Prevalence, Consequences and Prevention of Riboflavin Deficiency

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Riboflavin

Characteristics

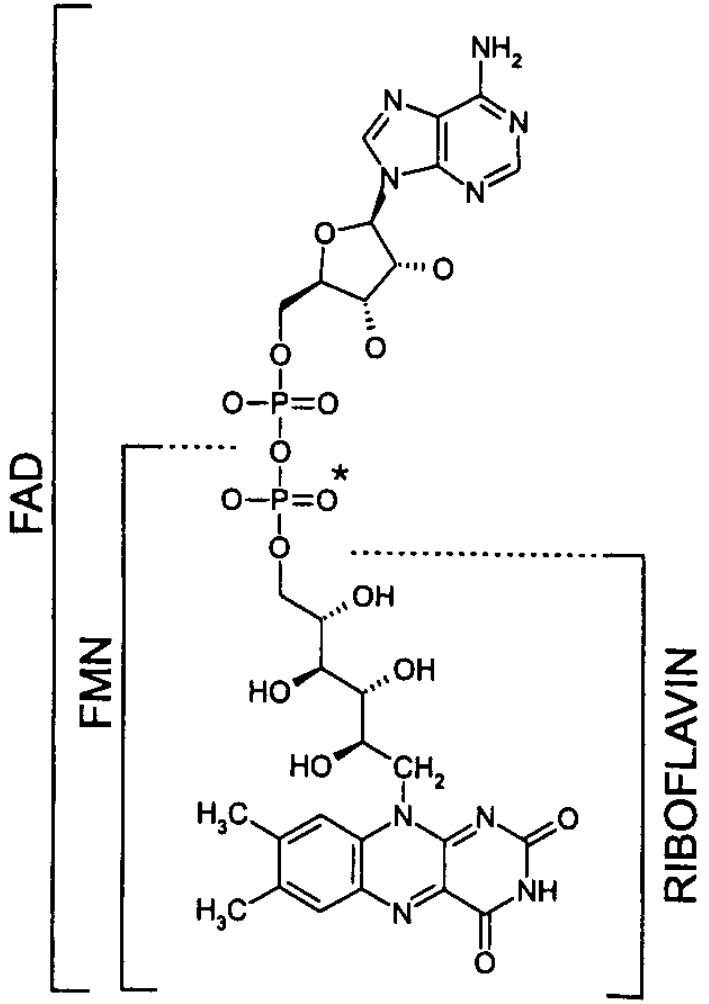
- Heat stable, water soluble, light sensitive.
- Not stored. Excreted in urine.
- Consumed as FMN and FAD.
- Biologically active as:

  FMN (flavin mononucleotide)

  FAD (flavin adenine dinucleotide)

  Cofactors for many enzymes

EAR 0.9-1.1 RDA 1.1-1.3



Some cofactor functions of FMN and FAD

			Comment
FAD	Energy metabolism	Succinate dehydrogenase Dihydrolipoyl dehydrogenase	
	FA oxidation	Fatty acyl CoA dehydrogenase	
	Purine metabolism	Xanthine dehydrogenase	
	Folate metabolism	MTHF reductase	↑ req. if C677T genotype
	Tryp → niacin	Kynurenine mono-oxidase	
	GSSG → GSH	Glutathione reductase	EGRAC test
	Neurotransmitters	Monoamine oxidase	
FMN	B6 metabolism	Pyridoxine PO4 oxidase	Ribo def. → ↓PALP
	Respiratory chain	NADH dehydrogenase	

Physiological effects

- Clinical deficiency: stomatitis, glossitis, cheilosis
- Anemia/abnormal Fe metabolism (IOM)
- Increase in tHcy and BP in MTHFR 677TT genotype

Possible effects – a few examples:

- Anti-inflammatory (animal studies)
- Reduction of migraine attacks
- Reduction of diabetes
- Prevention of cataracts
- Cisplatin adjuvant in cancer treatment

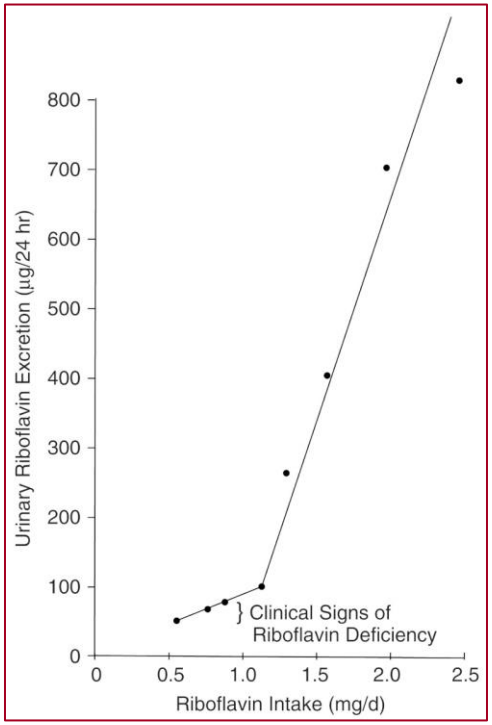
Status assessment

Erythrocyte glutathione reductase activity coefficient (EGRAC); conversion of GSSG to GSH with FAD addition.

- Cut-points usually 1.2-1.4 (low), ≥1.4 (deficient)…but used variably
- Tedious assay, cannot be used in G6PD deficiency
- Cited as a "functional" assay. Relation to other functions?

Urinary riboflavin; inflection point where tissues saturated ≡ intake 1.1-1.6 (1.4, Guo 2016) mg/d.

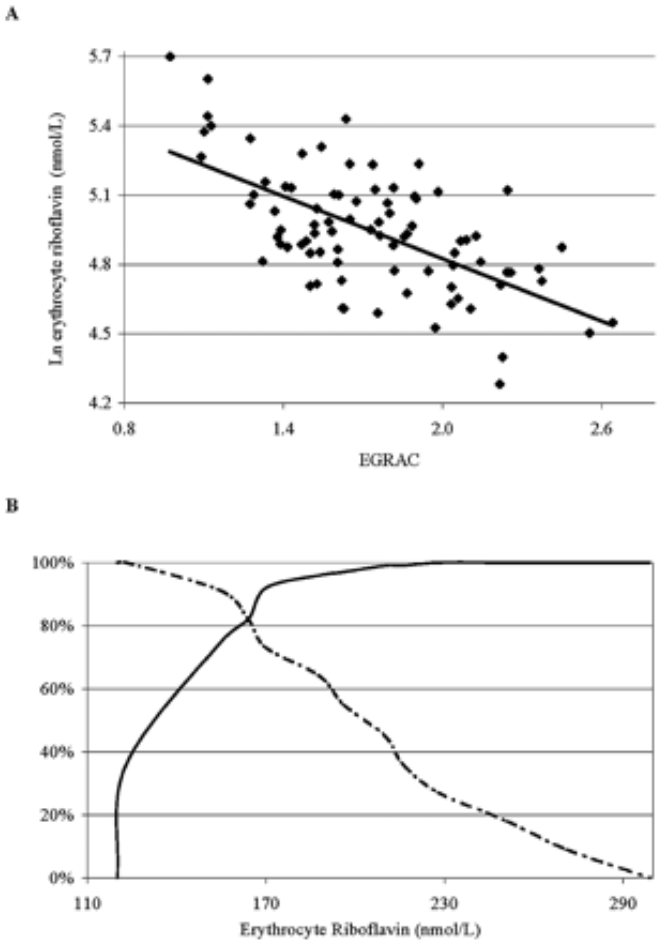
Status marker if intake >1.1 mg/d & EGRAC ≤1.3.



- Plasma not correlated with EGRAC.
- RBC FMN+FAD. <270 nmol/L (IOM). Too high?

Erythrocyte Riboflavin for the Detection of Riboflavin Deficiency in Pregnant Nepali Women

Graham et al., Clin. Chem. 2005



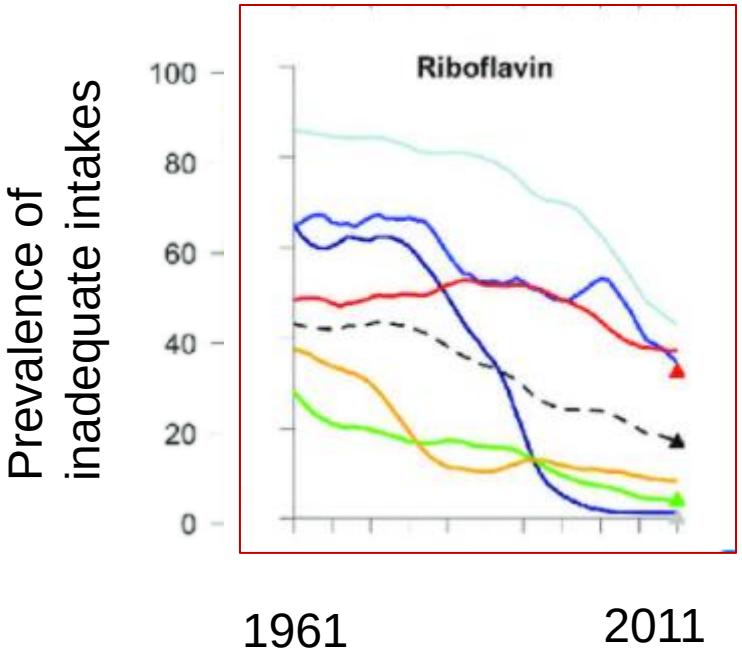
Correlation between RBC riboflavin and EGRAC

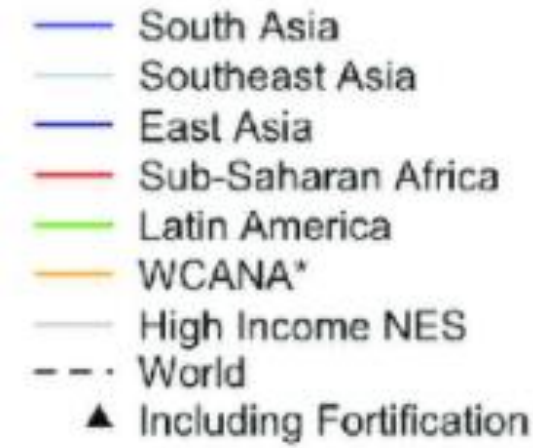
RBC riboflavin 170 nmol/L detected 92% of cases with EGRAC ≥1.4.

HPLC enabled 2x rate of sample analysis and was less variable than EGRAC.

RBC riboflavin may be less sensitive to short term supplements.

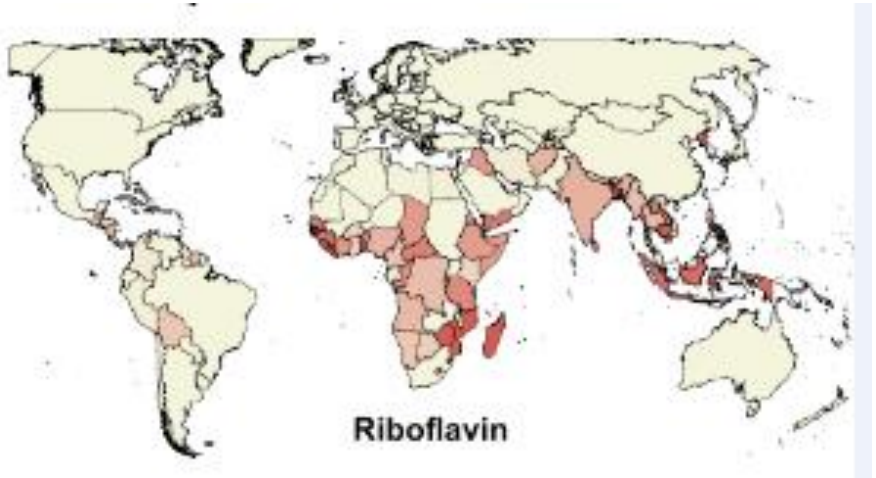
Global trends and prevalence of inadequate intakes of riboflavin (<EAR)





Mostly FAO food balance sheets, 5-year moving average

Beal, T et al. PLoS ONE 2017



Prevalence of deficiency
(EGRAC 1.3-1.39 and ≥1.4, or RBC <170 nmol/L)
(note: older studies 70-100% deficient in LIC)

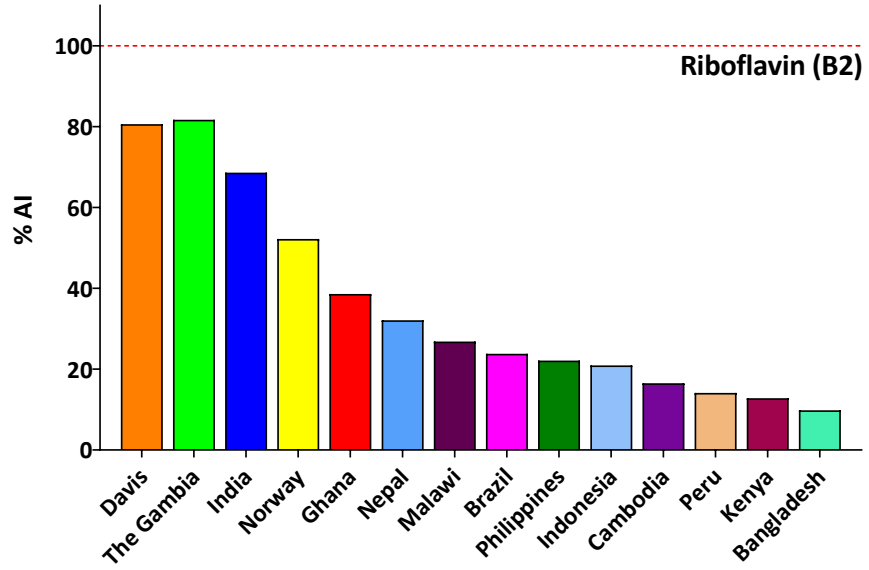
Author, y	Location	Group	% Suboptimal 1.3-1.39	% Deficient ≥1.40
Abrams 2003	Botswana	6-11 y		40
Siekmann 2003	Kenya	5-14 y		33
Graham 2005	Nepal	Preg night-blind		60
Rohner 2007	Cote d'Ivoire	5-15 y		65 (>1.2)
Whitfield 2015	Cambodia u/r	20-45 y women	9-10	78-81
Aljaadi 2019	Malaysia	19-45 y women	19	71
Whitfield 2015	Vancouver	20-45 y women		70 (≥1.3)
Aljaadi 2019	Vancouver	19-45 y women	32	40
McAnena	Ireland NANS (2008-2010)	18-64 y 18-39 y women	27 25	35 45
Moore 2019	Ireland TUDA	60-102 y	19	29
NDNS	UK (2014-2016)	19-64 y	65 (adults) 74-89 (11-18 y)	? Included in >1.3

Human milk

- FAD (60%), free (30%) + other flavin derivatives (sum <10%)
- Analysis: LC-FLD, LC-MS/MS

Reference riboflavin (B2) concentrations in human milk

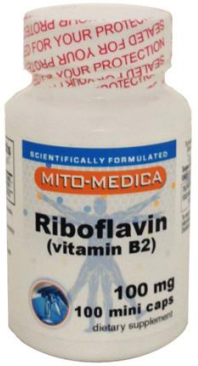
Source	Conc. [mg/L]	Comments
IOM	0.35	1 study (n=5). First to include FAD (1990)
EFSA	0.36	Mean of 3 studies, Spain & USA. Range 0.24-0.48
UK	0.31	5-center study (old)



AI 0.3 mg/d IOM, 0.4 mg EFSA

Riboflavin in breast milk, Davis, CA

Many prenatal supplements contain 20-40 mg, 10-25 x RDA



Causes of deficiency

- Low intake animal source foods: *mg/100g*
  - Salmon 0.5, egg 0.5, pork 0.3, milk 0.17, beef 0.1
  - Whole wheat 0.16, white flour 0.04, corn 0.08, rice 0.05.
- Milk and dairy main source in Western diets. Also RTE cereals.
- Status affected by milk intake, e.g.

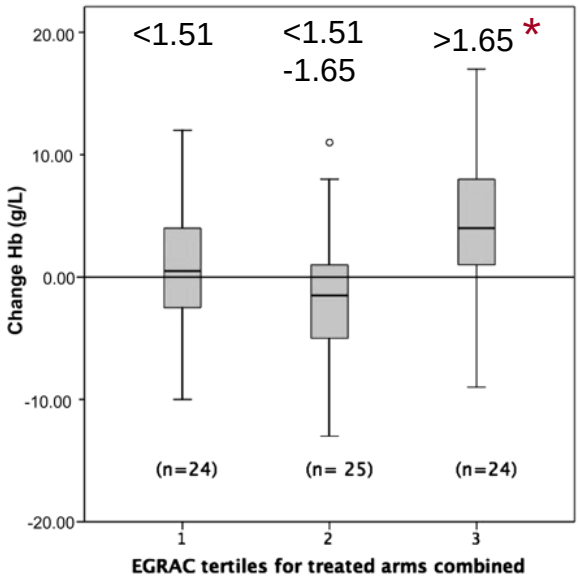
  27% deficient if <1 c/week (New York 1980)

  71% suboptimal/deficient if no/low intake, vs. 51% if 2 servings/day (Ireland 2018).
- Vegetarians/omnivores 10% deficient, vegans >30% (Austria 2006)

- Malabsorption (IBD, celiac or small bowel disease, resections)

Riboflavin and hematologic status

- Riboflavin related to Fe status, and supplements ↑ Hb in LIC.
- RIBOFEM RCT in UK (Powers 2011), because 41% elderly and 95% adolescent girls had EGRAC >1.3, and 15% women >1.65 (NDNS).
- Recruited women <250 mL milk/d; 121 (≈50%) with EGRAC >1.4.
- 0, 2, 4 mg riboflavin for 6 wk. EGRAC ↓ -0.25 with 2 mg, -0.37 with 4 mg.



Hb (and RBC) only ↑ in >1.65 tertile.

No sig. changes in ferritin, TfR, ZPP

No ↑ Fe absorption!

Does ribo mobilize Fe from ferritin (FMN oxidoreductase)?

Setting recommended intakes; IOM (1998)

- 0.5-0.6 mg/d → clinical deficiency
- 0.8 mg/d → ↓ urine but no clinical deficiency
- 0.8-1.1 mg/d → marked ↑ urine
- 1.3 mg/d → normal EGRACs most studies

- So EAR = 0.9 mg women, 1.1 men, assuming curvilinear increase in requirement from deficient to adequate (0.6 to 1.3 mg).

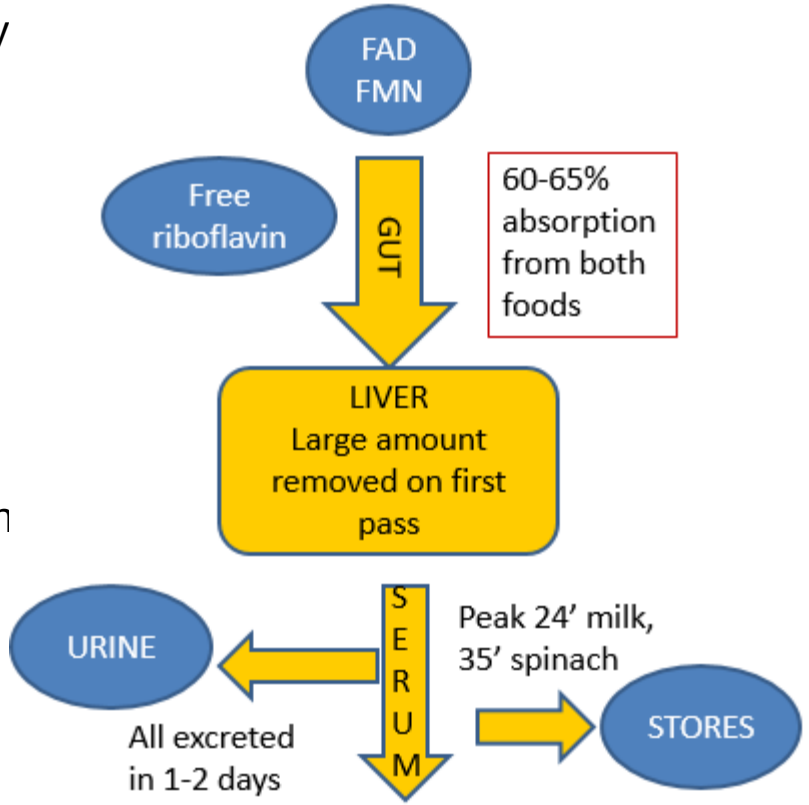
- NOTE: Studies 30-80 years old; only 1 study had 2 indicators; definition of abnormal EGRAC varied from >1.2 to >1.4; abnormal urine value <0.1 mg/d (1 study); studies used foods, some supplements for repletion.

- "95% bioavailability" cited but actual value 74% for 20 mg (Zempleni 1996)., and 60% from food (Dainty 2007)…….

Quantification of the bioavailability of riboflavin from foods by use of stable-isotope labels and kinetic modeling[1–3]

Jack R Dainty, Natalie R Bullock, Dave J Hart, Alan T Hewson, Rufus Turner, Paul M Finglas, and Hilary J Powers

- NDNS in UK – 54-90% biochemical deficiency (EGRAC)
- 50% adolescent girls 2 SDs <RNI
- Prevalence low intake always << biochemical deficiency
- Overestimate of bioavailability or wrong EGRAC cutpoint?
- Prior studies used high doses of free riboflavin (not FMN and FAD in food), and rate of appearance in serum/urine.
- Synthesized 13C-milk (free riboflavin) and 15N-spinach (FMN).
- 60% absorbed from both foods.
- "EGRAC cut-point must be wrong". But – bioavailability overestimated too.



Am J Clin Nutr 2007

Setting recommended intakes; EFSA (2017)

- Primary biomarker is inflection point of urinary excretion.
- Occurs at intake 1.13 to 1.4 mg/d (4 studies - 2 old).
- Based on mean weighted intake at urine inflection point (4 studies)

  AR = 1.3 mg/d.
- No gender differences.
- EGRAC ≤1.3 = adequate status "all population groups".
- Relationship intake and EGRAC only useful as supporting evidence; at same intake, EGRAC higher in observational studies than in experiments.

- Reviewed data on intake and health outcomes 1990-2014; no studies useful to set recommendations. (Blood pressure not included).
- Unable to quantify effects of activity or MTHFR C677T polymorphism.
- Assumed 95% bioavailability (Zempleni).

Are EGRAC cut-points wrong?

- Prevalence of low intake always much less than prevalence of EGRAC ≥ 1.3 or 1.4; e.g.
  - Ireland intake 1.6 mg/d. 33% EGRAC 1.2-1.4, 12% >1.4; and 49% ≥1.2 in elderly (Madigan 1998).
  - Ireland intake 2.5 mg/d (only 18% <EAR); 61% EGRAC ≥1.3 (Kehoe 2017).
  - UK intake 1.5-2 mg/d; 65% EGRAC ≥1.4 (NDNS).
- Supplements increase Hb and RBC only if EGRAC >1.65 tertile at baseline.
- Need evaluation against other functional outcomes.

Prevention of deficiency

- Adequate milk/dairy product intake.
- Fortified RTE cereals and weaning foods.
- 62 countries have “fortification standards” for riboflavin but mostly “restoration/enrichment” of refined cereals.
- In USA, FDA mandates 1.8 mg/lb. refined wheat flour.
- L. plantarum fermentation e.g. soy milk.
- Riboflavin supplements; rapidly restore status. 6 mg/d reduced EGRACs ≥1.4 from 60% to 6% in 6 weeks.
- Maternal supplement appears same day in breast milk.
- Trials with riboflavin in MMN; do lower EGRAC.
- EGRACs fall rapidly to baseline after supplements stopped. Adequate daily intakes important.

Recommendations

- Update data on relationships between riboflavin intake, EGRAC, urinary riboflavin inflection point and RBC riboflavin.
- Review/revise cut-points.
- Reconsider bioavailability – 65%, not 95%?
- Include additional functional outcomes (anemia? hypertension?).
- Quantify requirements if MTHFR C677T polymorphism.