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One-carbon metabolism in children with marasmus and kwashiorkor

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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 µmol/L (± 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.

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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

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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 consistentcy of this pattern indicates that kwashiorkor is fundamentally a problem of poor nutrition. The first formal description of kwashiorkor[12] 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-

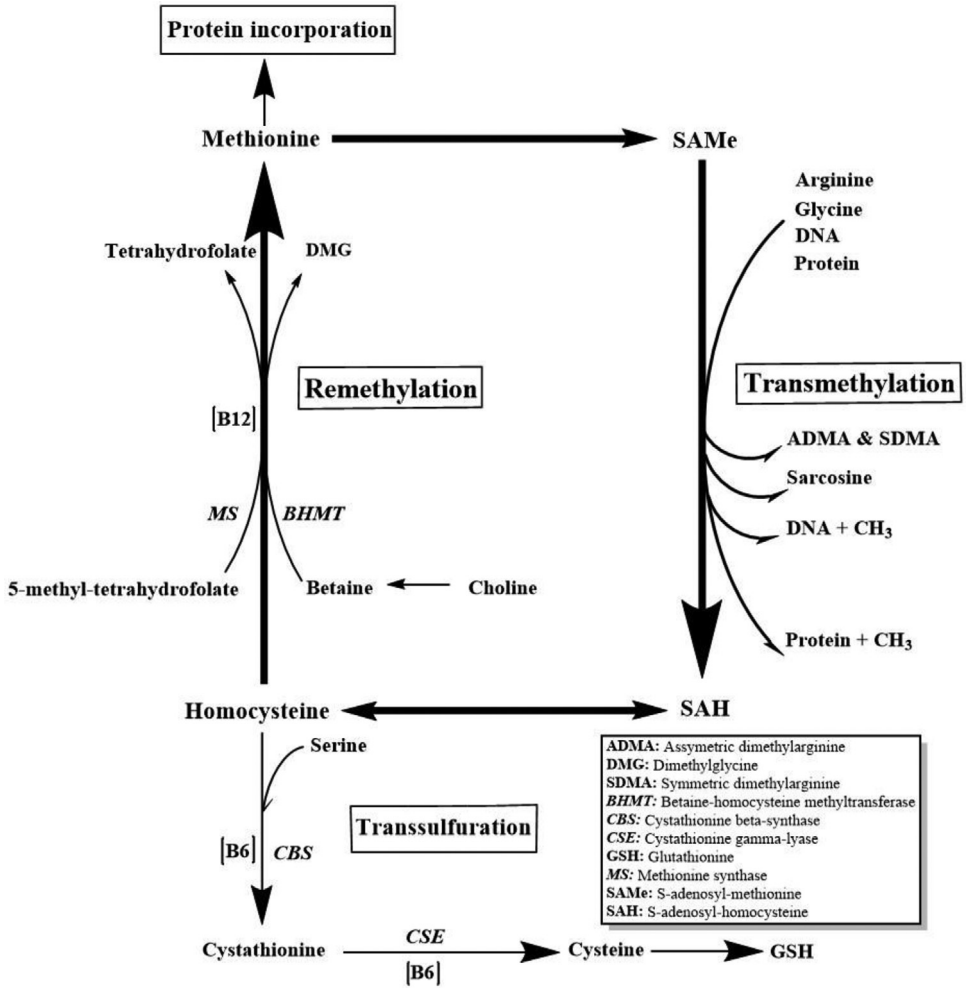

Figure 1. One-carbon metabolism schematic, adapted with permission.[170]

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) $< 11{\cdot}5$ 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 $< 12{\cdot}5$ 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, ($\sim$ 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 & anthropometry[1]					
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 outcome[7]					
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. $P > 0{\cdot}05$).
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. $P > 0{\cdot}05$), 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.

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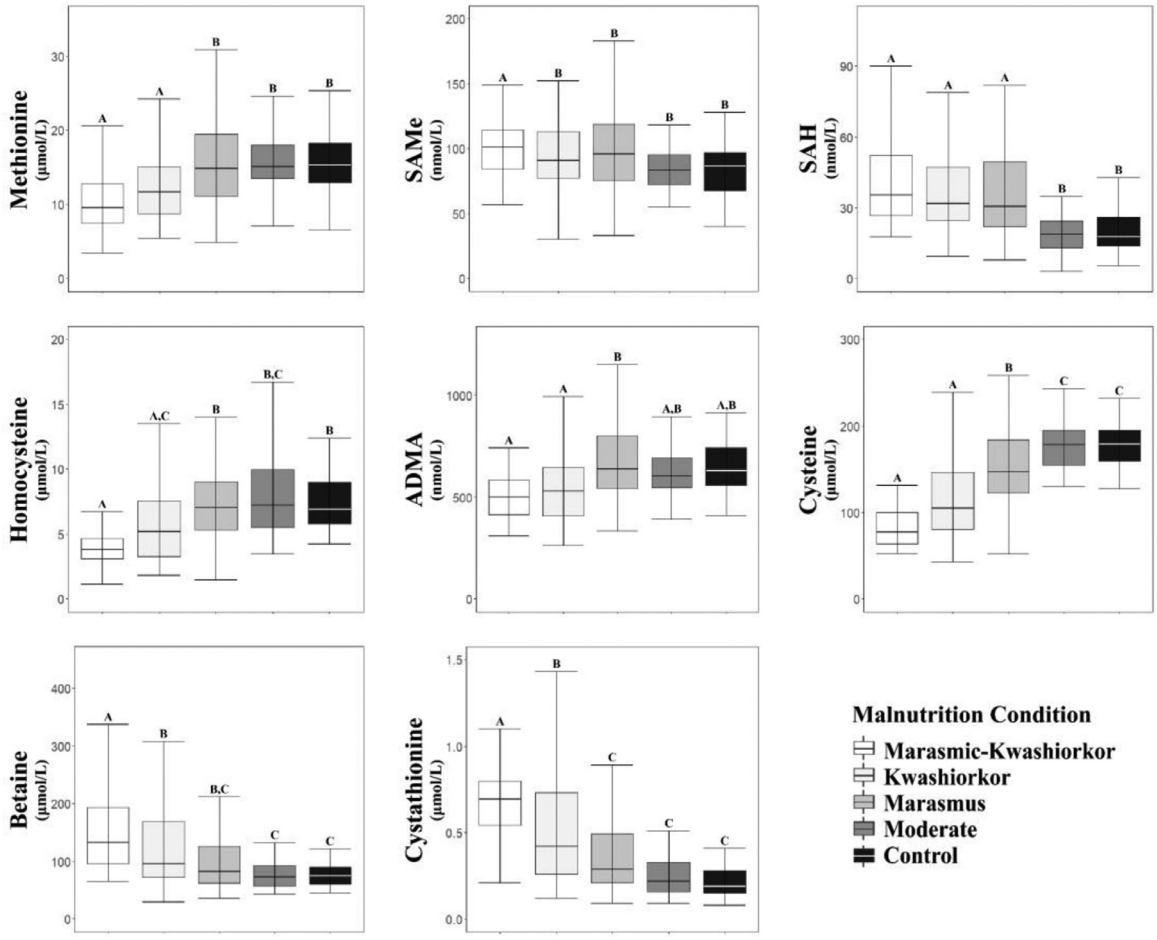

Figure 2. One-carbon metabolites in serum: Subject groups are reflected by gray-scale differences according to the legend: kwashiorkor (N = 94), marasmic-kwashiorkor (43), marasmus (N = 118), moderate acute malnutrition (N = 56) controls (N = 46). Box plots depict serum concentrations (y axis) of methionine, 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 $P > 0{\cdot}05$), 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 $\geq$ 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

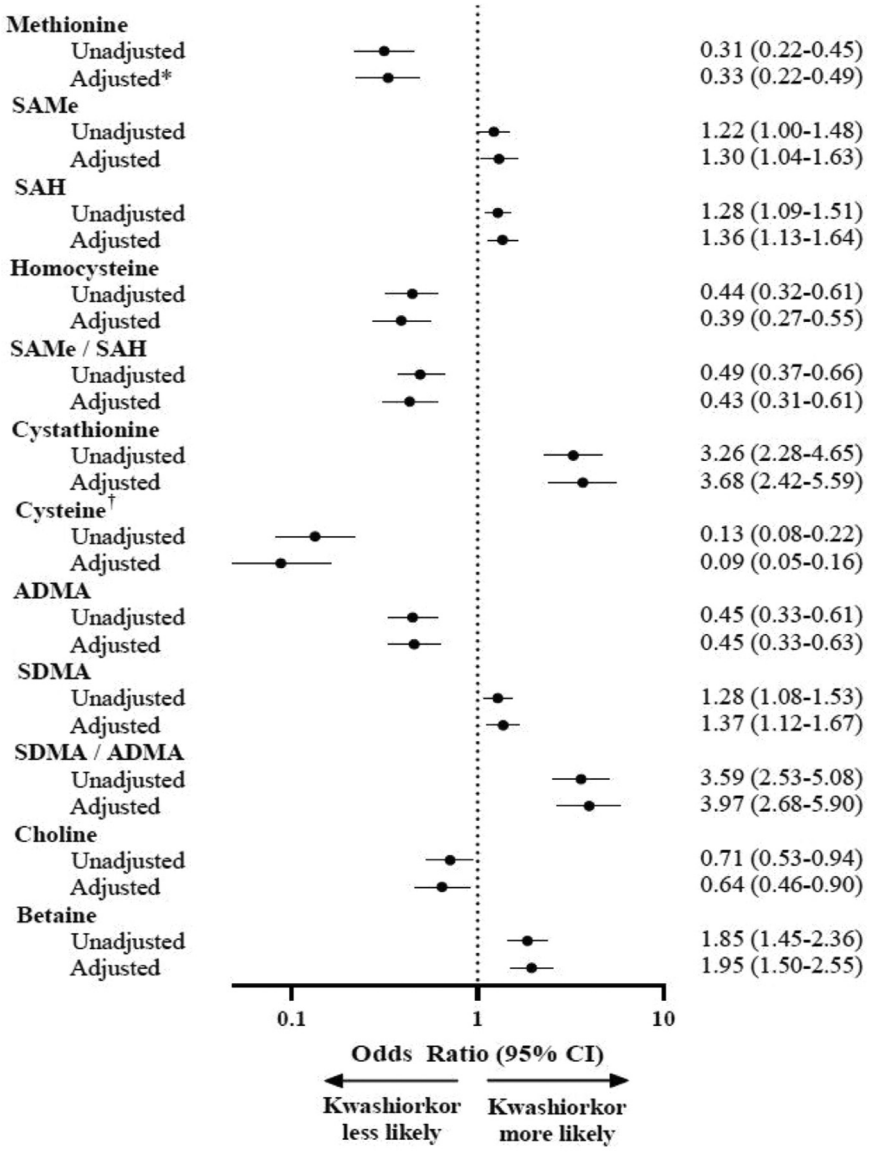

Figure 3. Covariate regression analysis: Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression. Depicted ORs and CIs reflect interquartile range effects for the prediction of kwashiorkor (including marasmic-kwashiorkor), before and after adjusting for mean upper 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). † 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 distritubted asymmetrically across participant groups

Feature	1CNDDs	Kwashiorkor
Organ changes		
Liver steatosis	↑[24]	↑[88]
Pancreatic atrophy	↑[89]	↑[88]
Exocrine pancreas *fxn.*	↓[90]	↓[91]
Intestinal thickness	↓[48,92]	↓[93]
Intestinal permeability	↑[94]	↑[95]
Intestinal inflammation	↑[96]	↑[97]
Skin disturbances	↑[98,99]	↑[100]
Cellular immune *fxn.*	↓[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 ($P \leq 0{\cdot}0025$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 ($P < 0{\cdot}0001$) 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 ($P \leq 0{\cdot}02$). 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 transmethylase 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 ($P = 0{\cdot}047$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 ($P < 0{\cdot}0001$) and marasmic-kwashiorkor ($P = 0{\cdot}00032$), relative to marasmus (Figure 2 and Table 2). Cysteine was also lower in kwashiorkor ($P < 0{\cdot}0001$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 ($P < 0{\cdot}01$) and methionine ($P < 0{\cdot}01$). 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 ($P \leq 0{\cdot}012$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 kwasiorkor.[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 ($P = 0{\cdot}034$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 ($P < 0{\cdot}0001$). This has been reported previously.[55,56] Cysteine was well correlated with homocysteine ($P < 0{\cdot}01$) 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 ($P \leq 0{\cdot}0019$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$) 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 ($P < 0{\cdot}05$), 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 $< 0{\cdot}01$.

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 ($P < 0{\cdot}0001$) and marasmic-kwashiorkor ($P < 0{\cdot}0001$), 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 downregulated 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 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 transmethylation activity in kwashiorkor[57] support the hypothesis that PEMT activity is suppressed in kwashiorkor, a disturbance that is expected to increases 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 malnutritrion.[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 glycosaminoglycans[172] 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.

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Supplementary materials

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

References

1 WHO Child Growth Standards and the Identification of Severe Acute Malnutrition in Infants and Children: A Joint Statement by the World Health Organization and the United Nations Children's Fund [Internet]. Geneva: World Health Organization; 2009. [cited 2016 Sep 28]. (WHO Guidelines Approved by the Guidelines Review Committee). Available from: http://www.ncbi.nlm.nih.gov/books/NBK200775/.
2 Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet Lond Engl* 2013;**382**(9890):427–51.
3 Frison S, Checchi F, Kerac M. Omitting edema measurement: how much acute malnutrition are we missing? *Am J Clin Nutr* 2015;**102**(5):1176–81.
4 Briend A. Kwashiorkor: still an enigma-the search must go on. CMAM Forum Technical Brief [Internet]; [cited 2016 Jun 30]. Available from: http://fr.cmamforum.org/Pool/Resources/Kwashiorkor,-still-an-enigma-CMAM-Forum-Dec-2014.pdf.
5 Manary MJ, Heikens GT, Golden M. Kwashiorkor: more hypothesis testing is needed to understand the aetiology of oedema. *Malawi Med J J Med Assoc Malawi* 2009;**21**(3):106–7.
6 Kismul H, Van den Broeck J, Lunde TM. Diet and kwashiorkor: a prospective study from rural DR Congo. *PeerJ* 2014;**2**:e350.
7 Sullivan J, Ndekha M, Maker D, Hotz C, Manary MJ. The quality of the diet in Malawian children with kwashiorkor and marasmus. *Matern Child Nutr* 2006;**2**(2):114–22.
8 Liu T, Howard RM, Mancini AJ, Weston WL, Paller AS, Drolet BA, et al. Kwashiorkor in the United States: fad diets, perceived and true milk allergy, and nutritional ignorance. *Arch Dermatol* 2001;**137**(5):630–6.
9 Tierney EP, Sage RJ, Shwayder T. Kwashiorkor from a severe dietary restriction in an 8-month infant in suburban Detroit, Michigan: case report and review of the literature. *Int J Dermatol* 2010;**49**(5):500–6.
10 Mori F, Serranti D, Barni S, Pucci N, Rossi ME, de Martino M, et al. A kwashiorkor case due to the use of an exclusive rice milk diet to treat atopic dermatitis. *Nutr J* 2015;**14**:83.
11 Carvalho NF, Kenney RD, Carrington PH, Hall DE. Severe nutritional deficiencies in toddlers resulting from health food milk alternatives. *Pediatrics* 2001;**107**(4):E46.
12 Williams CD. A nutritional disease of childhood associated with a maize diet. *Arch Dis Child* 1933;**8**(48):423–33.
13 Trowell HC. Pellagra in African children. *Arch Dis Child* 1937;**12**(70):193–212.
14 Waterlow JC. Kwashiorkor revisited: the pathogenesis of oedema in kwashiorkor and its significance. *Trans R Soc Trop Med Hyg* 1984;**78**(4):436–41.
15 Dean RFA. The treatment of kwashiorkor with milk and vegetable proteins. *Br Med J* 1952;**2**(4788):791–6.
16 Thompson MD. Comparison of Milk and Soya Beans in the treatment of kwashiorkor in Uganda. *Br Med J* 1955;**2**(4952):1366–9.

17 Morlese JF, Forrester T, Badaloo A, Del Rosario M, Frazer M, Jahoor F. Albumin kinetics in edematous and nonedematous protein-energy malnourished children. *Am J Clin Nutr* 1996;**64**(6):952–9.
18 Golden MH, Golden BE, Jackson AA. Albumin and nutritional oedema. *Lancet Lond Engl* 1980;**1**(8160):114–6.
19 Richardson BD, Du Plessis JP, Rose EF. Serum albumin and protein energy malnutrition in black preschool children in Transkei. *South Afr Med J Suid Afr Tydskr Vir Geneeskd* 1979;**55**(4):113–4.
20 Montgomery RD. The relation of oedema to serum protein and pseudocholinesterase levels in the malnourished infant. *Arch Dis Child* 1963;**38**(200):343–8.
21 Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut microbiomes of Malawian Twin Pairs discordant for kwashiorkor. *Science* 2013;**339**(6119):548–54.
22 Alexander HD, Engel RW. The Importance of choline in the prevention of nutritional edema in rats fed low-protein diets three figures. *J Nutr* 1952;**47**(3):361–74.
23 Caudill MA, Wang JC, Melnyk S, Pogribny IP, Jernigan S, Collins MD, et al. Intracellular S-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. *J Nutr* 2001;**131**(11):2811–8.
24 Caballero F, Fernández A, Matías N, Martínez L, Fucho R, Elena M, et al. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. *J Biol Chem* 2010;**285**(24):18528–36.
25 Ducros V, Belva-Besnet H, Casetta B, Favier A. A robust liquid chromatography tandem mass spectrometry method for total plasma homocysteine determination in clinical practice. *Clin Chem Lab Med* 2006;**44**(8):987–90.
26 Arning E, Bottiglieri T. Quantitation of S-Adenosylmethionine and S-adenosylhomocysteine in plasma using liquid chromatography-electrospray tandem mass spectrometry. *Methods Mol Biol Clifton NJ* 2016;**1378**:255–62.
27 Inoue-Choi M, Nelson HH, Robien K, Arning E, Bottiglieri T, Koh WP, et al. One-carbon metabolism nutrient status and plasma S-adenosylmethionine concentrations in middle-aged and older Chinese in Singapore. *Int J Mol Epidemiol Genet* 2012;**3**(2):160–73.
28 Butler LM, Arning E, Wang R, Bottiglieri T, Govindarajan S, Gao YT, et al. Prediagnostic levels of serum one-carbon metabolites and risk of hepatocellular carcinoma. *Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol* 2013;**22**(10):1884–93.
29 Semba RD, Shardell M, Sakr Ashour FA, Moaddel R, Trehan I, Maleta KM, et al. Child stunting is associated with low circulating essential amino acids. *EBioMedicine* 2016;**6**:246–52.
30 Di Giovanni V, Bourdon C, Wang DX, Seshadri S, Senga E, Versloot CJ, et al. Metabolomic changes in serum of children with different clinical diagnoses of malnutrition. *J Nutr* 2016;**146**(12):2436–44.
31 Wilke C.O. ggridges: Ridgeline Plots in "ggplot2" [Internet]. 2021 [cited 2021 Nov 17]. Available from: https://CRAN.R-project.org/package=ggridges
32 Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 1952;**47**(260):583–621.
33 Jr FEH. rms: Regression Modeling Strategies [Internet]. 2021 [cited 2021 Nov 17]. Available from: https://CRAN.R-project.org/package=rms
34 Pearson K. Notes on the history of correlation. *Biometrika* 1920;**13**(1):25–45.
35 Shivapurkar N, Poirier LA. Tissue levels of S-adenosylmethionine and S-adenosylhomocysteine in rats fed methyl-deficient, amino acid-defined diets for one to five weeks. *Carcinogenesis* 1983;**4**(8):1051–7.
36 Yeo EJ, Wagner C. Purification and properties of pancreatic glycine N-methyltransferase. *J Biol Chem* 1992;**267**(34):24669–74.
37 Luka Z, Capdevila A, Mato JM, Wagner C. A glycine N-methyltransferase knockout mouse model for humans with deficiency of this enzyme. *Transgenic Res* 2006;**15**(3):393–7.
38 Deguchi T, Barchas J. Inhibition of transmethylations of biogenic amines by S-adenosylhomocysteine enhancement of transmethylation by adenosylhomocysteinase. *J Biol Chem* 1971;**246**(10):3175–81.
39 Duerre JA, Briske-Anderson M. Effect of adenosine metabolites on methyltransferase reactions in isolated rat livers. *Biochim Biophys Acta BBA Gen Subj* 1981;**678**(2):275–82.
40 Tryndyak VP, Han T, Muskhelishvili L, Fuscoe JC, Ross SA, Beland FA, et al. Coupling global methylation and gene expression profiles reveal key pathophysiological events in liver injury induced by a methyl-deficient diet. *Mol Nutr Food Res* 2011;**55**(3):411–8.
41 Rowling MJ, McMullen MH, Chipman DC, Schalinske KL. Hepatic glycine N-methyltransferase is up-regulated by excess dietary methionine in rats. *J Nutr* 2002;**132**(9):2545–50.
42 Cook RJ, Horne DW, Wagner C. Effect of dietary methyl group deficiency on one-carbon metabolism in rats. *J Nutr* 1989;**119**(4):612–7.
43 Renwick SB, Snell K, Baumann U. The crystal structure of human cytosolic serine hydroxymethyltransferase: a target for cancer chemotherapy. *Struct Lond Engl* 1998;**6**(9):1105–16.
44 Innis SM, Hasman D. Evidence of choline depletion and reduced betaine and dimethylglycine with increased homocysteine in plasma of children with cystic fibrosis. *J Nutr* 2006;**136**(8):2226–31.
45 Di Pasqua LG, Berardo C, Rizzo V, Richelmi P, Croce AC, Vairetti M, et al. MCD diet-induced steatohepatitis is associated with alterations in asymmetric dimethylarginine (ADMA) and its transporters. *Mol Cell Biochem* 2016;**419**(1–2):147–55.
46 Elshorbagy AK, Valdivia-Garcia M, Refsum H, Smith AD, Mattocks DAL, Perrone CE. Sulfur amino acids in methionine-restricted rats: hyperhomocysteinemia. *Nutrition* 2010;**26**(11):1201–4.
47 Riedijk MA, Stoll B, Chacko S, Schierbeek H, Sunehag AL, van Goudoever JB, et al. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. *Proc Natl Acad Sci U S A.* 2007;**104**(9):3408–13.
48 Bauchart-Thevret C, Stoll B, Chacko S, Burrin DG. Sulfur amino acid deficiency upregulates intestinal methionine cycle activity and suppresses epithelial growth in neonatal pigs. *Am J Physiol Endocrinol Metab* 2009;**296**(6):E1239–50.
49 Gary JD, Clarke S. RNA and protein interactions modulated by protein arginine methylation. *Prog Nucleic Acid Res Mol Biol* 1998;**61**:65–131.
50 Brooks ER, Langman CB, Wang S, Price HE, Hodges AL, Darling L, et al. Methylated arginine derivatives in children and adolescents with chronic kidney disease. *Pediatr Nephrol Berl Ger* 2009;**24**(1):129–34.
51 Fleck C, Janz A, Schweitzer F, Karge E, Schwertfeger M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in renal failure patients. *Kidney Int* 2001 Feb 1;**59**:S14–8.
52 Davies JN. Renal lesions in kwashiorkor. *Am J Clin Nutr* 1956;**4**(5):539–42.
53 Golden M, Ramdath DD, Brooks S, Taylor E. Effacement of glomerular foot processes in kwashiorkor. *Lancet* 1990;**336**(8729):1472–4.
54 Klahr S, Alleyne GA. Effects of chronic protein-calorie malnutrition on the kidney. *Kidney Int* 1973;**3**(3):129–41.
55 Jackson AA. Blood glutathione in severe malnutrition in childhood. *Trans R Soc Trop Med Hyg* 1986;**80**(6):911–3.
56 Jackson AA. Glutathione in kwashiorkor. *Am J Clin Nutr* 2002;**76**(3):495–6.
57 Jahoor F, Badaloo A, Reid M, Forrester T. Sulfur amino acid metabolism in children with severe childhood undernutrition: methionine kinetics. *Am J Clin Nutr* 2006;**84**(6):1400–5.
58 Augoustides-Savvopoulou P, Luka Z, Karyda S, Stabler SP, Allen RH, Patsiaoura K, et al. Glycine N -methyltransferase deficiency: a new patient with a novel mutation. *J Inherit Metab Dis* 2003;**26**(8):745–59.
59 Barić I, Fumić K, Glenn B, Ćuk M, Schulze A, Finkelstein JD, et al. S-adenosylhomocysteine hydrolase deficiency in a human: a genetic disorder of methionine metabolism. *Proc Natl Acad Sci* 2004;**101**(12):4234–9.
60 Stabler S, Lindenbaum J, Savage D, Allen R. Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. *Blood* 1993;**81**(12):3404–13.
61 Lapidus N, Minetti A, Djibo A, Guerin PJ, Hustache S, Gaboulaud V, et al. Mortality risk among children admitted in a large-scale nutritional program in Niger, 2006. *PLoS One* 2009;**4**(1):e4313.

62 Gernaat HBPE, Dechering WHJC, Voorhoeve HWA. Mortality in severe protein-energy malnutrition at nchelenge, Zambia. *J Trop Pediatr* 1998;**44**(4):211–7.
63 Trehan I, Goldbach HS, LaGrone LN, Meuli GJ, Wang RJ, Maleta KM, et al. Antibiotics as part of the management of severe acute malnutrition. *N Engl J Med* 2013;**368**(5):425–35.
64 Feigin RD, Klainer AS, Beisel WR, Hornick RB. Whole-blood amino acids in experimentally induced typhoid fever in man. *N Engl J Med* 1968;**278**(6):293–8.
65 Feigin RD, Dangerfield HG. Whole blood amino acid changes following respiratory-acquired Pasteurella tularensis infection in man. *J Infect Dis* 1967;**117**(4):346–51.
66 Feigin RD, Haymond MW. Circadian periodicity of blood amino acids in the neonate. *Pediatrics* 1970;**45**(5):782–91.
67 Feigin RD, Klainer AS, Beisel WR. Circadian periodicity of blood amino-acids in adult men. *Nature* 1967;**215**(5100):512–4.
68 Feigin RD, Klainer AS, Beisel WR. Factors affecting circadian periodicity of blood amino acids in man. *Metabolism* 1968;**17**(9):764–75.
69 Jahoor F. Effects of decreased availability of sulfur amino acids in severe childhood undernutrition. *Nutr Rev* 2012;**70**(3):176–87.
70 Jahoor F, Badaloo A, Reid M, Forrester T. Protein kinetic differences between children with edematous and nonedematous severe childhood undernutrition in the fed and postabsorptive states. *Am J Clin Nutr* 2005;**82**(4):792–800.
71 Reid M, Badaloo A, Forrester T, Morlese JF, Frazer M, Heird WC, et al. *In vivo* rates of erythrocyte glutathione synthesis in children with severe protein-energy malnutrition. *Am J Physiol Endocrinol Metab* 2000;**278**(3):E405–12.
72 Badaloo A, Reid M, Forrester T, Heird WC, Jahoor F. Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. *Am J Clin Nutr* 2002;**76**(3):646–52.
73 Arroyave G, Wilson D, Funes CD. BéHAR Mois. The free amino acids in blood plasma of children with kwashiorkor and marasmus. *Am J Clin Nutr* 1962;**11**(5):517–24.
74 Holt LE, Snyderman S, Norton P, Roitman E, Finch J. The plasma aminogram in kwashiorkor. *Lancet* 1963;**282**(7322):1343–8.
75 Ittyerah TR, Pereira SM, Dumm ME. Serum amino acids of children on high and low protein intakes. *Am J Clin Nutr* 1965;**17**:11–4.
76 Tryndyak VP, Han T, Muskhelishvili L, Fuscoe JC, Ross SA, Beland FA, et al. Coupling global methylation and gene expression profiles reveal key pathophysiological events in liver injury induced by a methyl-deficient diet. *Mol Nutr Food Res* 2011;**55**(3):411–8.
77 Nguyen D, Hsu JW, Jahoor F, Sekhar RV. Effect of increasing glutathione with cysteine and glycine supplementation on mitochondrial fuel oxidation, insulin sensitivity, and body composition in older HIV-infected patients. *J Clin Endocrinol Metab* 2014;**99**(1):169–77.
78 Jiang X, Yan J, West AA, Perry CA, Malysheva OV, Devapatla S, et al. Maternal choline intake alters the epigenetic state of fetal cortisol-regulating genes in humans. *FASEB J Off Publ Fed Am Soc Exp Biol* 2012;**26**(8):3563–74.
79 Dominguez-Salas P, Moore SE, Cole D, da Costa K-A, Cox SE, Dyer RA, et al. DNA methylation potential: dietary intake and blood concentrations of one-carbon metabolites and cofactors in rural African women. *Am J Clin Nutr* 2013;**97**(6):1217–27.
80 Schulze KV, Swaminathan S, Howell S, Jajoo A, Lie NC, Brown O, et al. Edematous severe acute malnutrition is characterized by hypomethylation of DNA. *Nat Commun* 2019;**10**(1):1–13.
81 Niculescu MD, Zeisel SH. Diet, methyl donors and dna methylation: interactions between dietary folate, methionine and choline. *J Nutr* 2002;**132**(8):2333S–5S.
82 Manary MJ, Leeuwenburgh C, Heinecke JW. Increased oxidative stress in kwashiorkor. *J Pediatr* 2000;**137**(3):421–4.
83 Golden MH, Ramdath D. Free radicals in the pathogenesis of kwashiorkor. *Proc Nutr Soc* 1987;**46**(1):53–68.
84 Sekhar RV, Patel SG, Guthikonda AP, Reid M, Balasubramanyam A, Taffet GE, et al. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation1-4. *Am J Clin Nutr* 2011;**94**(3):847–53.
85 Beau JP, Sy A. [Vitamin e supplementation in Senegalese children with kwashiorkor]. *Sante Montrouge Fr* 1996;**6**(4):209–12.
86 Ciliberto H, Ciliberto M, Briend A, Ashorn P, Bier D, Manary M. Antioxidant supplementation for the prevention of kwashiorkor in Malawian children: randomised, double blind, placebo controlled trial. *BMJ* 2005;**330**(7500):1109.
87 Finkelstein JD, Martin JJ. Homocysteine. *Int J Biochem Cell Biol* 2000;**32**(4):385–9.
88 Davies JN. The pathology of dietary liver disease in tropical Africa. *Ann N Y Acad Sci* 1954;**57**(6):714–21.
89 Lombardi B, Rao NK. Acute hemorrhagic pancreatic necrosis in mice. Influence of the age and sex of the animals and of dietary ethionine, choline, methionine, and adenine sulfate. *Am J Pathol* 1975;**81**(1):87–100.
90 Gilliland L, Steer ML. Effects of ethionine on digestive enzyme synthesis and discharge by mouse pancreas. *Am J Physiol* 1980;**239**(5):G418–26.
91 Sauniere JF, Sarles H, Attia Y, Lombardo A, Yoman TN, Laugier R, et al. Exocrine pancreatic function of children from the Ivory Coast compared to French children. effect of kwashiorkor. *Dig Dis Sci* 1986;**31**(5):481–6.
92 Bressenot A, Pooya S, Bossenmeyer-Pourie C, Gauchotte G, Germain A, Chevaux J-B, et al. Methyl donor deficiency affects small-intestinal differentiation and barrier function in rats. *Br J Nutr* 2013;**109**(4):667–77.
93 Stransky E. Nutritional Dystrophy. *Br Med J* 1950;**1**(4666):1370–1.
94 Longo L, Tonin Ferrari J, Rampelotto PH, Hirata Dellavia G, Pasqualotto A, P Oliveira C, et al. Gut dysbiosis and increased intestinal permeability drive microRNAs, NLRP-3 inflammasome and liver fibrosis in a nutritional model of non-alcoholic steatohepatitis in adult male sprague dawley rats. *Clin Exp Gastroenterol* 2020;**13**:351–68.
95 Brewster DR, Manary MJ, Menzies IS, O'Loughlin EV, Henry RL. Intestinal permeability in kwashiorkor. *Arch Dis Child* 1997;**76**(3):236–41.
96 Matthews DR, Li H, Zhou J, Li Q, Glaser S, Francis H, et al. Methionine- and choline-deficient diet-induced nonalcoholic steatohepatitis is associated with increased intestinal inflammation. *Am J Pathol* 2021;**191**(10):1743–53.
97 Attia S, Versloot CJ, Voskuijl W, van Vliet SJ, Di Giovanni V, Zhang L, et al. Mortality in children with complicated severe acute malnutrition is related to intestinal and systemic inflammation: an observational cohort study. *Am J Clin Nutr* 2016;**104**(5):1441–9.
98 Hirakawa DA, Baker DH. Sulfur amino acid nutrition of the growing puppy: determination of dietary requirements for methionine and cystine. *Nutr Res* 1985;**5**(6):631–42.
99 Strieker MJ, Werner A, Morris JG, Rogers QR. Excess dietary cystine intensifies the adverse effect of a methionine deficiency in the cat. *J Anim Physiol Anim Nutr* 2006;**90**(11–12):440–5.
100 Heilskov S, Rytter MJH, Vestergaard C, Briend A, Babirekere E, Deleuran MS. Dermatosis in children with oedematous malnutrition (Kwashiorkor): a review of the literature. *J Eur Acad Dermatol Venereol* 2014;**28**(8):995–1001.
101 Courrèges MC, Benencia F, Uceda A, Monserrat AJ. Effect of dietary choline deficiency on immunocompetence in Wistar rats. *Nutr Res* 2003;**23**(4):519–26.
102 Geefhuysen J, Rosen EU, Katz J, Ipp T, Metz J. Impaired cellular immunity in kwashiorkor with improvement after therapy. *Br Med J* 1971;**4**(5786):527–9.
103 Alexander HD, Sauberlich HE. The influence of lipotropic factors on the prevention of nutritional edema in the rat. *J Nutr* 1957;**61**(3):329–41.
104 Robinson JL, Bartlett RK, Harding SV, Randell EW, Brunton JA, Bertolo RF. Dietary methyl donors affect *in vivo* methionine partitioning between transmethylation and protein synthesis in the neonatal piglet. *Amino Acids* 2016;**48**(12):2821–30.
105 Wainfan E, Dizik M, Stender M, Christman JK. Rapid appearance of hypomethylated DNA in livers of rats fed cancer-promoting, methyl-deficient diets. *Cancer Res* 1989;**49**(15):4094–7.
106 Corredor C, Mansbach C, Bressler R. Carnitine depletion in the choline-deficient state. *Biochim Biophys Acta BBA Lipids Lipid Metab* 1967;**144**(2):366–74.
107 Hammond KD, Tobiansky R, Abrahams OL. Serum carnitine in children with kwashiorkor. *Ann Trop Paediatr* 1987;**7**(3):214–6.
108 Veteläinen R, Van Vliet A, Van Gulik TM. Essential pathogenic and metabolic differences in steatosis induced by choline or methione-choline deficient diets in a rat model. *J Gastroenterol Hepatol* 2007;**22**(9):1526–33.
109 Kurup GM, Kurup PA. Metabolism of glycosaminoglycans in rats during methionine deficiency and administration of excess methionine. *J Biosci* 1982;**4**(1):95–104.

110 Faubion WA, Camilleri M, Murray JA, Kelly P, Amadi B, Kosek MN, et al. Improving the detection of environmental enteric dysfunction: a lactulose, rhamnose assay of intestinal permeability in children aged under 5 years exposed to poor sanitation and hygiene. *BMJ Glob Health* 2016;**1**(1):e000066.

111 Sos J, Kemeny T. On the mode of action of methionine deficiency. *Acta Physiol Acad Sci Hung* 1960;**17**:355–60.

112 Bandsma RHJ, Voskuijl W, Chimwezi E, Fegan G, Briend A, Thitiri J, et al. A reduced-carbohydrate and lactose-free formulation for stabilization among hospitalized children with severe acute malnutrition: a double-blind, randomized controlled trial. *PLoS Med* 2019;**16**(2):1–19. [cited 2021 Apr 9] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6390989/.

113 Pooya S, Blaise S, Moreno Garcia M, Giudicelli J, Alberto JM, Guéant-Rodriguez RM, et al. Methyl donor deficiency impairs fatty acid oxidation through PGC-1α hypomethylation and decreased ER-α, ERR-α, and HNF-4α in the rat liver. *J Hepatol* 2012;**57**(2):344–51.

114 Doherty JF, Golden MH, Brooks SE. Peroxisomes and the fatty liver of malnutrition: an hypothesis. *Am J Clin Nutr* 1991;**54**(4):674–7.

115 Machado MV, Michelotti GA, Xie G, Almeida Pereira T, de Almeida TP, Boursier J, et al. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PLoS One* 2015;**10**(5):e0127991.

116 Truswell AS, Hansen JDL, Watson CE, Wannenburg P. Relation of serum lipids and lipoproteins to fatty liver in kwashiorkor. *Am J Clin Nutr* 1969;**22**(5):568–76.

117 Park HS, Jeon BH, Woo SH, Leem J, Jang JE, Cho MS, et al. Time-dependent changes in lipid metabolism in mice with methionine choline deficiency-induced fatty liver disease. *Mol Cells* 2011;**32**(6):571–7.

118 Badaloo AV, Forrester T, Reid M, Jahoor F. Lipid kinetic differences between children with kwashiorkor and those with marasmus. *Am J Clin Nutr* 2006;**83**(6):1283–8.

119 Tan KH, Meyer DJ, Ketterer B. Lipid peroxidation in choline-methionine deficiency. *Free Radic Res Commun* 1987;**3**(1–5):273–8.

120 Lenhartz H, Ndasi R, Anninos A, Böttticher D, Mayatepek E, Tetanye E, et al. The clinical manifestation of the kwashiorkor syndrome is related to increased lipid peroxidation. *J Pediatr* 1998;**132**(5):879–81.

121 Mu Y, Ogawa T, Kawada N. Reversibility of fibrosis, inflammation, and endoplasmic reticulum stress in the liver of rats fed a methionine-choline-deficient diet. *Lab Investig J Tech Methods Pathol* 2010;**90**(2):245–56.

122 Palladini G, Di Pasqua LG, Berardo C, Siciliano V, Richelmi P, Perlini S, et al. Animal models of steatosis (NAFLD) and steatohepatitis (NASH) exhibit hepatic lobe-specific gelatinases activity and oxidative stress. *Can J Gastroenterol Hepatol* 2019;**2019**:5413461.

123 Sauerwein RW, Mulder JA, Mulder L, Lowe B, Peshu N, Demacker PN, et al. Inflammatory mediators in children with protein-energy malnutrition. *Am J Clin Nutr* 1997;**65**(5):1534–9.

124 Gillman T, Gillman J. Hepatic damage in infantile pellagra: and its response to vitamin, liver and dried stomach therapy as determined by repeated liver biopsies. J Am Med Assoc. 1945 Sep 1;129(1):12–9.

125 Noga AA, Zhao Y, Vance DE. An unexpected requirement for phosphatidylethanolaminen-methyltransferase in the secretion of very low density lipoproteins. *J Biol Chem* 2002;**277**(44):42358–65.

126 Yao ZM, Vance DE. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J Biol Chem* 1988;**263**(6):2998–3004.

127 Davenport C, Yan J, Taesuwan S, Shields K, West AA, Jiang X, et al. Choline intakes exceeding recommendations during human lactation improve breast milk choline content by increasing PEMT pathway metabolites. *J Nutr Biochem* 2015;**26**(9):903–11.

128 Resseguie ME, da Costa KA, Galanko JA, Patel M, Davis IJ, Zeisel SH. Aberrant estrogen regulation of PEMT results in choline deficiency-associated liver dysfunction. *J Biol Chem* 2011;**286**(2):1649–58.

129 Song J, da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, et al. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). *FASEB J Off Publ Fed Am Soc Exp Biol* 2005;**19**(10):1266–71.

130 Nakatsuka A, Matsuyama M, Yamaguchi S, Katayama A, Eguchi J, Murakami K, et al. Insufficiency of phosphatidylethanolamine N-methyltransferase is risk for lean non-alcoholic steatohepatitis. *Sci Rep* 2016;**6**(1):21721.

131 Bruckman FS, Peters JP. The plasma proteins in relation to blood hydration: V. serum proteins and malnutritional or cachectic edema. *J Clin Invest* 1930;**8**(4):591–5.

132 Keys A, Taylor HL, Mickelsen O, Henschel A. Famine edema and the mechanism of its formation. *Science* 1946;**103**(2683):669–70.

133 Fiorotto M, Coward WA. Pathogenesis of oedema in protein-energy malnutrition: the significance of plasma colloid osmotic pressure. *Br J Nutr* 1979;**42**(1):21–31.

134 Minchiotti L, Galliano M, Caridi G, Kragh-Hansen U, Peters T. Congenital analbuminaemia: molecular defects and biochemical and clinical aspects. *Biochim Biophys Acta* 2013;**1830**(12):5494–502.

135 Curry FE, Michel CC, Phillips ME. Effect of albumin on the osmotic pressure exerted by myoglobin across capillary walls in frog mesentery. *J Physiol* 1987;**387**:69–82.

136 Michel CC, Phillips ME. Steady-state fluid filtration at different capillary pressures in perfused frog mesenteric capillaries. *J Physiol* 1987;**388**:421–35.

137 Henry CB, Duling BR. TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. *Am J Physiol Heart Circ Physiol* 2000;**279**(6):H2815–23.

138 Fleck A, Raines G, Hawker F, Trotter J, Wallace PI, Ledingham IM, et al. Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet Lond Engl* 1985;**1**(8432):781–4.

139 Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 2010;**87**(2):198–210.

140 Golden MH. Nutritional and other types of oedema, albumin, complex carbohydrates and the interstitium - a response to Malcolm Coulthard's hypothesis: oedema in kwashiorkor is caused by hypo-albuminaemia. *Paediatr Int Child Health* 2015;**35**(2):90–109.

141 Yang X, Meegan JE, Jannaway M, Coleman DC, Yuan SY. A disintegrin and metalloproteinase 15-mediated glycocalyx shedding contributes to vascular leakage during inflammation. *Cardiovasc Res* 2018;**114**(13):1752–63.

142 Stahl K, Gronski PA, Kiyan Y, Seeliger B, Bertram A, Pape T, et al. Injury to the endothelial glycocalyx in critically Ill patients with COVID-19. *Am J Respir Crit Care Med* 2020;**202**(8):1178–81.

143 van den Berg BM, Vink H, Spaan JAE. The endothelial glycocalyx protects against myocardial edema. *Circ Res* 2003;**92**(6):592–4.

144 Gouverneur M, Broekhuizen L, Meuwese M, Mooij H, Stroes E, Vink H. Sulfated glycosaminoglycans restore glycocalyx barrier properties of cultured endothelial cells in hyperglycemia. *FASEB J* 2008;**22**(S2):83.

145 Henry CB, Duling BR. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. *Am J Physiol* 1999;**277**(2):H508–14.

146 Amadi B, Fagbemi AO, Kelly P, Mwiya M, Torrente F, Salvestrini C, et al. Reduced production of sulfated glycosaminoglycans occurs in Zambian children with kwashiorkor but not marasmus. *Am J Clin Nutr* 2009;**89**(2):592–600.

147 Bistrup A, Bhakta S, Lee JK, Belov YY, Gunn MD, Zuo FR, et al. Sulfotransferases of two specificities function in the reconstitution of high endothelial cell ligands for L-selectin. *J Cell Biol* 1999;**145**(4):899–910.

148 Hoffmann L, Brauers G, Gehrmann T, Häussinger D, Mayatepek E, Schliess F, et al. Osmotic regulation of hepatic betaine metabolism. *Am J Physiol Gastrointest Liver Physiol* 2013;**304**(9):G835–46.

149 Gopalan C, Venkatachalam PS, Srikantia SG. Body composition in nutritional edema. *Metabolism* 1953;**2**:335–43.

150 Henschel A, Mickelsen O. Plasma volume and thiocyanate space in famine edema and recovery. *Am J Physiol* 1947;**150**(1):170–80.

151 Nuss ET, Tanumihardjo SA. Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. *Adv Nutr Bethesda Md* 2011;**2**(3):217–24.

152 Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* 2003;**133**(5):1302–7.

153 Kristensen KHS, Wiese M, Rytter MJH, Özçam M, Hansen LH, Namusoke H, et al. Gut microbiota in children hospitalized with oedematous and non-oedematous severe acute malnutrition in Uganda. *PLoS Negl Trop Dis* 2016;**10**(1):e0004369.

154 Salomon JB, Gordon JE, Scrimshaw NS. Studies of diarrheal disease in Central America. X. Associated chickenpox, diarrhea and kwashiorkor in a highland Guatemalan village. *Am J Trop Med Hyg* 1966;**15**(6):997–1002.

155 Forrester TE, Badaloo AV, Boyne MS, Osmond C, Thompson D, Green C, et al. Prenatal factors contribute to the emergence of kwashiorkor or marasmus in severe undernutrition: evidence for the predictive adaptation model. *PLoS One* 2012;**7**(4). [Internet] Apr 30 [cited 2020 Apr 8] Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3340401/.

156 Yajnik CS, Deshpande SS, Panchanadikar AV, Naik SS, Deshpande JA, Coyaji KJ, et al. Maternal total homocysteine concentration and neonatal size in India. *Asia Pac J Clin Nutr* 2005;**14**(2):179–81.

157 Coulter JB, Hendrickse RG, Lamplugh SM, Macfarlane SB, Moody JB, Omer MI, et al. Aflatoxins and kwashiorkor: clinical studies in Sudanese children. *Trans R Soc Trop Med Hyg* 1986;**80**(6):945–51.

158 McMillan A, Renaud JB, Burgess KMN, Orimadegun AE, Akinyinka OO, Allen SJ, et al. Aflatoxin exposure in Nigerian children with severe acute malnutrition. *Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc* 2018;**111**:356–62.

159 Fitzpatrick MC, Kurpad AV, Duggan CP, Ghosh S, Maxwell DG. Dietary intake of sulfur amino acids and risk of kwashiorkor malnutrition in eastern democratic republic of the Congo. *Am J Clin Nutr* 2021(nqab136). https://doi.org/10.1093/ajcn/nqab136. [Internet] May 8 [cited 2021 May 27].

160 Marshall KG, Howell S, Reid M, Badaloo A, Farrall M, Forrester T, et al. Glutathione S-transferase polymorphisms may be associated with risk of oedematous severe childhood malnutrition. *Br J Nutr* 2006;**96**(2):243–8.

161 Marshall KG, Howell S, Badaloo AV, Reid M, Farrall M, Forrester T, et al. Polymorphisms in genes involved in folate metabolism as risk factors for oedematous severe childhood malnutrition: a hypothesis-generating study. *Ann Trop Paediatr* 2006;**26**(2):107–14.

162 Hernandez-Vargas H, Castelino J, Silver MJ, Dominguez-Salas P, Cros M-P, Durand G, et al. Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in the Gambia. *Int J Epidemiol* 2015;**44**(4):1238–48.

163 Chen Y, Liu Y, Zhou R, Chen X, Wang C, Tan X, et al. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep* 2016;**6**:19076.

164 Kambale KJ, Ali ER, Sadiki NH, Kayembe KP, Mvumbi LG, Yandju DL, et al. Lower sulfurtransferase detoxification rates of cyanide in konzo-a tropical spastic paralysis linked to cassava cyanogenic poisoning. *Neurotoxicology* 2017;**59**:256–62.

165 Yajnik CS, Deshmukh US. Fetal programming: maternal nutrition and role of one-carbon metabolism. *Rev Endocr Metab Disord* 2012;**13**(2):121–7.

166 Tanaka T, Scheet P, Giusti B, Bandinelli S, Piras MG, Usala G, et al. Genome-wide association study of vitamin B6, vitamin B12, folate, and homocysteine blood concentrations. *Am J Hum Genet* 2009;**84**(4):477–82.

167 Manger MS, Taneja S, Strand TA, Ueland PM, Refsum H, Schneede J, et al. Poor folate status predicts persistent diarrhea in 6- to 30-month-old north Indian children. *J Nutr* 2011 Dec;**141**(12):2226–32.

168 Baumann-Dudenhoeffer AM, D'Souza AW, Tarr PI, Warner BB, Dantas G. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. *Nat Med* 2018;**24**(12):1822–9.

169 McBreairty LE, Robinson JL, Harding SV, Randell EW, Brunton JA, Bertolo RF. Betaine is as effective as folate at re-synthesizing methionine for protein synthesis during moderate methionine deficiency in piglets. *Eur J Nutr* 2016;**55**(8):2423–30.

170 Robinson JL, McBreairty LE, Randell EW, Brunton JA, Bertolo RF. Restriction of dietary methyl donors limits methionine availability and affects the partitioning of dietary methionine for creatine and phosphatidylcholine synthesis in the neonatal piglet. *J Nutr Biochem* 2016;**35**:81–6.

171 Humayun MA, Turner JM, Elango R, Rafii M, Langos V, Ball RO, et al. Minimum methionine requirement and cysteine sparing of methionine in healthy school-age children. *Am J Clin Nutr* 2006;**84**(5):1080–5.

172 Roediger WE, Waterlow J. New views on the pathogenesis of kwashiorkor: methionine and other amino acids. *J Pediatr Gastroenterol Nutr* 1995;**21**(2):130–6.

173 Robinson JL, Bertolo RF. The pediatric methionine requirement should incorporate remethylation potential and transmethylation demands. *Adv Nutr Bethesda Md* 2016;**7**(3):523–34.

174 Kroening GH, Pond WG. Methionine, choline and threonine interrelationships for growth and lipotropic action in the baby pig and Rat. *J Anim Sci* 1967;**26**(2):352–7.

175 Schutte J, De Jong J, Smink W, Pack M. Replacement value of betaine for DL-methionine in male broiler chicks. *Poult Sci* 1997;**76**(2):321–5.

176 Pillai PB, Fanatico AC, Blair ME, Emmert JL. Homocysteine remethylation in broilers fed surfeit choline or betaine and varying levels and sources of methionine from eight to twenty-two days of age. *Poult Sci* 2006;**85**(10):1729–36.

177 Zhan XA, Li JX, Xu ZR, Zhao RQ. Effects of methionine and betaine supplementation on growth performance, carcase composition and metabolism of lipids in male broilers. *Br Poult Sci* 2006;**47**(5):576–80.

178 Mudd SH, Poole JR. Labile methyl balances for normal humans on various dietary regimens. *Metabolism* 1975;**24**(6):721–35.

179 King JH, Kwan STC, Bae S, Klatt KC, Yan J, Malysheva OV, et al. Maternal choline supplementation alters vitamin B-12 status in human and murine pregnancy. *J Nutr Biochem* 2019;**72**:108210.

180 Melse-Boonstra A, Holm PI, Ueland PM, Olthof M, Clarke R, Verhoef P. Betaine concentration as a determinant of fasting total homocysteine concentrations and the effect of folic acid supplementation on betaine concentrations. *Am J Clin Nutr* 2005;**81**(6):1378–82.

181 Yaikhomba T, Poswal L, Goyal S. Assessment of iron, folate and vitamin B12 status in severe acute malnutrition. *Indian J Pediatr* 2015;**82**(6):511–4.

182 Osifo OA, Laditan AA, Parmentier Y, Gerard P, Nicolas HP. Clinical significance of serum transcobalamins in protein-energy malnutrition. *Clin Nutr Edinb Scotl* 1983;**2**(2):87–91.

183 Macdougall LG, Ross GIM. Serum vitamin B12 concentrations in kwashiorkor and marasmus. *J Pediatr* 1960;**57**(4):589–93.

184 Khalil M, Tanios A, et al. Serum and red cell folates, and serum vitamin B12 in protein calorie malnutrition. *Arch Dis Child* 1973;**48**(5):366.

185 Engel RW. Anemia and edema of chronic choline deficiency in the rat. *J Nutr* 1948;**36**(6):739–49.

186 Alexander HD, Sauberlich HE. The influence of lipotropic factors on the prevention of nutritional edema in the rat. *J Nutr* 1957;**61**(3):329–41.

187 Sauberlich HE. Studies with the use of Co60-labeled vitamin B12 on the interrelationship of choline and vitamin B12 in rats with nutritional edema. *J Nutr* 1959;**69**(3):309–17.

188 May T, Klatt KC, Smith J, Castro E, Manary M, Caudill MA, et al. Choline supplementation prevents a hallmark disturbance of kwashiorkor in weanling mice fed a maize vegetable diet: hepatic steatosis of undernutrition. *Nutrients* 2018;**10**(5).

189 Heilskov S, Rytter MJH, Vestergaard C, Briend A, Babirekere E, Deleuran MS. Dermatosis in children with oedematous malnutrition (Kwashiorkor): a review of the literature. *J Eur Acad Dermatol Venereol JEADV* 2014;**28**(8):995–1001.

190 Savino W, Dardenne M, Velloso LA. Dayse Silva-Barbosa S. The thymus is a common target in malnutrition and infection. *Br J Nutr* 2007;**98 Suppl 1**:S11–6.

191 Smythe PM, Brereton-Stiles GG, Grace HJ, Mafoyane A, Schonland M, Coovadia HM, et al. Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. *Lancet Lond Engl* 1971;**2**(7731):939–43.

192 Harland PS. Tuberculin reactions in malnourished children. *Lancet Lond Engl* 1965;**2**(7415):719–21.

193 Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens A-C, Sayadi S, et al. Massive increase, spread, and exchange of extended spectrum β-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2011;**53**(7):677–85.

194 Isaack H, Mbise RL, Hirji KF. Nosocomial bacterial infections among children with severe protein energy malnutrition. *East Afr Med J* 1992;**69**(8):433–6.

195 Ma EH, Bantug G, Griss T, Condotta S, Johnson RM, Samborska B, et al. Serine Is an Essential Metabolite for Effector T Cell Expansion. Cell Metab. 2017; 7;25(2):345–357.

196 Roy DG, Chen J, Mamane V, Ma EH, Muhire BM, Sheldon RD, et al. Methionine metabolism shapes T helper cell responses through regulation of epigenetic reprogramming. *Cell Metab* 2020;**31**(2):250–66.

197 Albrecht LV, Bui MH, De Robertis EM. Canonical Wnt is inhibited by targeting one-carbon metabolism through methotrexate or methionine deprivation. *Proc Natl Acad Sci* 2019;**116**(8):2987–95.
198 Council on foods and nutrition. *JAMA J Am Med Assoc* 1953;**153** (14):1280.
199 Gonzales GB, Njunge JM, Gichuki BM, Wen B, Ngari M, Potani I, et al. Albumin-dependent and independent mechanisms in the syndrome of kwashiorkor [Internet]. 2021 Jun [cited 2022 Jan 4] p. 2021.05.31.21257914. Available from:https://www.medrxiv.org/content/10.1101/2021.05.31.21257914v1.