

Trouble Shooting Health

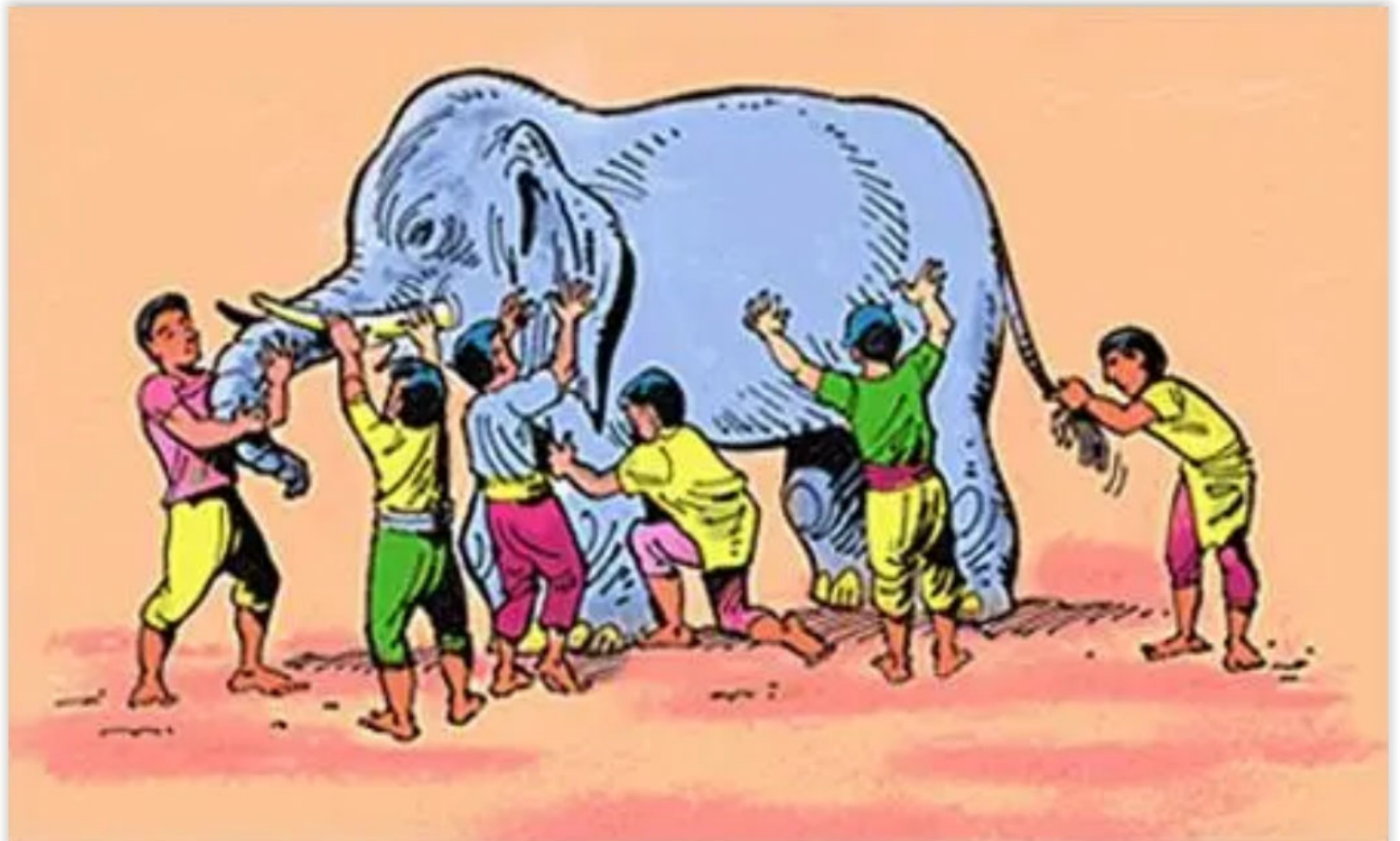
Chemical Engineer and a B12 patient



Dr. Ir. Peter J. Daudey



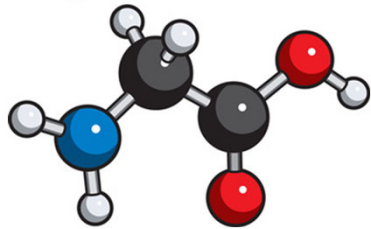
What is it?...



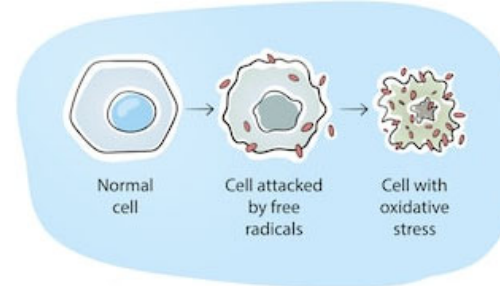
In short....

- Bowel issues after a trip (2011)
- MS-type symptoms
- SIBO? Study together with B12 Institute
- Gabriela Hadiwinoto: B12 Biochemistry
 - Glycine and Glutathione deficiency
 - Vicious cycles discovered
- Acidosis
 - Leads to microbiome shift
 - Methanol from pectins, source of ROS
- Macro-B12 explanation

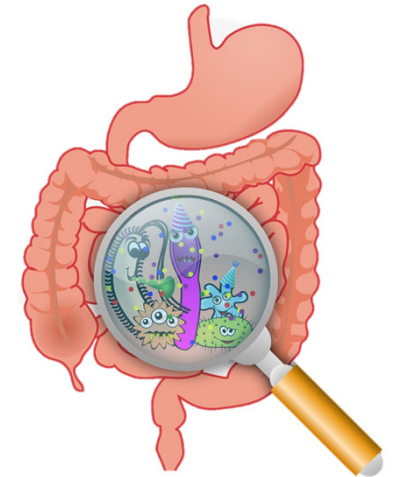
Hypotheses



Glycine deficiency: Protein damage and collagen issues



Glutathione deficiency: Activation of B12; high oxidative stress



Acidosis, Microbiome switch, loss of methanol detox to methane

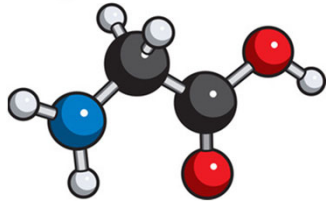


Formaldehyde: Protein/enzyme damage, acidosis

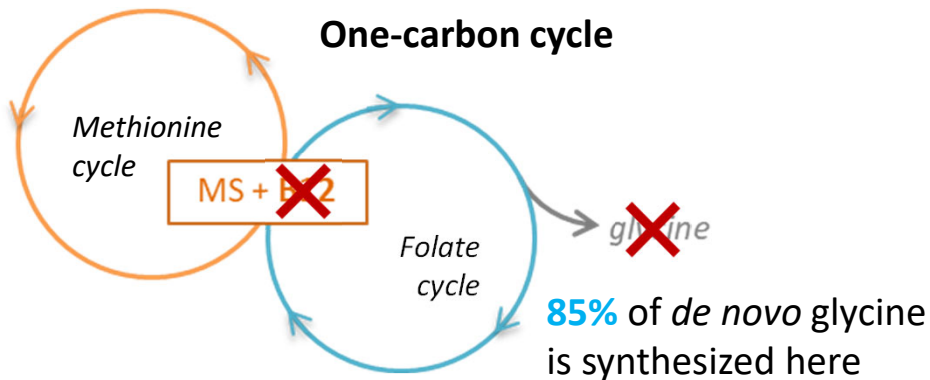


Glycine Deficiency

What is glycine and why is it **important** yet **overlooked**?

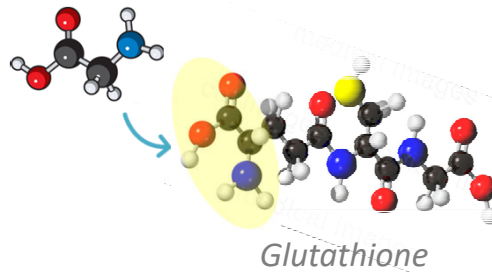


- Smallest amino acid
- The building block of all proteins (e.g. DNA, B12 enzymes, binders)
- Considered non-essential



B12 deficiency leads to folate cycle block,
folate cycle block leads to glycine synthesis block

Glycine deficiency **consequences**:

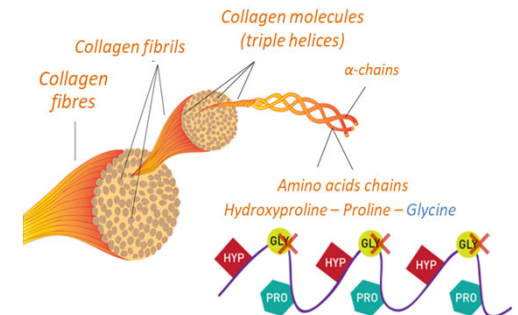


1. Glutathione synthesis failure

- Glutathione deficiency
 - Essential for fighting ROS!
- GSH synthesis top priority!!**

2. Collagen synthesis failure

- Affects muscles, tendons, bone, veins, joints
- Ageing, wrinkles



3. Other issues

- Sleep deprivation (NMDA receptor)
- Protein point mutations – Synthesis rate

Glycine Deficiency Biomarkers and Treatment



Proposed biomarkers



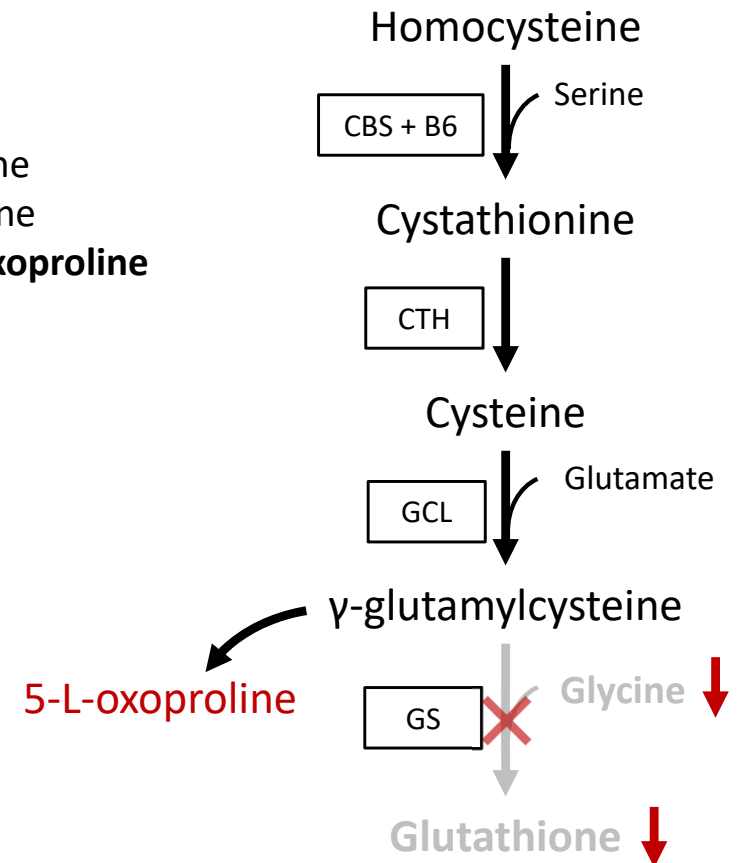
- Plasma glycine
- Urinary glycine
- Serum 5-L-oxoproline

Proposed treatment



Glycine supplementing

- Glycine demand depends on age
- Needs to be used **together with N-acetyl cysteine** (for Glutathione)
- Proper dose is yet to be determined



CBS = cystathionine beta synthase; CTH = cystathionine γ -lyase;
GCL = glutamate-cysteine ligase; GS = glutathione synthase

M. F. McCarty, et al., *Ochsner J.*, vol. 18, no. 1, pp. 81–87, 2018.

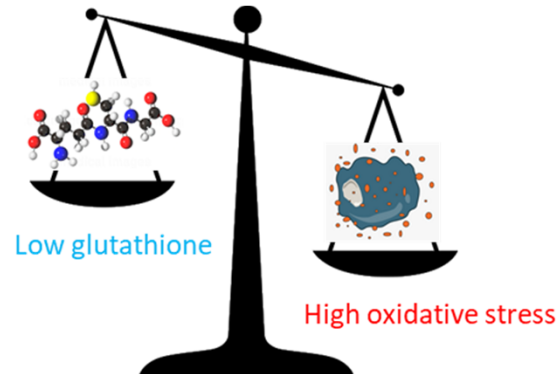
U. Heresco-Levy, et al., *Arch. Gen. Psychiatry*, vol. 56, no. 1, pp. 29–36, Jan. 1999.

High Oxidative Stress and Glutathione Deficiency

The notorious vicious cycle:

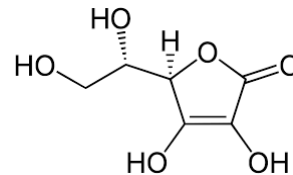
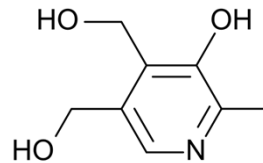
Glutathione is the major antioxidant;

- Glutathione deficiency leads to oxidative stress,
- Oxidative stress exacerbates glutathione deficiency

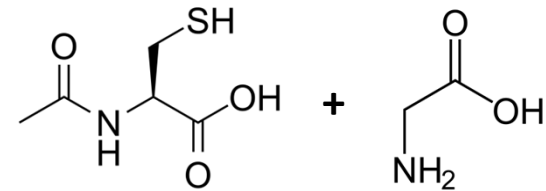


Possible biomarkers: blood test

- Reduced glutathione (GSH)
- Oxidized glutathione (GSSG)
- GSH:GSSG ratio
- Malondialdehyde (MDA)
- C-reactive protein (CRP)



Proposed treatment



- N-Acetylcysteine (NAC) and Glycine
 - For *de-novo* synthesis of GSH
 - Take both to avoid Oxoproline
- Vitamin B6 (pyridoxine)
 - Transsulphuration pathway
 - Leads to Cysteine/Taurine/GSH
 - Requires Glycine to avoid Oxoproline (B6 toxicity!!)
- Vitamin C (ascorbic acid)
 - To support the antioxidant action
 - The recycling of ascorbic acid from the deactivated form (dehydroascorbic acid) requires GSH

E. Ho et al., *Redox Biology*, vol. 1, no. 1. Elsevier B.V., pp. 483–491, 2013.

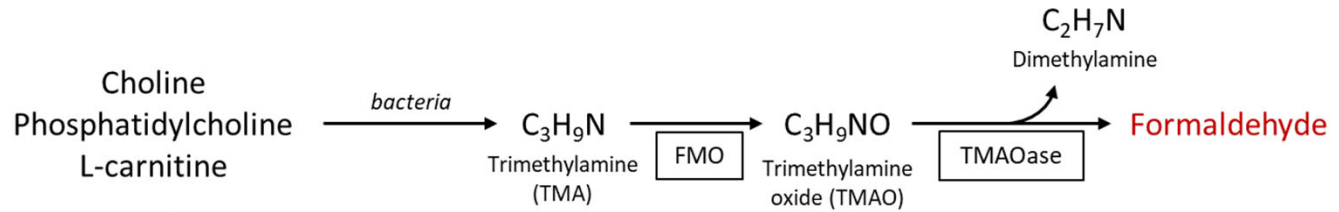
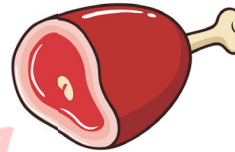
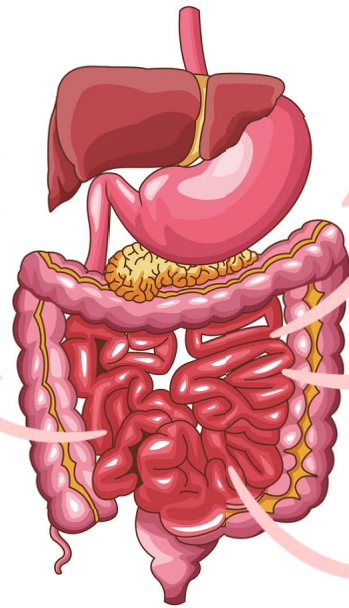
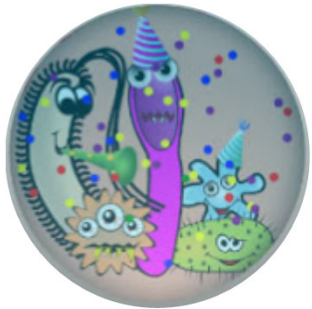
R. V. Sekhar et al., *Am. J. Clin. Nutr.*, vol. 94, no. 3, pp. 847–853, 2011.

J. M. May et al., *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1528, no. 2–3, pp. 159–166, Oct. 2001.

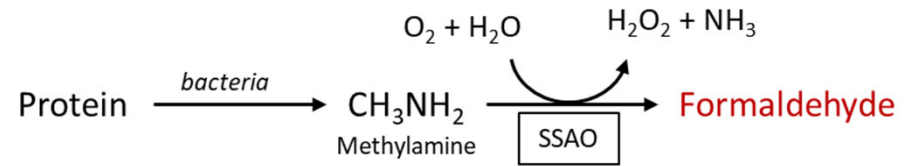
Intestinal bacterial dysbiosis

- Colon pH (acidosis)
- Diet, over-eating

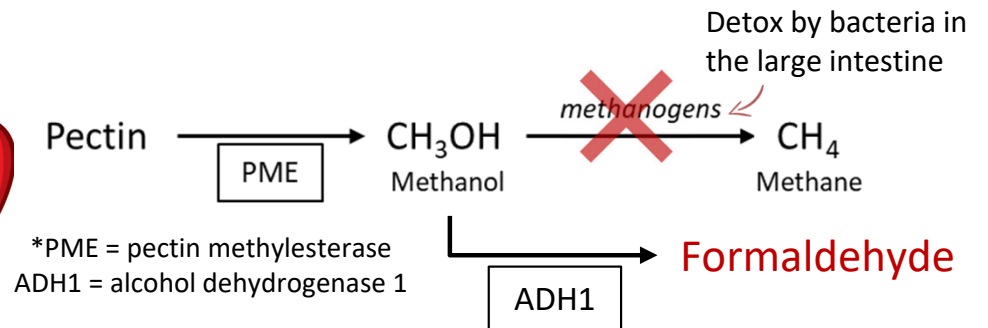
The **shift** of bacteria in the intestines will lead to **toxin production** from food



*FMO = flavin monooxidase; TMAOase = trimethylamine oxide aldolase



*SSAO = semicarbazide-sensitive amine oxidase



*PME = pectin methylesterase
ADH1 = alcohol dehydrogenase 1

J. R. Ussher, et al., *Atherosclerosis*, vol. 231, no. 2, pp. 456–461, 2013.
S. Dedeurwaerder, et al., *Planta*, vol. 229, no. 2, pp. 311–321, Jan. 2009.
J. O'sullivan, et al., doi: 10.1016/S0161-813X(03)00117-7.

Bacterial Dysbiosis Biomarkers and Treatment

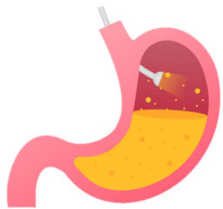
Common biomarkers/analytical tools



- First indication: the symptoms (diarrhoea, constipation, bloating, flatulence, stomach pain, etc.)



- Stool microbiome analysis
Cannot differentiate bacteria from the small or the large intestine



- Endoscopy
To see the gastrointestinal tract condition; invasively uncomfortable

Proposed (indirect) biomarkers: bacterial toxins



- Methanol breath test
- Blood and urinary TMA and TMAO
- Urinary pH and formic acid (*formed from formaldehyde*)

Proposed treatments



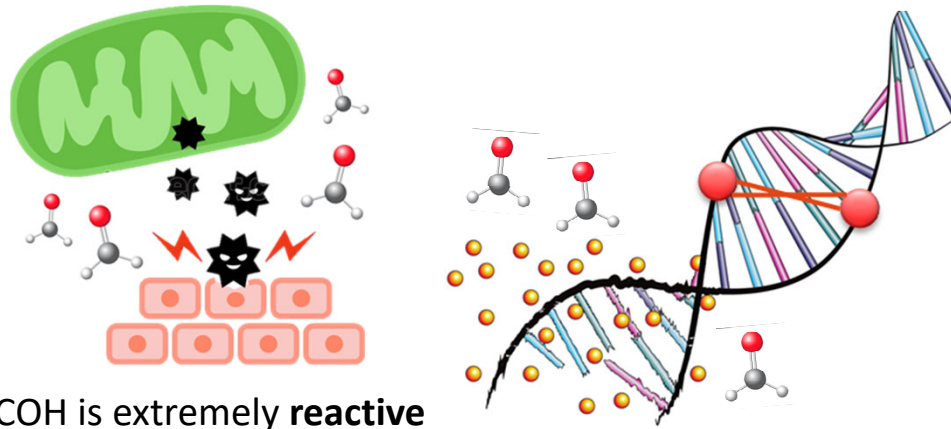
- Diet plan
Involve a nutritionist; Avoid food for bacteria e.g. reduce protein intake, avoid pectin-containing foods, lower carbohydrate, etc. intake.

- Antibiotics
*e.g. Rifaximin
Needed for severe case, to eradicate pathogens*

- Probiotics?
- **Correct Colon pH**



Damages by Formaldehyde



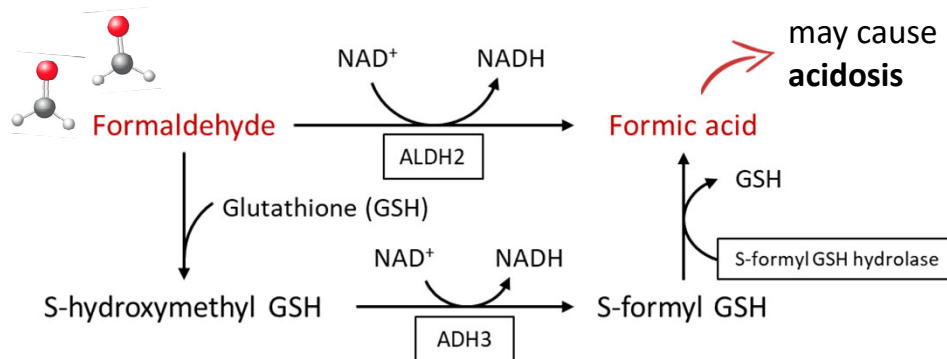
HCOH is extremely **reactive**

HCOH causes **DNA damages:**

- Mono-adduct
- Crosslinking
- Hypermethylation

Glucose oxidation inhibited:

- Lactic acidosis



HCOH detoxification to formic acid **requires glutathione**

Immune responses,
see next slide

S. L. MacAllister, et al.,
Chemico-Biological Interactions,
May 2011, vol. 191, no. 1–3, pp.
308–314.

M. Kawanishi, et al., *Front.*
Environ. Sci., vol. 2, no. SEP, p.
36, Sep. 2014.

Immune response on formaldehyde?

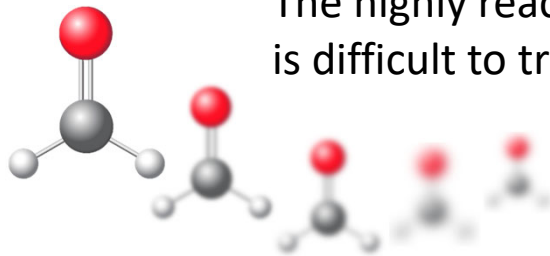
- Agglomeration of proteins:
 - Macro B12 (Wolffenbuttel)
 - No cellular uptake
 - Functional B12 deficiency
 - Glycine deficiency → GSH deficiency
 - Albumine, prolactin may also agglomerate
 - Su, Monte (2016) suggest formaldehyde as cause
 - Explains the triggering of the immune system!

2022 Wolffenbuttel, Bruce H R; Muller Kobold, Anneke C.; et al.; Macro-B12 masking B12 deficiency
2013 Fahie-Wilson, Michael; Smith, Thomas P.; Determination of prolactin. The macroprolactin problem
2016 Su, Tao; Monte, Woodrow C.; et al.; Formaldehyde as a Trigger for Protein Aggregation and Potential Target for Mitigation of Age-Related, Progressive Cognitive Impairment

Acidosis

- Formic Acidosis from formaldehyde detox
 - The oxidation of formic acid to CO₂ is slow
 - Na-formate in urine (loss of sodium bicarbonate)
- Slow-down of glucose oxidation: Lactic acidosis
 - Oxidative Phosphorylation
 - Formaldehyde reacts with Cytochrome C
 - Causes Hypoxia and Lactic acidosis
 - Mitochondrial acidosis
- B12 related: MMA, Oxoproline
- Acidosis leads to ammonia loss:
 - From protein/glycine break-down (Glycine Cleavage System)
 - Triggers protein wasting and obesity (protein:fat ratio)

Formaldehyde Biomarkers and Treatment



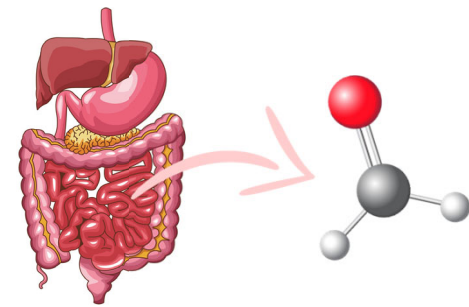
The highly reactive formaldehyde is difficult to trace

Proposed (indirect) biomarkers



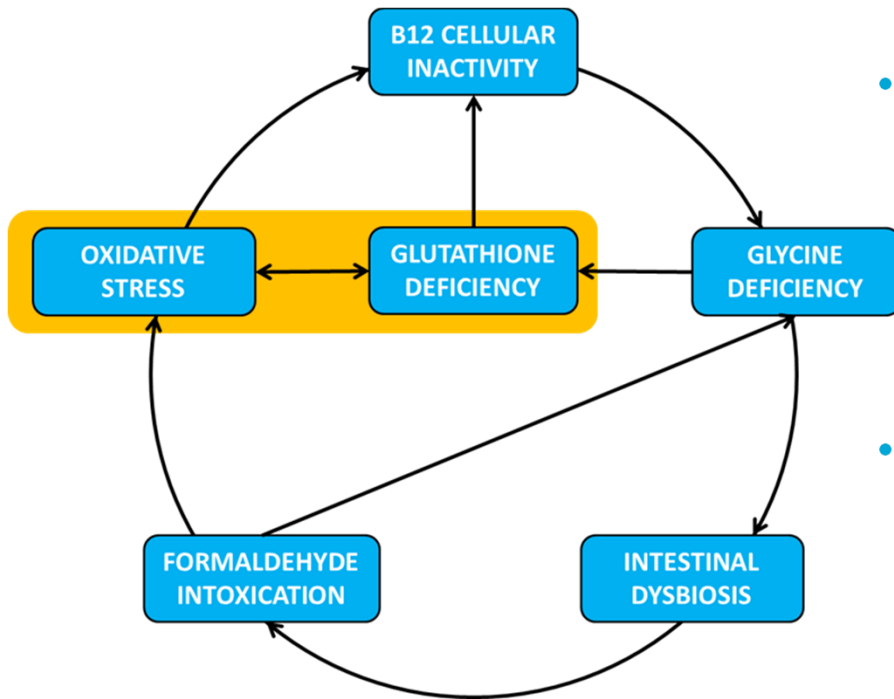
- Urine **formic acid and lactic acid**
Products of formaldehyde
- Urine **pH**
Acidic urine (pH < ~5.0) indicates acidosis
- **Breath analysis**
Methanol and methylamines, FH precursors
- **CRP** *Indicates inflammation*

Proposed treatment



- **Resolve the intestinal bacterial dysbiosis**
 - Correct **body** acidity in order to eliminate **colon** acidity
 - Use Na-citrate and K-citrate, check urine pH
 - Diet: cut back on meat
 - Diet: cut back on sugars

Conclusion – Vicious Cycle – Formaldehyde



- Main trigger of B12 deficiency is **macro-B12, blocking cellular incorporation**
 - Glutathione is a key metabolite in the metabolism of B12
 - For B12 intracellular activation (MMACHC)
 - As the major antioxidant to reduce ROS (formaldehyde)
- ### Glycine deficiency
- Essential for glutathione synthesis
 - To keep the intestinal health
- Gradual Formaldehyde intoxication
 - Main source of oxidative stress
 - Causing acidosis (formic/lactic)
 - Colon dysbiosis, loss of protecting bacteria: Methanobacters leading to methanol from pectins
 - Other:
 - Osteoporosis
 - Protein point defects and synthesis rate
 - Loss of ammonia for neutralization
 - Overwhelms the folate pool and Glutathione

Recommendations for treatment

- Develop protocol against acidosis
 - Optimum gut health
 - May also be implicated in Osteoporosis, CKD, cancer
- Develop screening method based on urine metabolomics
 - Useful for finding deficiencies (Glycine, GSH, etc)
 - Develop Glycine, NAC and vitamin protocols
- Develop protocol against oxidative stress
 - Include dietary measures

Acknowledgements

- **Dr Woodrow Monte**
 - Worked lifelong on methanol – formaldehyde
 - Suggested the role of formaldehyde in protein agglomeration
- **Gabriela Hadiwinoto MSc, EngD**
 - Spent 1 year on B12 mechanisms
 - Sponsored by Clara Plattèl of the B12 Institute

Thank you!



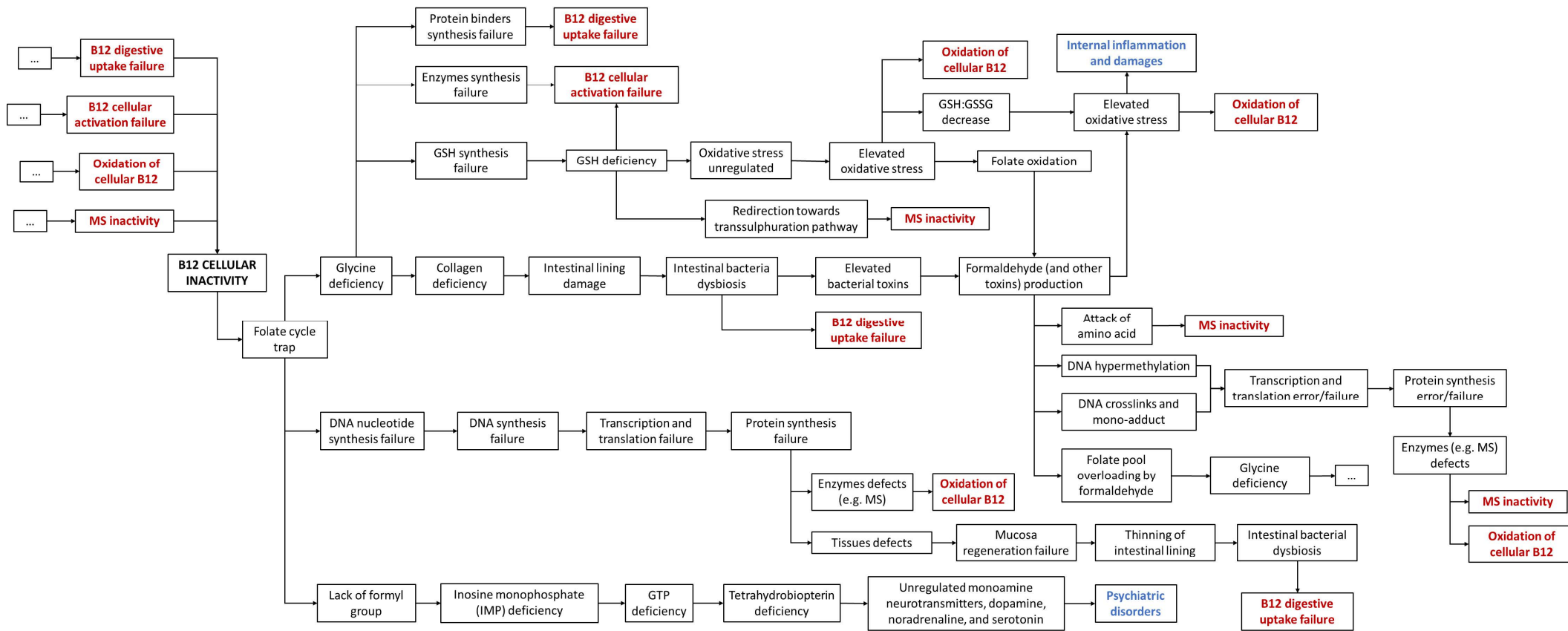
Ferdi Rizkiyanto

AT THE BOOKENDS OF LIFE: PREGNANCY, CHILDHOOD, ADULTS, AND THE ELDERLY

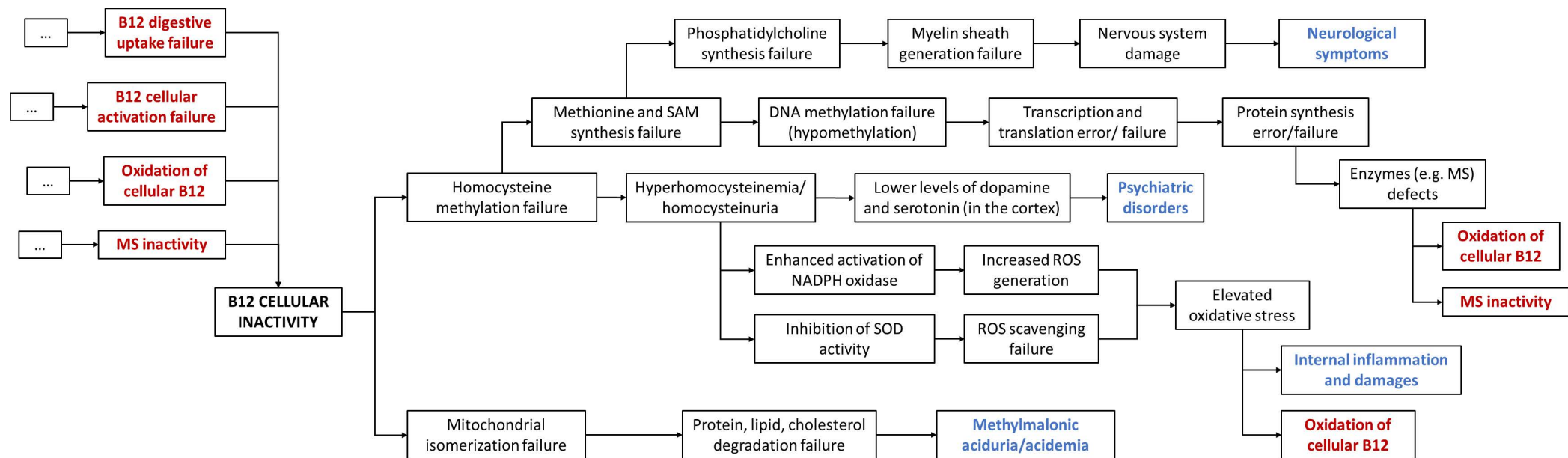
B12 related Research and Development Directions

Organizational	Have working party on B12
	Organize series of web meetings
	Discuss topics and priorities
	Publicity, Training, Advocacy
Research	Study effects on Glycine Deficiency
	Maternal B12, Folate and Glycine, potential cause of obesitas?
	Immunology of Macro B12
	Acidosis causes and consequences
Treatment	Life style advice, work with dietitians
	Measuring deficiencies
	Treatment protocols

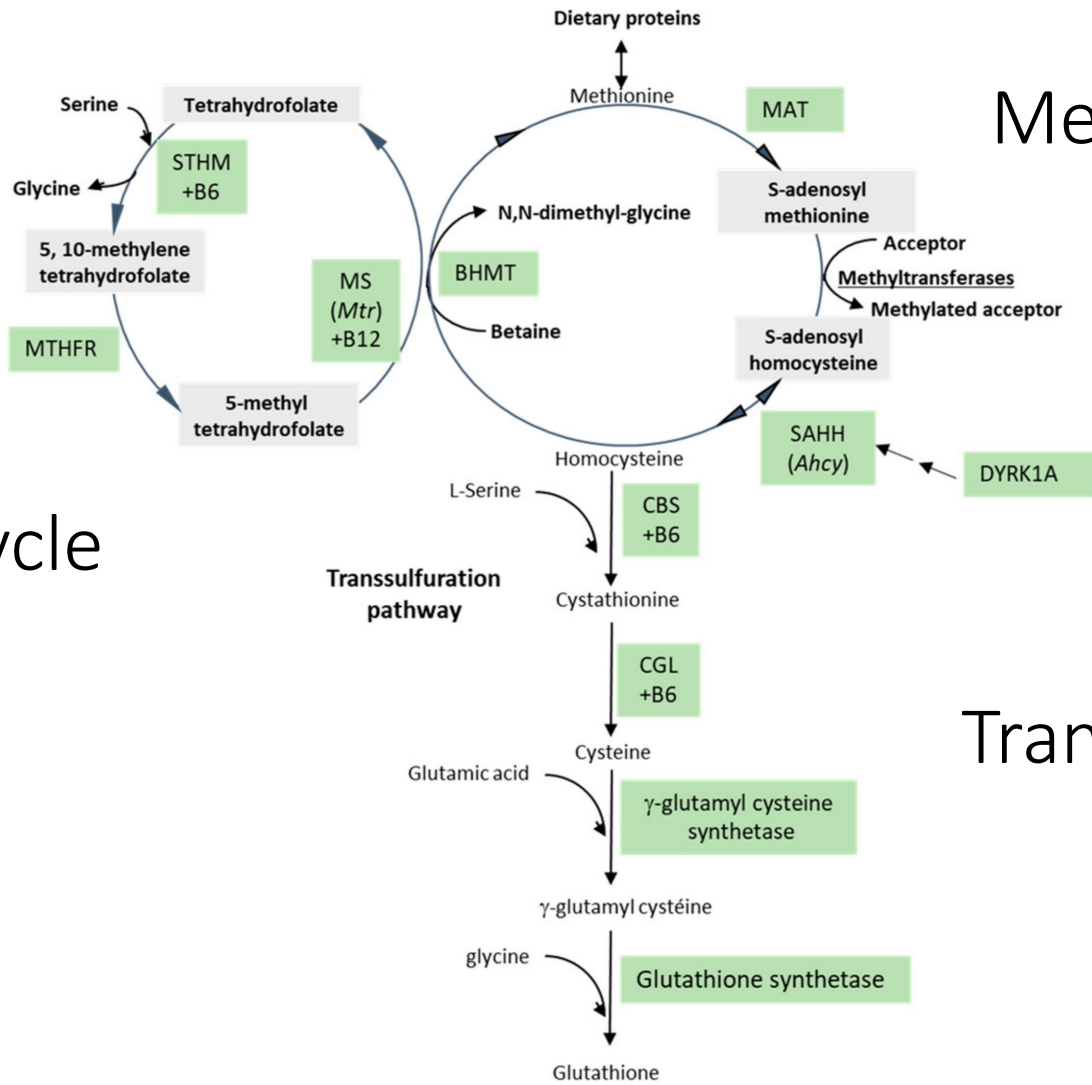
Overview of hypotheses Gabriela - 1



Overview of hypotheses Gabriela - 2



Folate cycle



Methylation

Transsulphuration