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
  - **<u>Wasting</u>**: a continuous process resulting in weight losss.
  - 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

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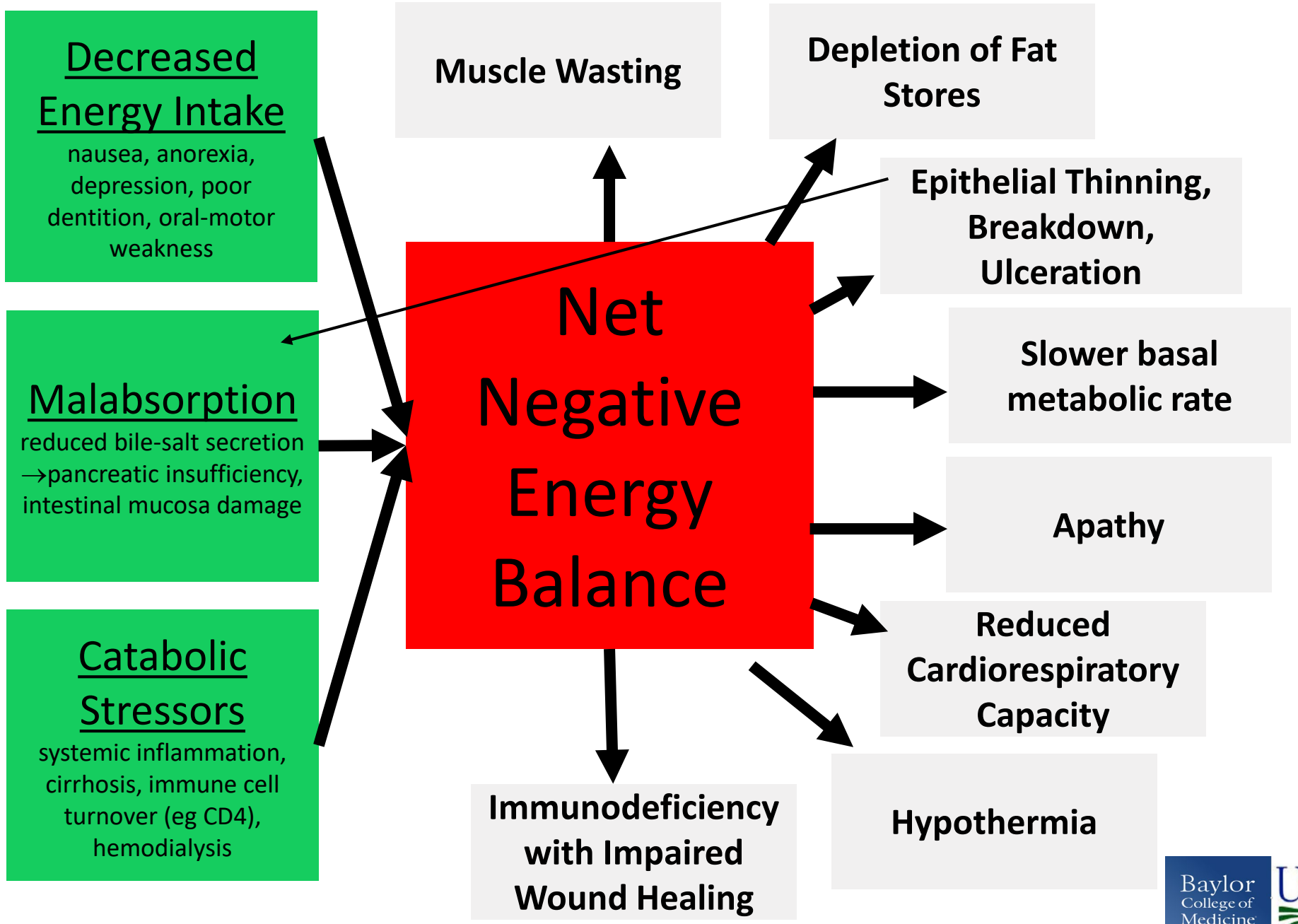
WASTING


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 (eg CD4), hemodialysis
Net Negative Energy Balance
Muscle Wasting
Depletion of Fat Stores
Epithelial Thinning, Breakdown, Ulceration
Slower basal metabolic rate
Apathy
Reduced Cardiorespiratory Capacity
Hypothermia
Immunodeficiency with Impaired Wound Healing

Marasmus

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Kwashiorkor

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Kwashiorkor

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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.

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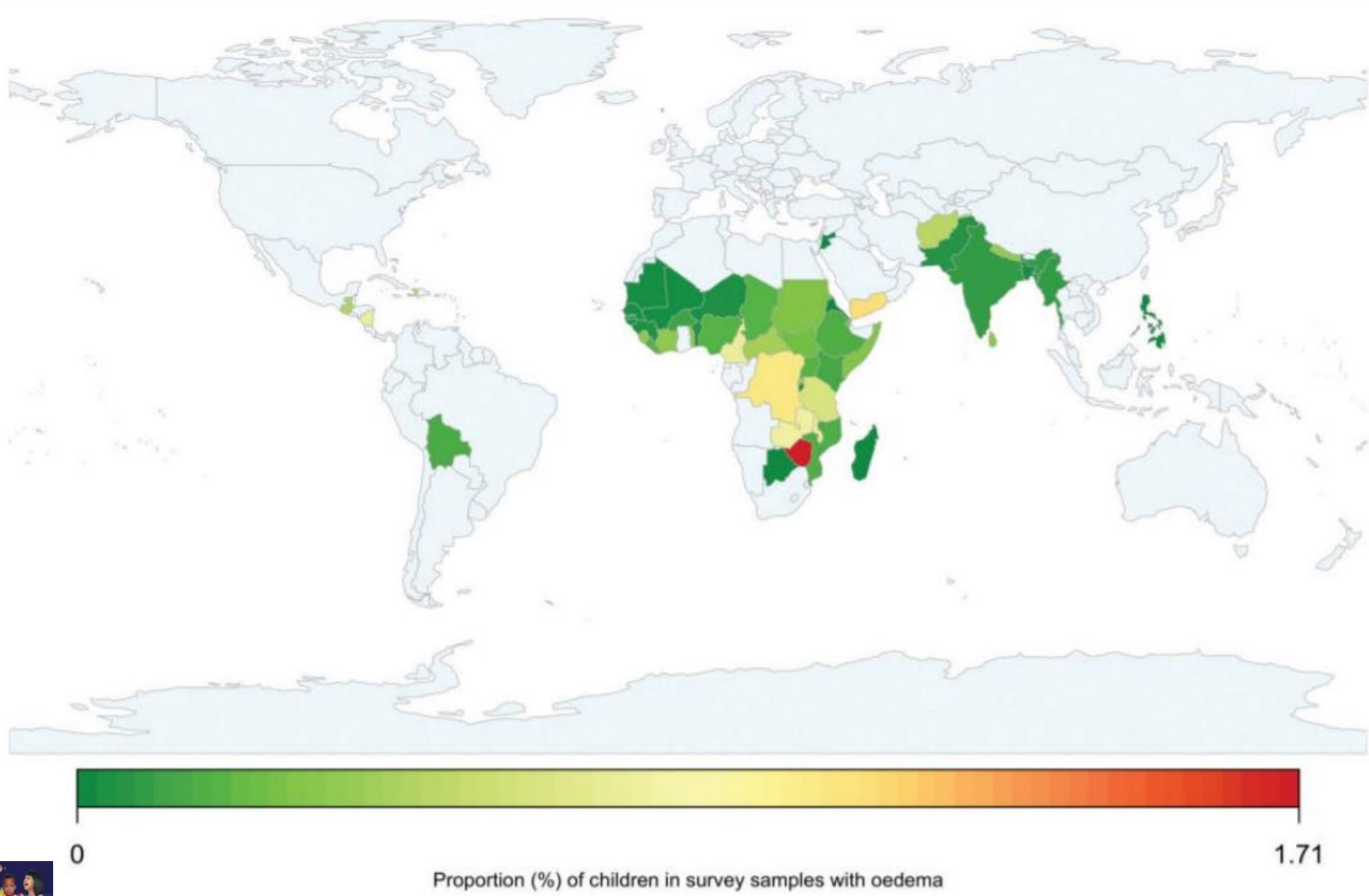
Global distribution of kwashiorkor



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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.

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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**

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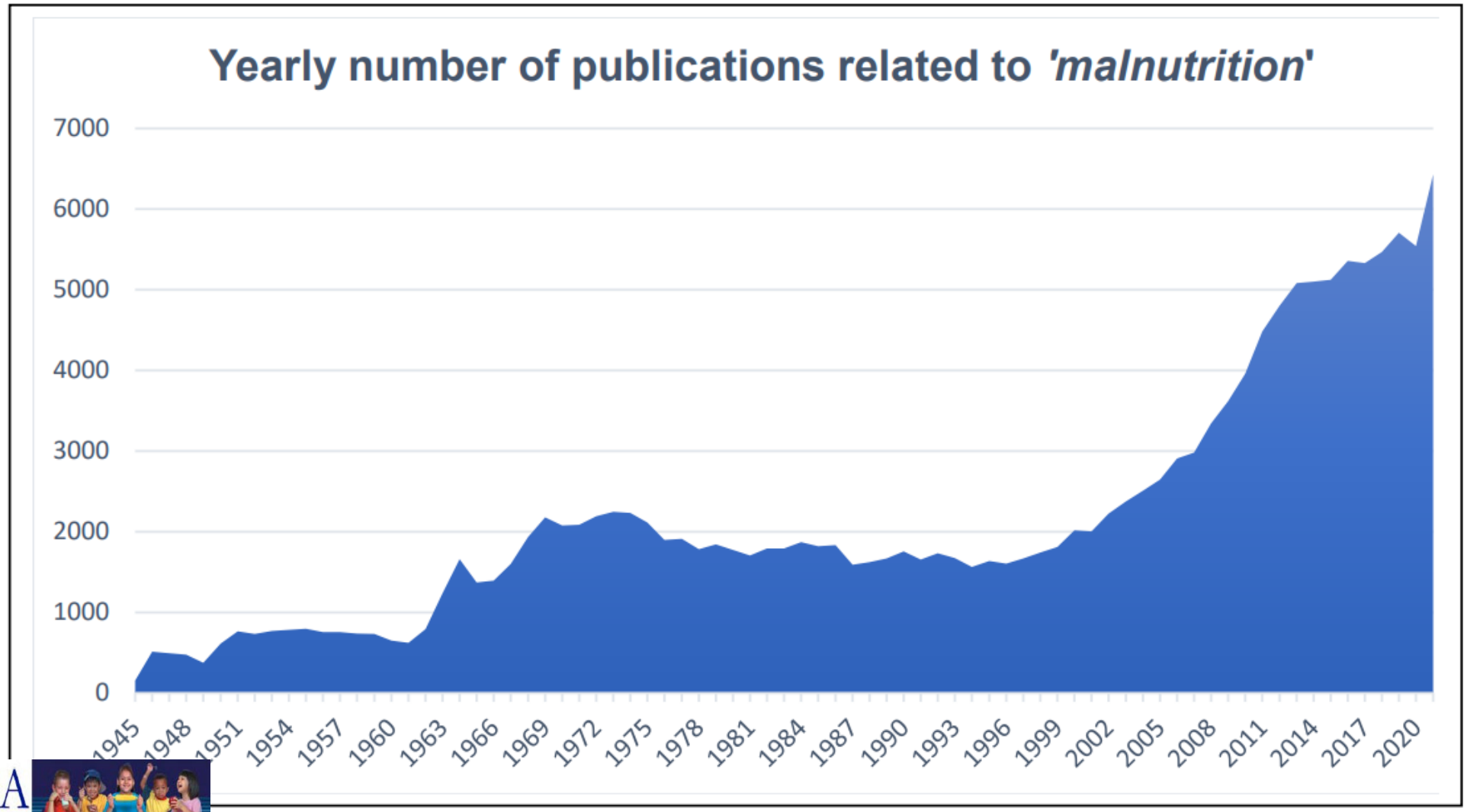
Kwashiorkor as a neglected tropical disease



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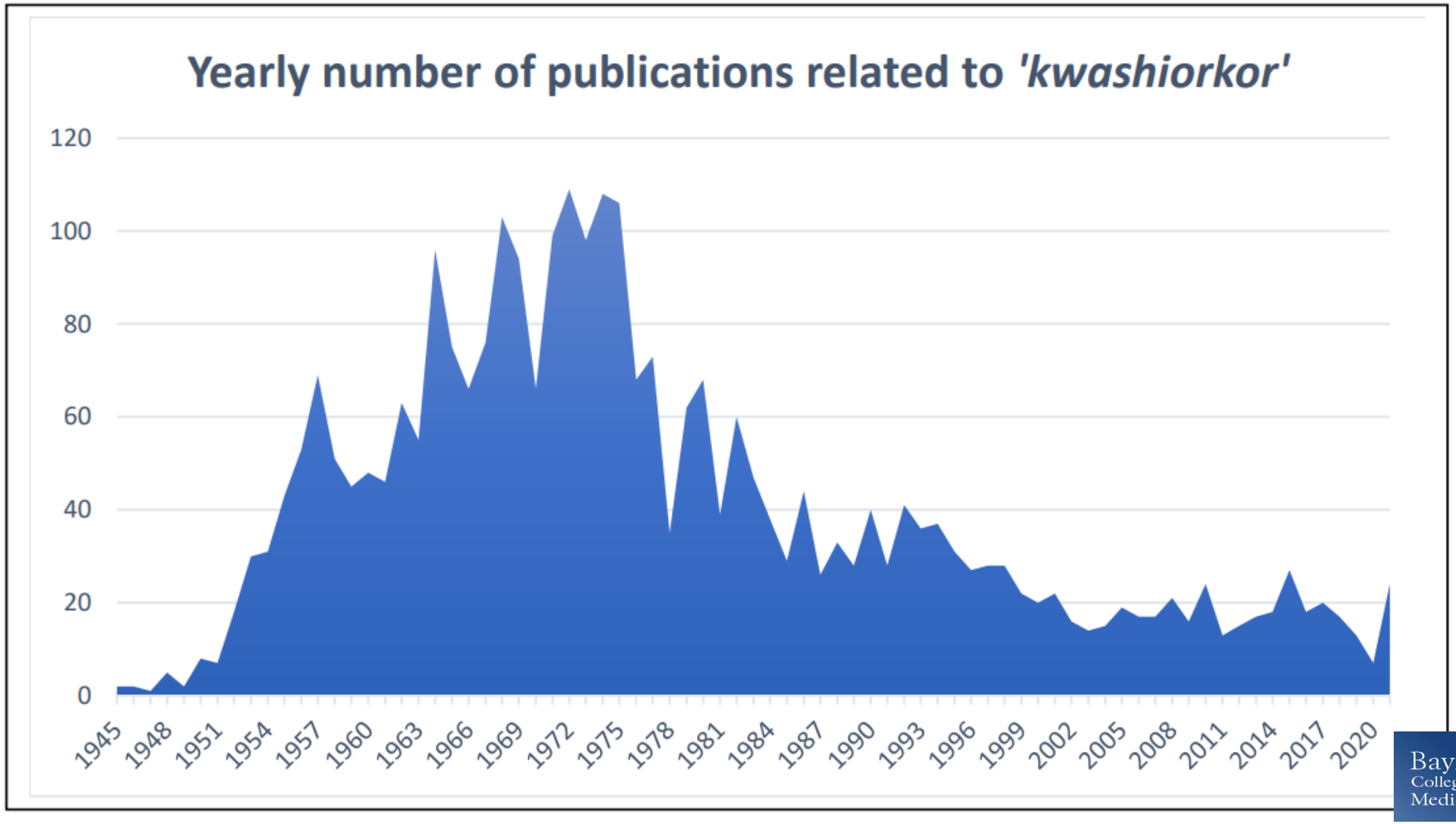
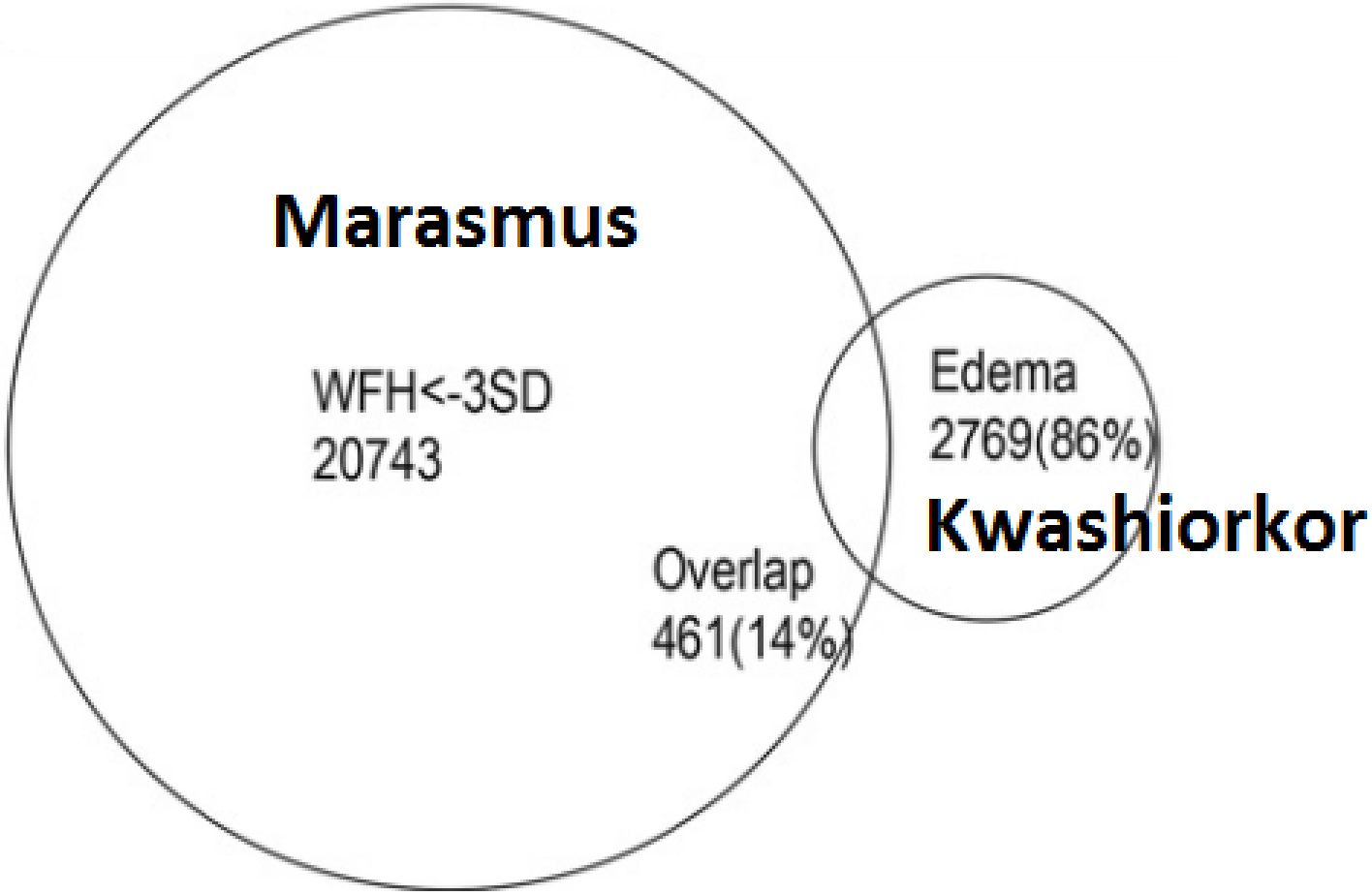
Kwashiorkor as a neglected tropical disease



Is kwashiorkor associated with wasting?

- 14% of kwashiorkor is associated with marasmus
- 64% of kwashiorkor cases are not associated with clinically relevant weight loss



Frison S, Checchi F, Kerac M. Omitting edema measurement: how much acute malnutrition are we missing? Am J Clin Nutr. 2015 Nov 1;102(5):1176–81.

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What are kwashiorkor's distinctives?

- ***Kwashiorkor: "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

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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).

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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?)

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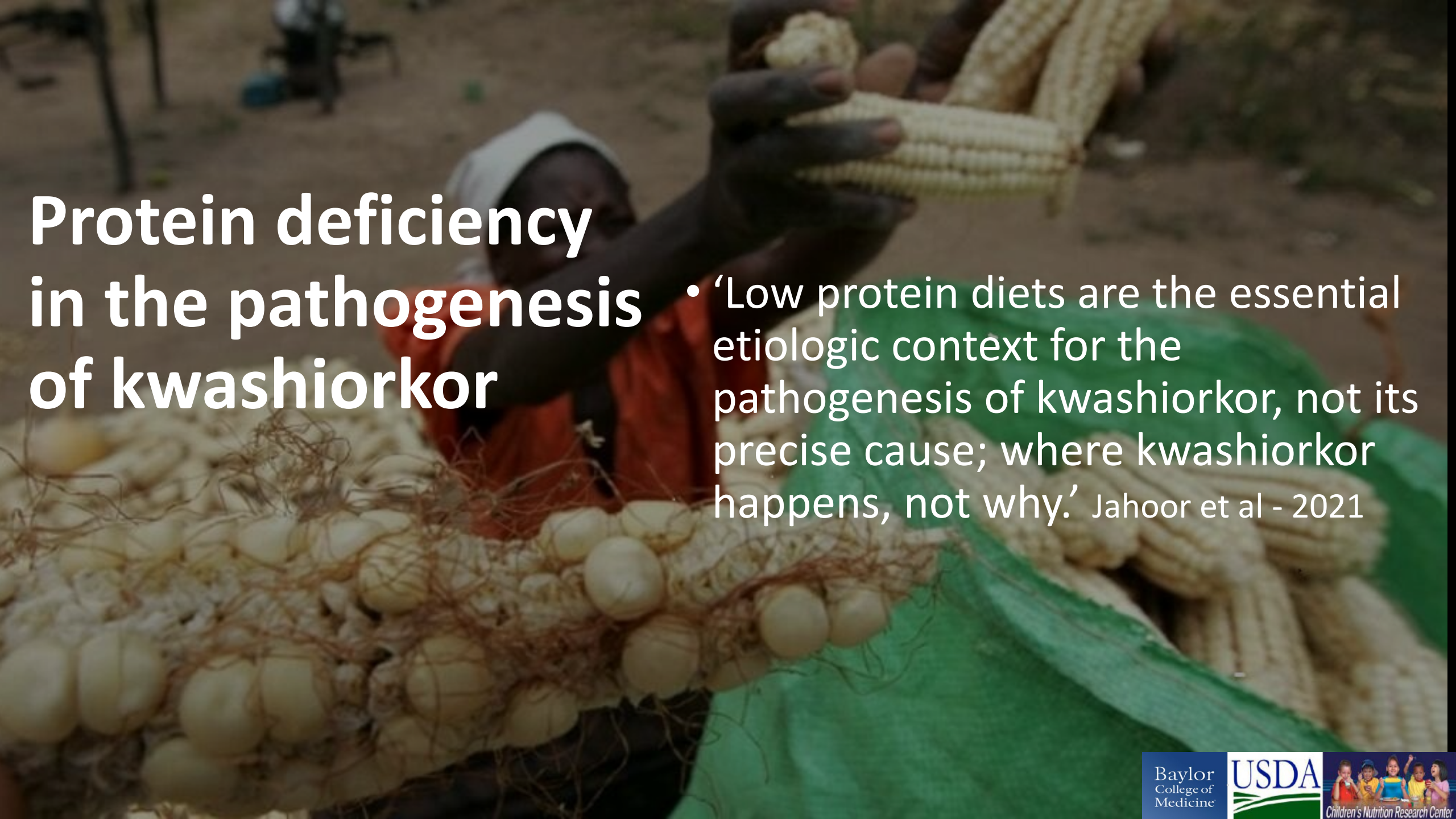
Protein deficiency in the pathogenesis of kwashiorkor

- ‘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



Oxidative stress in kwashiorkor

Observations in 1987

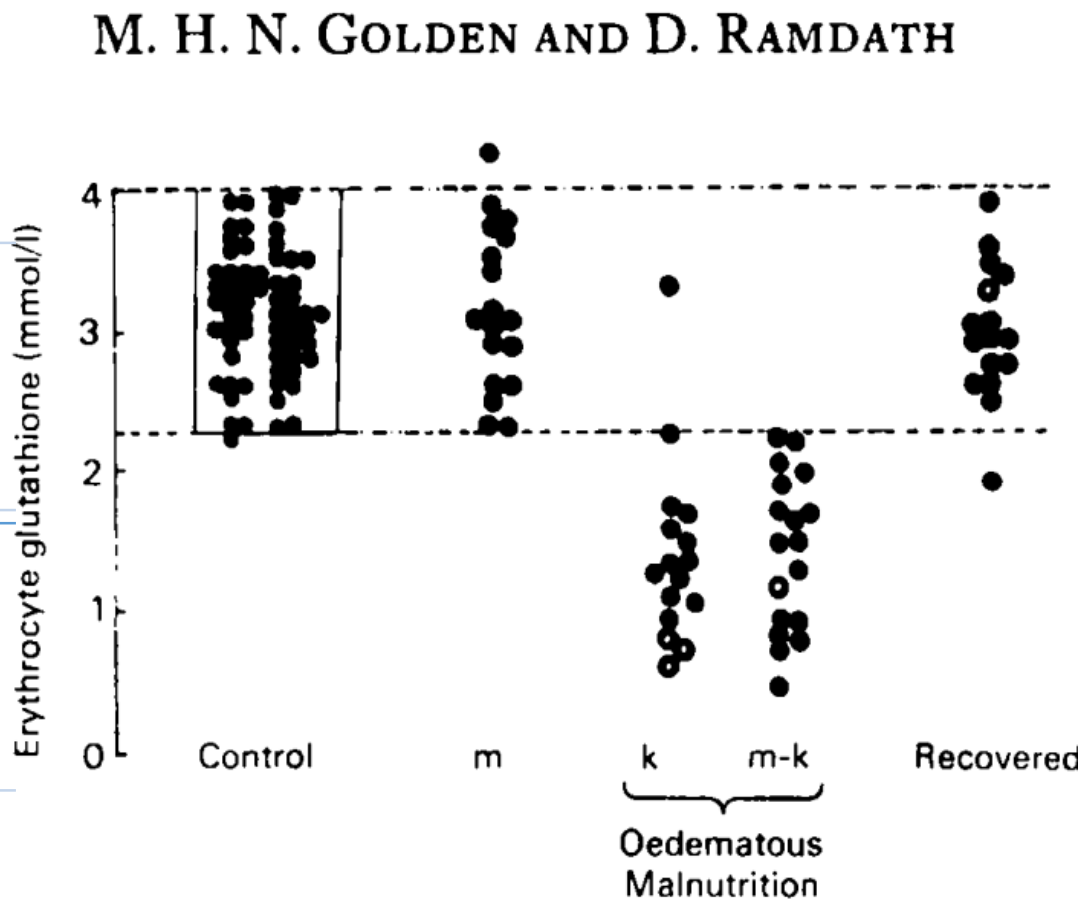
Decreased erythrocyte glutathione (GSH)

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.



Free radicals in the pathogenesis of kwashiorkor. Proc Nutr Soc. 1987. M H Golden, D Ramdath

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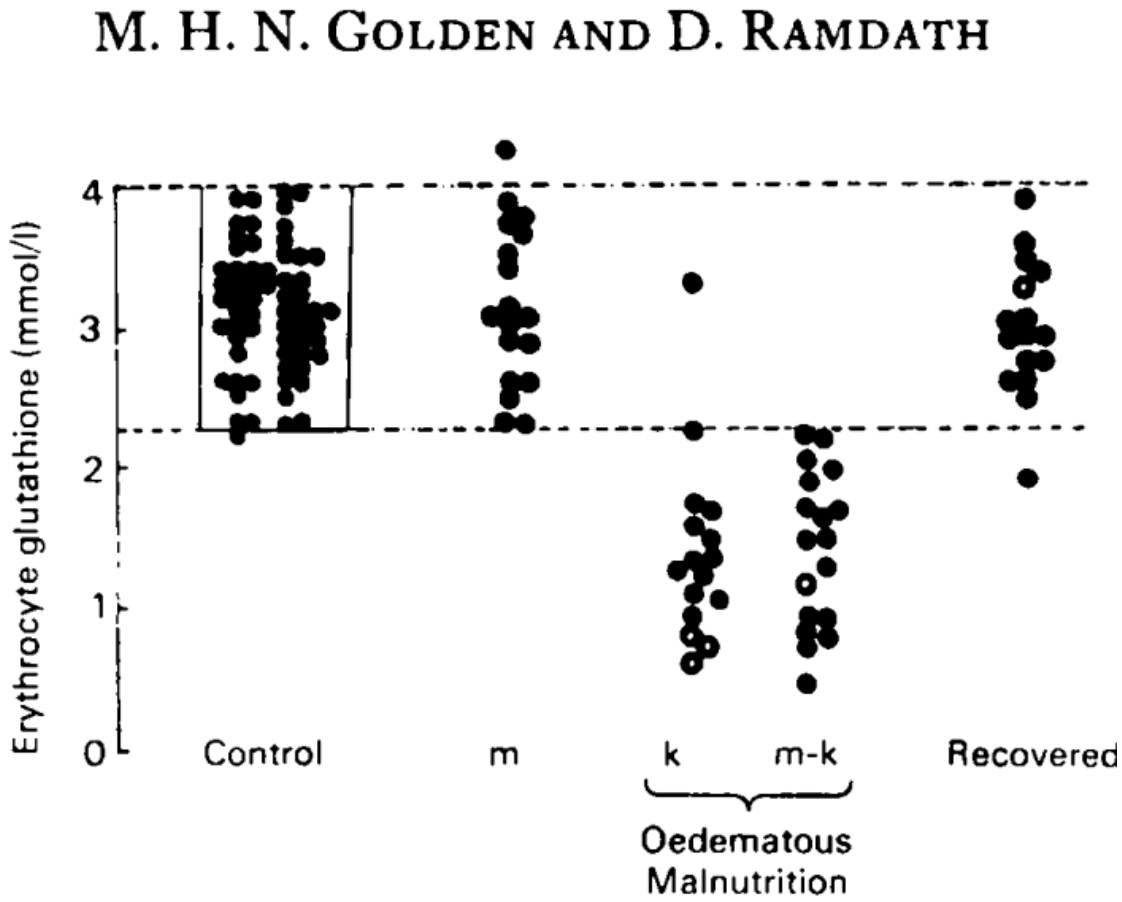
Oxidative stress in kwashiorkor

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



1. *Antioxidant supplementation for the prevention of kwashiorkor in Malawian children: randomized, double blind, placebo-controlled trial. Clinical Trial BMJ. 2005. Ciliberto et al.*
2. *Free radicals in the pathogenesis of kwashiorkor. M H Golden, D Ramdath. Proc Nutr Soc. 1987*

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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.

Aflatoxins and kwashiorkor: a study in Sudanese children. Br Med J. 1982. R G Hendrickse, et al.

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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

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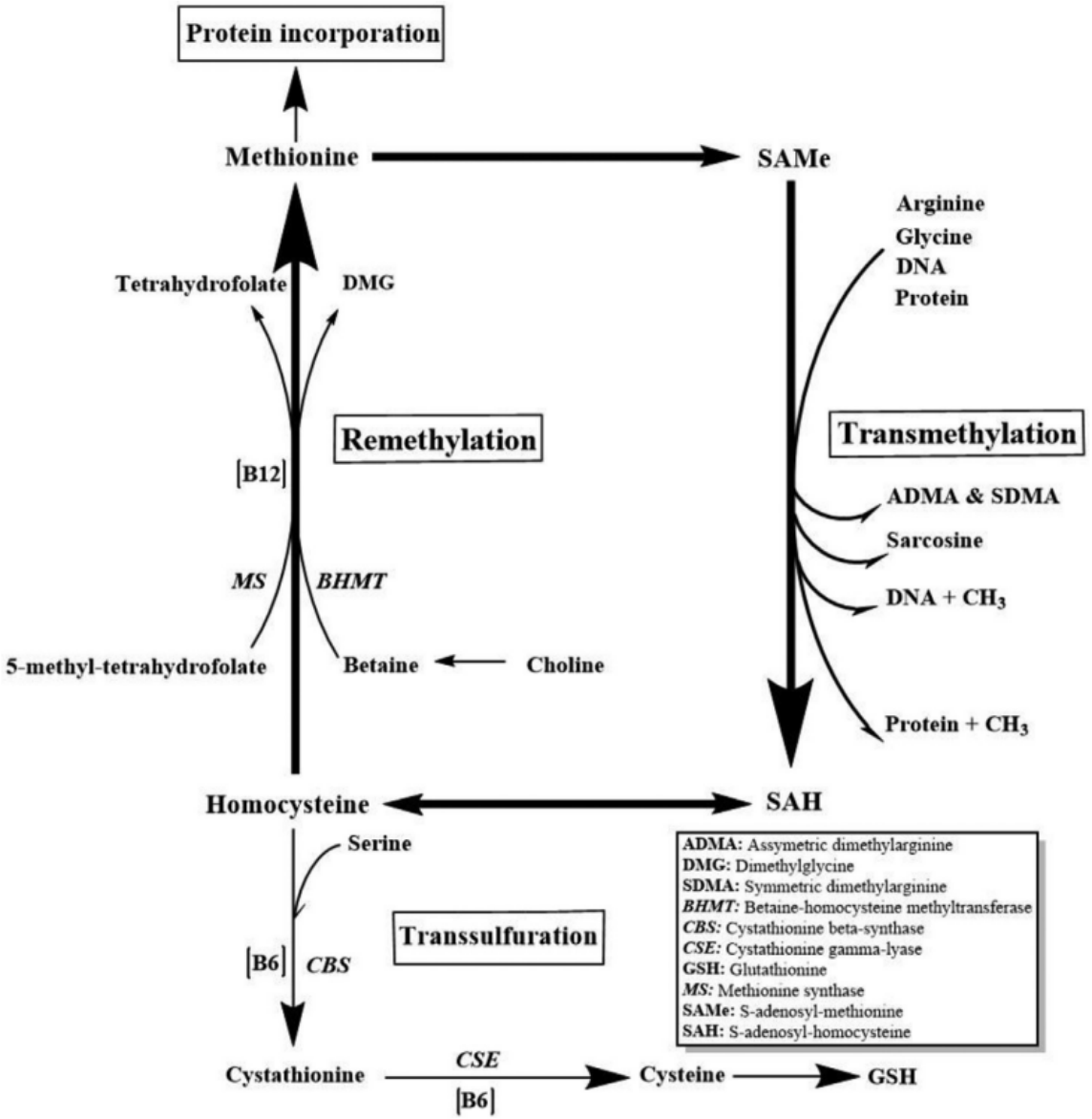
Kwashiorkor's risk factors offer insights into its pathogenesis

- Genetic polymorphisms associated with kwashiorkor
  - Glutathione S-transferase polymorphisms[1]
  - Folate enzyme polymoprhisms[2]



1. Glutathione S-transferase polymorphisms may be associated with risk of oedematous severe childhood malnutrition. Br J. Nutr 2006. Marshall et al.

2. Polymorphisms in genes involved in folate metabolism as risk factors for oedematous severe childhood malnutrition: A hypothesis-generating study. Ann Trop Paediatr 2006;26. Marshall et al.

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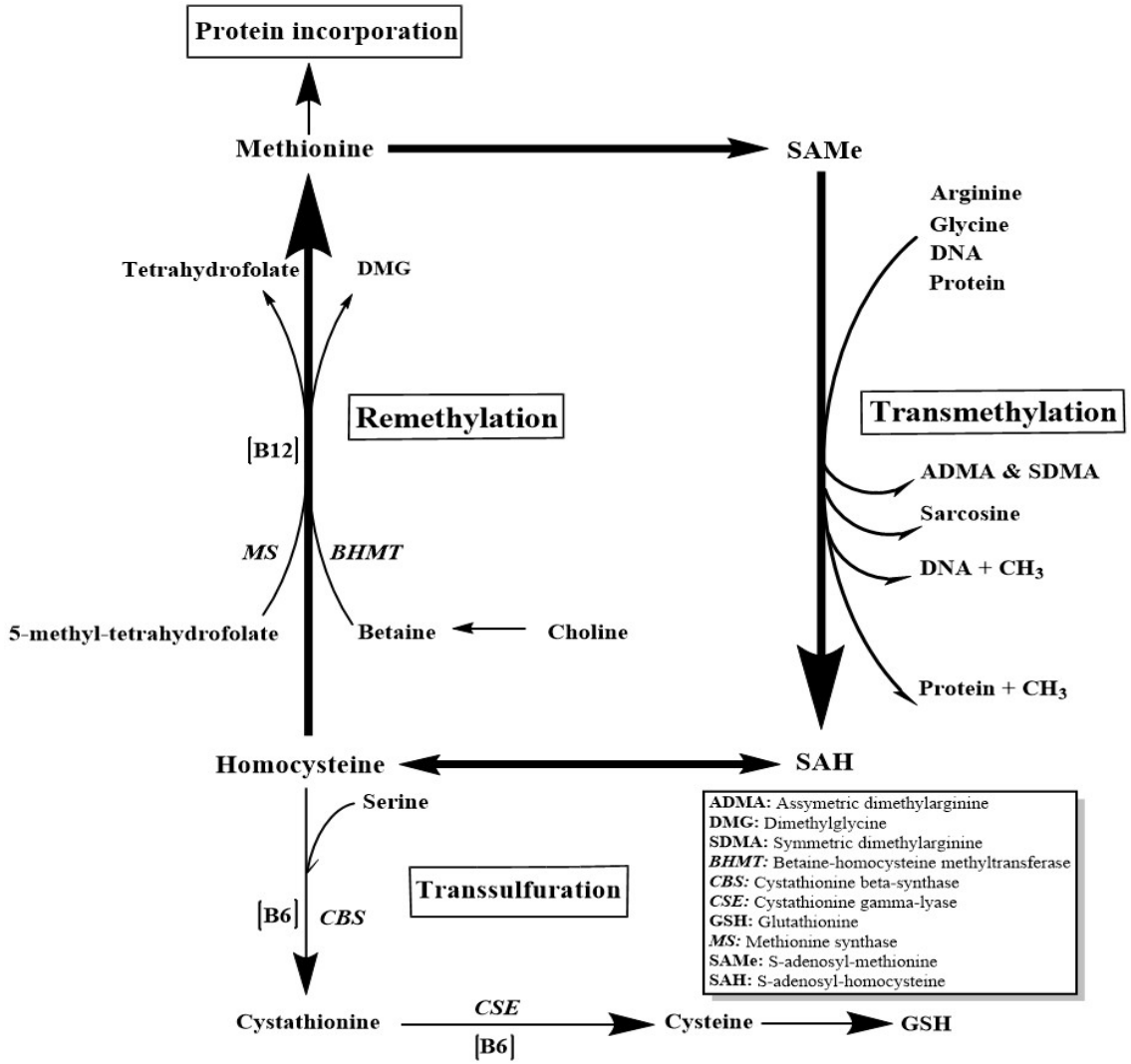
One-carbon metabolism disturbances are implicated in the pathogenesis of kwashiorkor

- ***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



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Molecular changes in kwashiorkor resemble one-carbon nutrient deficient diets (1CNDDs)

Molecular changes	1CNDDs	Kwashiorkor
Transmethylation	↓	↓
DNA methylation	↓	↓
Plasma carnitine	↓	↓
Plasma cysteine	↓	↓
Plasma glutathione	↓	↓
Sulfated GAGs	↓	↓
Plasma albumin	↓	↓
Plasma triglycerides	↓	↓
Fatty acid oxidation	↓	↓
Lipid peroxidation	↑	↑
'*Oxidative stress*'	↑	↑
Metalloproteinase-2	↑	↑
Plasma TNF-α	↑	↑

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Molecular changes in kwashiorkor resemble one-carbon nutrient deficient diets (1CNDDs)

Feature	1CNDDs	Kwashiorkor
Organ changes		
Liver steatosis	↑	↑
Pancreatic atrophy	↑	↑
Exocrine pancreas *fxn.*	↓	↓
Intestinal thickness	↓	↓
Intestinal permeability	↑	↑
Intestinal inflammation	↑	↑
Skin disturbances	↑	↑
Cellular immune *fxn.*	↓	↓
Edema	↑	↑

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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

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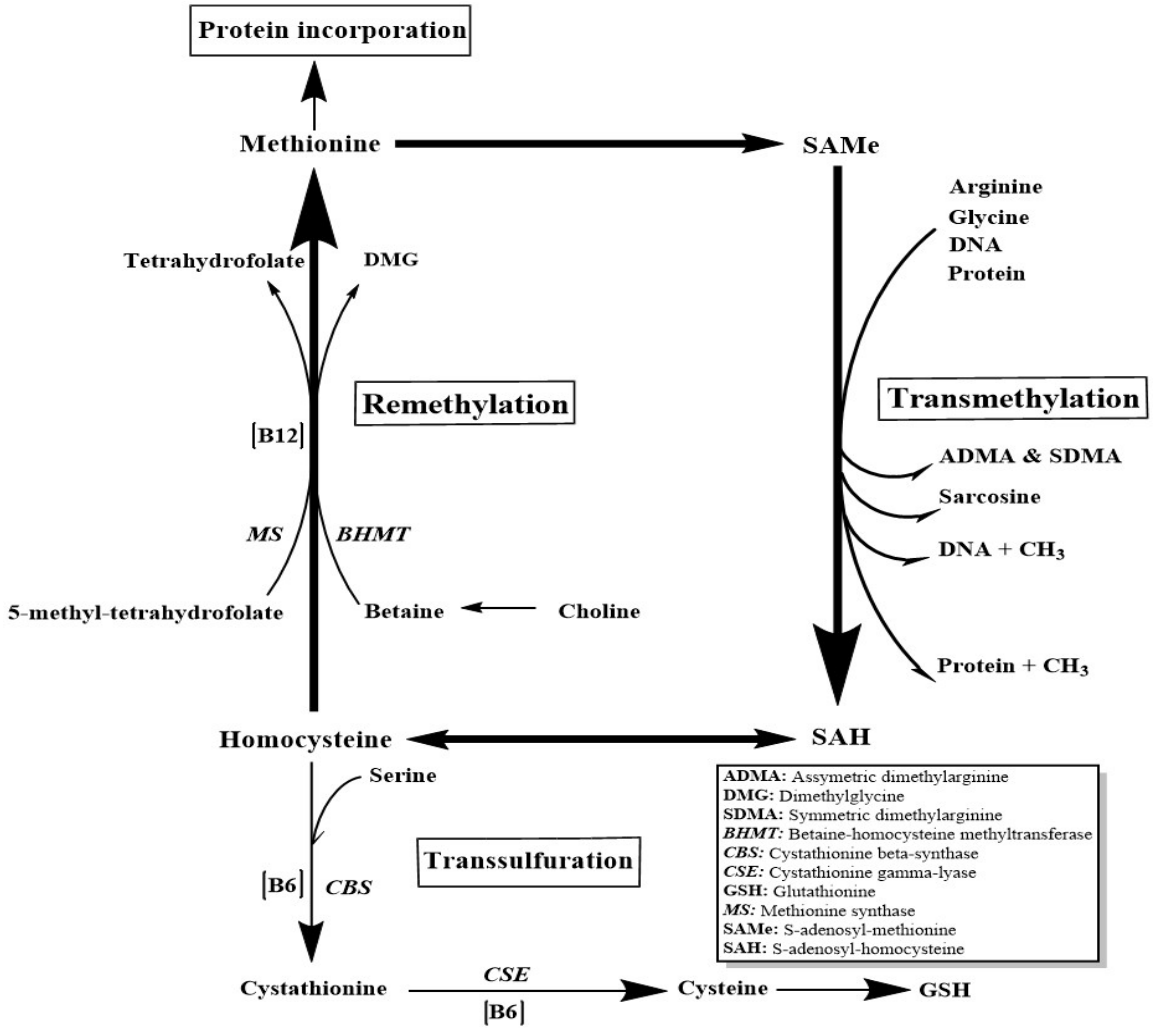
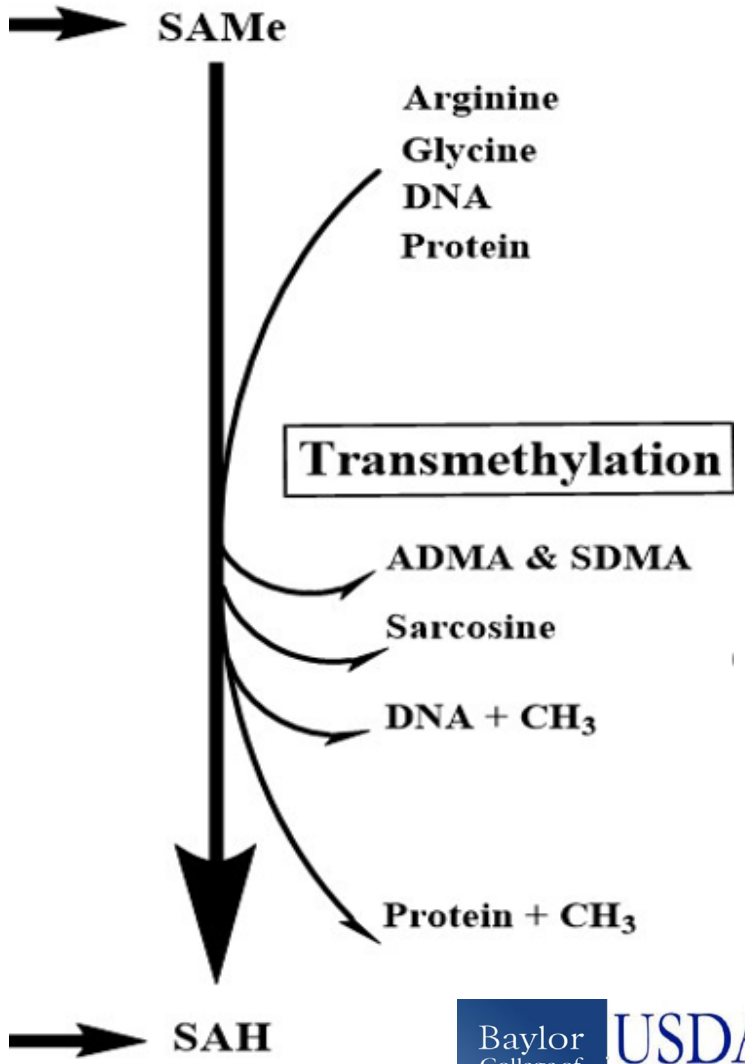
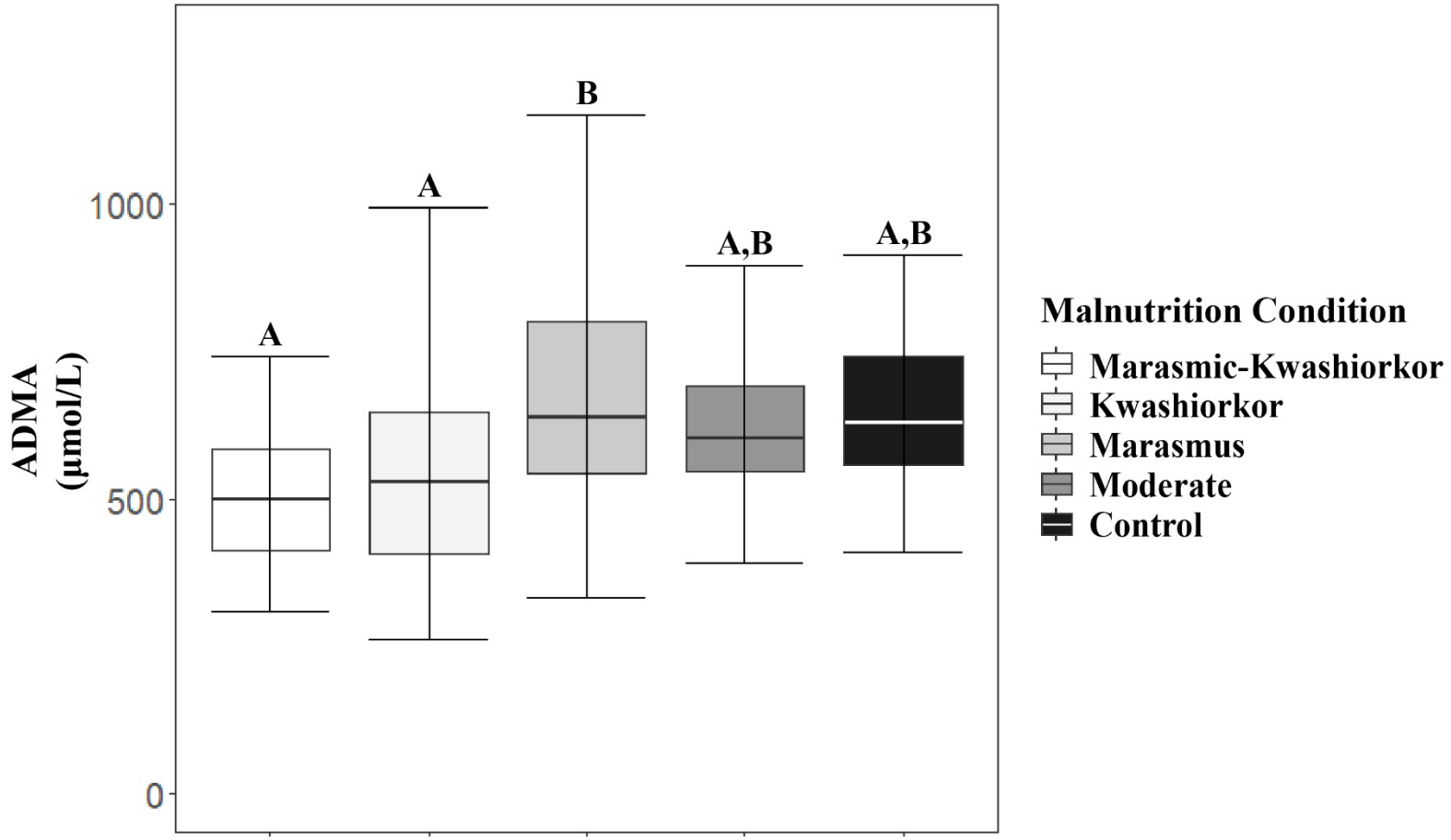
Transmethylation in marasmus & kwashiorkor





Asymmetric dimethyl arginine in marasmus & kwashiorkor



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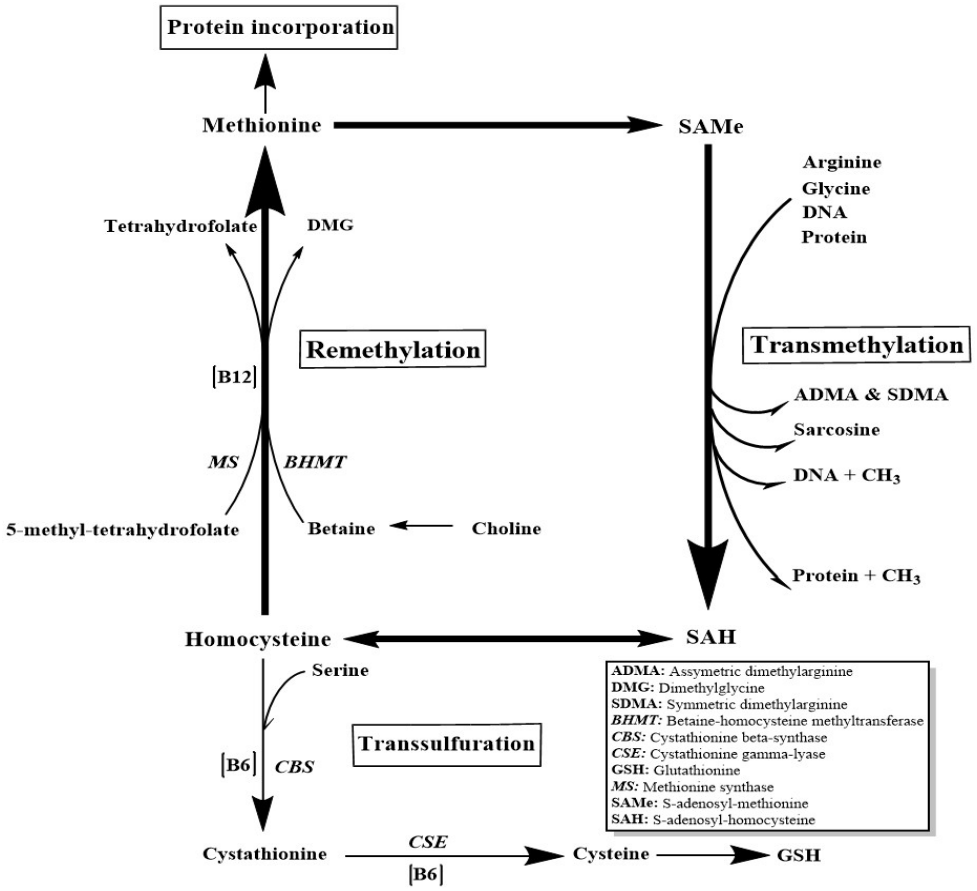
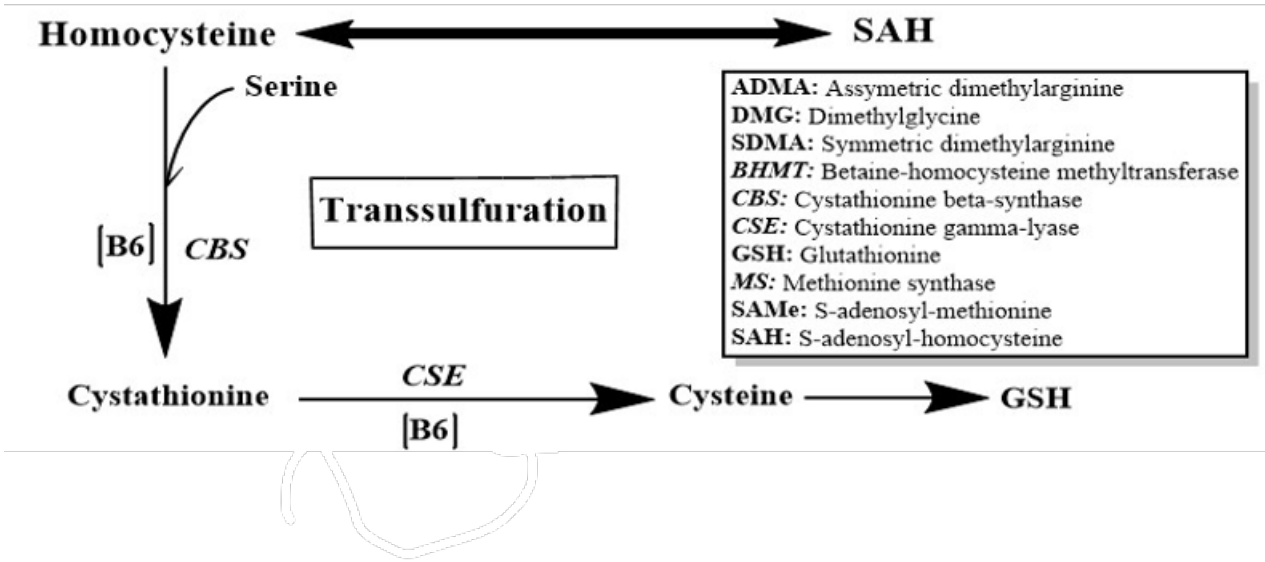
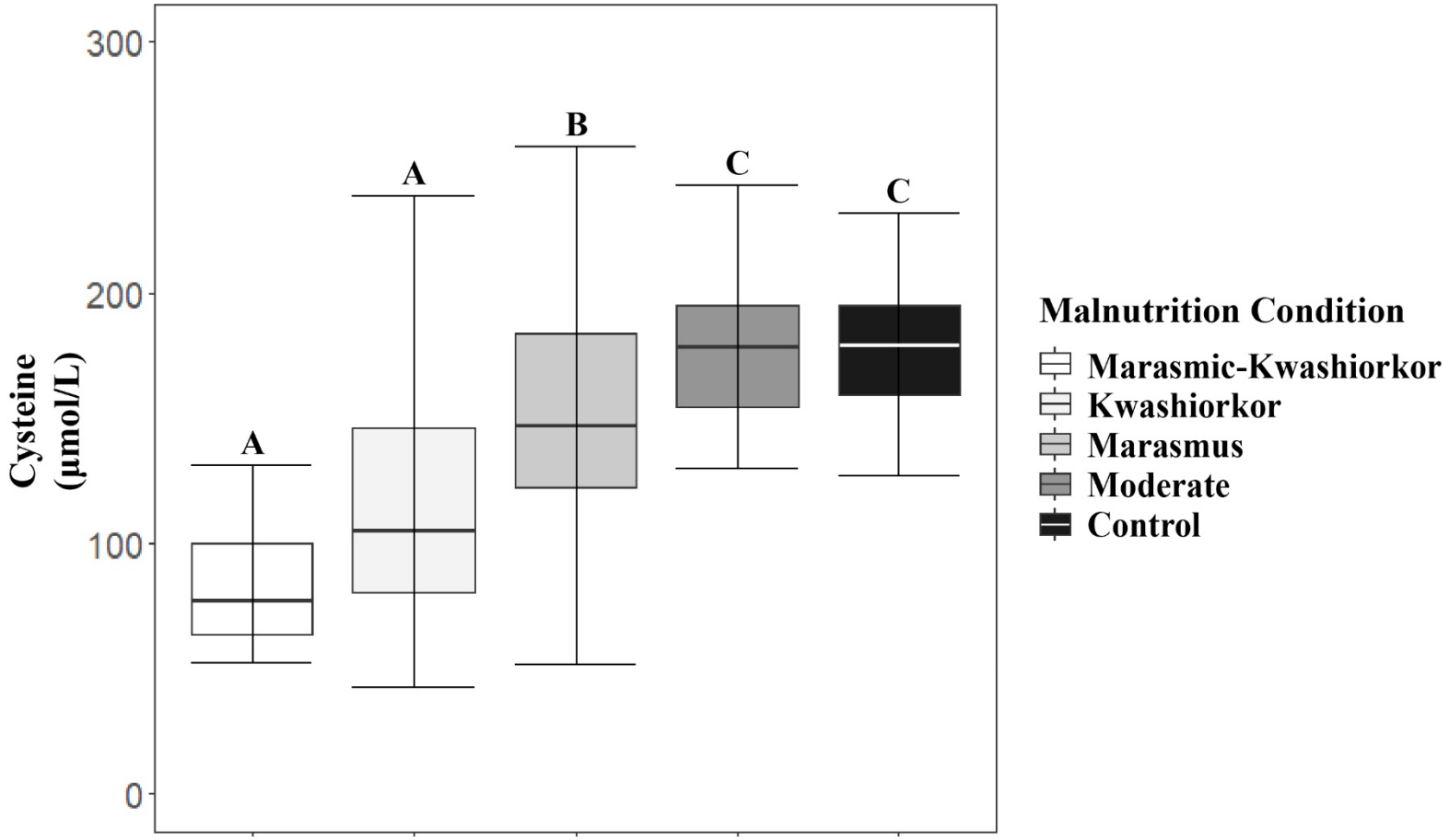
Transsulfuration in marasmus & kwashiorkor





Cysteine in marasmus & kwashiorkor



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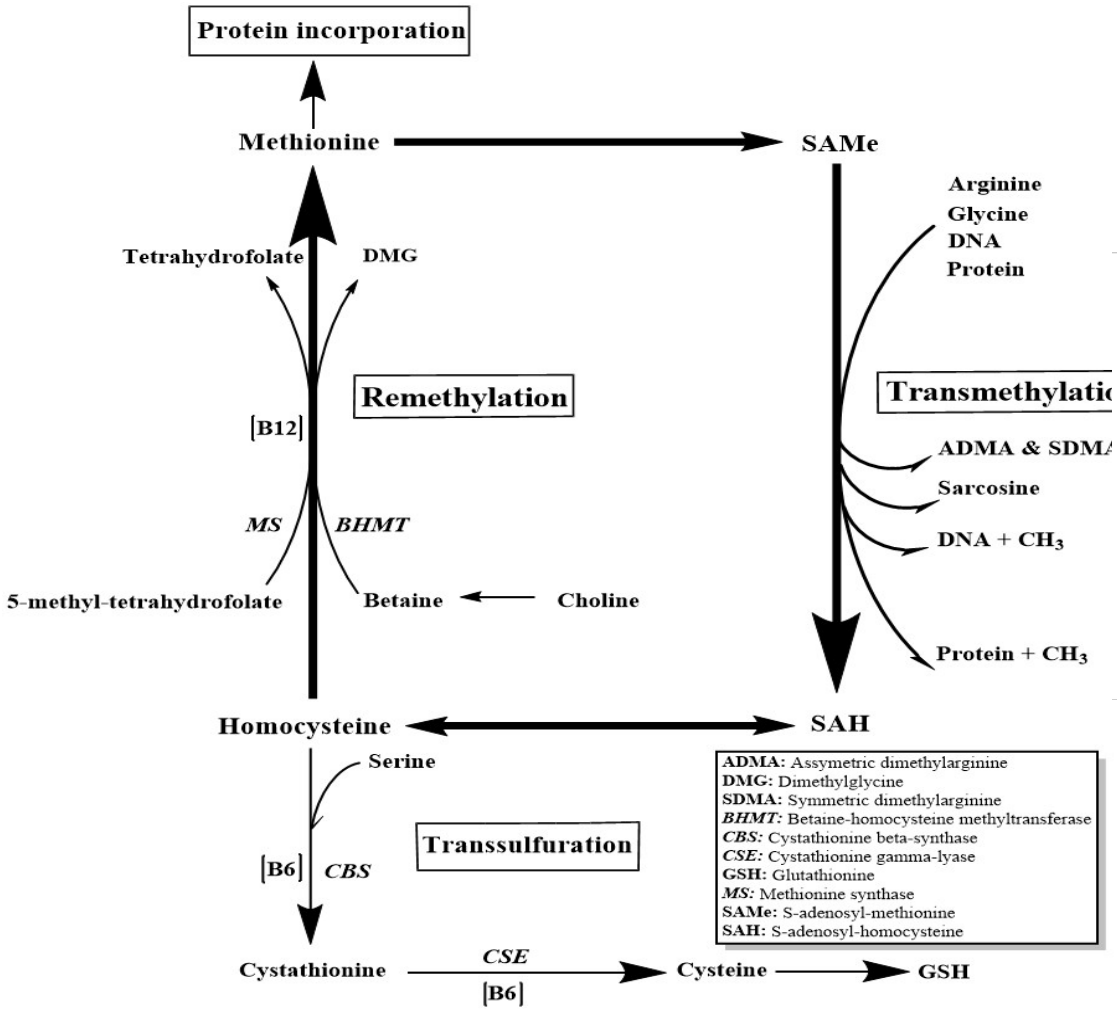
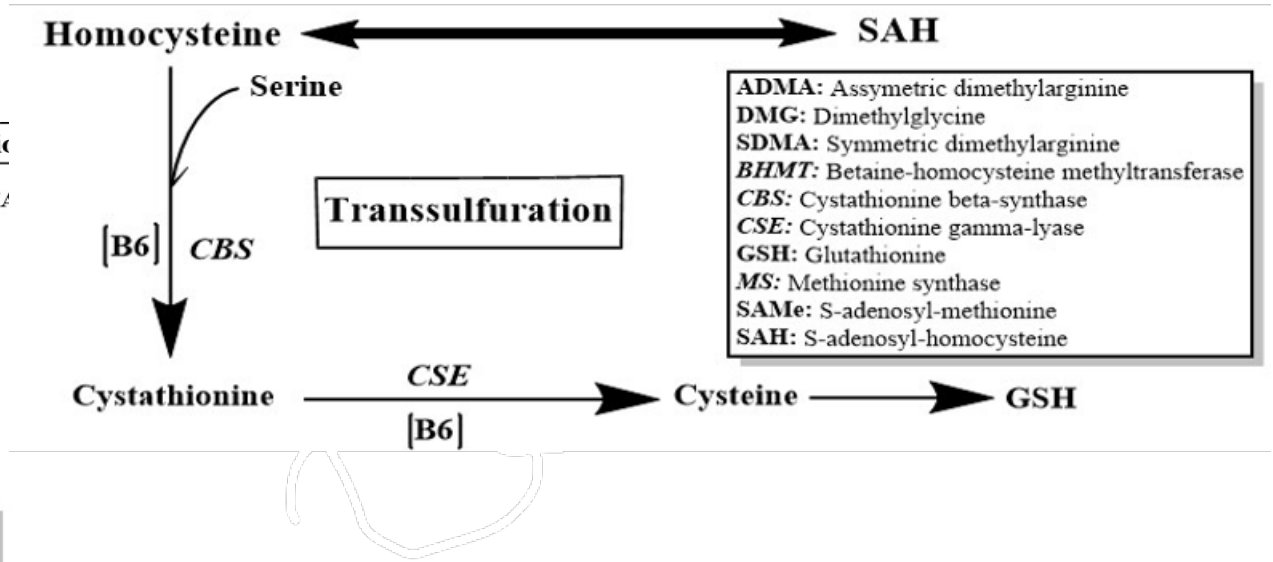
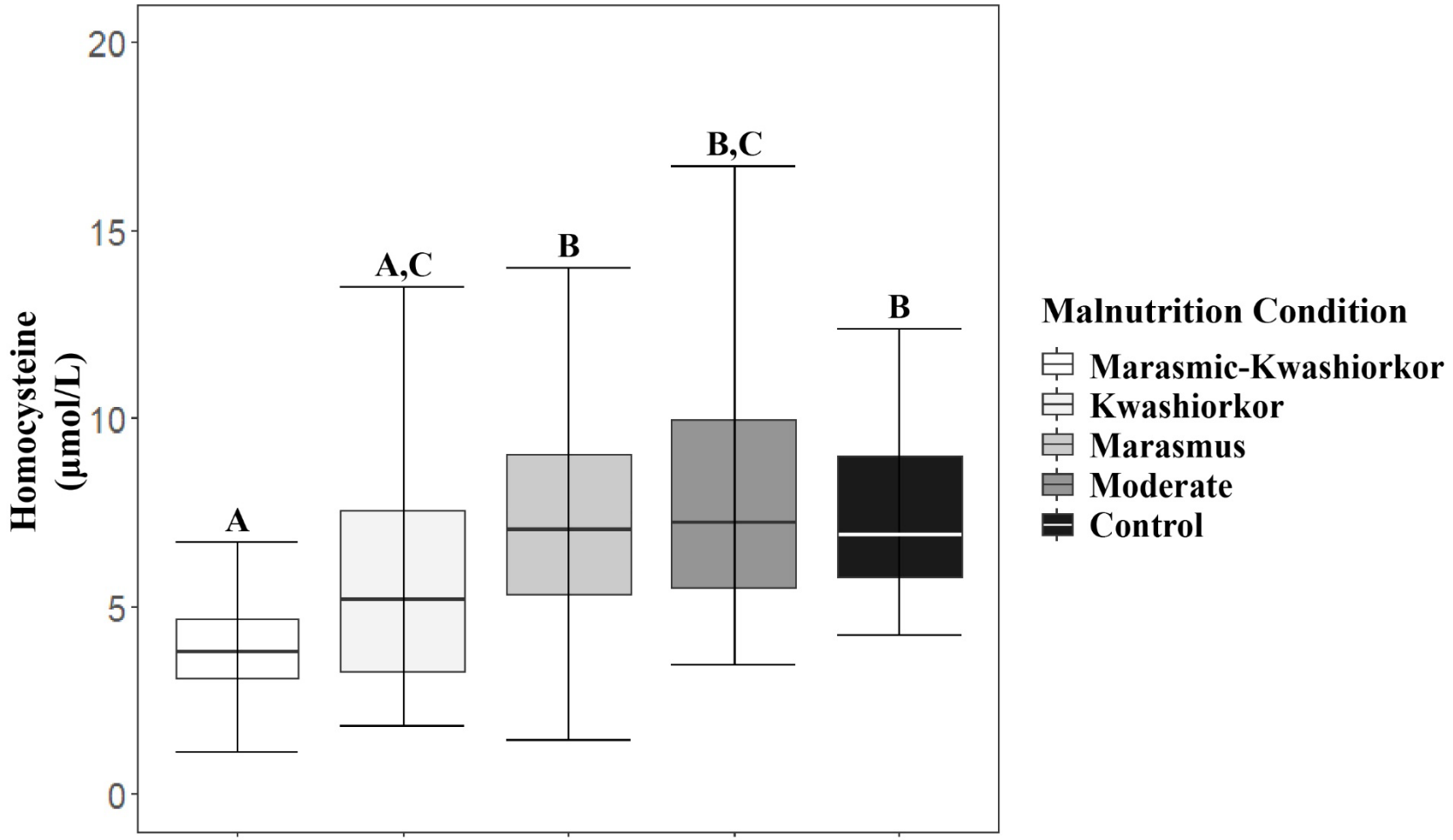
Transsulfuration in marasmus & kwashiorkor

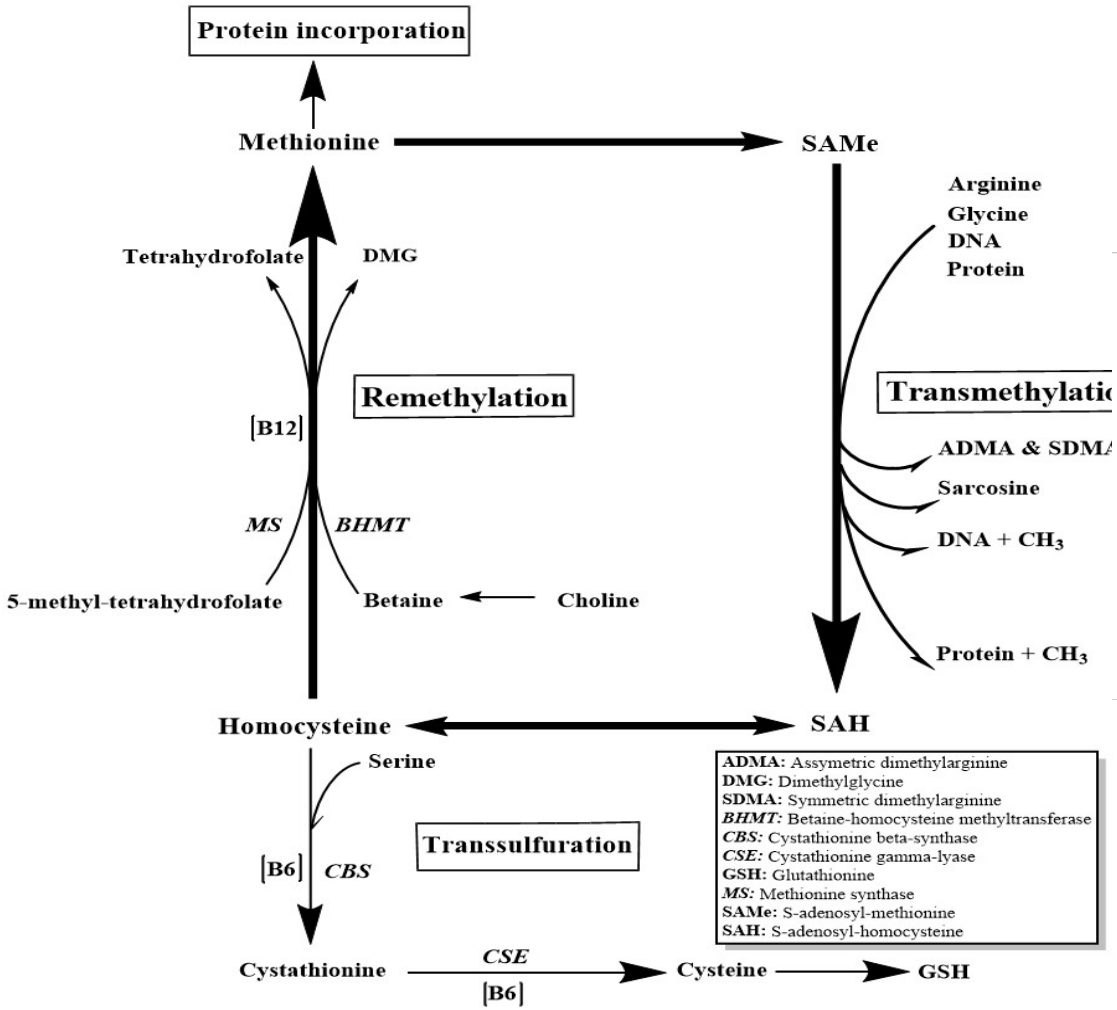


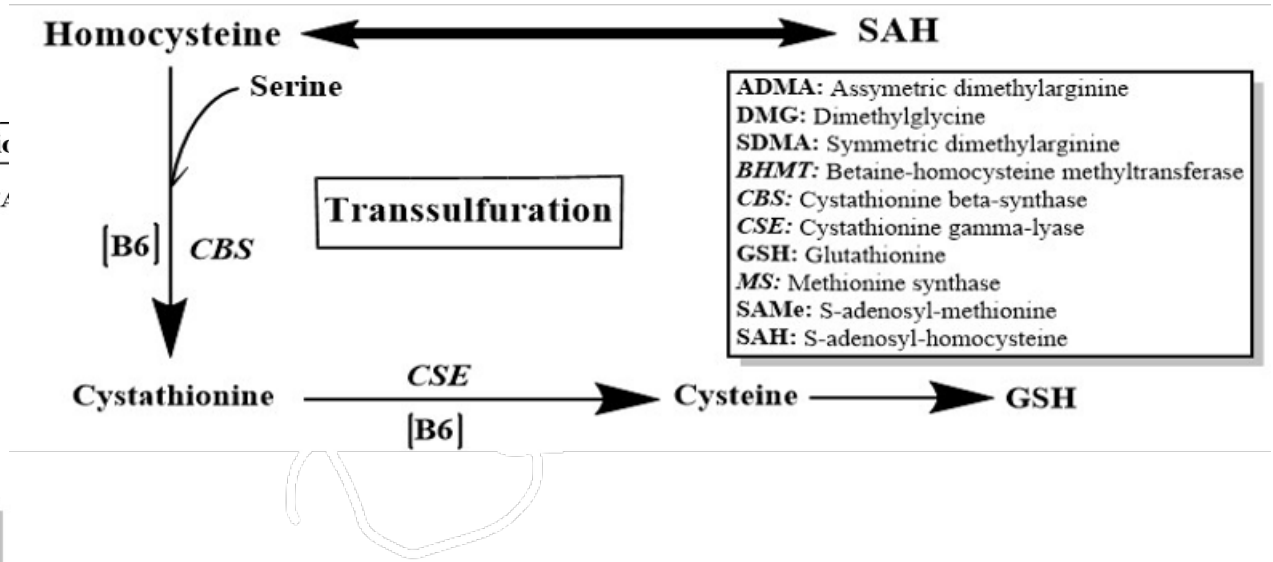


Homocysteine in marasmus & kwashiorkor

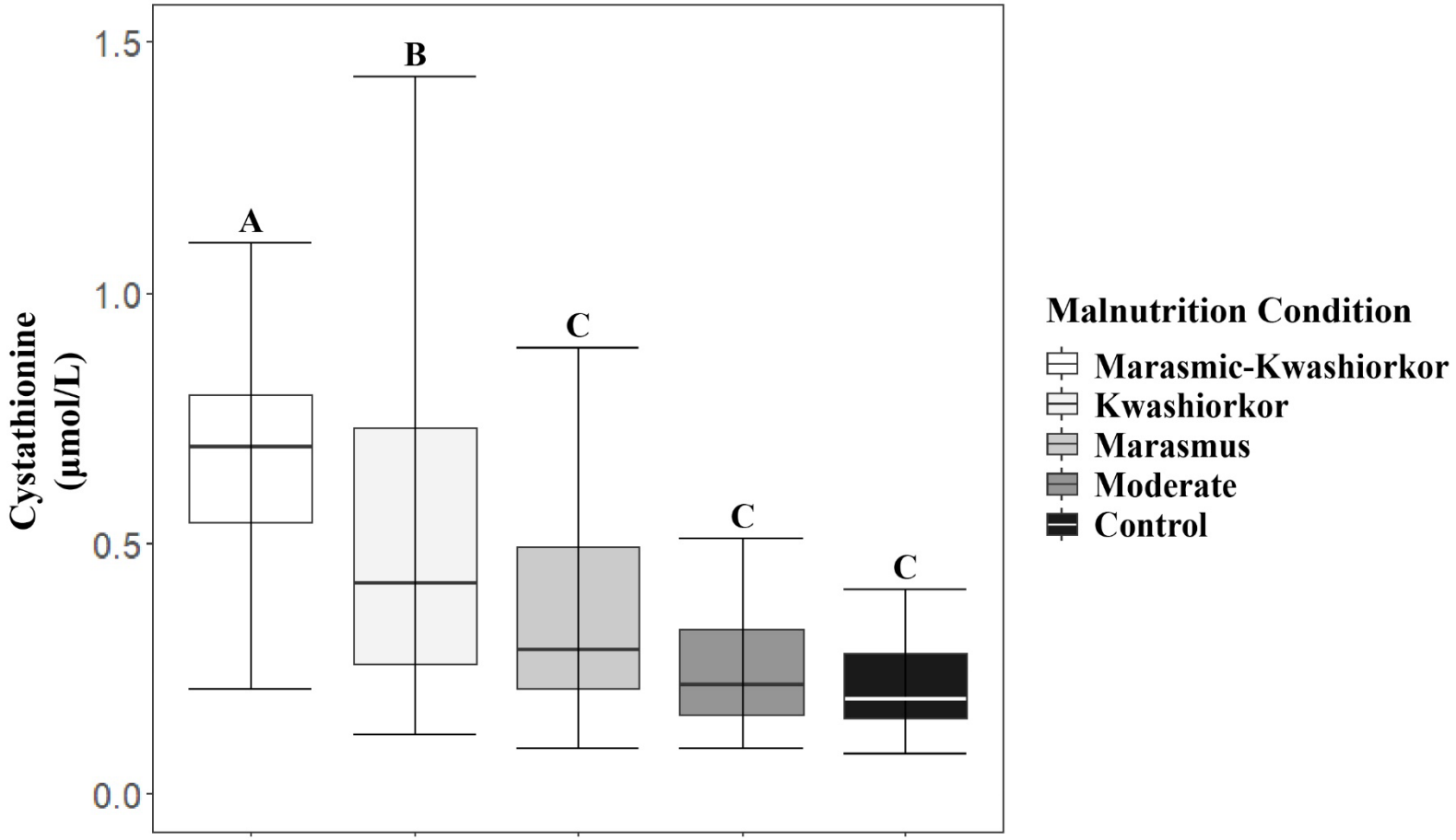


Transsulfuration in marasmus & kwashiorkor

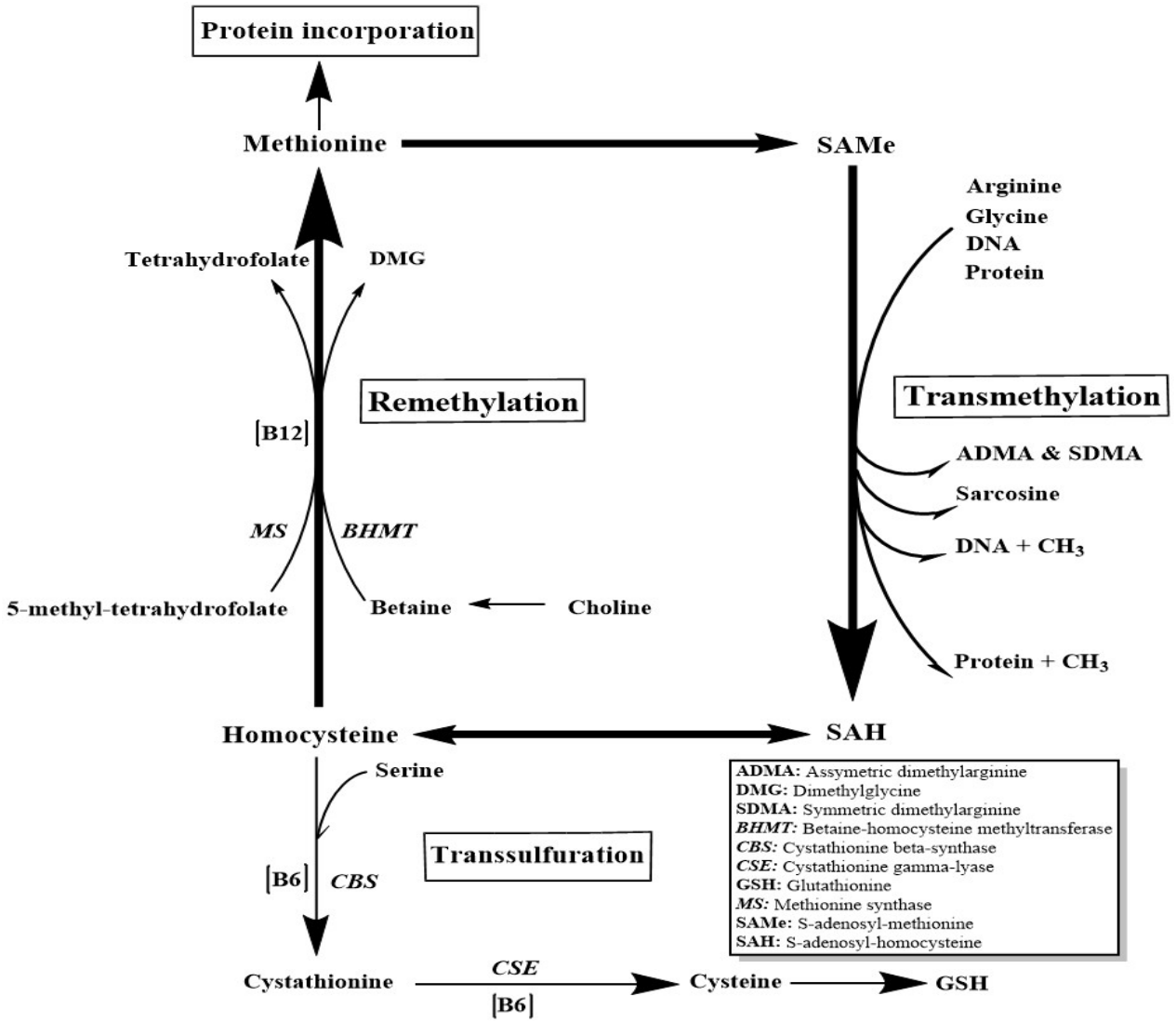


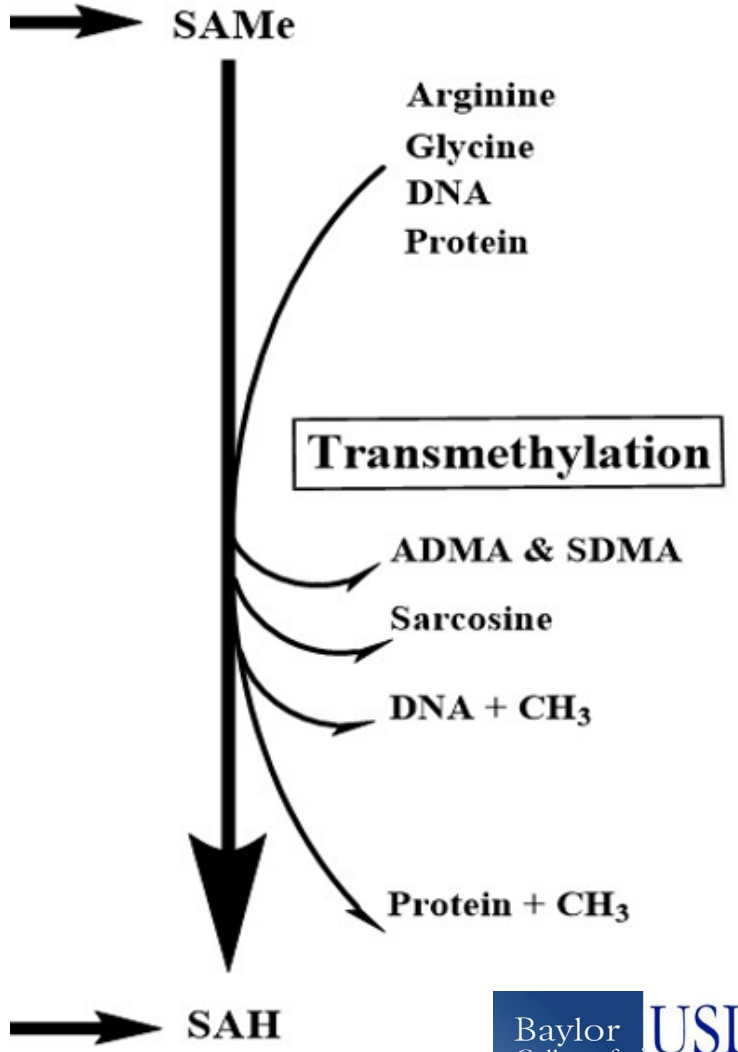


Cystathionine in marasmus & kwashiorkor



Methionine cycle in marasmus & kwashiorkor





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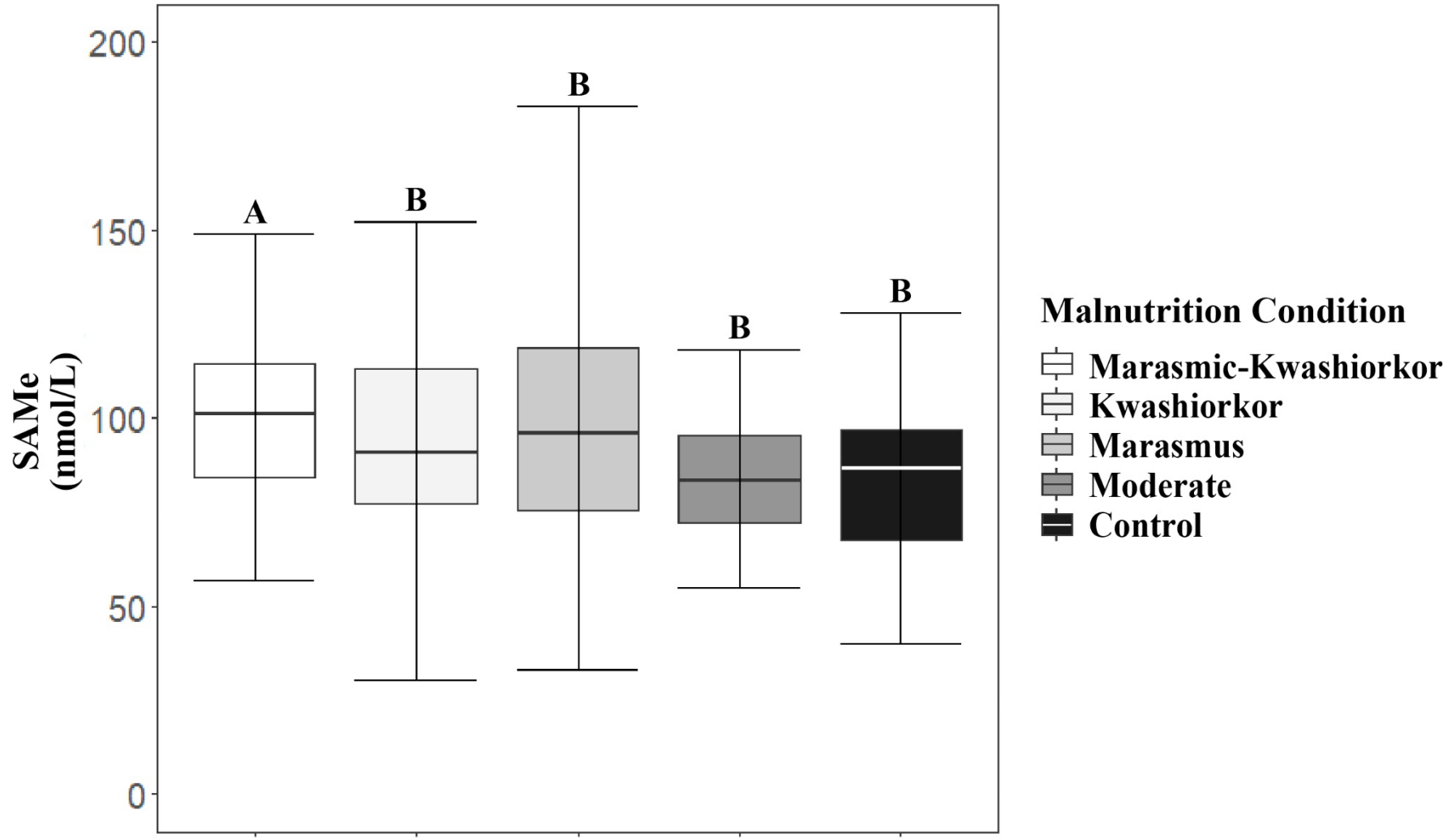
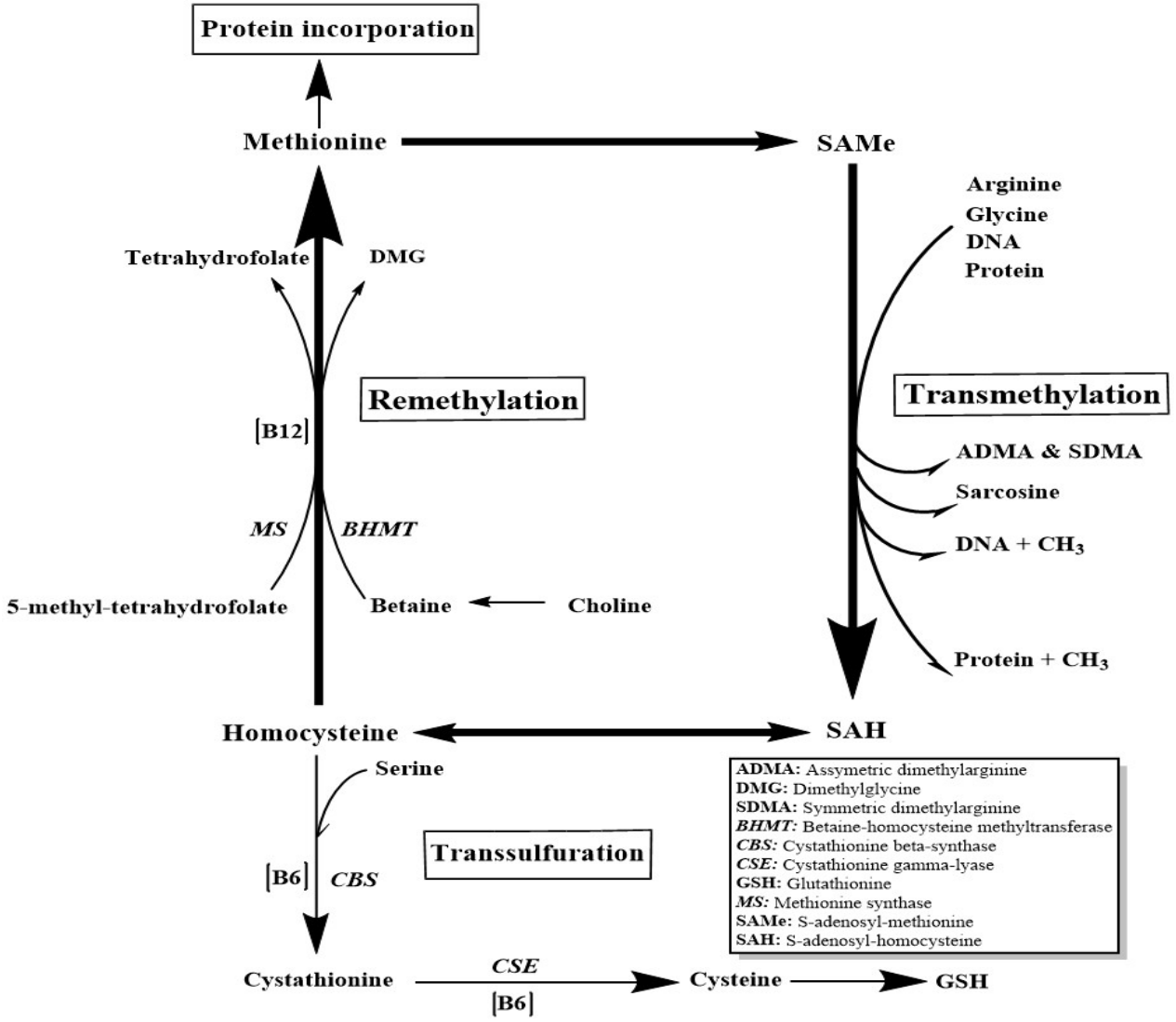
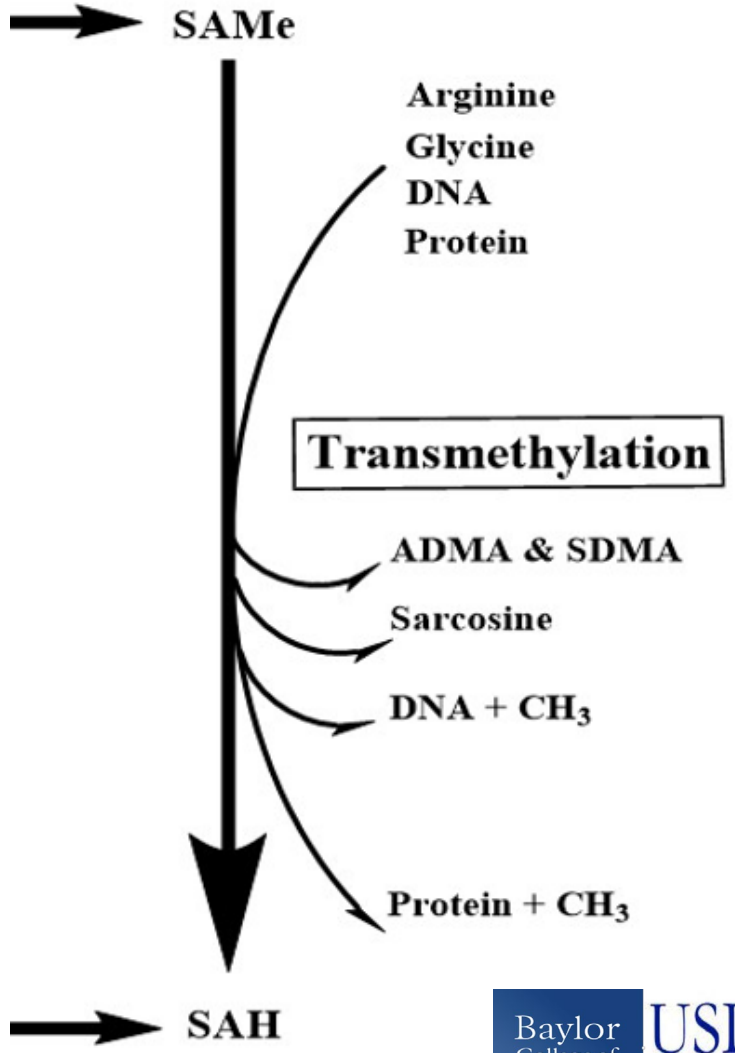
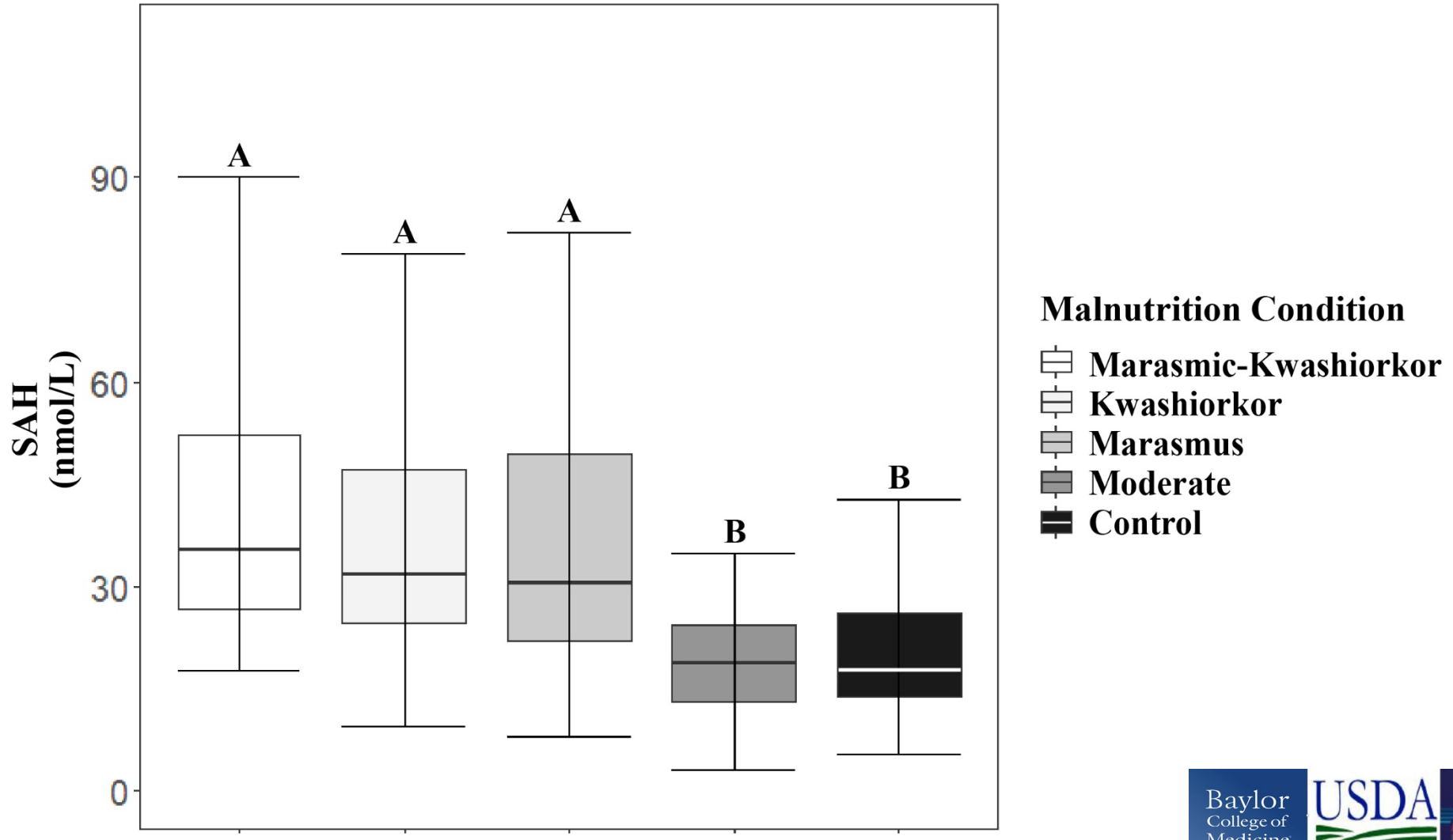


Methionine cycle in marasmus & kwashiorkor





S-adenosyl Homocysteine (SAH) in marasmus and kwashiorkor



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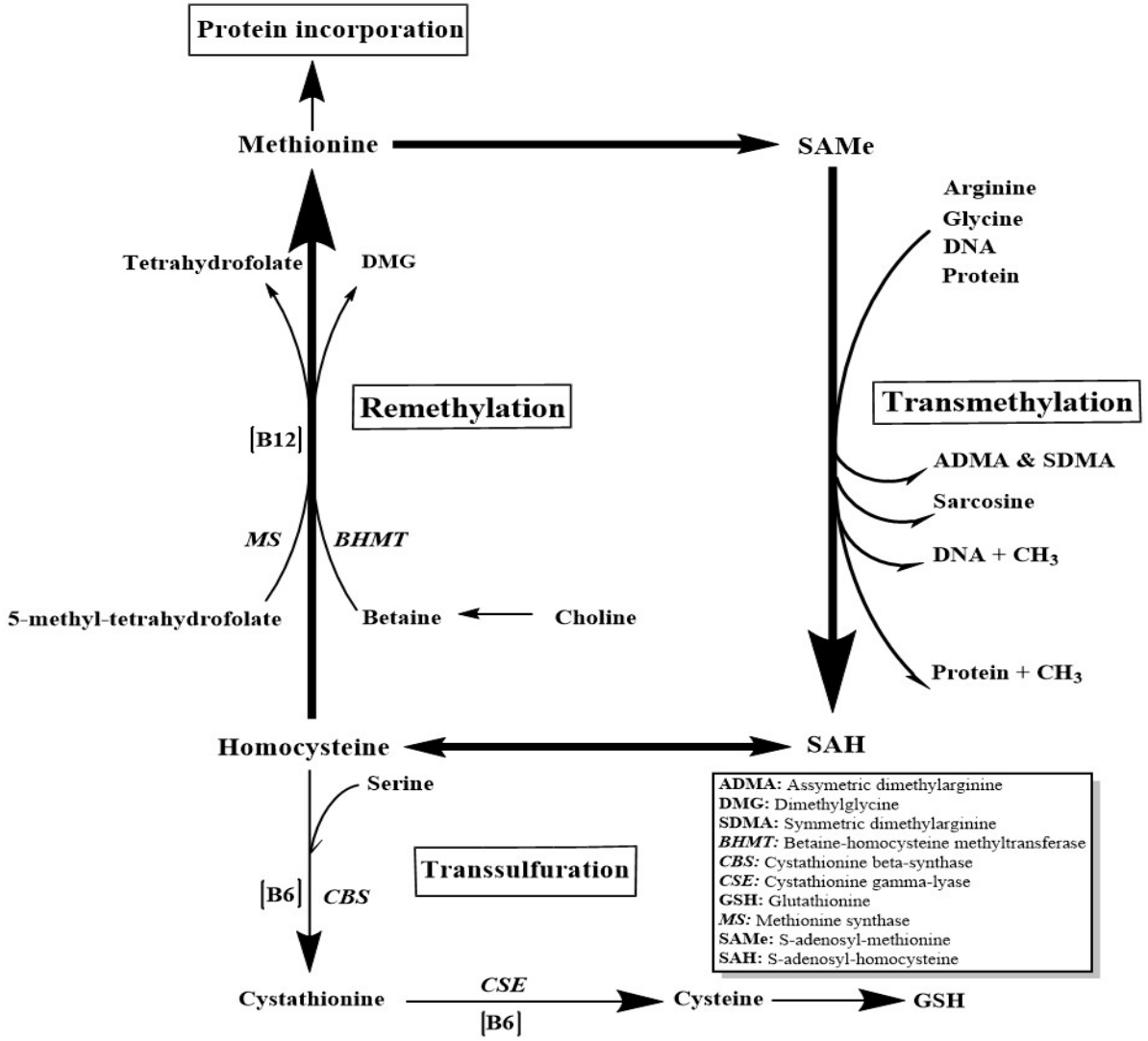
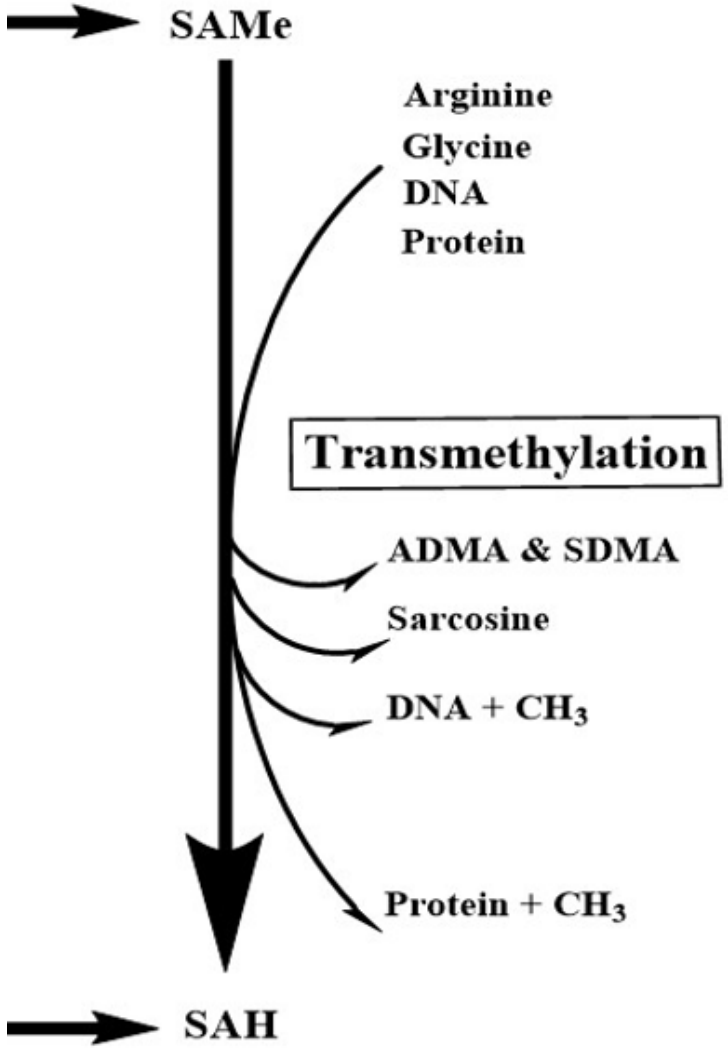
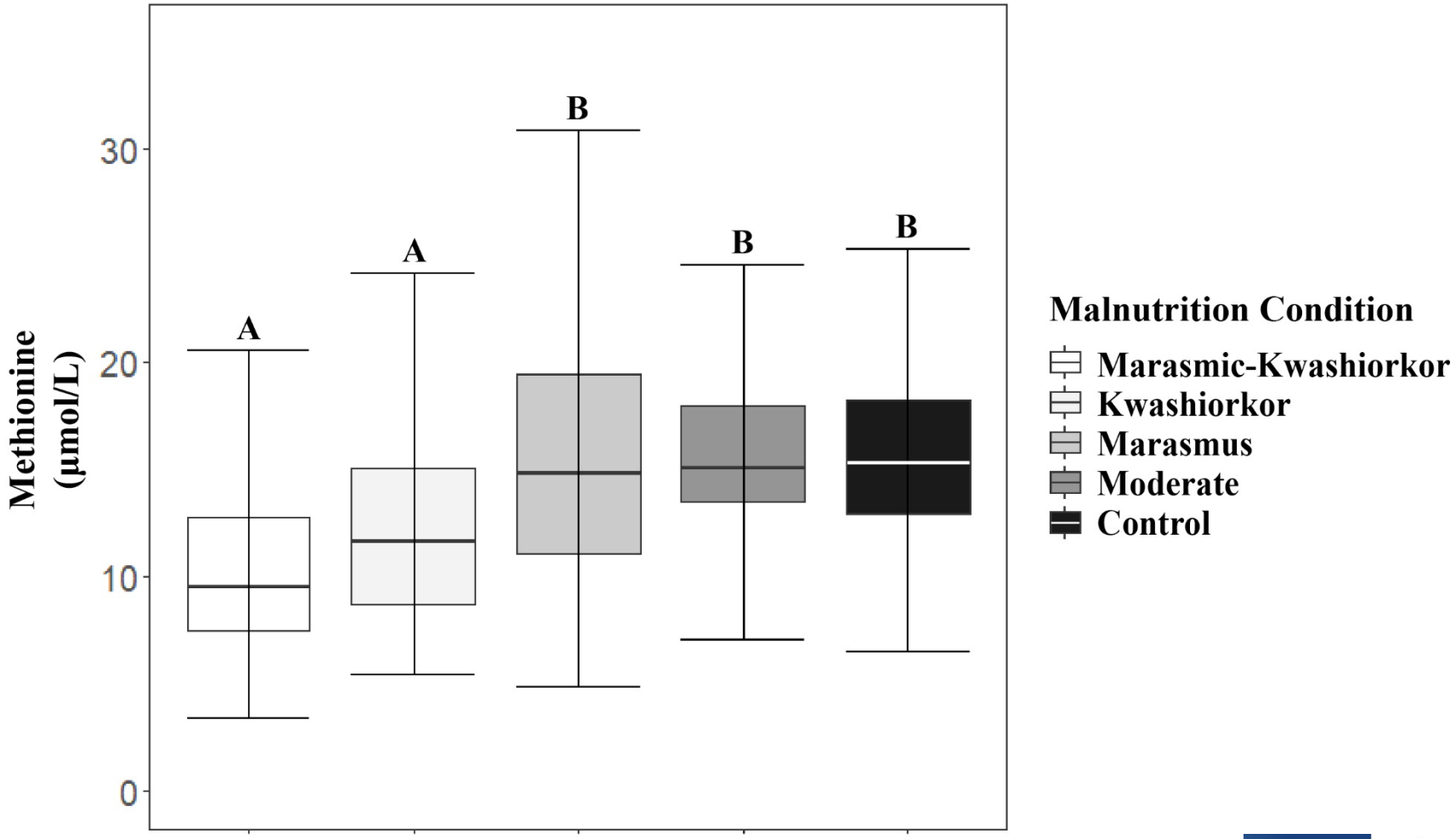
Methionine cycle in marasmus & kwashiorkor





Methionine in marasmus and kwashiorkor



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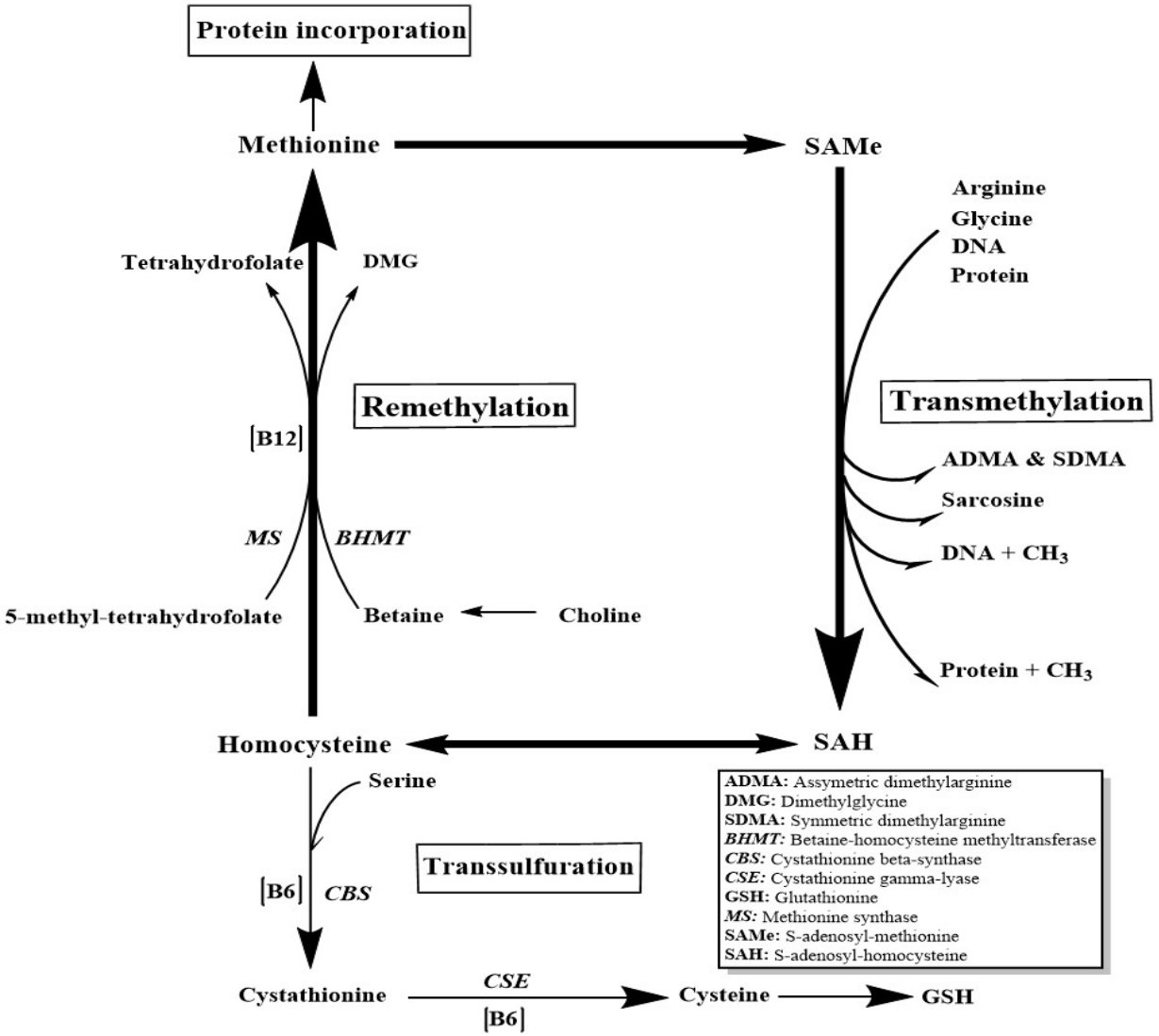
Methyl donors & vitamin co-factors in marasmus & kwashiorkor

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



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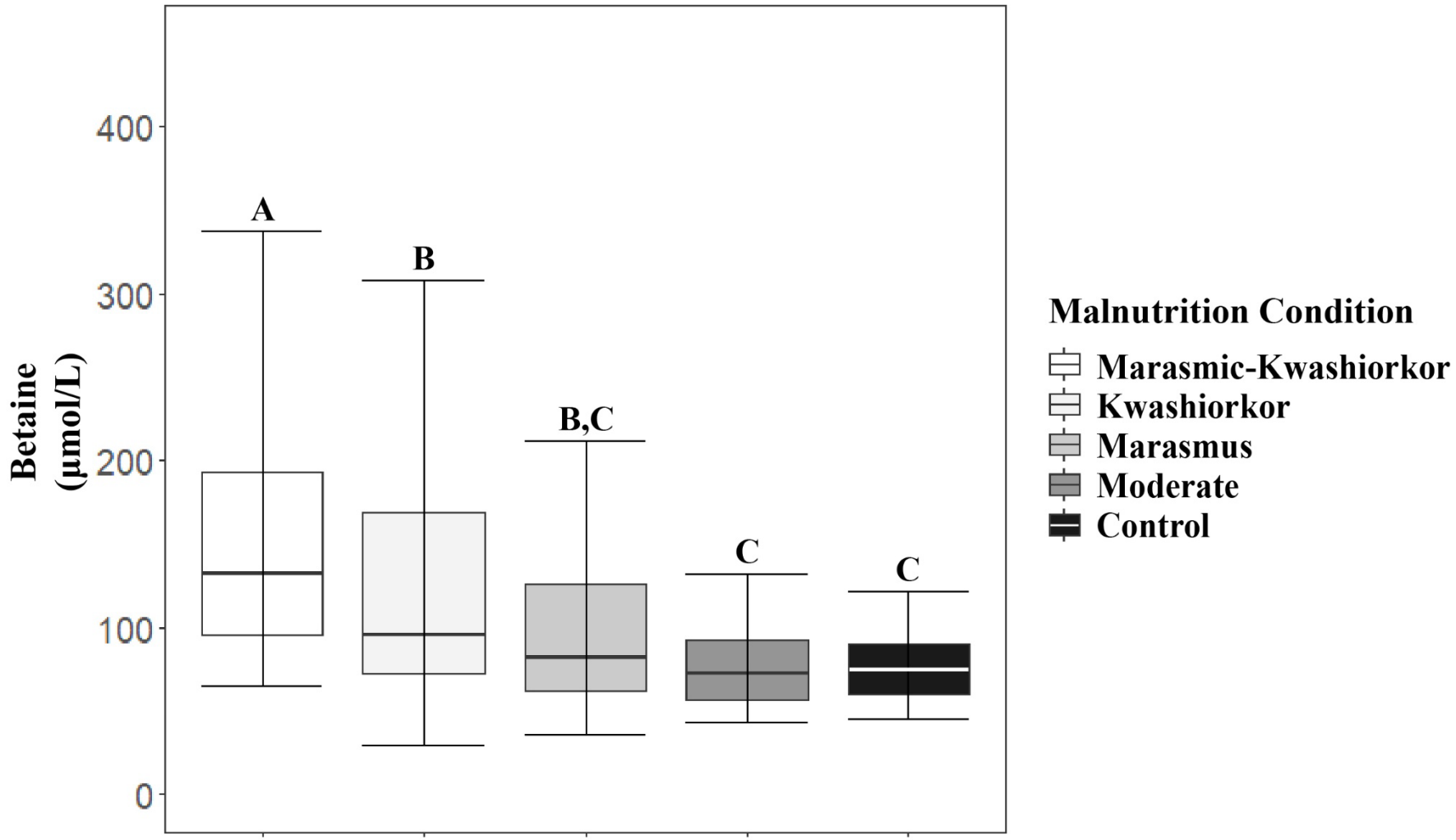
Betaine in marasmus & kwashiorkor



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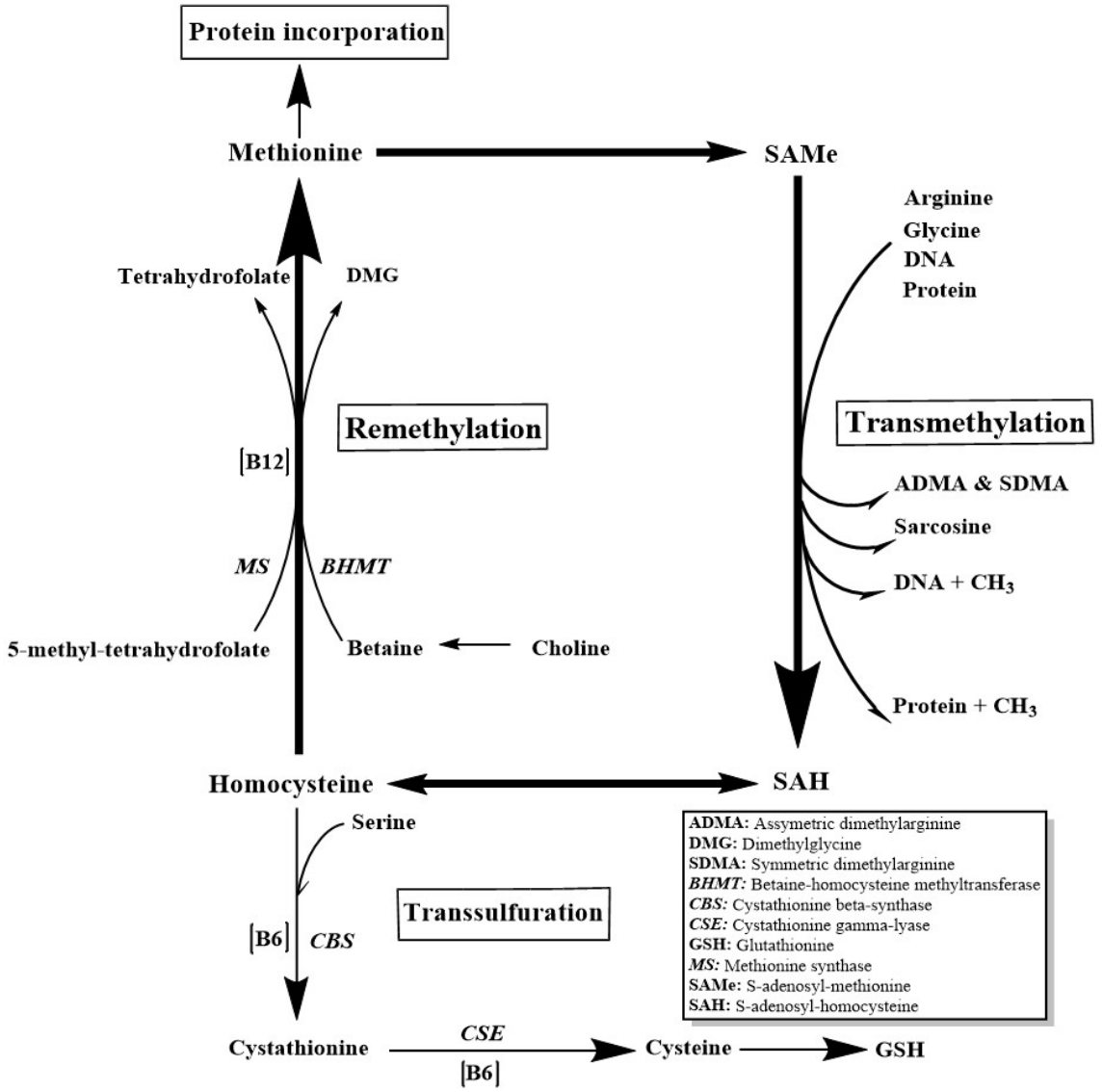
One-Carbon metabolism in kwashiorkor relative to marasmus

- **Kwashiorkor's distinguishing features include:**
  - Transsulfuration disturbances
  - ↓ Methionine
  - ↓ Homocysteine
  - ↓ Cysteine
  - ↓ADMA
  - ↓ SAMe:SAH



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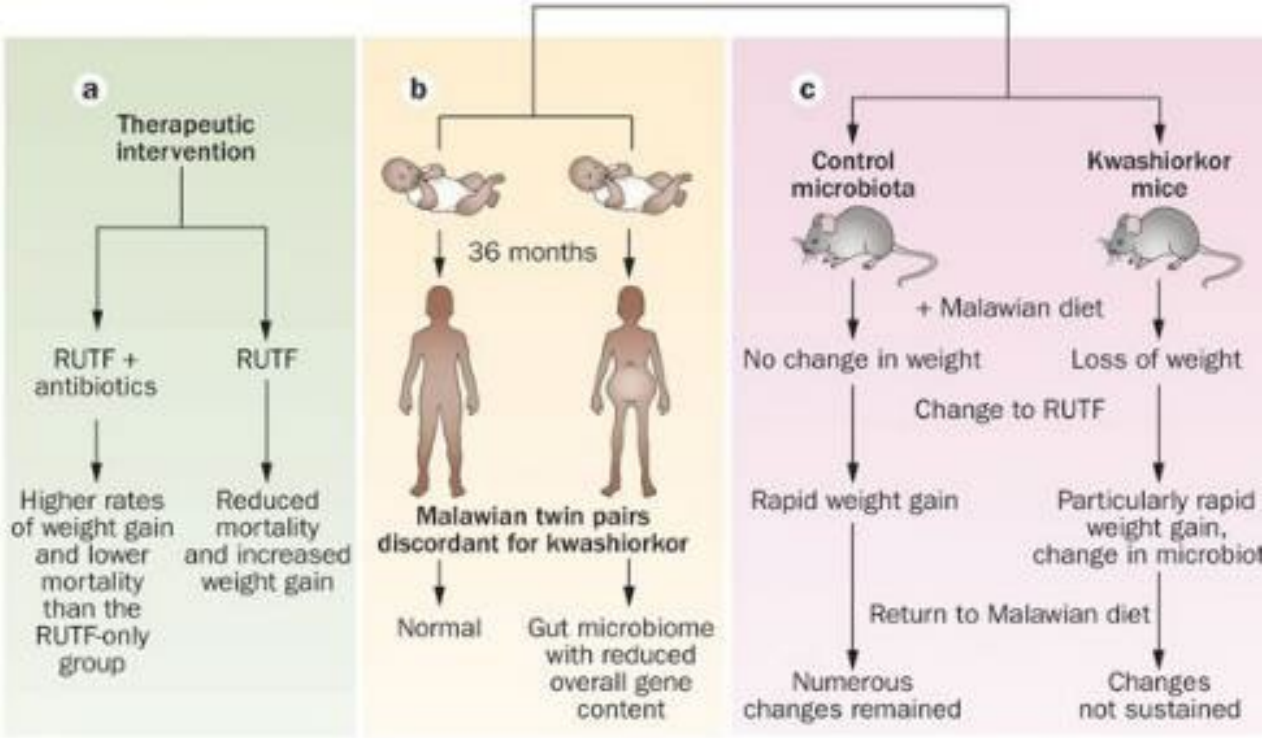
The gut microbiome in kwashiorkor

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

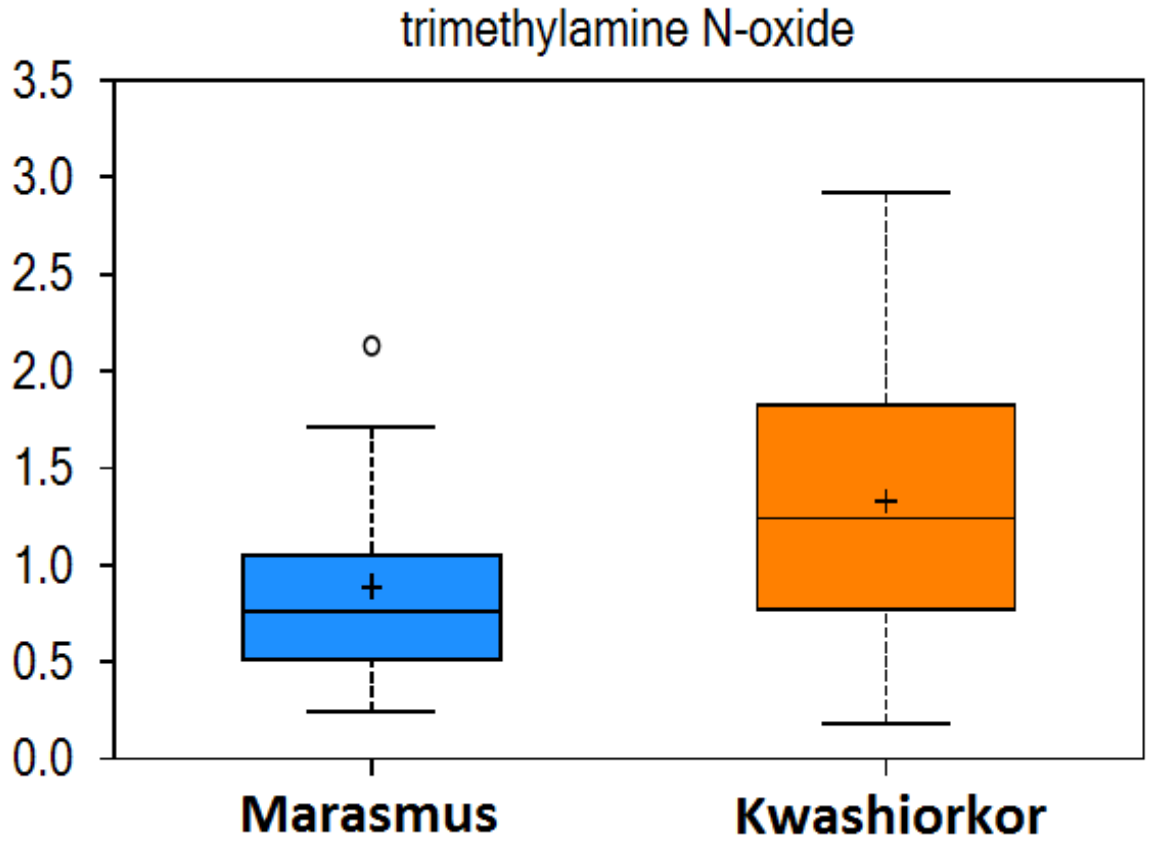


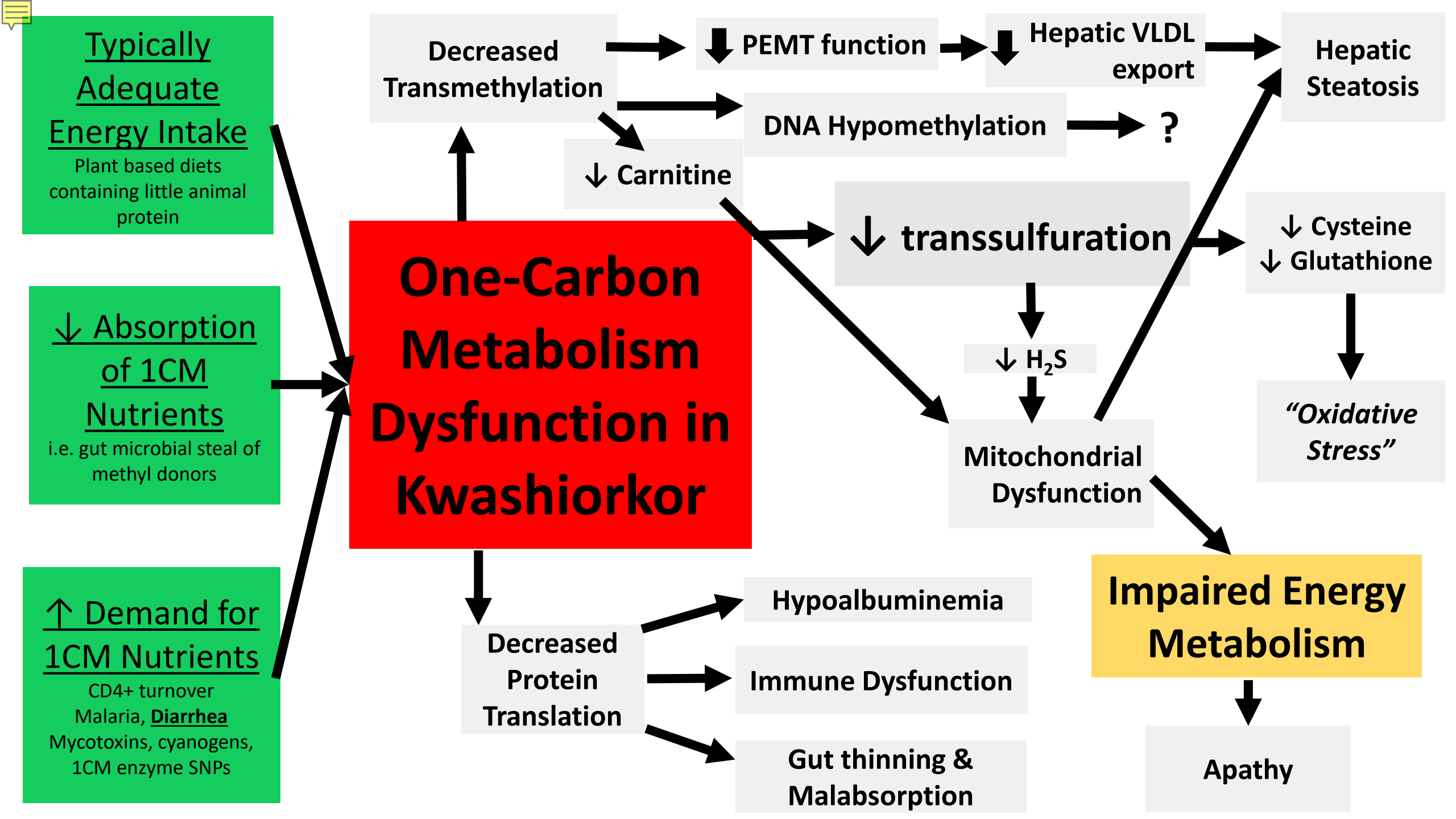
Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science. 2013. Smith et al.

Antibiotics for uncomplicated severe malnutrition. N Engl J Med. 2013. Trehan et al.

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.

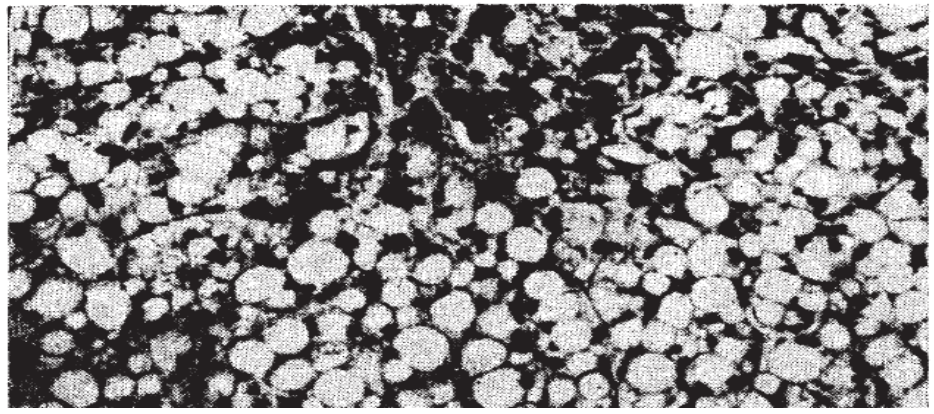


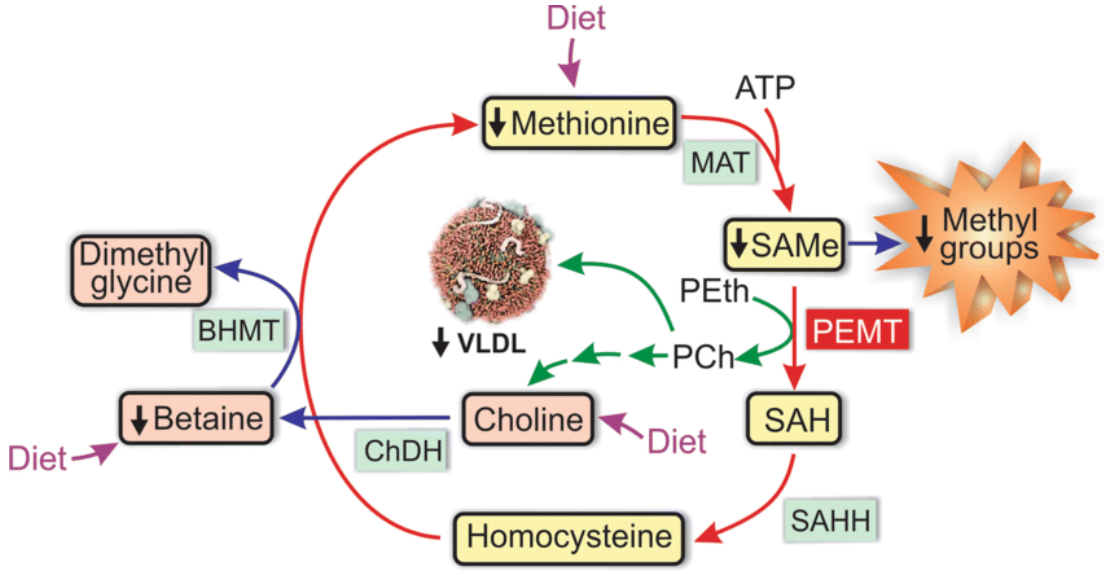

Typically Adequate Energy Intake
Plant based diets containing little animal protein
↓ Absorption of 1CM Nutrients
i.e. gut microbial steal of methyl donors
↑ Demand for 1CM Nutrients
CD4+ turnover
Malaria, Diarrhea
Mycotoxins, cyanogens, 1CM enzyme SNPs
One-Carbon Metabolism Dysfunction in Kwashiorkor
Decreased Transmethylation
PEMT function
Hepatic VLDL export
Hepatic Steatosis
DNA Hypomethylation
?
↓ Carnitine
↓ transsulfuration
↓ Cysteine
↓ Glutathione
↓ H_2S
"Oxidative Stress"
Mitochondrial Dysfunction
Impaired Energy Metabolism
Decreased Protein Translation
Hypoalbuminemia
Immune Dysfunction
Gut thinning & Malabsorption
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?





1. A nutritional disease of childhood associated with a maize diet. Arch Dis Child. 1933. Williams CD.
2. Molecular pathways of nonalcoholic fatty liver disease development and progression. Cell Mol Life Sci. 2018 Oct 20. Bessone et al.

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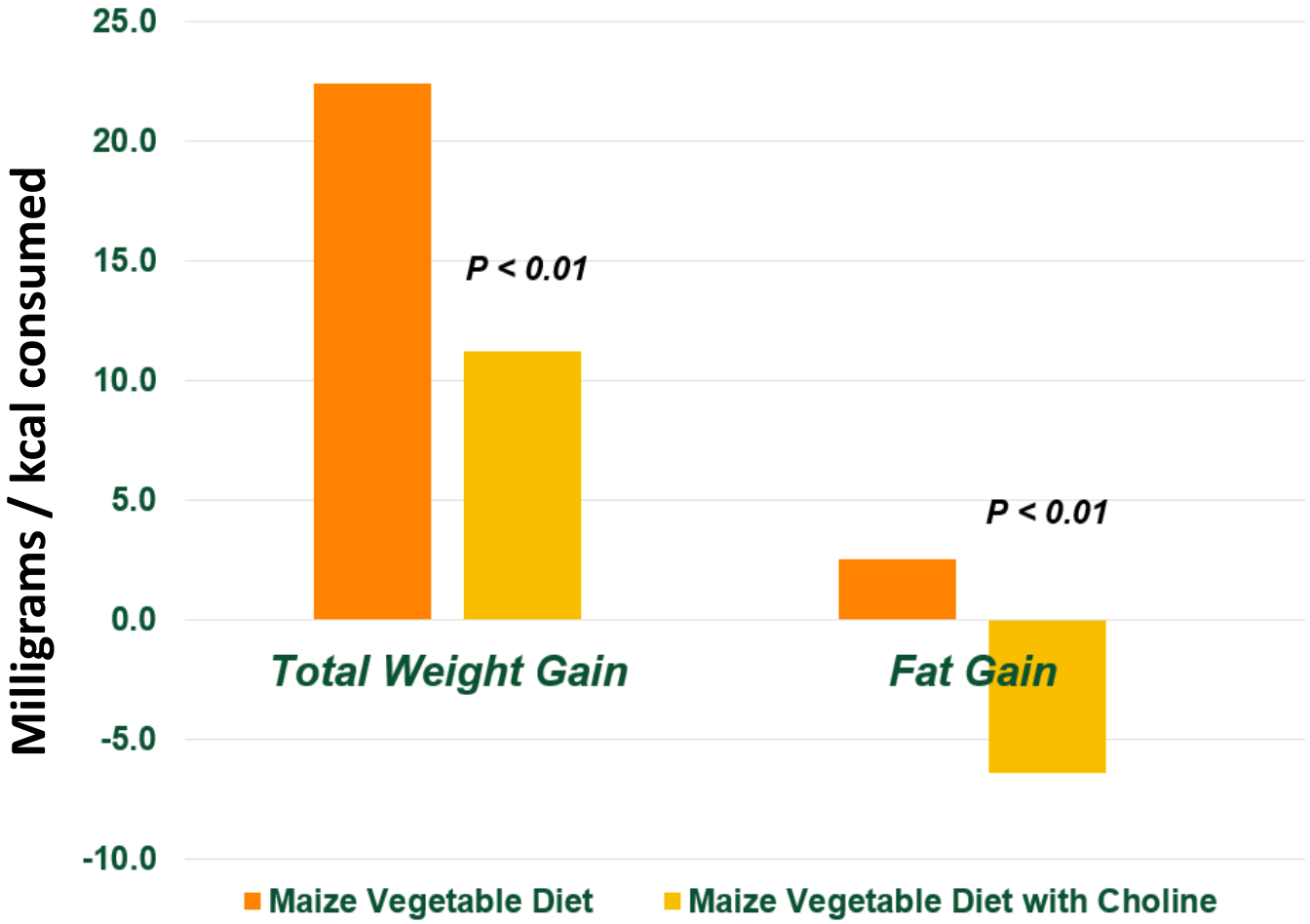
Weight gain: MVD vs. MVD + choline

- ***Reduced fat gain*** in mice fed a choline supplemented MVD is consistent with improved energy metabolism after choline administration.



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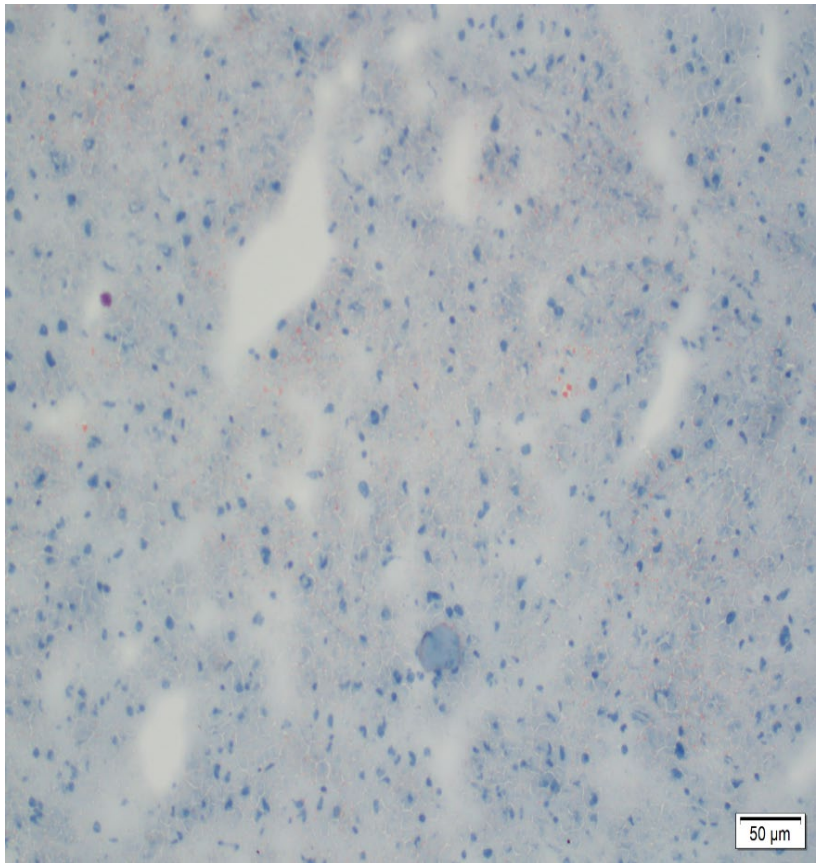
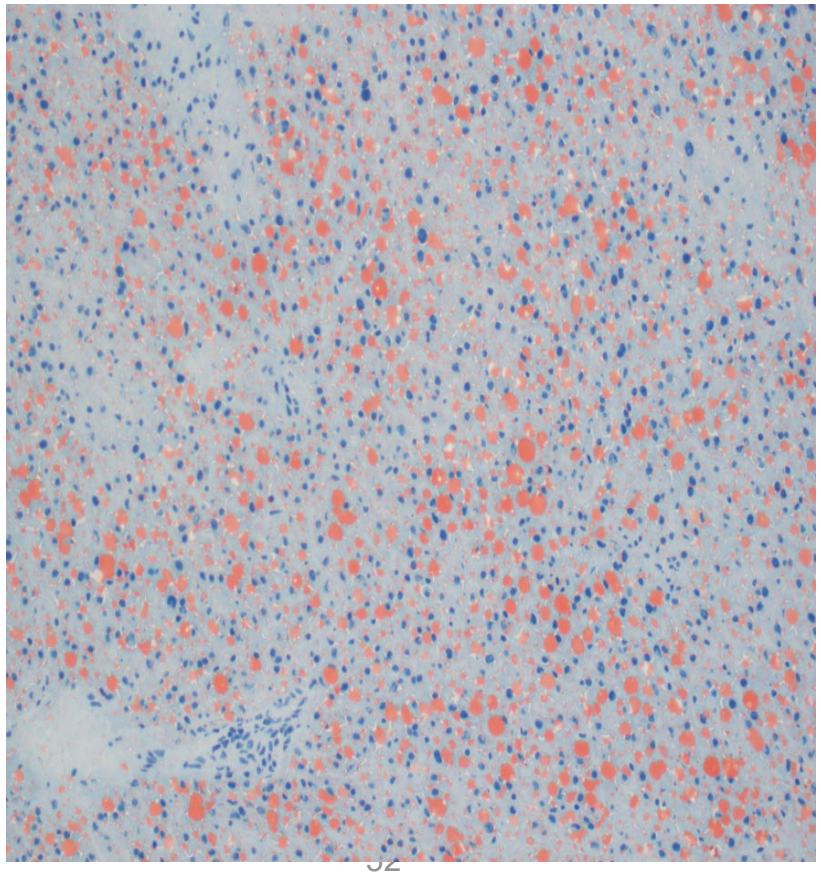
Maize Vegetable Diet (MVD) with & without choline

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

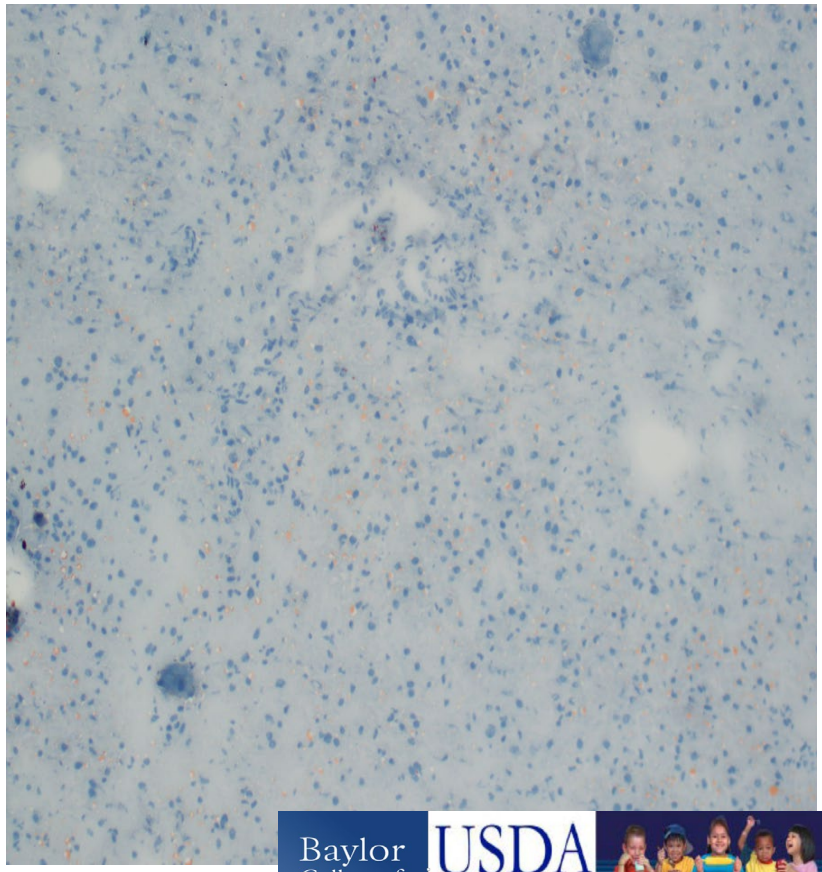
Control + choline



MVD



MVD + choline



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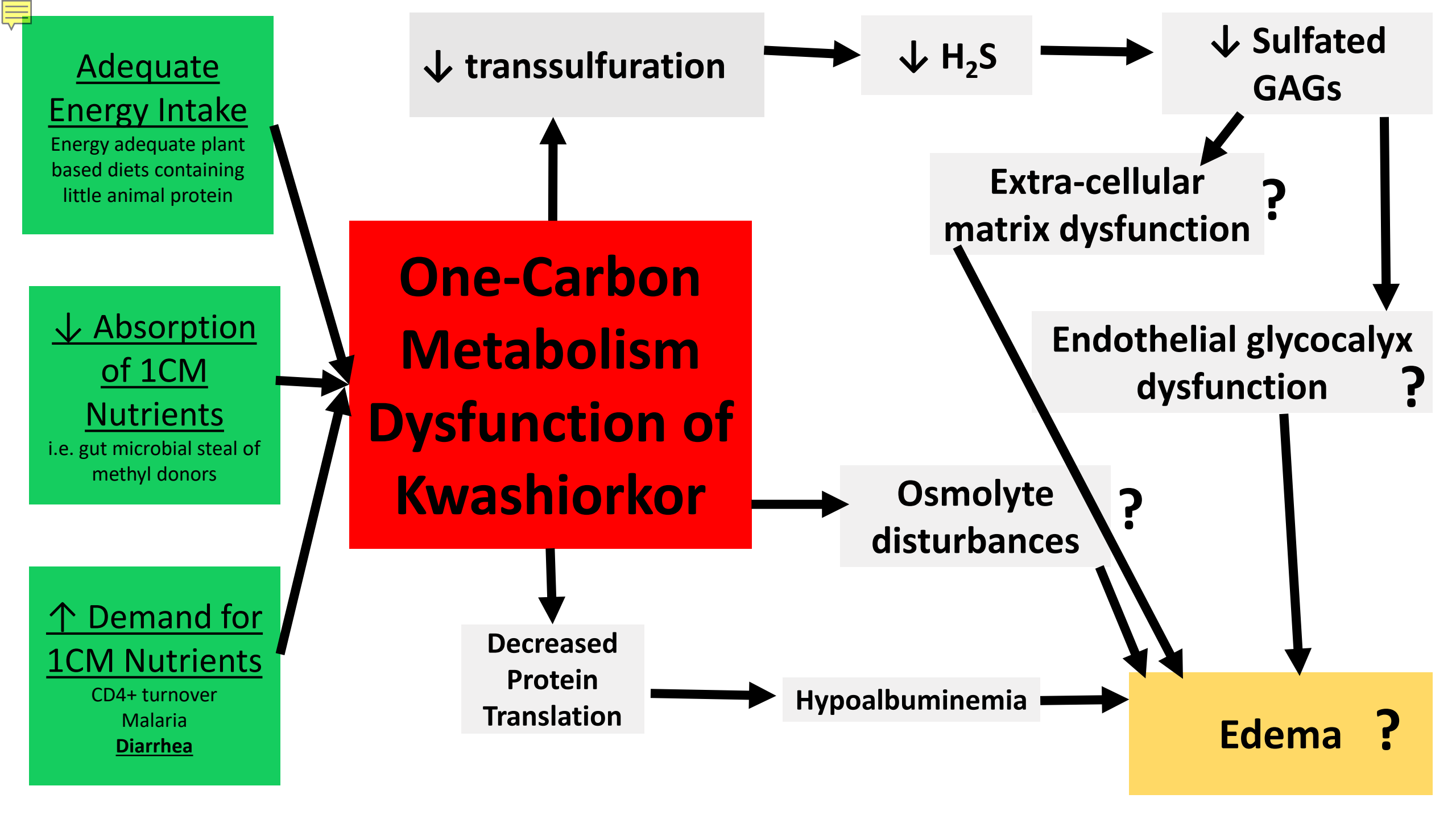
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CD4+ turnover
Malaria
Diarrhea
One-Carbon Metabolism Dysfunction of Kwashiorkor
↓ transsulfuration
↓ H_2S
↓ Sulfated GAGs
Extra-cellular matrix dysfunction ?
Endothelial glycocalyx dysfunction ?
Osmolyte disturbances ?
Decreased Protein Translation
Hypoalbuminemia
Edema ?

One-carbon metabolism dysfunction in the pathogenesis of nutritional edema

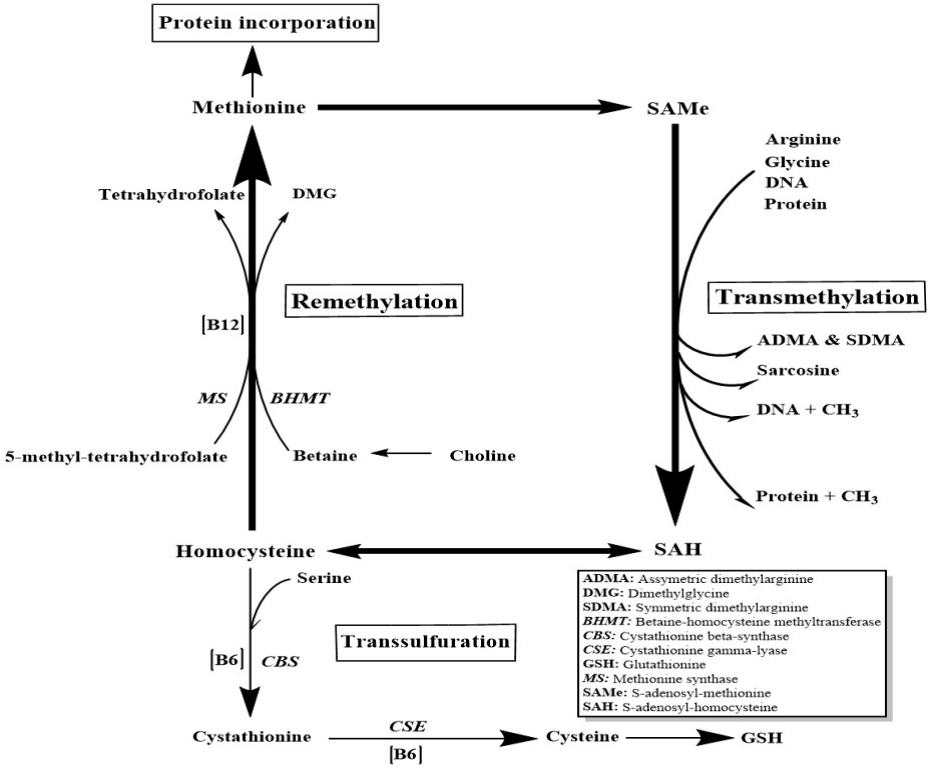
Nutrients known to reduce nutritional edema in animal models

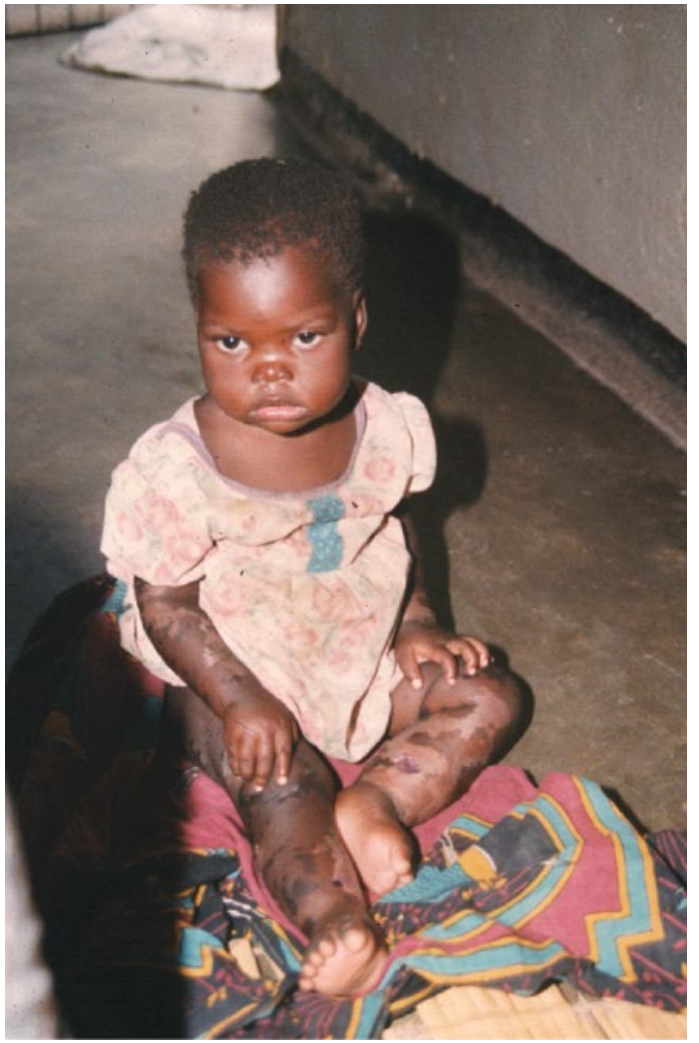
Partial

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

Complete

- Cobalamin + folate. Alexander, 1956
- Choline. Alexander, 1952
- Methionine. Alexander, 1956





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Protein quality in the pathogenesis of kwashiorkor

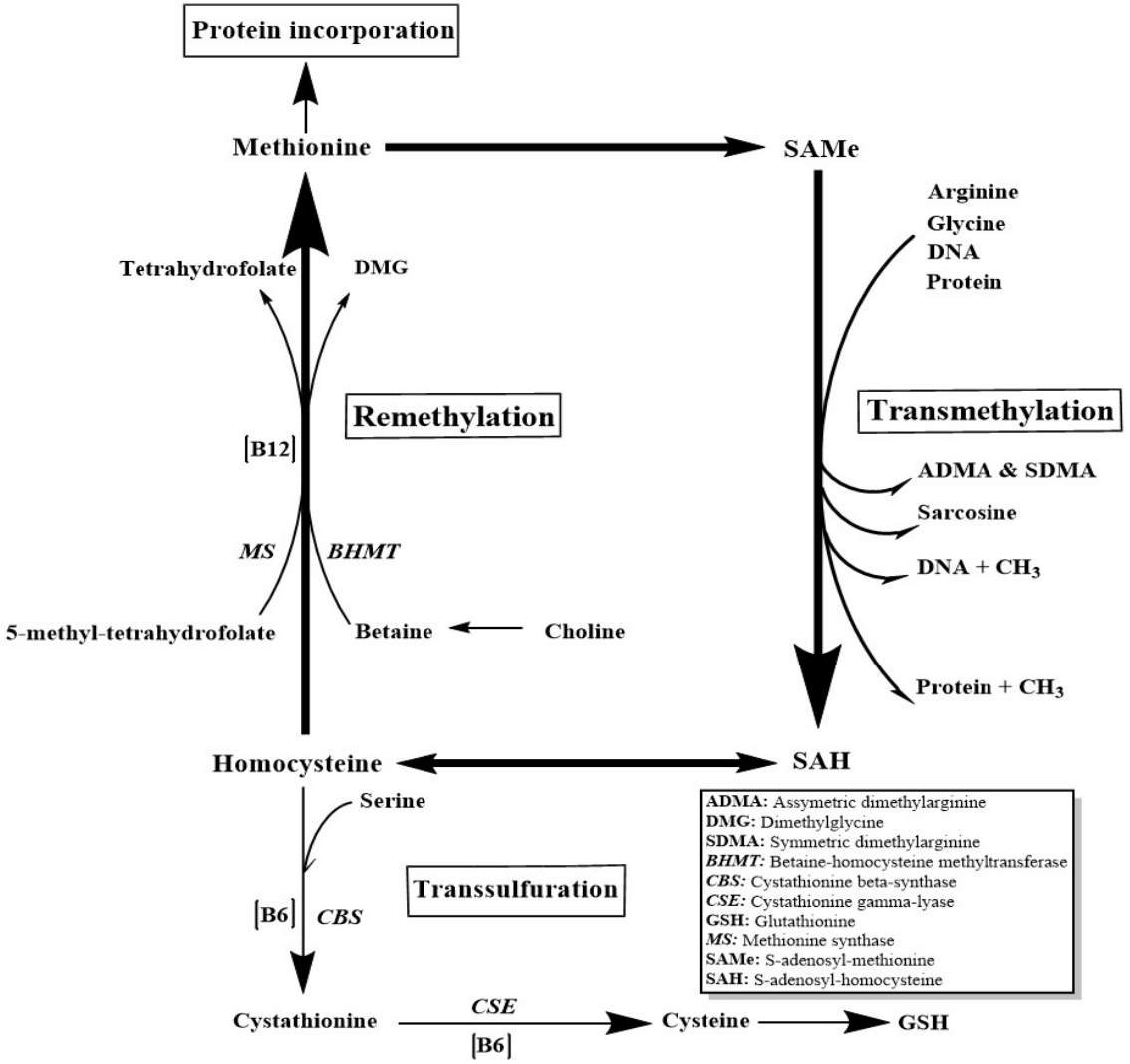
- Protein quality is limited to the availability of the least represented essential amino acid

- 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

Methionine ↔ One-carbon metabolism

- ***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.



Methionine One-carbon metabolism

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.

Implications for cognition

SAM is associated with subsequent cognitive impairment

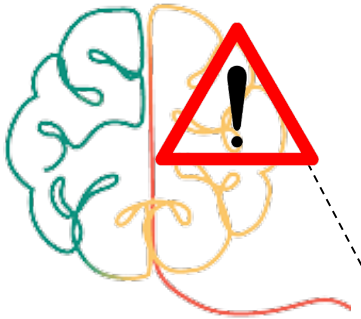
Children who have recovered from SAM score 2-3 standard deviations below age-normalized expectations as assessed by Malawi Developmental Assessment Tool (MDAT)

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

Cognitive outcomes in SAM have received little attention

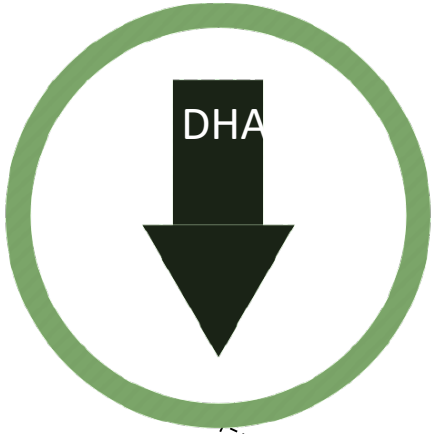
RUTF was designed to maximize weight gain; pre-clinical data show that ignoring fat composition has downsides with regards cognitive recovery

Summary of a winding story

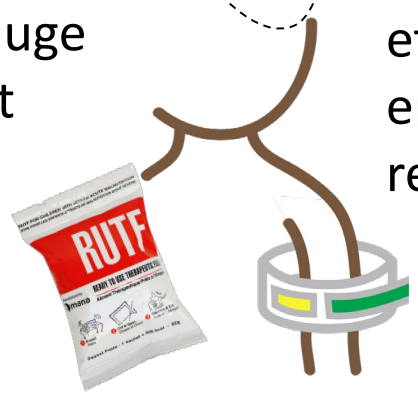


Children with SAM are vulnerable and experience a huge cognitive insult

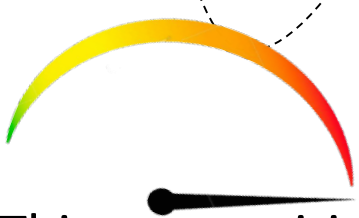
RUTF was optimized to efficiently provide energy for physical recovery



Children treated with standard RUTF experience a drop in plasma and RBC membrane DHA levels



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



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

Improved PUFA RUTF Study

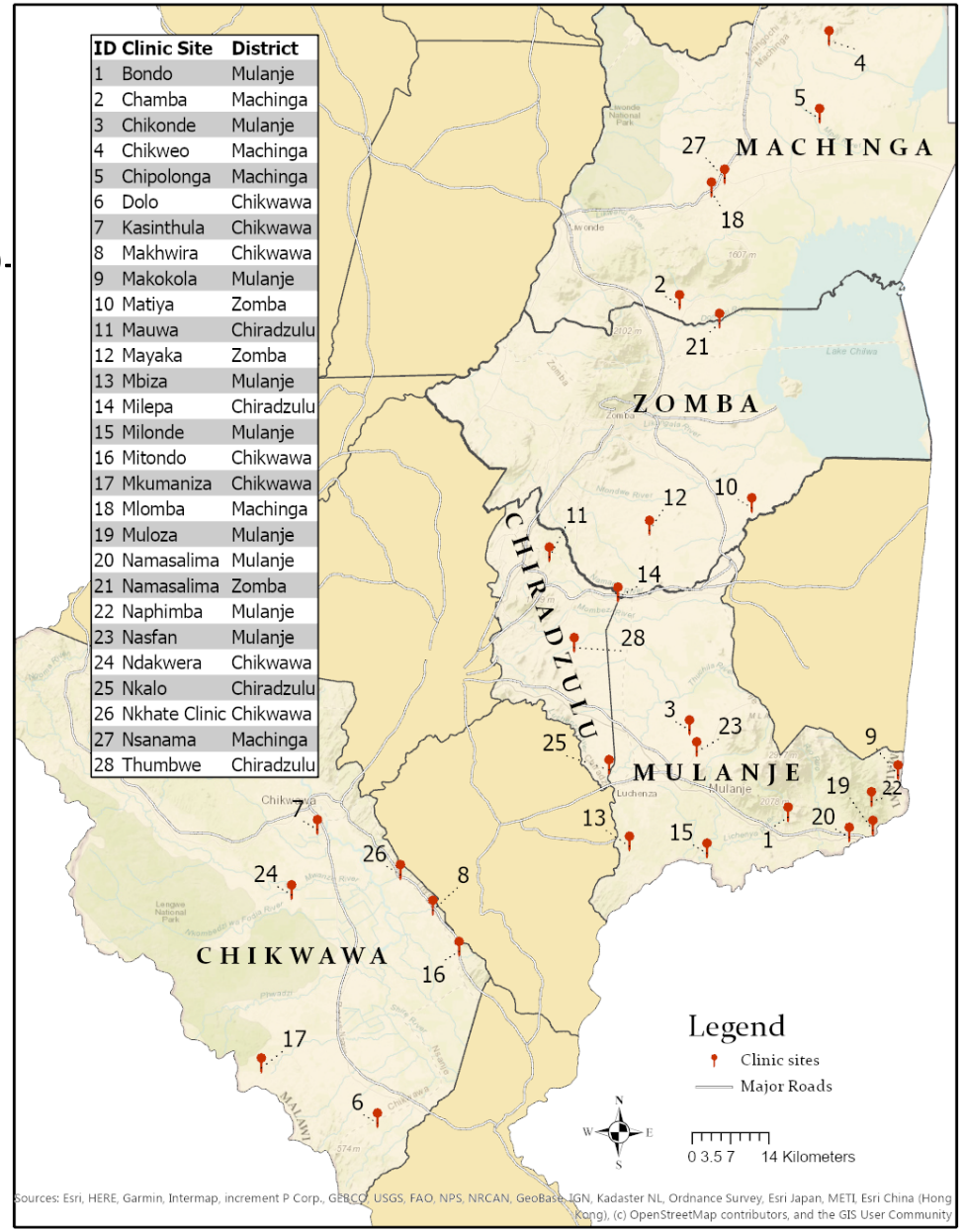
The UN's international food regulatory body, Codex Alimentarius, was set to meet in late 2021 to set guidelines for fatty acid content of RUTF

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.



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

Washington University in St. Louis
School of Medicine

PROJECT PEANUT BUTTER

Baylor College of Medicine

USDA

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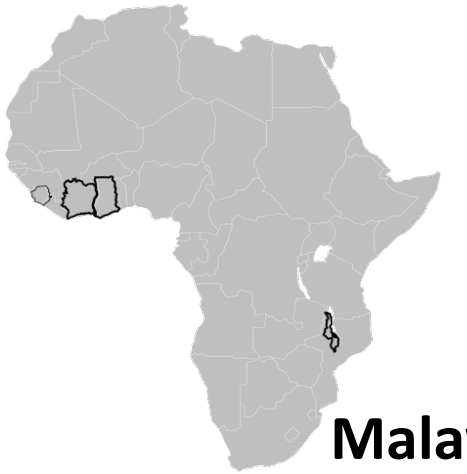
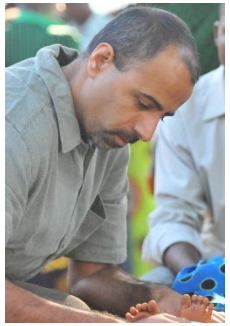
NIH National Institutes of Health

Neil Hanchard, MD PhD
Associate professor, NIH

Kevin Stephenson, MD
Instructor, Department of Medicine



Malawi
Sierra Leone



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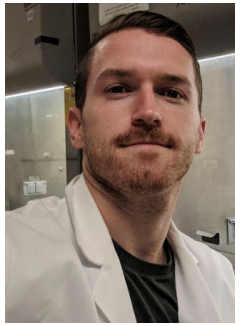

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