Maternal Micronutrient Status and Intake: Effects on Human Milk Composition

Lindsay H. Allen

USDA, ARS Western Human Nutrition Research Center,

University of California-Davis

Issues

- Human milk is definitely best source of nutrients for young infants!
- But questions remain:
  - Poor data on micronutrient content:
    - Many nutrients, uncertain how/when to collect, milk matrix, effects of maternal status.
  - Does it supply enough nutrients for first 6 months?
  - What if mother has poor diet?
  - Are supplements needed for lactating women?
  - For their infants?

Micronutrient groups in lactation

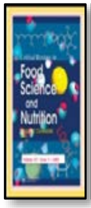
(Allen 1994, 2018)

Group I	**Group II**
Milk MN ∝ to maternal status, infant depleted. Supplements can ↑ MN in milk.	Milk MN independent of maternal status, mother depleted. Supplements no effect on milk.
B-1, B-2, B-6, B-12 **A, D, K, E** **Choline** **Iodine** **Selenium**	**Folate** **Calcium** **Iron, copper, zinc**

Few data available for setting Adequate Intakes for infants and lactation (Allen et al., 2018)

Nutrient	Value used by IOM, /L	Data	Range/other studies
B1 (mg)	0.21±0.4	Only study, source unknown	
B2 (mg)	0.35 (0.31-0.51)	n=5, USA	Only 1 study with valid method
Niacin (mg)	1.8 (1.2-2.8)	n=23 (UK, 16-244 d)	Only 1 study
Pantoth.	1.7 mg/d	2 studies (UK, USA)	Range: 2.2-2.5mg/L, but higher values includes women consuming supplements
B6 (mg)	0.13 (0.07-0.18)	n=6 (USA , 3 wk to 30 mo)	Intakes were <RDA (0.24, 0.31 if intake >RDA)
Biotin	5 µg/d	3 studies	Range: 3.8-7ug/L (different methods)
B12 (ug)	0.42 (0.01-1.47)	n=9 (Brazil, 4 d to 3 mo)	0.31 (vegan), 0.34, 0.91 (suppl)
Choline (mg)	125mg/d	2 studies	160-200 mg/L
C (mg)	50mg	8 studies, n=12-200 /study	Range 34-83mg/L if no supplement

Development of analytical methods

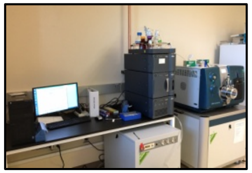


Critical Reviews in Food Science and Nutrition

Analyzing B-vitamins in Human Milk: Methodological Approaches

DANIELA HAMPEL and LINDSAY H. ALLEN

USDA, ARS Western Human Nutrition Research Center, Davis, California, USA



Journal of Chromatography B, 903 (2012) 7–13

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Ultra-performance liquid chromatography tandem mass-spectrometry (UPLC–MS/MS) for the rapid, simultaneous analysis of thiamin, riboflavin, flavin adenine dinucleotide, nicotinamide and pyridoxal in human milk

Daniela Hampel[a,*], Emily R. York[a,b], Lindsay H. Allen[a]

Free thiamin, riboflavin, FAD, nicotinamide, pyridoxal, pyridoxine, biotin, pantothenic acid via UPLC-MS/MS

Food Chemistry 153 (2014) 60–65

Contents lists available at ScienceDirect

Food Chemistry

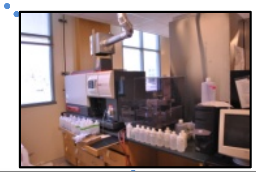
journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Competitive chemiluminescent enzyme immunoassay for vitamin B12 analysis in human milk

Daniela Hampel[a,b,*], Setareh Shahab-Ferdows[a], Joseph M. Domek[a], Towfida Siddiqua[a,b,c], Rubhana Raqib[c], Lindsay H. Allen[a,b]

B12 via CPBA



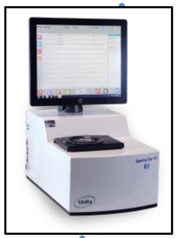
Iron, copper, zinc, calcium, potassium, magnesium, phosphorus, sodium via ICP-AES

5 platforms

6 methodological approaches

≈1 mL of milk

+ omics on 20 uL



Carbohydrates, fat, protein via NIRS

Unity Scientific — Neonatal Analyzer Package — EQUIPMENT SERIES

Received: 13 February 2017 | Revised: 27 June 2017 | Accepted: 12 July 2017

DOI: 10.1111/mcn.12503

WILEY | Maternal & Child Nutrition

ORIGINAL ARTICLE

The effects of a lipid-based nutrient supplement and antiretroviral therapy in a randomized controlled trial on iron, copper, and zinc in milk from HIV-infected Malawian mothers and associations with maternal and infant biomarkers

Daniela Hampel[2,1] | Setareh Shahab-Ferdows[1] | Erik Gertz[1] | Valerie L. Flax[3] | Linda S. Adair[3] | Margaret E. Bentley[3] | Denise J. Jamieson[4] | Gerald Tegha[5] | Charles S. Chasela[5,6] | Debbie Kamwendo[5] | Charles M. van der Horst[7] | Lindsay H. Allen[1,2]

Chromatographia (2012) 75:241–252

DOI 10.1007/s10337-012-2193-9

ORIGINAL

Rapid Isocratic HPLC Method and Sample Extraction Procedures for Measuring Carotenoid, Retinoid, and Tocopherol Concentrations in Human Blood and Breast Milk for Intervention Studies

Tami Turner · Betty J. Burri

Vitamin A and E via HPLC-DAD

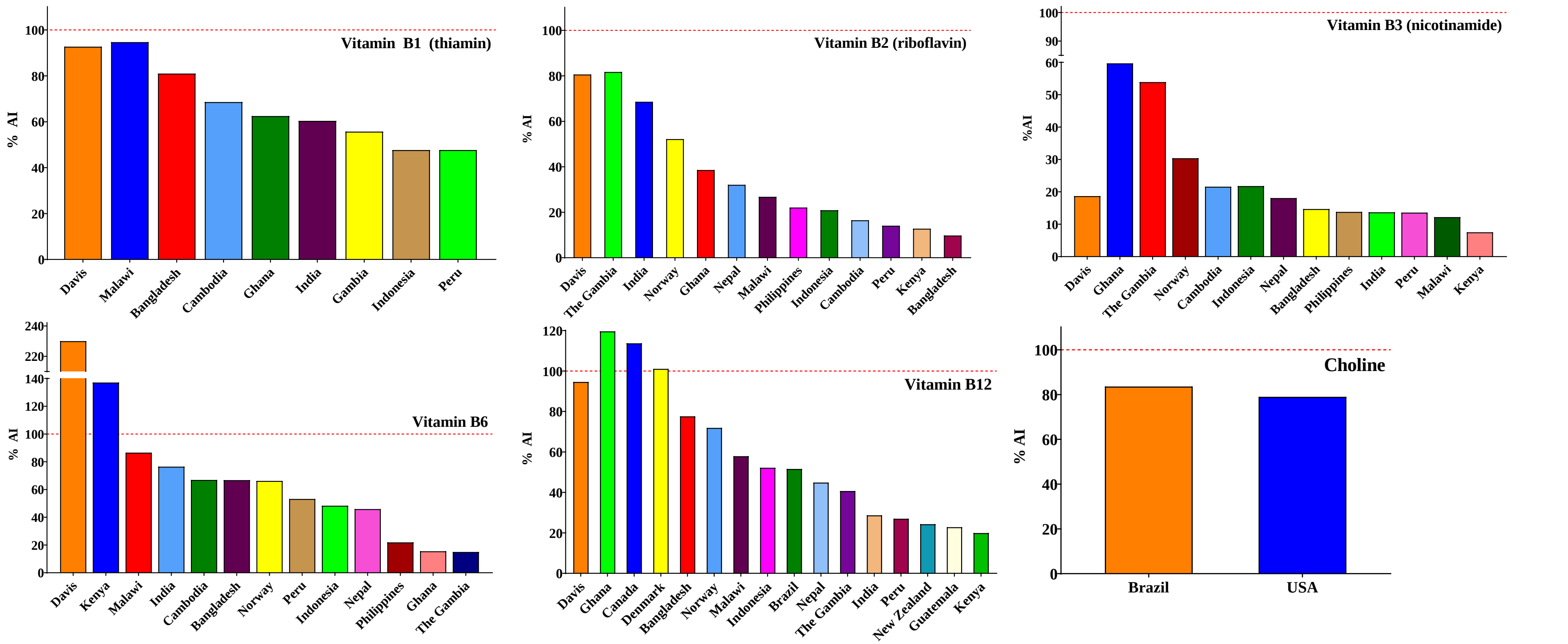
PLOS | ONE

RESEARCH ARTICLE

Thiamin and Riboflavin in Human Milk: Effects of Lipid-Based Nutrient Supplementation and Stage of Lactation on Vitamer Secretion and Contributions to Total Vitamin Content

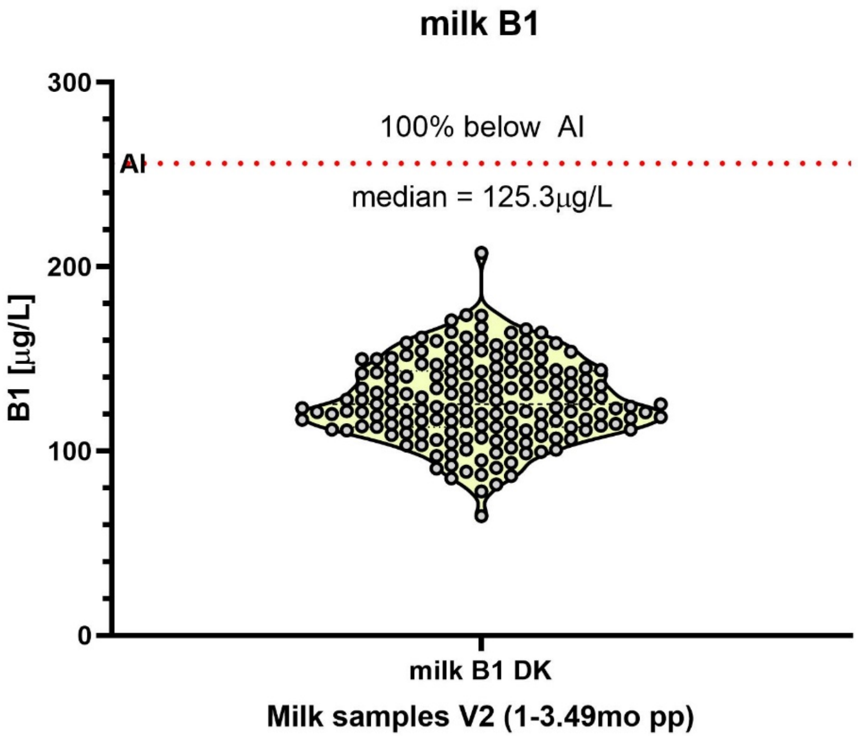
Total thiamin via HPLC-FLD

B-vitamin concentrations in milk vs. Adequate Intake (AI) value



- Many recent measurements of milk vitamins globally show median levels below current values used to set AIs for infants

Emerging new data to inform AIs



Milk thiamin in a High Income Country

All values well below the AI (median 125 ug/L vs AI 210 ug/L. Recommended intake for infants is higher than needed.

Note AI was set based on 1 study (source unknown).

Supplementation trials (fortified fish sauce) in Cambodia increased milk content from 66% up to 100% of the IOM AI concentration.

However our results suggest 66% below may actually be close to normal!

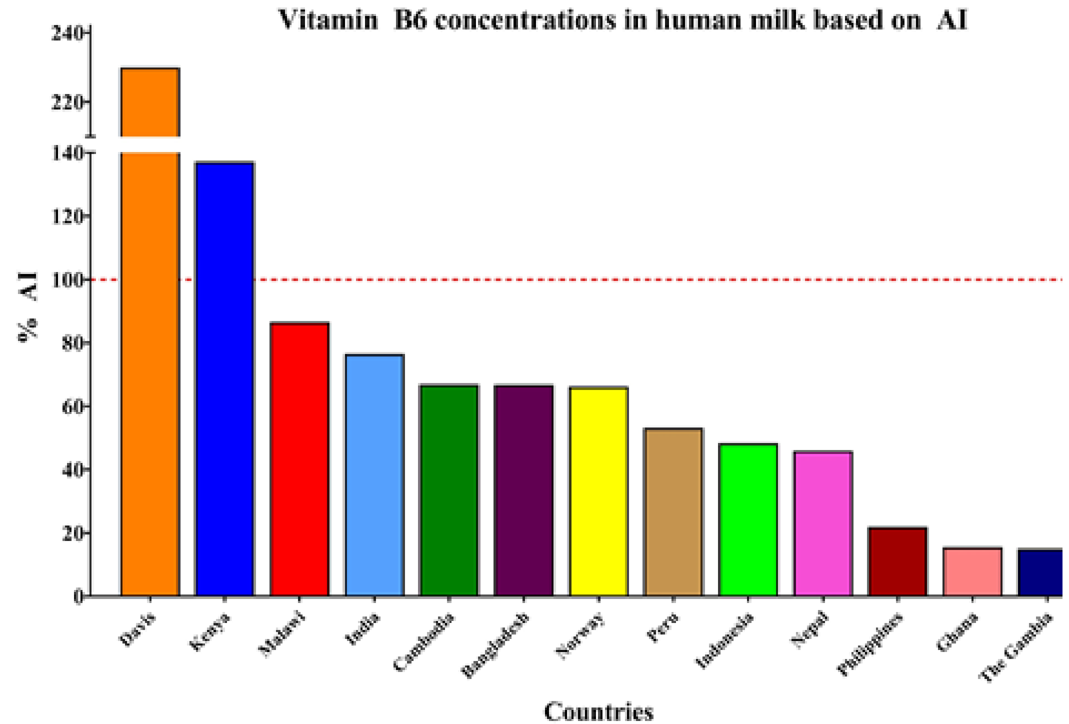
Vitamin B-6

- Neither global nor US deficiency prevalence known.
- NHANES: low serum PLP in 11% supplement users, 24% non-users.
- Serum PLP correlates and maternal intake rapidly increases milk B6.
- AI value milk 0.13 mg/L; <60% AI most LIC.
- 40% Egyptian mothers had milk B-6 <0.1 mg/L, abnormal infant behavior.
- Davis, 0.3 mg/L. High in prenatal supplements?

≈60% prenatal suppl. USA >RDA for B6.

ACOG: 10-25 mg B6, 3-4x/d, ± doxylamine, to treat nausea.

(RDA pregnancy 1.9 mg/d).

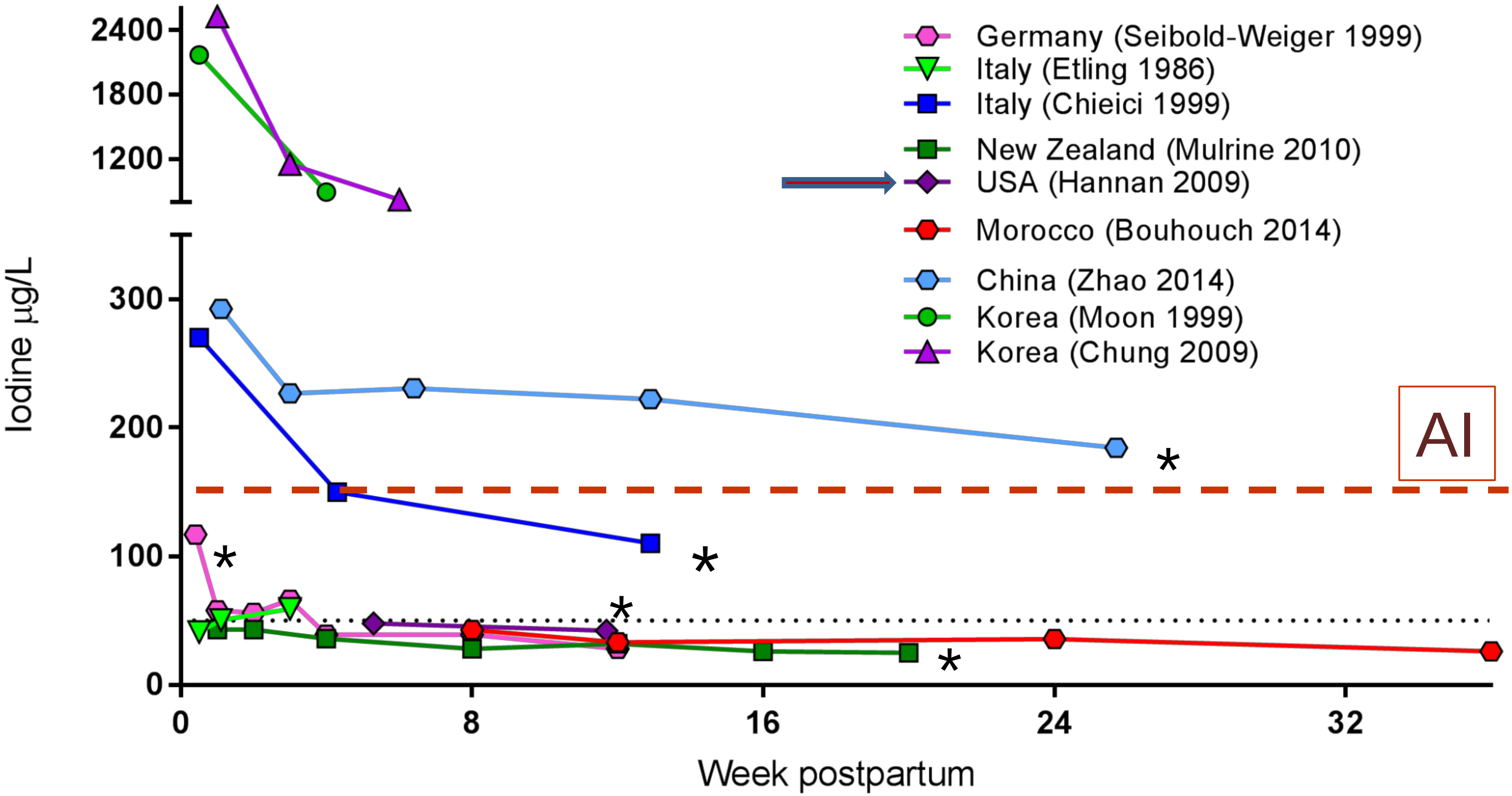


Iodine in Human Milk: A Systematic Review

Dror and Allen, Adv. Nutr. 2018

- Rapid fall in first weeks.
- Varies 100-fold across studies.
- Very sensitive to maternal intake and supplements.
- Values can be low even with USI.
- Older analytical methods unreliable.
- Current debate about infant requirements and need for maternal supplementation in lactation.

Longitudinal studies on milk iodine: mixed exposure to USI* (Dror & Allen, 2018)



***Hannan (2009): 31 Mexican-American women in Texas

Iodine deficiency

- Breast milk iodine parallels maternal U iodine.
- Review of 57 studies: iodine in milk =
  - 13-18 ug/L in women with goiter
  - 9-32 ug/L where prevalence of goiter high
  - >90 ug/L where effective salt iodization
- Low iodine in breast milk very common.
- WHO: give 250 ug/d to lactating women in areas of moderate/severe iodine deficiency if iodized salt reaches <50% households.
- APA and ATA (2014) recommend 150 ug/d supplemental iodine in lactation.

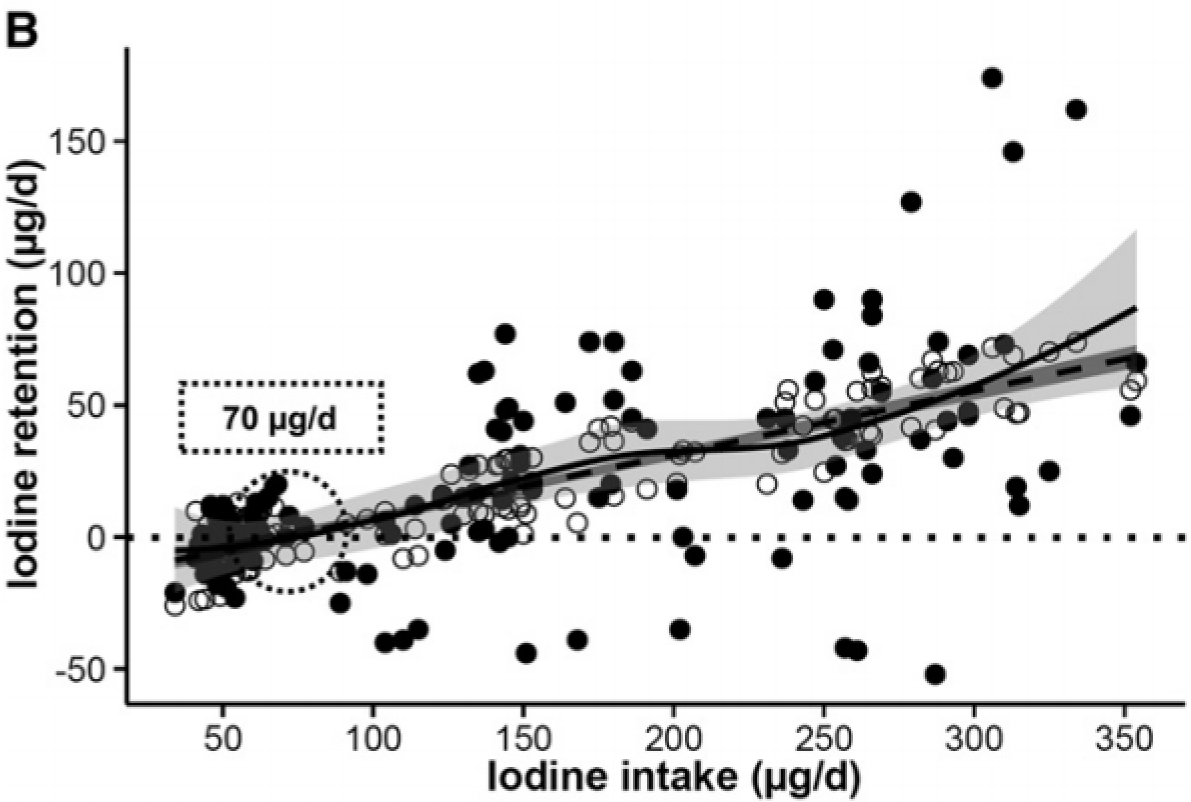
Assessing iodine status in lactation

- Current cutpoint is maternal UIC <100 ug/L lactation (vs. <150 ug/L pregnancy) (WHO 2007).
- Requirements similar in lactation vs pregnancy but in lactation UI is diverted to milk.
- "WHO's ≥100 ug/L UIC for lactation is inappropriate";
- iodine preferentially partitioned into milk when maternal intake/status is low (Dold et al. 2017).
- Low intakes, ≈70% total excreted milk, 30% urine.
- Higher intakes, ≈40% total excreted milk, 60% urine.
- BMIC best measure of iodine status lactation.

Iodine requirements lower than currently?

- IOM's AI for infants based on 1984 study, n=37, 14d to 3.5 y lactation.
- Assumes BMIC 146 ug/L so infant obtains 114 ug/d from milk.
- **EAR lactation 209 ug/d** (BM 114 + NPNL 95 ug/d).
- New: infant requires 72 ug/d for balance (= BMIC ≥92 ug/L).
- So EAR should be 95 + 72 = **167 ug/d**.

Infant is in iodine balance at intake 70 ug/d = 92 ug/L BMIC, but use BMIC 171 (60-465) ug/L to evaluate population status.



The American Journal of Clinical Nutrition, Volume 104, Issue 3, September 2016, Pages 620–628,
https://doi.org/10.3945/ajcn.116.134049

Dold et al., 2016

Weaning infants risk ID because iodized salt and milk contribute little dietary iodine; in Switzerland infants sufficient (UIC >100) only if fortified complementary foods

BM iodine (40-140 µg/L) from maternal dietary iodine

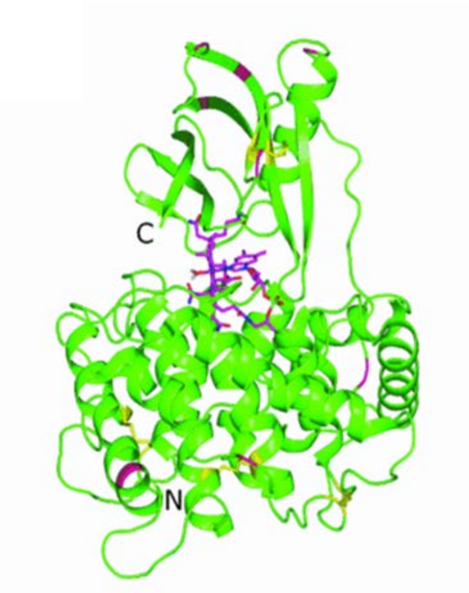
Home-prepared complementary foods very low in iodine

No added salt or cow's milk in the 1st year

Systematic review: vitamin B12

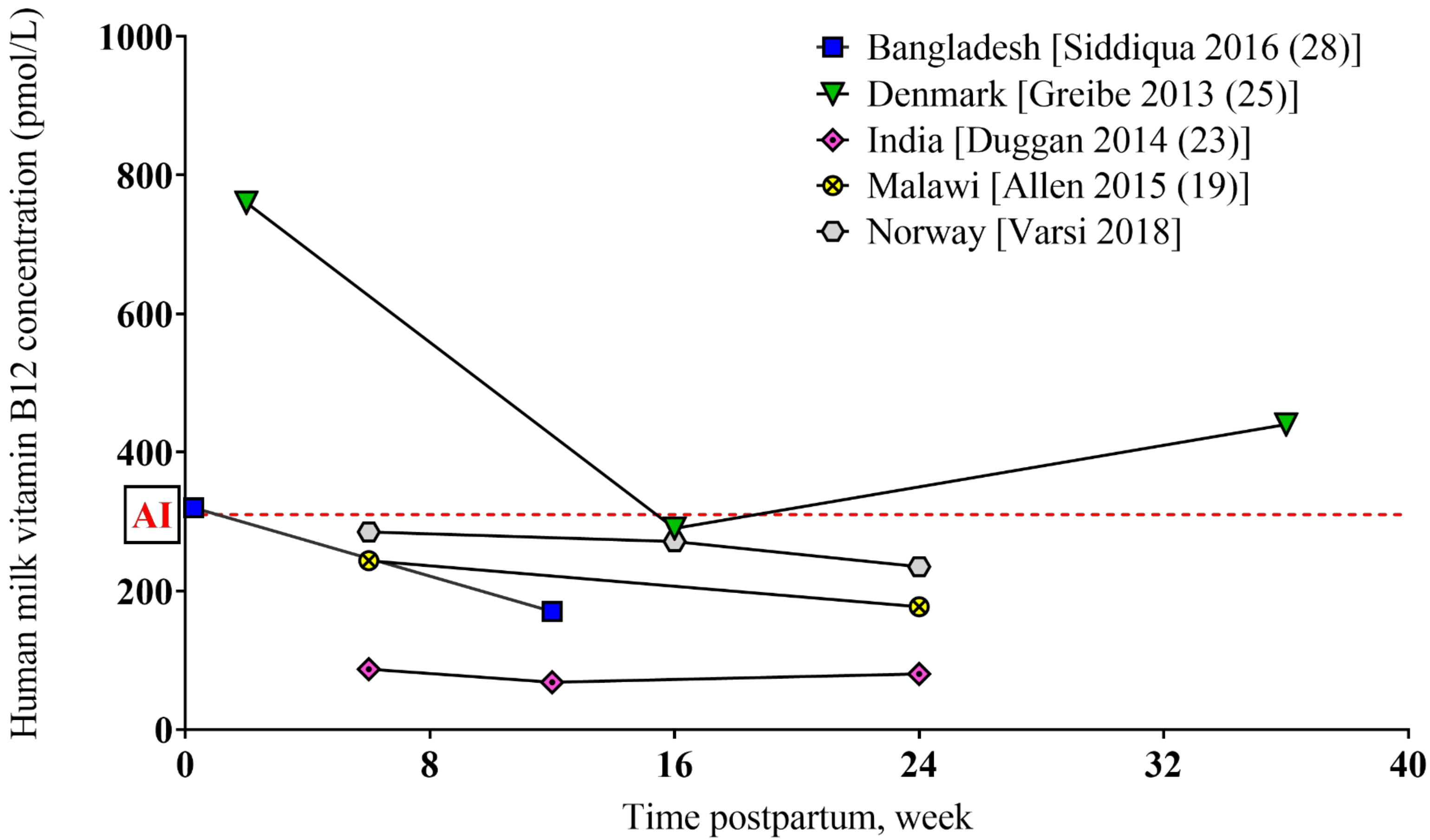
Dror & Allen, 2018

- 26 studies, but half in LIC, MIC or deficiency.
- 7/26 used invalid analytical method – uncertain removal of binding to haptocorrin.

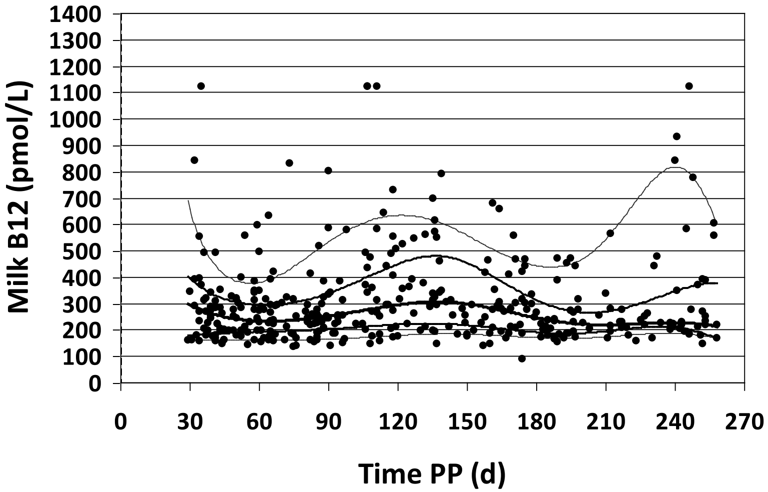


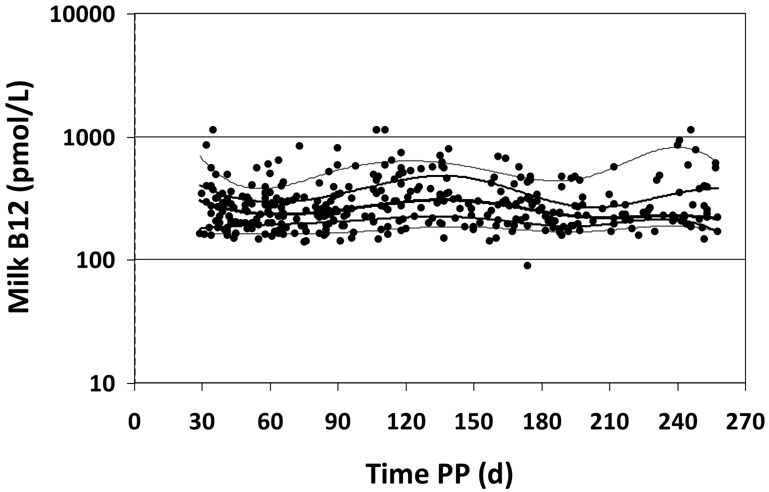
- Wide range of values across studies.
- Large decrease early lactation.
- Milk B12 correlates with maternal intake and maternal and infant status.

Longitudinal data on milk B12: valid methods, unsupplemented women



Breastmilk B12 across lactation in a high income country **5 Nov 2019**

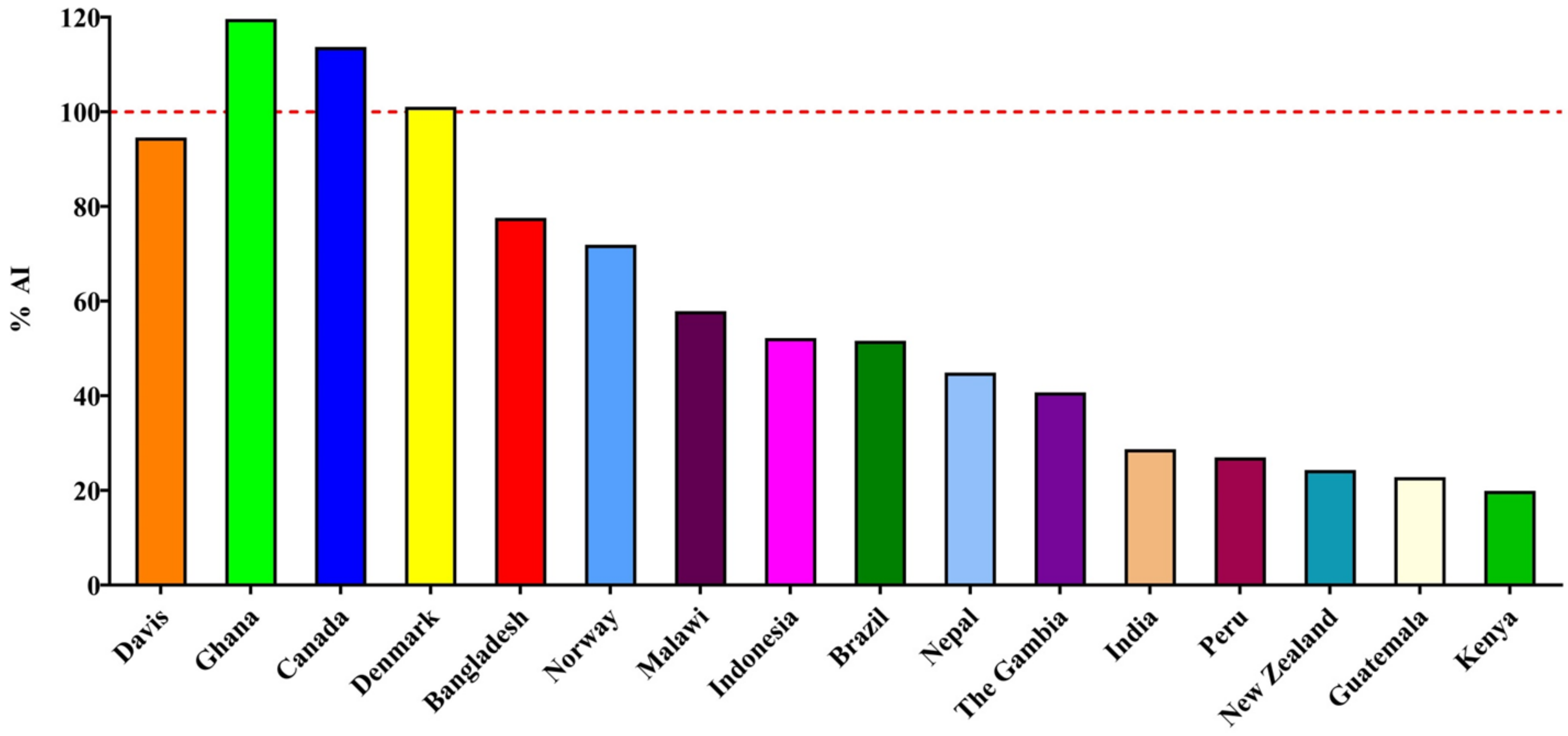




Concentrations appear to be stable across 9 months. Disagrees with some previous Scandinavian reports showing marked fall around 4 to 6 months.

Log scale

Global values for milk B12: analyses from the Allen lab
Median values as % of Adequate Intake value (310 pmol/L)

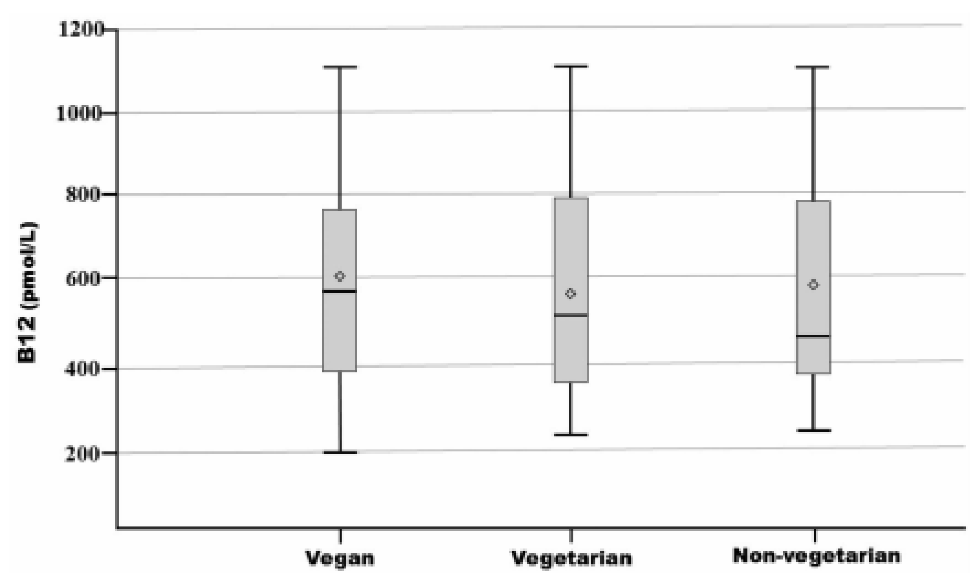


% Infants with symptoms, in case studies of B-12 deficiency (Dror & Allen)

	Mother pern. anemia (n=18)	Mother vegan (n=30)
Wt <10 pcle	93	89
L <10 pcle	83	60
Head <10 pcle	91	77
Hypotonia	61	63
Developmental delay	56	60
Lethargy	50	63
Slow/abnl EEG	50	33
Not able to sit alone	33	43
Convulsions/tremors	33	23
Cerebral atrophy	28	37
Irritable	20	28
Not smiling	11	23

Vitamin B-12 content in breast milk of vegan, vegetarian, and nonvegetarian lactating women in the United States

Roman Pawlak,[1] Paul Vos,[2] Setareh Shahab-Ferdows,[3] Daniela Hampel,[3,4] Lindsay H. Allen,[3,4] and Maryanne Tigchelaar Perrin[5]



74 women N. Carolina

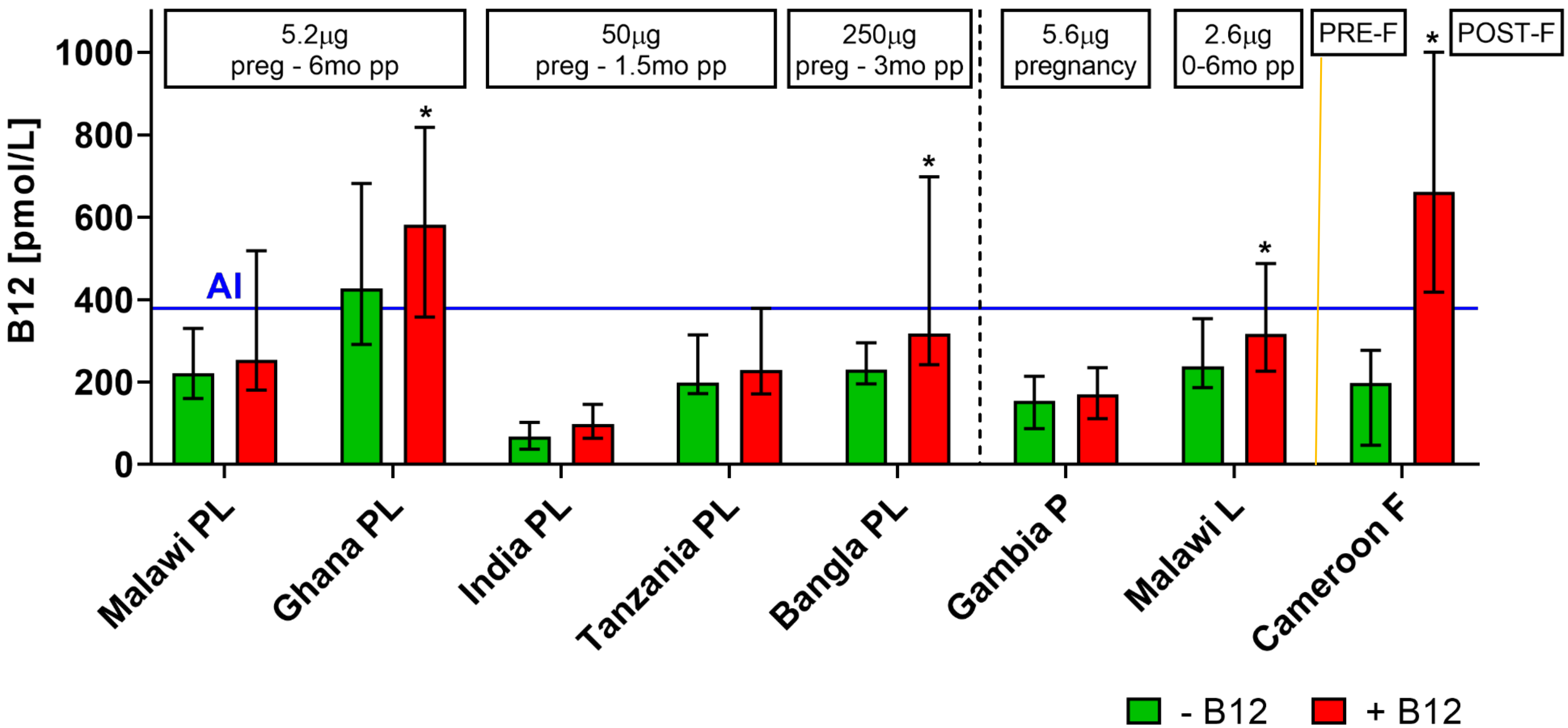
Prevalence low sB12 15-19% all groups

No differences BMIC

Supplement use:
46% vegans
27% vegetarians
3% non-vegetarians

- Virtually no data on B12 (or other nutrient) status in vegetarians/vegans in US.
- Likely more conscious of need for supplements, fortified foods.

Effects of B12 interventions on milk B12, 3-6 months



- Sig. ↑ milk B12 only in Ghana & Bangladesh (P+L), Malawi (L)
- Overall, ↑ only 33 (15 to 154) pmol/L (AI value 310 pmol/L)
- Dose not important
- Fortification most effective.

Continuum of mother-child B12 depletion in Guatemala (3 studies)

Maternal depletion in pregnancy

↓

Low B12 stores in infant at birth?
& in breast milk

↓

Infant depletion by 3 months

↓

Depletion at 7 and 12 months (r=0.49)

Breastfed freq (-)

Cows milk (+)

↓ (r=0.54)

Depletion at 21 months (and still correlated with 12 month maternal B12)

↓ weight, length, motor development

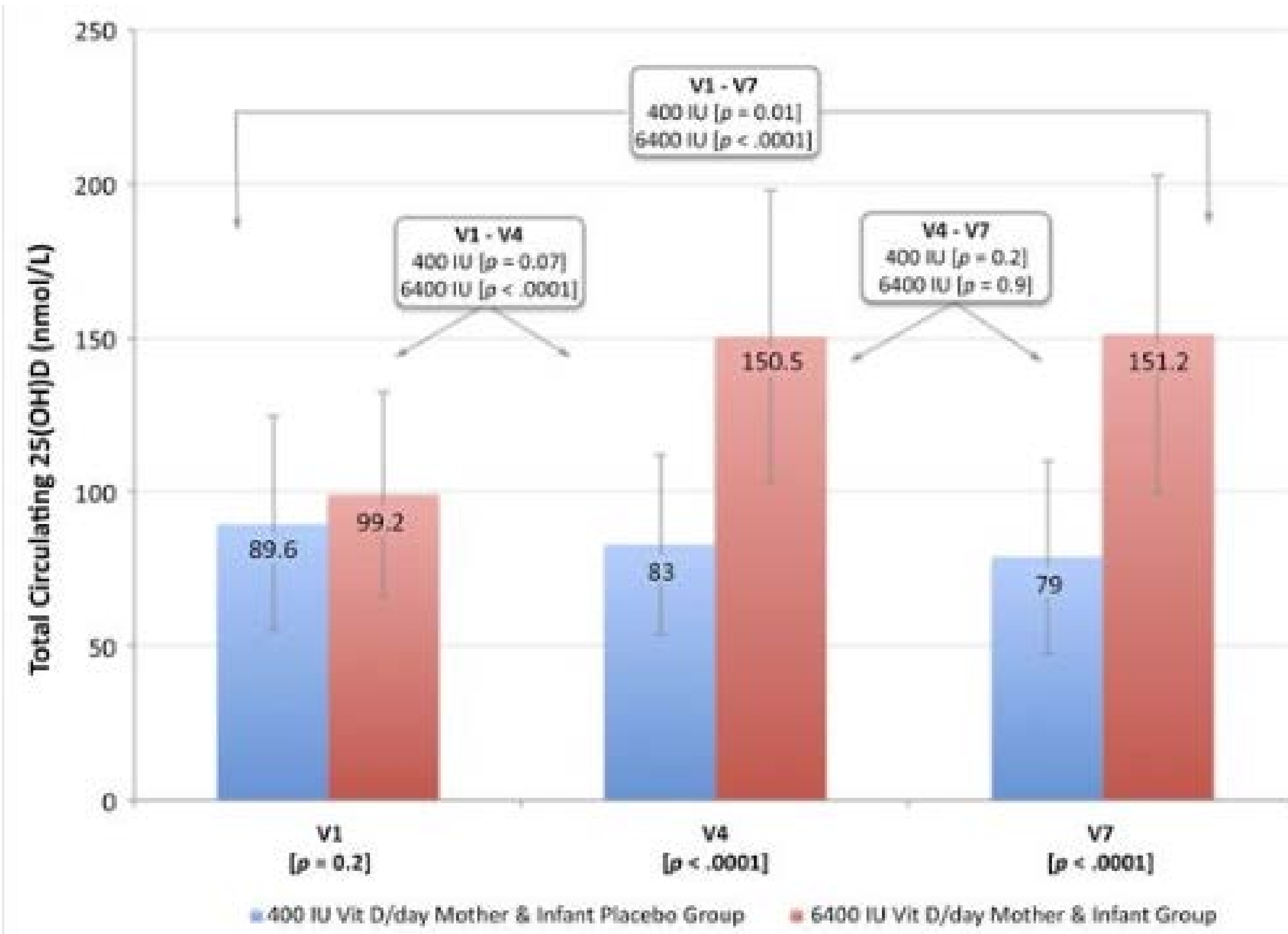
Conclusions: B12 interventions

- Important to ensure good maternal status throughout pregnancy, but one-a-day supplements may not be very effective.
- Dose unimportant for maternal, milk and infant response (2 – 250 ug/d).
- Early pregnancy sB12 predicts infant B12 at 4-6 mo postpartum regardless of supplement dose in pregnancy + lactation.
- In lactation, single dose or one-a-day supplement little effect on milk or infant status.
- Current maternal intake is not main influence on milk B12.
- Complementary foods do increase infant B12 status.
- More effective approach may be small repeated maternal doses to mother (e.g. fortified flour causes a 300% greater increase in milk vs. supplements).

Vitamin D

- Human milk is very poor source of vitamin D (<50 IU/L).
- Infants born with low stores. Depend on milk and sun.
- AAP recommends direct supplementation of infants starting in early days of life;
  - 400 IU/day if breastfed
  - None if weaned to D-fortified formula, or to D-fortified milk after age 12 mo., or
  - 2 h sun/week with only face and hands exposed, or
  - 30 minutes/week wearing only a diaper
- New: If mother supplemented 6400 IU/d (UL is 4000 IU) will supply infant enough VD in her milk so no need for infant drops; adherence to drops is poor. Risks?
- Higher risk of maternal deficiency and low milk D if low sunlight exposure, darker skin, urban, air pollution.

High dose vitamin D to lactating mother increases plasma vitamin D in infant



400 IU/d to mother

6400 IU/d to mother

4 months

7 months

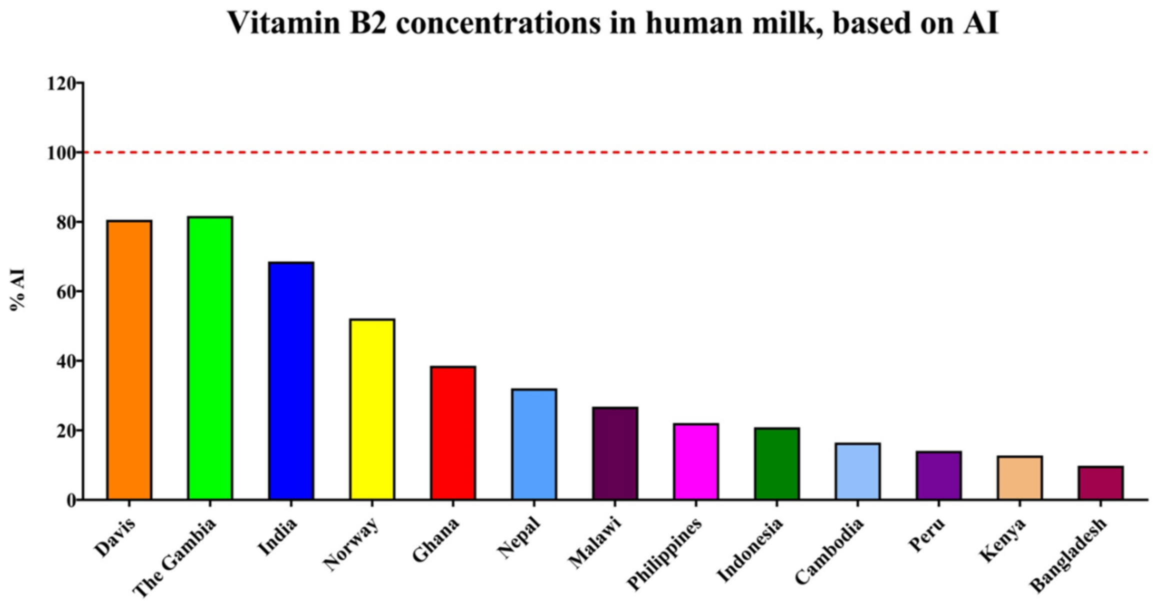
Hollis BW et al., Pediatrics 2015,136:4, doi:10.1542/peds.2015-1669

No data on many micronutrients e.g. riboflavin

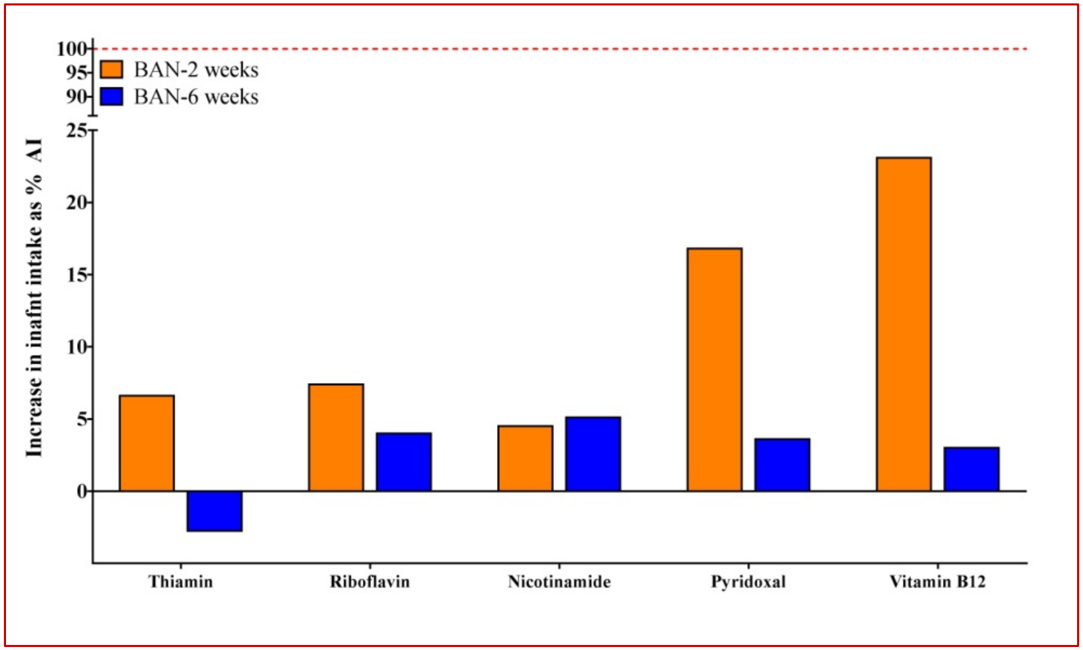
- 25-60% depletion in adults in UK, Canada, Europe, Irish National Survey. NO DATA FOR USA.
- Low dairy product and egg intake.
- In severe deficiency infant has poor growth & development.
- Milk levels affected by maternal deficiency and supplements
  – faster and to greater extent than other nutrients.

Our data:

10-20% AI in most LICs



How much does maternal supplementation affect infant intake?



Malawi, EBF infants. Daily LNS from ≈ birth to 6 months.

Increased infant intake by:

5-23% of AI after 2 weeks

but only 0-5% at 6 weeks.

(ARVs eliminated increase).

MILQ study

- To establish Reference Values for concentration of each milk nutrient across first 9 months lactation.
- Well-nourished (but not supplemented) mothers.
- 4 countries, same methods and selection criteria
- Supported by data on diets, maternal and infant status, milk volume, plasma and milk infants, microbiome…

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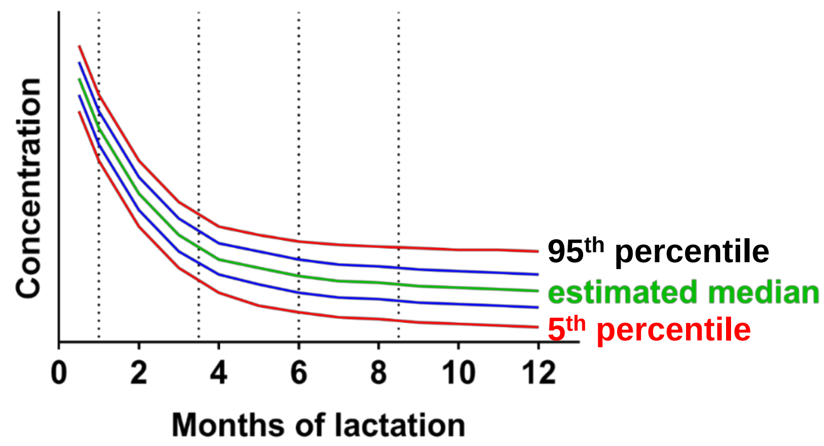

Summary

- Very poor data on micronutrient status in lactating women, infants and milk concentrations in US.
- Maternal and infant requirements and cut-points uncertain.
- Milk MN greatly affected by maternal status.
- Ongoing MILQ study will provide Reference Values for milk micronutrients and relationship to maternal and infant status in unsupplemented, well-nourished women (in 2022?) using efficient, valid methods.
- National study being considered (Casavale et al., 2019), but who to sample? When in lactation? Effects supplements/ and fortification?
- Maternal status pre-pregnancy may be critical for milk micronutrients; not much of daily supplements appears in milk?

Collaborators in milk research

WHNRC:

Setti Shahab-Ferdows

Daniela Hampel

Julianne Saracha

Juliana Haber

Janet Peerson

USDA Agricultural Research Service

Denmark: E. Nexo, D. Lildballe, K. Michaelsen, K. Eriksen, S. Hilario Christensen

Gambia: S. Moore, A. Doel

Ghana: K. Dewey, iLiNS team

Guatemala: M. Ramirez, N. Solomons

India: C. Duggan, A. Kurpad

Indonesia: L. Houghton, R. Gibson

Kenya: A. Williams, C. Stewart. C. Chantry

Bangladesh: M. Islam, R. Raqib, T. Siddiqua

Brazil: G. Kac, E. Barros, D. Farias

Cambodia: K. Whitfield

Cameroon: R. Engle-Stone, K. Brown

Canada: T. Green, P. Chebaya

Malawi: P. Bentley, L. Adair, V. Flax, K. Dewey, P. Ashorn

New Zealand: T. Green

Norway: T. Strand, S. Henjum

Peru: T. Gyorkos, L. Mofid

Philippines: M. Haskell

Tanzania: W. Fawzi

USA: M. Perrin, R. Pawlak, J. Smilowitz

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MiLQ Mothers, Infants and Lactation Quality