic-kwashiorkor were numerically higher relative to marasmus, MAM, and controls (Table 2). However, this difference was only significant in the case of kwashiorkor ($P \leq 0.02$). Glycine to sarcosine ratios are subject to the activity of glycine N-methyltransferase (GNMT),^{36,37} which by sinking SAME derived methyl groups into the sarcosine pool regulates SAME availability and SAME to SAH ratios.²³ Higher glycine to sarcosine ratios in kwashiorkor may reflect lower GNMT activity. Ratios of SAH to homocysteine were higher in kwashiorkor and marasmic-kwashiorkor ($P < 0.0001$), relative to MAM and controls (Table 2). Higher ratios of SAH to homocysteine may reflect more limited SAH hydrolase activity. SAH hydrolase catalyzes the conversion of SAH to homocysteine. Hence, suppression of SAH hydrolase preserves SAH. SAH is a potent inhibitor of transmethylation enzymes.³⁸ Thus, suppression of SAH hydrolase causes both SAH and SAME to accumulate intracellularly.³⁹ Together, these observations are consistent with the hypothesis that GNMT and SAH hydrolase are suppressed in kwashiorkor and marasmic-kwashiorkor. Experimental models indicate that these SAME regulatory enzymes are broadly influenced by one-carbon nutrients. For example, transcription of SAH hydrolase is suppressed by deficiencies of methionine, choline, and folate.⁴⁰ Similarly, GNMT transcription is stimulated by excess methionine,⁴¹ whereas GNMT activity is suppressed by choline deficiency.⁴²

Remethylation

5-methyltetrahydrofolate (MTHF) is a reduced form of folate. It is a direct cofactor for the remethylating activity

of methionine synthase, which converts homocysteine to methionine (Figure 1). PLP is the active isomer of vitamin B6. It is necessary for the activity of serine hydroxyl methyl transferase (SHMT),⁴³ which catalyzes the methylation of tetrahydrofolate. Although PLP and MTHF were numerically lower in marasmic-kwashiorkor compared to all other participant groups, these differences were not statistically significant (Table 2). Nor was homocysteine higher. Homocysteine rises when remethylation is limiting.⁴⁴ Choline, sarcosine, glycine, and serine, which furnish labile methyl groups for the remethylation of homocysteine, were not well differentiated in kwashiorkor and marasmic-kwashiorkor, relative to other participants (Table 2). In contrast, betaine, a methyl donor and intracellular osmolyte, was notably higher in kwashiorkor ($P = 0.047$) and marasmic-kwashiorkor ($P < 0.0001$), relative to controls (Table 2).

One-carbon metabolism synthetic function

One-carbon metabolism's synthetic activities decline when one-carbon nutrient intake is deficient. For instance, methionine deficiency causes reduced serum concentrations of ADMA and cysteine, products of transmethylation and transsulfuration respectively.^{45–48} In this single time point observational study we used serum concentrations of ADMA and cysteine as proxy measures of one-carbon metabolism synthetic activity. ADMA was lower in kwashiorkor ($P < 0.0001$) and marasmic-kwashiorkor ($P = 0.00032$), relative to marasmus (Figure 2 and Table 2). Cysteine was also lower in kwashiorkor ($P < 0.0001$) and marasmic-kwashiorkor ($P < 0.0001$), relative to other participant groups. Among all participants with edematous malnutrition (i.e. either kwashiorkor or marasmic-kwashiorkor, Supplemental Figure 11), both ADMA and cysteine were well correlated with homocysteine ($P < 0.01$) and methionine ($P < 0.01$). Importantly, both ADMA and its enantiomer, SDMA, are formed by the sequential methylation of arginine.⁴⁹ However, SDMA is mainly excreted by the kidneys. Therefore, its serum concentration tends to increase as kidney function declines. This causes SDMA to ADMA ratios to rise.^{50,51} To our knowledge, this report of higher SDMA to ADMA ratios in kwashiorkor ($P \leq 0.012$) and marasmic-kwashiorkor ($P < 0.0001$), relative to other participant groups, is the first published characterization of SDMA to ADMA ratios in edematous malnutrition. Higher SDMA to ADMA ratios suggest that renal dysfunction is a frequent complication of kwashiorkor and marasmic-kwashiorkor. These observations correspond with past reports of glomerular injury and renal dysfunction in kwashiorkor.^{52–54}

Transsulfuration

The transsulfuration pathway supports the transfer of sulfur from homocysteine to numerous vital molecules. Homocysteine is thus an essential substrate for synthesis of transsulfuration pathway products, including

cysteine and glutathione (Figure 1). Homocysteine was lower in kwashiorkor ($P = 0.034$) and marasmic-kwashiorkor ($P < 0.0001$), relative to marasmus (Figure 2, Table 2). We did not measure glutathione in this investigation, due to the logistical constraints associated with its proper collection and preservation in the field. However, we did observe that cysteine was markedly lower in kwashiorkor and marasmic-kwashiorkor ($P < 0.0001$). This has been reported previously.^{55,56} Cysteine was well correlated with homocysteine ($P < 0.01$) in both kwashiorkor and marasmic-kwashiorkor (Supplemental Figs. 9–11). Notably, ratios of homocysteine to cysteine were not higher in kwashiorkor and marasmic-kwashiorkor (Table 2). This ratio rises when homocysteine flux through the transsulfuration pathway is impaired. These observations correspond with the past observation that flux of labeled methionine through the transsulfuration pathway is similar in kwashiorkor and marasmus.⁵⁷ Unexpectedly, we observed that cystathionine, a transsulfuration intermediate, was higher in kwashiorkor ($P \leq 0.0019$) and marasmic-kwashiorkor ($P < 0.0001$) relative to other participants. The cause cannot be determined from these data. Serum cystathionine rises in the setting of SAH hydrolase deficiency and GNMT deficiency, heritable syndromes of one-carbon metabolism dysfunction,^{58,59} as well as during folate and cobalamin deficiencies, nutritionally mediated conditions of one-carbon metabolism dysfunction.⁶⁰

Marasmic-kwashiorkor

The combination of nutritional edema with severe wasting is referred to as marasmic-kwashiorkor. Separate consideration of this condition is relevant because children with marasmic-kwashiorkor tend to die more often than children with uncomplicated marasmus or kwashiorkor without wasting.^{61–63} We enrolled fewer participants with marasmic-kwashiorkor ($N = 43$) than marasmus ($N = 118$), or kwashiorkor without severe wasting ($N = 94$). This distribution is similar to the observations of prior studies in the same population.⁶³ The character of one-carbon disturbances in marasmic-kwashiorkor was similar to that observed in participants with kwashiorkor without wasting (Table 2). However, the magnitude of one-carbon disturbances in marasmic-kwashiorkor was generally greater. Although children with marasmic-kwashiorkor comprised a minority of participants, five of six confirmed deaths occurred in this group (Table 1). The sixth death occurred in a child who had kwashiorkor without wasting. Each death reportedly occurred after a brief medical illness. The precise cause of death could not be ascertained in any of these six cases.

Metabolite associations adjusted for covariates

Logistic regression was used to assess the association of one-carbon metabolites with the presence nutritional

edema (i.e. kwashiorkor or marasmic-kwashiorkor) after adjusting for age, sex, wasting (i.e. WHZ and MUAC), stunting (i.e. HAZ), diarrhea, and fever. These findings are represented in [Figure 3](#) and Supplemental Figure 5, which depict predictive odds ratios (ORs) and 95% CIs associated with an increase of each metabolic parameter from its 25th to 75th percentile (i.e. interquartile range effect). Among those metabolites that were significantly altered in kwashiorkor and marasmic-kwashiorkor, we observed that methionine, homocysteine, cystathionine, cysteine, and ADMA were consistently associated with kwashiorkor and marasmic-kwashiorkor ($P < 0.05$), in both adjusted and unadjusted regression models. ANOVA plots demonstrating the relative importance of each variable during regression are located in Supplemental Figs. 6–8. Linear correlations between each metabolic parameter, as well as MUAC, WHZ, and HAZ, offered additional insights into potential associations between wasting and individual metabolic parameters. These univariate correlations are depicted in Supplemental Figs. 9–16. Highlighted values reflect correlation coefficients with unadjusted P values < 0.01 .

Discussion

The idea that kwashiorkor may result from an essential nutrient deficiency was first proposed in 1933 by Cecily Williams, who suggested that “some amino acid... deficiency cannot be excluded as a cause.”¹² Various theories for kwashiorkor’s pathogenesis have since been proposed. However, none has been established.^{4,5} The aim of this study was to explore the hypothesis that kwashiorkor is a nutritional syndrome of one-carbon metabolism dysfunction that is precipitated by inadequate intake of certain one-carbon nutrients. The findings of this study offer insight for considering this idea. Importantly however, the interpretation of these findings is restrained by a number of limitations, particularly regarding conclusions about causality. Foremost among these is the study’s single time point cross-sectional design, which does not reveal which metabolic differences preceded the onset of different diagnoses of malnutrition. Additional challenges stemmed from the need to select controls who were distinguished primarily by the absence of acute malnutrition. Although controls were not acutely malnourished, a number of those who were recruited for this convenience sample were stunted or had acute health complaints. Another challenge was posed by the need to deliver prompt care. This necessarily prevented us from collecting fasting blood samples from untreated participants. Doing so would have required a dangerous and unethical delay of therapy. We are mindful that these limitations may have affected the observations of this study, as individual circulating amino acids and their metabolites are influenced by stunting, meals, and infections, as well as

their inherent circadian periodicities.^{29,64–68} The assessment of circulating metabolites in malnutrition is also complicated by the simultaneous occurrence of edema and wasting. This overlap leaves open the question of whether observed metabolic differences resulted from wasting or the underlying disturbances that precipitate edema. Additionally, in severe edema, both MUAC and weight may be positively skewed to the extent that marasmic-kwashiorkor is missed. Similarly, changes in body water partitioning in kwashiorkor may accentuate reductions of some molecules while masking accumulations of others. These challenges are common to any assessment of circulating metabolites in kwashiorkor. Nevertheless, despite these limitations, this exploration of one-carbon metabolites in malnourished children contributes testable hypotheses regarding the pathogenesis of kwashiorkor.

Most of what is known about one-carbon metabolism in acute malnutrition was learned in Jamaica. There it was discovered that *during treatment* kwashiorkor and marasmic-kwashiorkor are distinguished by one-carbon disturbances, including reduced transmethylation and slower methionine flux.^{57,69–72} Elsewhere it has been reported that kwashiorkor is differentiated from marasmus by lower circulating concentrations of molecules that depend on one-carbon metabolism for their generation, such as phosphatidylcholine and acylcarnitine species.³⁰ To date however, there has been no focused comparison of one-carbon metabolites in kwashiorkor and marasmus before treatment. The central process of one-carbon metabolism is the methionine cycle. This cycle sustains the synthesis of numerous transmethylation and transsulfuration products, many of which are critical for homeostasis ([Figure 1](#)). To assess the methionine cycle we measured its four intermediates: methionine, SAME, SAH, and homocysteine ([Figure 1](#)). The observation that methionine was lower in kwashiorkor ($P < 0.0001$) and marasmic-kwashiorkor ($P < 0.0001$), relative to marasmus, corresponds with previous reports.^{30,69,73–75} Unexpectedly, we observed that serum concentrations of methionine’s adenosylated analogues, SAME and SAH, were not lower in kwashiorkor and marasmic-kwashiorkor ([Table 2](#)). Various causes may be considered. For instance, in the case of marasmic-kwashiorkor, it is hypothesized that higher serum concentrations of SAME and SAH may reflect compensatory suppressions of GNMT and SAH hydrolase. These one-carbon regulatory enzymes are down-regulated in animals that are subjected to one-carbon nutrient deficient diets.^{42,76} Looking beyond the methionine cycle, we compared one-carbon synthetic activity across participant groups by assessing cysteine and ADMA, stable outputs of transsulfuration and transmethylation respectively ([Figure 1](#)). Both of these functional outputs were lower in kwashiorkor (including marasmic-kwashiorkor), relative to marasmus ([Figure 2](#)). Together these observations suggest that

one-carbon metabolism is relatively preserved in marasmus, whereas it is relatively dysfunctional in kwashiorkor. One-carbon metabolism dysfunction appears to be a distinguishing feature of kwashiorkor. *What are the likely precipitants of one-carbon metabolism dysfunction in kwashiorkor?*

Evidence of one-carbon metabolism dysfunction increased in a step-wise fashion across participant groups. It was not evident in controls and MAM, who were poorly differentiated from each other. Certain one-carbon differences were apparent in marasmus, relative to controls. These disturbances became more pronounced in kwashiorkor without severe wasting. However, the greatest one-carbon disturbances were observed in marasmic-kwashiorkor. Overall, this pattern is consistent with the interpretation that one-carbon metabolism dysfunction is a hallmark disturbance of edematous malnutrition (i.e. kwashiorkor and marasmic-kwashiorkor), but not marasmus. We also considered the possibility that serum concentrations of one-carbon metabolites are influenced by malnutrition or acute illness. To do so, we compared each one-carbon parameter in a multivariate regression model, which was adjusted for MUAC, WHZ, HAZ, age, sex, fever, and diarrhea. We also compared unadjusted univariate correlations, in order to assess the relationship of individual one-carbon metabolites with continuous measures of nutritional status (i.e. MUAC, WHZ, and HAZ), across participant groups (Supplemental Figs. 9–16). The observations of these multivariate and univariate correlative analyses suggest that one-carbon disturbances in malnutrition are not primarily attributable to age, sex, acute illness, stunting, or wasting. Rather, evidence of one-carbon metabolism dysfunction was most associated with lower serum concentration of methionine and its demethylated analogue, homocysteine.

Efficient remethylation of homocysteine to methionine is critical for one-carbon homeostasis (Figure 1). Nutrients that support remethylation limit the severity of the disturbances that stem from remethylation dysfunction, including DNA hypomethylation and phosphatidylcholine disturbances.^{77–79} Such disturbances are prominent in kwashiorkor (including marasmic-kwashiorkor).^{30,80} We therefore considered the possibility that one-carbon metabolism dysfunction in kwashiorkor results from impaired remethylation of homocysteine. Remethylation is supported by methyl donors and certain vitamin co-factors. However, measured serum concentrations of methyl donors (choline, glycine, sarcosine, and serine) and vitamin co-factors that support remethylation (PLP and MTHF), were not reduced in kwashiorkor or marasmic-kwashiorkor (Table 2). Neither were ratios of homocysteine to methionine, nor homocysteine itself, higher in kwashiorkor and marasmic-kwashiorkor. These two inverse indicators of remethylation function rise when remethylation is limiting.⁸¹ Overall, these observations do not suggest

that impaired remethylation of homocysteine to methionine is the main driver one-carbon metabolism dysfunction in kwashiorkor and marasmic-kwashiorkor. ADMA and cysteine, stable outputs of one-carbon metabolism, were well correlated with both methionine and homocysteine in kwashiorkor and marasmic-kwashiorkor (Supplemental Figure 11). Likewise, edema was correlated best with reductions of methionine and two of its metabolites, homocysteine and cysteine, in a multivariate regression model (Figure 3). Together, these observations are consistent with the hypothesis that methionine deficiency is essential for the pathogenesis of one-carbon metabolism dysfunction and edema in kwashiorkor.

It is established that kwashiorkor is distinguished from marasmus by lower circulating concentrations of cysteine and glutathione.¹⁰⁰ Both of these transsulfuration products have antioxidant properties.⁸⁴ However, it is not known whether these antioxidants are lower in kwashiorkor because of excess utilization or inadequate synthesis. When kwashiorkor's hallmark redox disturbances were first described, it was proposed that environmental 'oxidative stress' may drive excess use of cysteine and glutathione, thereby precipitating the kwashiorkor syndrome.¹⁰¹ However, follow-up clinical studies have not provided consistent support for this theory.^{85,86} Homocysteine is the source of the sulfur atoms that are present in cysteine and glutathione. As such, homocysteine is an essential substrate for the transsulfuration pathway. Like others, we observed that cysteine is lower in kwashiorkor (including marasmic-kwashiorkor) (Figure 2).^{56,82} More uniquely, we also observed that *homocysteine is lower in kwashiorkor*. To our knowledge this is the first published comparison of homocysteine status in malnourished children before treatment. Homocysteine was well correlated with both cysteine and methionine ($P < 0.01$) in kwashiorkor (including marasmic-kwashiorkor). The requirement for homocysteine is satisfied by its methylated precursor, methionine.⁸⁷ These observations are consistent with the hypothesis that redox disturbances in kwashiorkor result from homocysteine insufficiency, which is precipitated by methionine deficiency.

A unifying molecular driver for kwashiorkor's various organ lesions has not yet been identified. However, it is notable that kwashiorkor bears a striking resemblance to the pattern of organ and molecular perturbations precipitated by experimental one-carbon nutrient deficient diets in animals (Table 3). This phenotypic overlap suggests that nutritionally mediated systemic one-carbon metabolism dysfunction may drive the pathogenesis of kwashiorkor. A full consideration of all the sub-cellular mechanisms implicated by this concept falls beyond the scope of this discussion. Two are presented briefly here: fatty liver of undernutrition and edema. Children with kwashiorkor have fatty livers. This prominent visceral lesion persists even when

accompanied by severe wasting.^{88,124} *Why do skinny children have fatty livers?* Notably, assembly of the main vehicle for lipid export from the liver, very low-density lipoprotein (VLDL), requires phosphatidylcholine that is synthesized by phosphatidylethanolamine methyltransferase (PEMT), an enzyme that is prominently expressed in the liver.^{125,126} PEMT activity is sustained by methyl groups, particularly those derived from choline.¹²⁷ PEMT dysfunction leads to fatty liver disease in humans.^{128–130} Current observations and the past demonstration of reduced transmethylase activity in kwashiorkor⁵⁷ support the hypothesis that PEMT activity is suppressed in kwashiorkor, a disturbance that is expected to increase liver steatosis. It is hypothesized that nutritionally mediated suppression of PEMT is a critical driver in the pathogenesis of the characteristic fatty liver of undernutrition, which distinguishes kwashiorkor from marasmus. PEMT status in kwashiorkor and marasmus has not yet been characterized. The pathogenesis of edema in kwashiorkor is also uncertain.^{4,5} The hypothesis that edema in malnutrition is caused directly by protein deficiency, which suppresses plasma protein synthesis and hence intravascular oncotic pressure, was introduced more than a ninety years ago.¹³¹ Although this idea became popular, a number of subsequent observations conflict with this straight forward hypothesis. For instance, plasma concentrations of albumin, the leading constituent of intravascular oncotic pressure, are poorly correlated with the onset, resolution, and severity of edema in malnutrition.^{20,132} Nor is albumin synthesis lower in kwashiorkor relative to marasmus.¹⁷ However, despite these inconsistencies, albumin and oncotic disturbances are not entirely exonerated. Plasma albumin is often lower in kwashiorkor.¹¹² Lower albumin is often, but not always, associated with edema.^{133,134} The pathogenesis of edema in kwashiorkor may have more to do with albumin's redistribution into the interstitium than an absolute deficiency. Modern microanatomical studies of capillary ultrastructure suggest that edema is often the result of increased microvascular permeability to protein macromolecules, including albumin.^{135,136} Plasma proteins are normally retained within the vascular space by the endothelial glycocalyx. This negatively charged sieve like structure lines the luminal surface of blood vessels. Endothelial glycocalyx damage allows plasma proteins to escape from the microvasculature into the interstitium.^{137,138} The subsequent leveling of protein concentration gradients between the intravascular and interstitial environments permits fluid to flow from the vascular space into the interstitium.¹³⁹ Golden has proposed that endothelial glycocalyx damage may contribute to the pathogenesis of edema in kwashiorkor by allowing plasma proteins, including albumin, to leak into the interstitium.¹⁴⁰ Close consideration of this idea is warranted by various strands of evidence. Endothelial glycocalyx damage leads to tissue edema in a number of

conditions, including sepsis, myocardial ischemia, and COVID-19 associated lung injury.^{141–143} Importantly, the structural integrity of the endothelial glycocalyx is supported by sulfated glycosaminoglycans (GAGs),^{144,145} which are reduced in kwashiorkor.¹⁴⁶ Animal models of methionine deficiency deplete sulfated GAGs,¹⁰⁹ the synthesis of which depends on free sulfur derived from methionine.¹⁴⁷ *Does methionine deficiency contribute to the pathogenesis of edema in kwashiorkor by limiting sulfated GAG synthesis, thereby increasing endothelial permeability to plasma proteins such as albumin, and hence fluid escape from small vessels into the interstitium?* More study is needed on this topic.

Unexpectedly, we observed that serum betaine was markedly higher in kwashiorkor and marasmic-kwashiorkor. The cause is not apparent from these data. Dietary differences are not implicated, as participants reported consuming similar maize-based diets. A portion of the betaine pool is derived from the oxidation of choline. However, choline was not notably lower in kwashiorkor or marasmic-kwashiorkor. This suggests that higher betaine in kwashiorkor is not likely to be due to increased oxidation of dietary choline alone. Betaine has two roles. It is a methyl donor *and* a ubiquitous intracellular osmolyte.¹⁴⁸ Higher serum betaine may reflect the release of intracellular betaine. Regardless of the cause, higher extracellular betaine in kwashiorkor has the potential to alter osmolar gradients. This is predicted to favor the accumulation of extracellular fluid at the expense of intracellular fluid, as occurs in kwashiorkor.^{149,150} The possibility that osmolar disturbances contribute to the pathogenesis of edema in kwashiorkor warrants further study.

One-carbon metabolism may offer mechanistic insight into kwashiorkor's risk factors. Kwashiorkor's only established universal risk factor is consumption of monotonous high carbohydrate diets that provide low-quality protein.^{6,7} Such diets are often deficient in one-carbon nutrients.^{151,152} However, only a minority of children who consume these diets get kwashiorkor. Risk for kwashiorkor is multifactorial. Certain environmental determinants render some children more vulnerable to the ill-effects of their meager diets. Kwashiorkor's non-universal second hits include gut microbiota disturbances,^{21,153} acute infections,¹⁵⁴ antenatal metabolic programming,^{155,156} aflatoxin exposure,^{157,158} and cyanogens in cassava.¹⁵⁹ Polymorphisms in genes for enzymes that regulate one-carbon metabolism may impart additional risk.^{160,161} A shared molecular focus that is common to each of these risk factors has not been identified. It is intriguing however that kwashiorkor's known risk factors are each associated with one-carbon disturbances in other disease states.^{156,162–165} One-carbon stressors are expected to result in more frequent dysfunction in children who consume limited quantities of one-carbon nutrients. It is hypothesized that kwashiorkor's environmental

determinants increase one-carbon stress during the run-up before the acute syndrome by either increasing demand for specific one-carbon nutrients or by reducing their absorption from the diet. Importantly, certain one-carbon nutrient deficiencies may be more detrimental than others. The observations of this study support the possibility that methionine deficiency is essential for the pathogenesis of one-carbon metabolism dysfunction in kwashiorkor. This hypothesis is succinct but not simple, since demand for methionine and its metabolism are influenced by various one-carbon nutrients, which are in turn influenced by genetics,¹⁶⁶ antenatal programming,¹⁵⁶ infections,¹⁶⁷ dietary toxins,¹⁶² and the gut microbiome.^{163,168} One-carbon metabolism appears to offer a molecular framework for gathering kwashiorkor's genetic determinants, environmental risk factors, underlying biochemical disturbances, and organ level lesions into an integrated mechanistic disease model. Prospective studies are needed. In due course it may become established that kwashiorkor results from the accretion of various one-carbon stressors, the combined detriment of which precipitates methionine deficiency and the ensuing systemic one-carbon metabolism dysfunction that propagates the syndrome's unique pathophysiology. Such a discovery would illuminate the pathogenesis of kwashiorkor, while also guiding the development of better strategies for its alleviation.

These observations offer guidance for future research. For instance, methionine requirements for weaned children are not well characterized. One-carbon nutrient cross-talk influences demand for methionine in mammals.^{104,169,170} The primary human example of this phenomenon is the methionine sparing effect of cysteine.¹⁷¹ This is basis for Roediger's hypothesis that kwashiorkor results from inadequate intake of both sulfur amino acids, methionine and cysteine.¹⁷² Methyl groups may also influence methionine requirements.¹⁷³ For example, in animals it has been established that methionine is spared by the methyl donor choline.^{174–177} This effect is accentuated during methionine restriction.¹⁶⁹ We observed that methionine was well correlated with choline across participant groups (Supplemental Figs. 9–16), all of whom reported consuming maize based diets, which provide little methionine.^{151,152} This observation is consistent with the concept that choline may spare methionine in children. This hypothesis is founded on choline's support of remethylation in humans,¹⁷⁸ which is expected to be more relevant in children who consume methionine restricted diets. Specifically, choline's support of remethylation is expected to expand the quantity of methionine that is available for protein incorporation and transsulfuration by shrinking the quantity needed to sustain transmethylation (Figure 1). In addition to directly supporting homocysteine remethylation, methyl donors also interact with the four B vitamins

that sustain one-carbon metabolism: pyridoxine, folate, cobalamin, and riboflavin. Established human examples of cross-talk between these B vitamins and methyl donors include the sparing of cobalamin by choline,¹⁷⁹ sparing of betaine by folate,¹⁸⁰ and seasonal switching between folate and betaine dependent remethylation pathways.⁷⁹ The variable status of cobalamin, which is sometimes reduced in kwashiorkor, has been well described.^{181–184} However, a more comprehensive understanding of the interactions between B-vitamins, methyl donors, and methionine is needed. The likelihood that one-carbon nutrient cross-talk influences methionine requirements for undernourished children raises practical questions. For example: *do methyl donors spare methionine in children who consume little methionine?* If so, fortifying meager diets with methyl donors may reduce risk for kwashiorkor. This possibility is suggested by the fact that supplementation with choline, a potent source of methyl groups, prevents two of kwashiorkor's distinguishing features in animal models of undernutrition, liver steatosis and edema.^{22,185–188} One-carbon metabolism disturbances may also participate in the pathogenesis of kwashiorkor's characteristic skin changes. The hypothesis that methionine deficiency contributes to the pathogenesis of skin disturbances in kwashiorkor by limiting the synthesis of epidermal sulfated glycosaminoglycans¹⁷² has been reviewed elsewhere.¹⁸⁹ A topic with clinical immediacy is the need to develop a better understanding of the observed association between one-carbon dysfunction and mortality. Immune dysfunction in malnutrition is associated with increased risk for invasive bacterial infections^{190–192} and death.^{193,194} Five of the six confirmed deaths in this study occurred in children with marasmic-kwashiorkor. This observation corresponds with the findings of larger studies, which consistently demonstrate higher mortality in marasmic-kwashiorkor.^{61,63} One-carbon metabolism supports multiple elements of the immune system, including T cell proliferation, antibody production, and gut barrier integrity.^{48,92,195–197} Our observation that more severe one-carbon disturbances in marasmic-kwashiorkor were associated with a trend of higher mortality is consistent with the hypothesis that one-carbon metabolism dysfunction increases risk for immune dysfunction in malnutrition.

In summary, the findings of this study are relevant for considering the pathogenesis of kwashiorkor, a poorly understood and often lethal syndrome of childhood malnutrition. We observed that kwashiorkor is distinguished from marasmus by numerous one-carbon metabolite differences. The character of these differences suggests that kwashiorkor is a nutritional syndrome of one-carbon metabolism dysfunction. One-carbon metabolism appears to offer a molecular grammar for harmonizing kwashiorkor's risk factors and disturbances into a unified disease model. The mechanistic

complexities implied by this concept are balanced by a simple fact. Kwashiorkor only happens to children who eat meager diets. Cecily Williams was not wrong: kwashiorkor is fundamentally a problem of inadequate nutrition.¹⁹⁸ Inadequate intake of certain one-carbon nutrients may increase risk for kwashiorkor. The findings of this study implicate methionine deficiency in particular. Clinical trials are needed to test the hypothesis that kwashiorkor can be prevented by fortifying monotonous cereal-based diets with methionine, in combination with nutrients that support efficient methionine use, such as choline. Practical implications abound for the millions of children who are at risk for kwashiorkor and its often-lethal consequences.

Contributors

TM, FJ, and MM designed the investigation. TM, BH, LB, KS, SA, and GN conducted the field portions of this investigation. TB and EA conducted laboratory-based analyses. TM, MM, FJ, KM, NH, and TB participated in the design and execution of this investigation while also contributing essential staff, equipment, and materials. TM, KK, KS, and FJ conducted statistical analyses. TM, KK, KS, MM, and FJ verified the data underlying these observations. TM wrote the paper with contributions from all authors. TM and FJ have primary responsibility for this manuscript.

Disclaimers

The content presented here is the responsibility of the authors and does not necessarily represent the views of the NIH, the United States Department of Agriculture (USDA), the Children's Nutrition Research Center, the University of Malawi College of Medicine, Washington University in St. Louis School of Medicine, Baylor Scott and White Health, or Baylor College of Medicine.

Data sharing statement

Anonymized data underlying the findings described here have been posted to *Mendeley* (DOI: 10.17632/3s2h2fp4v8.1), a publicly accessible online repository.

Declaration of Interests

The authors have no conflicts or interests to disclose.

Sources of funding

This investigation was supported by the American College of Gastroenterology, the Hickey Family Foundation, NICHD: T32-HD071839-05, and the following USDA/ARS grants administered by the Children's Nutrition Research Center: 6250-51000-051-00D-I, 58-3092-5-001, 25-3471-5-302, 58-3092-5-00, and 3092-51000-057

Acknowledgments

We are grateful to the patients and families who participated in this study. Likewise, this work was made possible by numerous volunteers, nurses, health assistants, and laboratory staff. Adam Gillum assisted by contributing illustrations. José Mato PhD and Indi Trehan MD assisted in the conceptualization of this investigation. This investigation was supported by the Hickey Family Foundation, the American College of Gastroenterology, the NICHD, and by the Children's Nutrition Research Center, a USDA/ARS institution.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103791.

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