Committee on Scanning for New Evidence on Riboflavin

– Open session, Jan 4th, 2021

Riboflavin Status across the Life Span: Pregnancy and Early Life

Helene McNulty PhD RD

Presentation outline

- Metabolic roles of riboflavin and key nutrient interactions
- Assessment of riboflavin status
- Health impacts of riboflavin from pre-conception to adolescence

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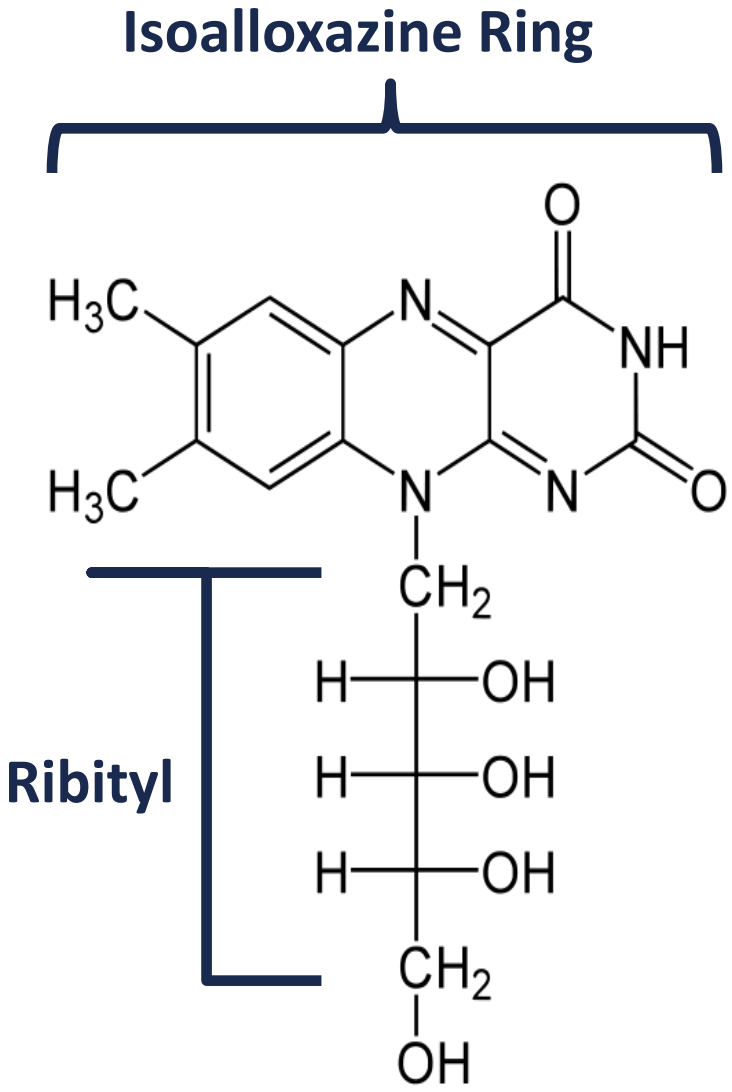
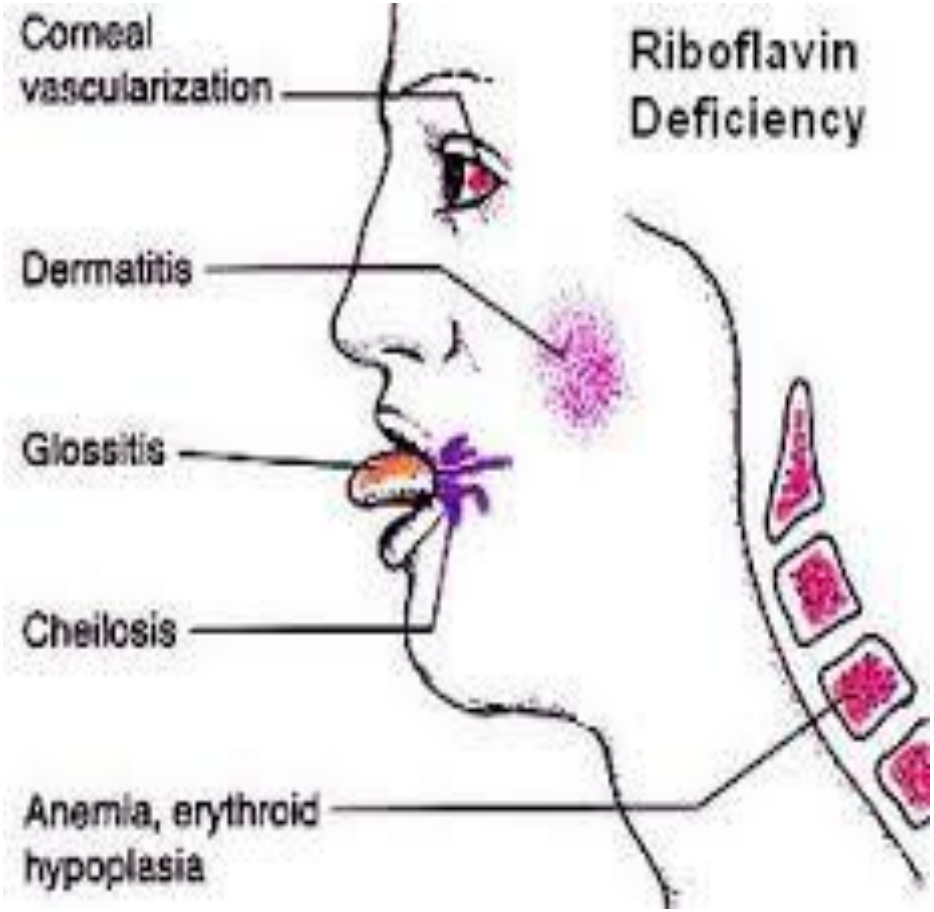
Riboflavin (vitamin B2)

7,8-dimethyl-10-ribityl-Isoalloxazine

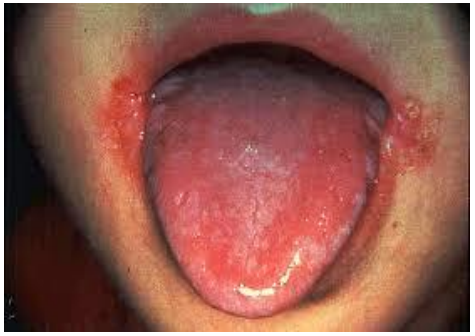


- Water soluble vitamin first identified in 1879 from milk whey
- Precursor for coenzymes:
  - Flavin adenine dinucleotide (**FAD**)
  - Flavin mononucleotide (**FMN**)
- Fundamental role in:
  - Oxidation-reduction reactions
  - Energy production
  - Cellular antioxidant function
  - Synthesis of niacin from tryptophan
  - **Metabolism of iron**
  - **One-carbon metabolism:** interactions with folate and vitamin B6
- **Food sources**: Widely distributed in small amounts; Important dietary sources are milk and dairy products
- **Biomarker status** is rarely measured, but deficient/low status may be widespread

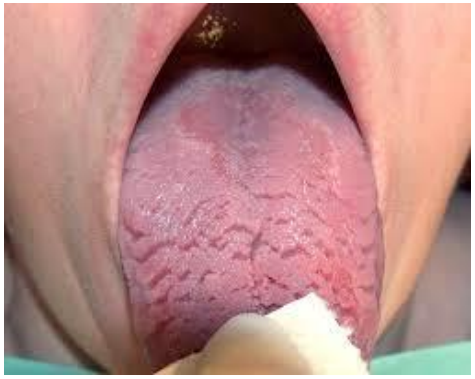
Riboflavin Deficiency



Cheilosis



Glossitis



Clinical signs of deficiency in humans appear at intakes of less than 0.5-0.6 mg/day[1]

[1]Food and Nutrition Board, Institute of Medicine. Riboflavin. Dietary Reference Intakes. 1998:87-122.

Assessment of riboflavin status

	Advantages	Disadvantages
EGRac Erythrocyte glutathione reductase activation coefficient	• Considered gold-standard • Functional assay • Stable for several years • Sensitive to small changes in riboflavin intake	• Requirement of specialized blood preparation procedures • Not routinely measured; lack of standardization • Less reliable in deficiency of glucose 6-phosphate dehydrogenase or ß-thalassemia
Urinary Riboflavin	• Direct test • High variability within and between subjects	• Reflects recent dietary intake • 24hr samples not convenient
Serum/Plasma/ Erythrocyte Riboflavin FAD FMN	• Direct test • Vitamers (riboflavin, FAD, FMN) are stable	• Affected by many factors • High variability in plasma/erythrocyte riboflavin within and between-subjects

McCormick and Greene (1994) Vitamins. In: Textbook of Clinical Chemistry

The American Journal of Clinical Nutrition

Am J Clin Nutr 2009;89(suppl):1960S–80S.

Studies of biomarker responses to intervention with riboflavin: a systematic review[1–5]

Leane Hoey, Helene McNulty, and JJ Strain

Background: National survey data of erythrocyte glutathione reductase activation coefficient (EGRac) indicate that suboptimal riboflavin status may be a problem in all population age groups, but the cutoff for deficiency is controversial. In addition, the effectiveness of different biomarkers of riboflavin status has not been critically evaluated.

Objective: We aimed to assess the effectiveness of different biomarkers of riboflavin status through a systematic review of published riboflavin supplementation trials.

Design: We structured our search strategy on Ovid MEDLINE, EMBASE (Ovid), and Cochrane databases; formal inclusion and exclusion criteria; data extraction; validity assessment; and meta-analysis.

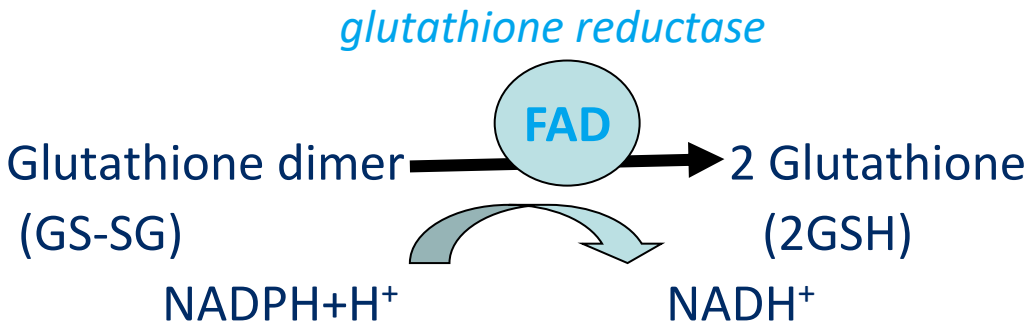
Results: Eighteen supplementation studies reporting up to 14 biomarkers were included. Sufficient data were available to show that EGRac (14 studies) and basal glutathione reductase activity (5 studies) were effective biomarkers of altered riboflavin intake ($P < 0.00001$), although substantial heterogeneity ($I^2 > 66\%$) that could not be explained by the subgroup analysis was observed. Plasma total homocysteine was not an effective biomarker of riboflavin status in the general population, but some evidence identified its potential usefulness specifically in those homozygous for a common polymorphism in the *MTHFR* gene.

Conclusions: The evidence suggests that EGRac is an effective biomarker of a change in riboflavin intake in populations with severe-to-normal baseline status. Studies of healthy populations that compare the response to low-dose supplementation among different age, sex, and *MTHFR* genotype groups are required to provide evidence for generating dietary riboflavin recommendations specific to different population subgroups. Further research into alternative biomarkers to EGRac is also required. *Am J Clin Nutr* 2009;89 (suppl):1960S–80S.

Assessment of biomarker status of riboflavin

Erythrocyte glutathione reductase activation coefficient (EGRac) assay

- Functional assay based on products of FAD dependent pathway:

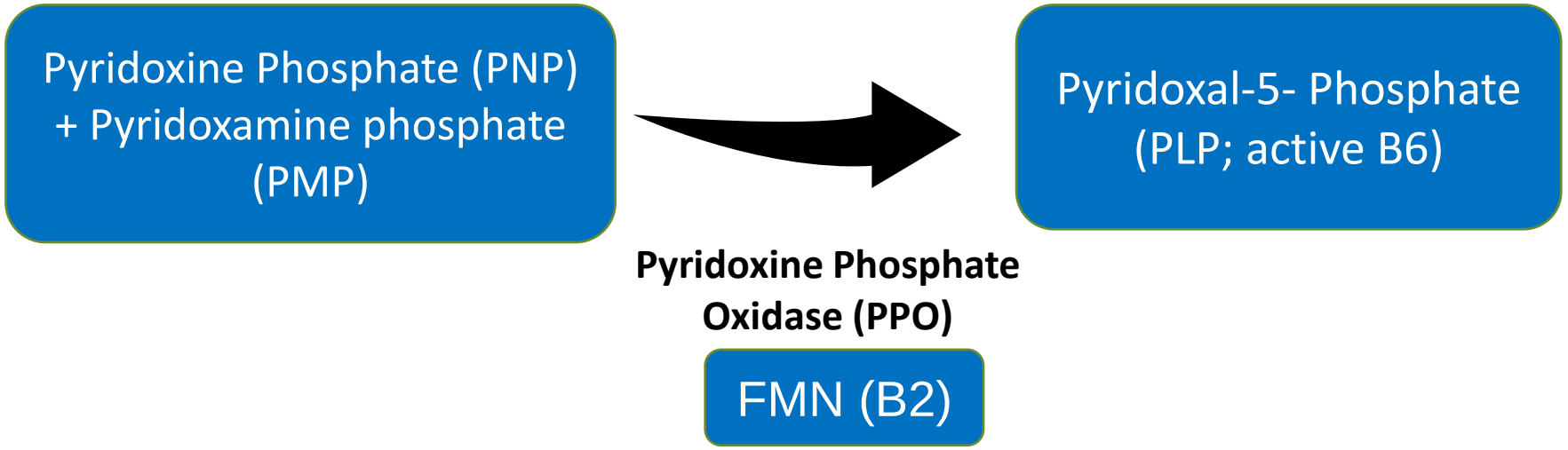


- Measures basal activity and enzyme activity with excess coenzyme

- Result is expressed as the activation coefficient (AC):

$$AC = \frac{\text{enzyme activity (with added coenzyme)}}{\text{basal enzyme activity (without added FAD)}}$$

Metabolic dependency of vitamin B6 on riboflavin



Metabolic dependency of vitamin B6 on riboflavin
Human study

Madigan et al. *Am J Clin Nutr* 1998;68:389–95

- Riboflavin supplementation at low or high dose (1.5 and 25 mg/d) resulted in a significant decrease in EGRac (i.e. improved riboflavin status)

- What was the *effect on PLP* of intervention with riboflavin?
  - Subgroup analysis of participants with low PLP at baseline:

	PLP (nmol/L)		
	Before	**After**	***p***
Riboflavin (1.6mg/d)	14.3 ± 8.1	20.7 ± 5.6	**0.035**
Riboflavin (25 mg/d)	14.1 ± 5.9	38.5 ± 25.4	0.054
Placebo	13.7 ± 2.2	15.5 ± 4.4	0.241

JPI a healthy diet for a healthy life

Ulster University

UCC University College Cork, Ireland Coláiste na hOllscoile Corcaigh

UBC THE UNIVERSITY OF BRITISH COLUMBIA

DERiVE

Aim: To develop **easily accessible riboflavin biomarkers** and demonstrate important **functional, gene-nutrient** and **health effects** of optimal riboflavin status.

BBSRC bioscience for the future

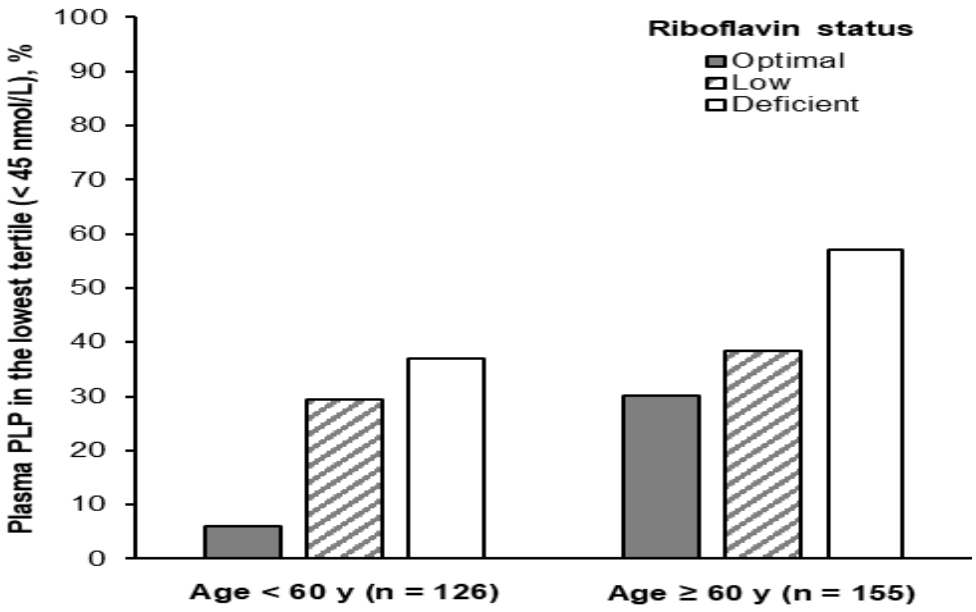
Science Foundation Ireland sfi For what's next

CIHR IRSC Canadian Institutes of Health Research Instituts de recherche en santé du Canada

JPI a healthy diet for a healthy life

Early results

WP3: Functional effects of riboflavin status (UU, UCC, UBC**)**:
Riboflavin status was an independent determinant of vitamin B6 status (plasma PLP) across adulthood in a large sample of 6000 Irish adults (Jarrett *et al*; In preparation)



Jungert et al; J Nutr 2020 150(10):2699-2706

Findings similar to the unpublished results of Jarret et al were recently reported (Jungert et al 2020)

Figure shows the **Proportion of females with vitamin B-6 status in the lowest tertile of plasma PLP according to riboflavin status**.

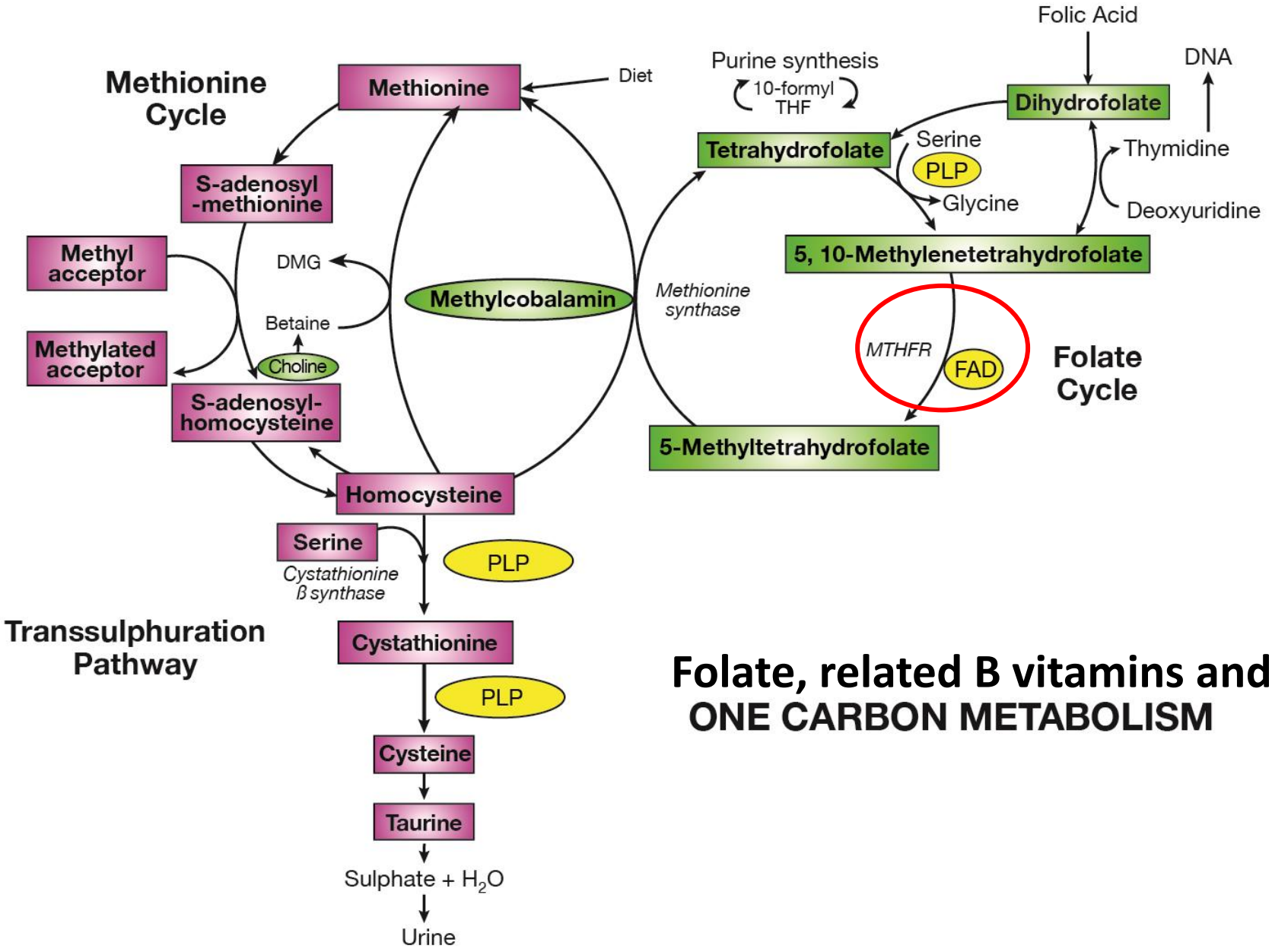
The y-axis represents the proportion of plasma PLP concentrations in the lowest tertile of PLP values (i.e. PLP <45.0 nmol/L) for study population).

Riboflavin in pregnancy

- Considered important for
  - Maternal health in pregnancy
  - Fetal development
- Dietary riboflavin recommendations (EFSA[1]):
  - Non-pregnancy: 1.6 mg/d
  - Pregnancy: 1.9 mg/d
- Population-based data from UK (NDNS) and Ireland (NANS) show
  - High rates of riboflavin deficiency as measured by EGRac
  - Women of reproductive age: subgroup with lowest intakes and highest rates of riboflavin deficiency
- Under-investigated in pregnancy but
  - one study of pregnant women in Zimbabwe reported that riboflavin deficient mothers (at 32 GW) were more likely to develop preeclampsia than those riboflavin-adequate (29% v 8%; OR 4.7, 95% CI 1.8 –12.2, P < .00).

[1] EFSA (2017) *EFSA Journal*; 15: 4919

[2] Wacker *et al*. (2000) *Obstet Gynecol*; 96: 38-44



Folate, related B vitamins and ONE CARBON METABOLISM

Methylenetetrahydrofolate reductase (MTHFR)

SUBSTRATE: 5,10 methylenetetrahydrofolate

PRODUCT: 5 methyltetrahydrofolate

COFACTOR: Flavin Adenine Dinucleotide (FAD)

PRECURSOR: **Riboflavin** (vitamin B2)

- Polymorphic mutations in the *MTHFR* gene
  - *MTHFR* 677C→T polymorphism
    - C to T substitution at base pair 677
    - Alanine/valine change in the amino acid sequence
    - Functionally defective enzyme

J Hypertens. 2010 Mar;28(3):478-86. doi: 10.1097/HJH.0b013e328334c126.

Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in *MTHFR*

Geraldine Horigan[a], Helene McNulty[a], Mary Ward[a], J.J. Strain[a], John Purvis[b] and John M. Scott[c]

Am J Clin Nutr. 2012 Mar;95(3):766-72. doi: 10.3945/ajcn.111.026245.

Riboflavin offers a targeted strategy for managing hypertension in patients with the *MTHFR* 677TT genotype: a 4-y follow-up[1–3]

Carol P Wilson, Mary Ward, Helene McNulty, J J Strain, Tom G Trouton, Geraldine Horigan, John Purvis, and John M Scott

Hypertension
Volume 61, Issue 6, June 2013, Pages 1302-1308
https://doi.org/10.1161/HYPERTENSIONAHA.111.01047

American Heart Association.

ORIGINAL ARTICLE - STRATIFIED TREATMENT OF HYPERTENSIONSTRATIFIED TREATMENT OF HYPERTENSION

Blood Pressure in Treated Hypertensive Individuals With the *MTHFR* 677TT Genotype Is Responsive to Intervention With Riboflavin

Findings of a Targeted Randomized Trial

Carol P. Wilson, Helene McNulty, Mary Ward, J.J. Strain, Tom G. Trouton, Birgit A. Hoeft, Peter Weber, Franz F. Roos, Geraldine Horigan, Liadhan McAnena, and John M. Scott

Optimal Nutrition for the Prevention of Hypertension in Pregnancy

OptiPREG

Aims

- To examine blood pressure in pregnancy in relation to the *MTHFR* C677T polymorphism
- To investigate the role of riboflavin as a modulator of the BP phenotype

Newer

- To investigate riboflavin status in relation to haemoglobin and risk of anemia

Inclusion criteria

✔ Singleton pregnancy in 1st trimester

Exclusion criteria

✗ High risk pregnancy

✗ Neural tube defect

✗ B-vitamin metabolism interfering medication

Findings from the OptiPREG study (so far)

Riboflavin status, blood pressure in pregnancy

- The *MTHFR* 677TT genotype is associated with higher BP in pregnant (as in non-pregnant) women
- Riboflavin deficiency
  - is an independent risk factor for hypertension in pregnancy;
  - exacerbates the genetic risk owing to the TT genotype in *MTHFR*
- Pilot data from an ongoing RCT show the potential for riboflavin supplementation as a prevention strategy for hypertension in pregnancy in women with the *MTHFR* 677TT genotype

Riboflavin status and Risk of anemia

- **low or deficient** riboflavin status was detected **in 68%** of pregnant women
- Lower riboflavin status at the 12th GW of pregnancy
  - was associated with lower Hb concentrations at 12th and 36th GW
  - Predicted an increased risk of anaemia by the 36th GW

Health Impacts of riboflavin in pregnancy

Hypertension in pregnancy

- **Affects** ~ 15% of pregnancies
- **Leads to** serious hypertensive disorders (e.g. pre-eclampsia) which are the major causes of fetal and maternal morbidity and mortality worldwide[1-2]
- **Pre-gestational hypertension** poses significantly higher risk of pre-eclampsia and other pregnancy complications, caesarean delivery and perinatal death[3]
- Nutritional factors known to influence BP and risk of hypertension and hypertension in pregnancy, *but a role for riboflavin in BP is not recognized* [4]

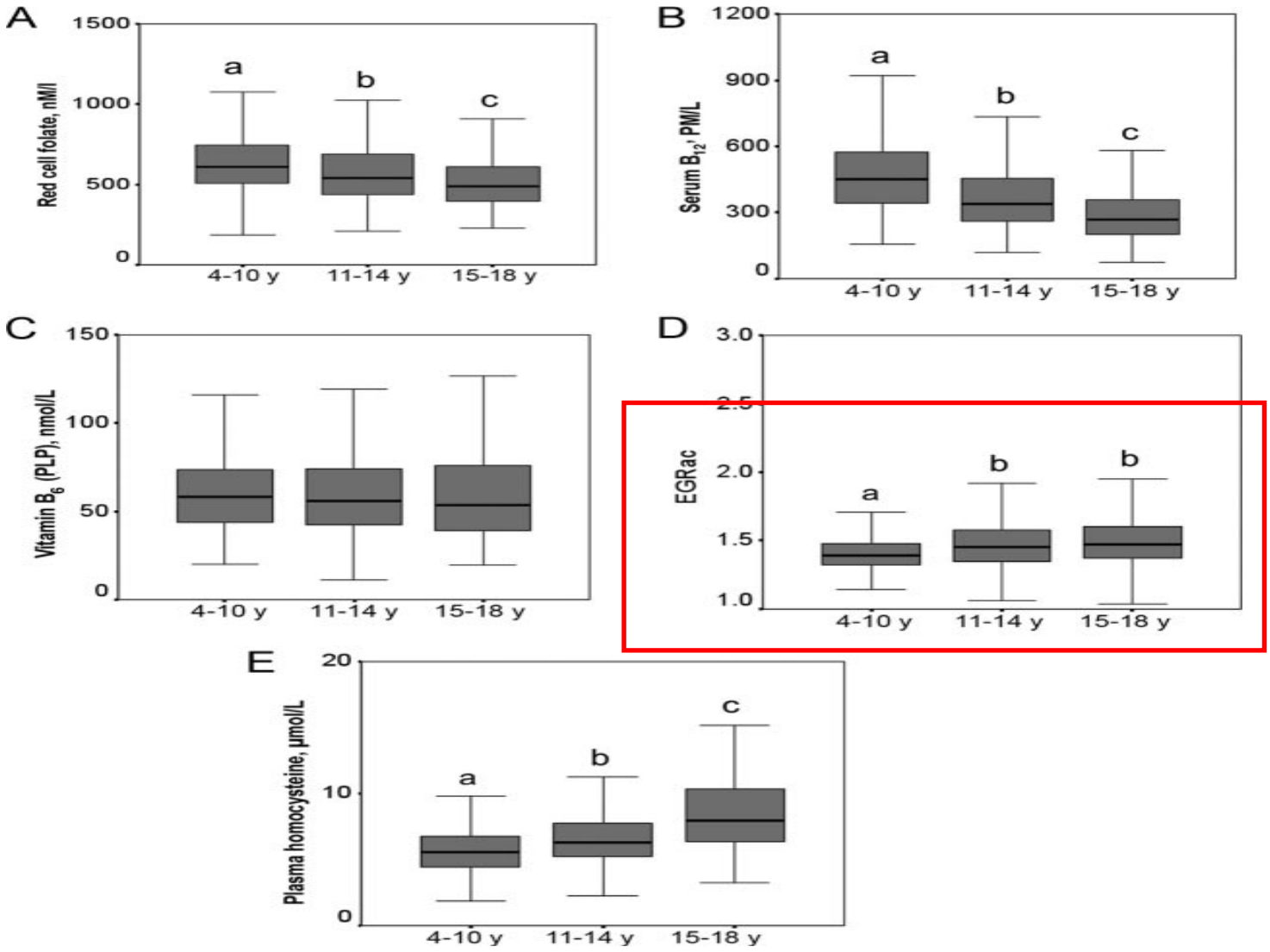
Anemia in pregnancy

- **Affects** 24- 46% of pregnancies (> 55 million globally); Iron deficiency is most common cause
- **Leads to** increased risk of stillbirth [5]; preterm delivery [6]; reduced birth weight [6]; postpartum haemorrhage[7]
- Riboflavin supplementation of young women was shown to enhance circulating haemoglobin concentrations and improve the response of iron deficiency anaemia to iron therapy[8]
- Maintenance of an optimal riboflavin status throughout pregnancy may improve Hb concentrations and reduce the risk of developing anaemia*, *but this needs to be confirmed in an RCT*

*Riboflavin deficiency may alter iron metabolism *through various mechanisms*: by impairing iron absorption, increasing intestinal loss of iron, and/or reducing the utilization of iron for the synthesis of haemoglobin

1. Waterstone et al. (2001) *BMJ* 322: 1089-1093
2. Slattery et al. (2008) *J Perinat Med* 36: 306-309
3. Bramham et al (2014) *BMJ*;348:g2301
4. Psara et al. (2020) *Biochimie* 173: 76-90
5. Nair et al (2017) *Br J Haematol* 179, 829-837
6. Rahman et al (2016) *Am J Clin Nutr* 103, 495-504
7. Nair et al (2016) *BMJ Glob Health* 1, 1-9
8. Powers et al (2011) *Am J Clin Nutr* 93, 1274-1284

Riboflavin status in childhood and adolescence (NDNS data)



Data from the National Dietary and Nutritional Survey of 2127 British children aged 4 to 18y

Kerr et al 2009 Pediatrics 123: 627-635

Riboflavin Status across the Life Span

Take-home messages and considerations for dietary requirements

- Riboflavin has important **metabolic interactions** with
  - vitamin B6
  - folate
  - Iron

- There are **health impacts of riboflavin status** across the lifespan; appears to be particularly important in pregnancy

- Riboflavin **has a novel role** in maintaining healthier blood pressure before and during pregnancy, *most notably* in those with common C677T polymorphism in *MTHFR* (affecting 10% of people worldwide)

- **Riboflavin deficiency in pregnancy** predisposes to
  - Higher risk of developing **hypertension** in pregnancy
  - Higher risk of developing **anemia** in pregnancy

- **Low/deficient riboflavin status much more common** than generally recognized, most notably in women on reproductive age in HICs as well as LMICs