One-carbon metabolism in marasmus and kwashiorkor

Insights from Malawi & Sierra Leone
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‘Acute malnutrition’

- Acute malnutrition: a disease category comprised of two separate conditions: wasting or kwashiorkor
- **Wasting**: a continuous process resulting in weight loss.
- Wasting can be either ‘moderate’ or ‘severe’; i.e. moderate acute malnutrition (MAM), or severe acute malnutrition (SAM).
Severe acute malnutrition

• **SAM is defined** by anthropometry or the presence of nutritional edema
  • *Marasmus* (wasting): mid-upper arm circumference measurement (MUAC) < 11.5 cm or a weight-for-height z-score (WHZ) < -3
  • *Kwashiorkor*: presence of bilateral pitting pedal edema

• **Etiology of SAM** is variable: multiple causes and contributors
  • Acute food insecurity, poor diet quality, chronic and recurrent infections
Decreased Energy Intake
- nausea, anorexia, depression, poor dentition, oral-motor weakness

Malabsorption
- reduced bile-salt secretion → pancreatic insufficiency, intestinal mucosa damage

Catabolic Stressors
- systemic inflammation, cirrhosis, immune cell turnover (e.g., CD4), hemodialysis

Net Negative Energy Balance
- Muscle Wasting
- Depletion of Fat Stores
- Epithelial Thinning, Breakdown, Ulceration
- Slower basal metabolic rate
- Apathy
- Reduced Cardiorespiratory Capacity
- Immunodeficiency with Impaired Wound Healing
- Hypothermia
Marasmus
Kwashiorkor
Kwashiorkor
Kwashiorkor as a neglected tropical disease

• Once global, the distribution of kwashiorkor is now tropical.

• Kwashiorkor is a condition of poverty that begets poverty.
  • Increased risk for neurocognitive development with reduced economic achievement later in life.
  • Increased risk for metabolic conditions later in life.

• Kwashiorkor receives little scientific attention.
Global distribution of kwashiorkor
Impact of kwashiorkor

**Uncertain incidence**: Hundreds of thousands of cases annually.

**Spotty distribution**: Burden of kwashiorkor is greatest in the rural peripheries of countries in Sub-Saharan Africa.

**Global impact**: Estimated to cause 25,000-40,000 deaths annually.

**Mortality**: Higher odds of death when kwashiorkor occurs with severe wasting, in areas where access to therapeutic feeding is limited.
Kwashiorkor as a neglected tropical disease

• The cause of kwashiorkor is uncertain.

• Mentions of kwashiorkor in a recent Lancet series on maternal and child nutrition: Zero

• Past & current Bill and Melinda Gates Foundation projects focused on kwashiorkor: Zero

• Number of current NIH grants mentioning kwashiorkor: One
Kwashiorkor as a neglected tropical disease
Kwashiorkor as a neglected tropical disease
Is kwashiorkor associated with wasting?

- 14% of kwashiorkor is associated with marasmus

- 64% of kwashiorkor cases are not associated with clinically relevant weight loss

What are kwashiorkor’s distinctives?

• **Kwashiorork: “the other SAM”**
• Defined by edema – not wasting
• Marked by additional disturbances
  • Apathy
  • Skin disturbances
  • Increased liver fat
  • Pancreas dysfunction
  • Gut wall thinning
  • Malabsorption
  • Increased mortality
What causes kwashiorkor?

- **Historical theory**: Kwashiorkor results from inadequate intake of total protein.

**Observations**
- Kwashiorkor is associated with low protein diets.
- Effective treatments for kwashiorkor provide high quality protein.
- Serum proteins are often reduced in kwashiorkor.
- *Multiple inconsistencies with the low protein hypothesis.*
Kwashiorkor is a syndrome of malnutrition associated with low protein diets

Low- and middle-income countries

• Maize, rice, & cassava

Higher income countries

• Rice milk
• Non-dairy coffee creamer
• Potatoes & juice
• Saltine crackers & Captain Crunch™

Kwashiorkor in the United States, Fad Diets, Perceived and True Milk Allergy, and Nutritional Ignorance, Theodore Liu, et al. 2001
Is kwashiorkor caused by protein deficiency?

Clinical inconsistencies with the low protein theory

• Broad overlap of serum albumin concentrations in kwashiorkor and marasmus

• Resolution of edema is not always correlated with increased serum albumin. (Golden & Jackson, 1980)

• Protein intake is poorly correlated with the resolution of edema (Golden and Jackson, 1982).
Is kwashiorkor caused by protein deficiency?

Modern food frequency questionnaires (FFQ) have not identified total protein intake as a risk determinant for kwashiorkor

• Sullivan et al (2006): FFQ of siblings (eggs & tomatoes)
• Lin et al (2007): prospective FFQ
• Kismul et al (2014): longitudinal FFQ (fruits & vegetables, β-carotene?)
Protein deficiency in the pathogenesis of kwashiorkor

• ‘Low protein diets are the essential etiologic context for the pathogenesis of kwashiorkor, not its precise cause; where kwashiorkor happens, not why.’ Jahoor et al - 2021
Observations in 1987

- Decreased erythrocyte glutathione (GSH)
- Proportion of oxidized GSH was not reduced

Proposed theory:

- The kwashiorkor syndrome is caused by an imbalance of ‘oxidative noxae’ and limited oxidative buffering.
- ‘Oxidative stress’ in kwashiorkor causes edema, fatty liver, & skin lesions.

Oxidative stress in kwashiorkor

**Trial of theory: 2003- 2004**
- Comparison of an anti-oxidant cocktail vs. placebo
- 2332 children completed trial
- 62 children developed kwashiorkor
  - Antioxidant group: 39 cases
  - Placebo group: 23 cases

Kwashiorkor’s risk factors offer insights into its pathogenesis

- Evidence of exposure to aflatoxins and reduced clearance of aflatoxins is more common in kwashiorkor, relative to marasmus.

Kwashiorkor’s risk factors offer insights into its pathogenesis

- Dietary risk factors for kwashiorkor in the DRC
  - High cyanogen cassava
  - Lower intake of sulfur amino acids (methionine & cysteine)

*Dietary intake of sulfur amino acids and risk of kwashiorkor malnutrition in eastern Democratic Republic of the Congo, Fitzpatrick et al. 2021*
Kwashiorkor’s risk factors offer insights into its pathogenesis

- Genetic polymorphisms associated with kwashiorkor
  - Glutathione S-transferase polymorphisms\(^1\)
  - Folate enzyme polymorphisms\(^2\)


One-carbon metabolism disturbances are implicated in the pathogenesis of kwashiorkor

- **One-carbon metabolism**: a tightly coupled network subject to numerous influences
  - Demand for one-carbon products
  - Genetics
  - Availability of dietary one-carbon nutrients
    - Diet quality
    - Microbiome
  - Multiple co-nutrient interactions
Molecular changes in kwashiorkor resemble one-carbon nutrient deficient diets (1CNDDs)

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<tr>
<th>Molecular changes</th>
<th>1CNDDs</th>
<th>Kwashiorkor</th>
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<td>Transmethylation</td>
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<tr>
<td>DNA methylation</td>
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<tr>
<td>Plasma carnitine</td>
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<tr>
<td>Plasma cysteine</td>
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<td>Sulfated GAGs</td>
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<td>Plasma albumin</td>
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<td>Plasma triglycerides</td>
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<td>Fatty acid oxidation</td>
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<td>Lipid peroxidation</td>
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<td>Metalloproteinase-2</td>
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<td>Plasma TNF-α</td>
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Molecular changes in kwashiorkor resemble one-carbon nutrient deficient diets (1CNDDs)

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<tr>
<th>Feature</th>
<th>1CNDDs</th>
<th>Kwashiorkor</th>
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<tr>
<td><strong>Organ changes</strong></td>
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<td>Liver steatosis</td>
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<td>Pancreatic atrophy</td>
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<td>Exocrine pancreas $fxn.$</td>
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<td>Intestinal thickness</td>
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<td>Intestinal permeability</td>
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<td>Intestinal inflammation</td>
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<tr>
<td>Skin disturbances</td>
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<tr>
<td>Cellular immune $fxn.$</td>
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<tr>
<td>Edema</td>
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Is kwashiorkor a syndrome of one-carbon metabolism dysfunction?

• **2016**: A clinical study to assess one-carbon metabolites in Malawian children, aged 6-59 months.

• Serum one-carbon metabolites quantified in 357 children:
  • Marasmic-kwashiorkor: $N = 43$
  • Kwashiorkor: $N = 94$
  • Marasmus: $N = 118$
  • Moderate wasting: $N = 56$
  • Controls: $N = 46$
Transmethylation in marasmus & kwashiorkor
Asymmetric dimethyl arginine in marasmus & kwashiorkor
Transsulfuration in marasmus & kwashiorkor
Cysteine in marasmus & kwashiorkor
Transsulfuration in marasmus & kwashiorkor
Homocysteine in marasmus & kwashiorkor
Transsulfuration in marasmus & kwashiorkor

Protein incorporation

Methionine → SAMe

Tetrahydrofolate

DMG

Arginine

Glycine

DNA

Protein

Remethylation

MS

BHMT

5-methyl-tetrahydrofolate

Betaine → Choline

Homocysteine → SAH

Homocysteine

Serine

Transsulfuration

[B6] CBS

Cystathionine

Cystathionine

CSE

[GSH]

Cysteine

SAH

Cysteine

GSH

ADMA: Asymmetric dimethylarginine
DMG: Dimethylglycine
SDMA: Symmetric dimethylarginine
BHMT: Betaine-homocysteine methyltransferase
CBS: Cystathionine beta-synthase
CSE: Cystathionine gamma-lyase
GSH: Glutathione
MS: Methionine synthase
SAMe: S-adenosyl-methionine
SAH: S-adenosyl-homocysteine
Cystathionine in marasmus & kwashiorkor

Malnutrition Condition
- Marasmic-Kwashiorkor
- Kwashiorkor
- Marasmus
- Moderate
- Control
Methionine cycle in marasmus & kwashiorkor

Protein incorporation

Methionine → SAMe

Remethylation

Methionine → SAMe

Transmethylation

SAMe → Arginine, Glycine, DNA, Protein

Transsulfuration

Homocysteine → Cystathionine

Cystathionine → Cysteine, GSH

ADMA: Asymmetric dimethylarginine
SDMA: Symmetric dimethylarginine
BHMT: Betaine-homocysteine methyltransferase
CBS: Cystathionine beta-synthase
CSE: Cystathionine gamma-lyase
GSH: Glutathione
MS: Methionine synthase
SAMe: S-adenosylmethionine
SAH: S-adenosylhomocysteine

Tetrahydrofolate → [B12] → DMG → Betaine → Choline

5-methyl-tetrahydrofolate → Remethylation

SAR

SAMe → ADMA & SDMA

Sarcosine → DNA + CH₃

Protein + CH₃

DNA + CH₃ → Protein

Arginine

Glycine

DNA

Protein
Methionine cycle in marasmus & kwashiorkor

![Graph showing SAMe levels in different malnutrition conditions.]
Methionine cycle in marasmus & kwashiorkor
S-adenosyl Homocysteine (SAH) in marasmus and kwashiorkor
Methionine cycle in marasmus & kwashiorkor

Protein incorporation

Methionine

DMG

Tetrahydrofolate

[\text{B12}]

Remethylation

5-methyl-tetrahydrofolate

Betaine

Choline

CH_3

Transsulfuration

Homocysteine

Serine

Cystathionine

CSE

[\text{B6}]

Sulphur

Cysteine

GSH

Transmethylation

SAMe

Arginine

Glycine

DNA

Protein

ADMA & SDMA

Sarcosine

DNA + CH_3

Protein + CH_3

SAH

ADMA: Asymmetric dimethylarginine
DMG: Dimethylglycine
SDMA: Symmetric dimethylarginine
BHMT: Betaine-homocysteine methyltransferase
CBS: Cystathionine beta-synthase
CSE: Cystathionine gamma-lyase
GSH: Glutathione
MS: Methionine synthase
SAMe: S-adenosylmethionine
SAH: S-adenosyl-homocysteine
Methionine in marasmus and kwashiorkor

![Box plot showing methionine levels in different malnutrition conditions.](image)
Methyl donors & vitamin co-factors in marasmus & kwashiorkor

• **Measured** vitamin co-factors and most methyl donors were not significantly reduced in kwashiorkor or marasmic-kwashiorkor, relative to other groups.

• **Exception**: betaine
Betaine in marasmus & kwashiorkor

Malnutrition Condition
- Marasmic-Kwashiorkor
- Kwashiorkor
- Marasmus
- Moderate
- Control
One-Carbon metabolism in kwashiorkor relative to marasmus

• Kwashiorkor’s distinguishing features include:
  • Transsulfuration disturbances
  • ↓ Methionine
  • ↓ Homocysteine
  • ↓ Cysteine
  • ↓ ADMA
  • ↓ SAMe:SAH
The gut microbiome in kwashiorkor

- Antibiotics reduce mortality in kwashiorkor
- ‘Delayed maturation’ of the gut microbiota was identified as a risk factor for kwashiorkor in Malawian twin pairs.


Do gut microbiota changes increase risk for one-carbon metabolism disturbances in kwashiorkor?

- **Trimethylamine N-oxide**: a gut flora-dependent metabolite that is derived from choline and L-carnitine
  - *Increased TMAO in kwashiorkor*: suggestive of increased gut microbial steal of dietary choline and carnitine.
Typically Adequate Energy Intake
Plant based diets containing little animal protein

↓ Absorption of 1CM Nutrients
i.e. gut microbial steal of methyl donors

↑ Demand for 1CM Nutrients
CD4+ turnover
Malaria, Diarrhea
Mycotoxins, cyanogens,
1CM enzyme SNPs

One-Carbon Metabolism Dysfunction in Kwashiorkor

Impaired Energy Metabolism
Apathy

Decreased Transmethylation

↓ PEMT function
↓ Hepatic VLDL export

DNA Hypomethylation

↓ transsulfuration

↓ H₂S

↓ Cysteine
↓ Glutathione

“Oxidative Stress”

Hepatic Steatosis

Hypoalbuminemia

Immune Dysfunction

Gut thinning & Malabsorption

Decreased Protein Translation

Hypalbuminemia

Mitochondrial Dysfunction

Impaired Energy Metabolism

Apathy
Fatty liver and one-carbon metabolism status

• Does consumption of a maize-vegetable diet (MVD) lead to hepatic steatosis?

• Does choline prevent steatosis associated with a MVD?

Reduced fat gain in mice fed a choline supplemented MVD is consistent with improved energy metabolism after choline administration.
Maize Vegetable Diet (MVD) with & without choline

Oil-red-O stained liver tissue after 14 days of feeding, in weanling mice

Control + choline  MVD  MVD + choline
Adequate Energy Intake
Energy adequate plant based diets containing little animal protein

↓ Absorption of 1CM Nutrients
i.e. gut microbial steal of methyl donors

↑ Demand for 1CM Nutrients
CD4+ turnover Malaria Diarrhea

One-Carbon Metabolism Dysfunction of Kwashiorkor

↓ transsulfuration

↓ H₂S

↓ Sulfated GAGs

Extra-cellular matrix dysfunction

Endothelial glycocalyx dysfunction

Osmolyte disturbances

Hypoalbuminemia

Edema
One-carbon metabolism dysfunction in the pathogenesis of nutritional edema

Nutrients known to reduce nutritional edema in animal models

Partial
- Cysteine (delay of edema) Luckner, 1938
- Cobalamin. Alexander, 1952

Complete
- Cobalamin + folate. Alexander, 1956
- Choline. Alexander, 1952
- Methionine. Alexander, 1956
Protein quality in the pathogenesis of kwashiorkor

• Protein quality is limited to the availability of the least represented essential amino acid

• Methionine is often the first limiting amino acid in maize-based diets

“The development of edema disease (sic) is not due to caloric malnutrition or vitamin deficiency, nor is it due solely to insufficient intake of protein; the cause of experimental nutritional edema is rather malnutrition with biologically inferior protein.” Luckner 1938
One-carbon metabolism is a tightly coupled network that is shaped by multiple co-nutrient interactions.

Demand for methionine is modified by numerous one-carbon nutrient interactions.

Choline influences methionine status: Serum choline is directly correlated with serum methionine status in Malawian children.
Is kwashiorkor a syndrome of one-carbon metabolism dysfunction caused by methionine deficiency?
Implications for children with kwashiorkor

• Concept

Outcomes in kwashiorkor will be improved by fortifying the current standard of care (i.e. RUTF) with methionine and methyl donors, such as choline.
Implications for children at risk for kwashiorkor

• Concept
  • Kwashiorkor can be prevented by fortifying meager diets with methionine and one-carbon nutrients, such as choline.
SAM is associated with subsequent cognitive impairment

Children who have recovered from SAM score 2-3 standard deviations below age-normalized expectations as assessed by Malawi Developmental Assessment Tool (MDAT)

Long-term cohort studies show worse school performance, behavioral problems, reduced economic achievement among SAM survivors

Cognitive outcomes in SAM have received little attention

*RUTF was designed to maximize weight gain; pre-clinical data show that ignoring fat composition has downsides with regards cognitive recovery*
Summary of a winding story

Children with SAM are vulnerable and experience a huge cognitive insult.

RUTF improved SAM treatment, leading to higher rates of recovery and lower mortality.

RUTF was optimized to efficiently provide energy for physical recovery.

This composition leads to huge excess of LA.

DHA is essential for brain development. Standard RUTF formulations may inadvertently reduce DHA bioavailability during SAM treatment.

Children treated with standard RUTF experience a drop in plasma and RBC membrane DHA levels.
Provision of RUTF made with high oleic peanuts (HO-RUTF) with or without added DHA (DHA-HO-RUTF) would improve cognition in children with SAM when compared with standard RUTF (S-RUTF)

Hypothesis:

Provision of RUTF made with high oleic peanuts (HO-RUTF) with or without added DHA (DHA-HO-RUTF) would improve cognition in children with SAM when compared with standard RUTF (S-RUTF)

Study Design:

Individually randomized, fully blinded, controlled clinical trial

Setting:

Rural Southern Malawi, at 28 clinics operated continuously by our research group for over a decade

Population:

Children 6mo – 5y of age diagnosed with SAM. Could not have known neurodevelopmental disorder.

The UN’s international food regulatory body, Codex Alimentarius, was set to meet in late 2021 to set guidelines for fatty acid content of RUTF

Improved PUFA RUTF Study
Study Conclusions Summary

No Differences
In rate of recovery, death, anthropometric growth, PSA scores

Fatty Acid Levels
Different RUTFs had expected effects on plasma fatty acid levels

Significant Difference
Children with RUTF made from HO peanuts with added DHA and EPA had better mean MDAT global, gross motor and social domain z-scores compared to children treated with standard. Each child added 6-15 IQ points with DHA RUTFs

Notes of Interest
• Effect estimate relatively stable across multiple subgroups
• MDAT scores steadily worsened as age at diagnosis went up
• Amount of DHA contained in average daily dose of RUTF was modest, 173 mg/d
• Production was straightforward and cost reasonable