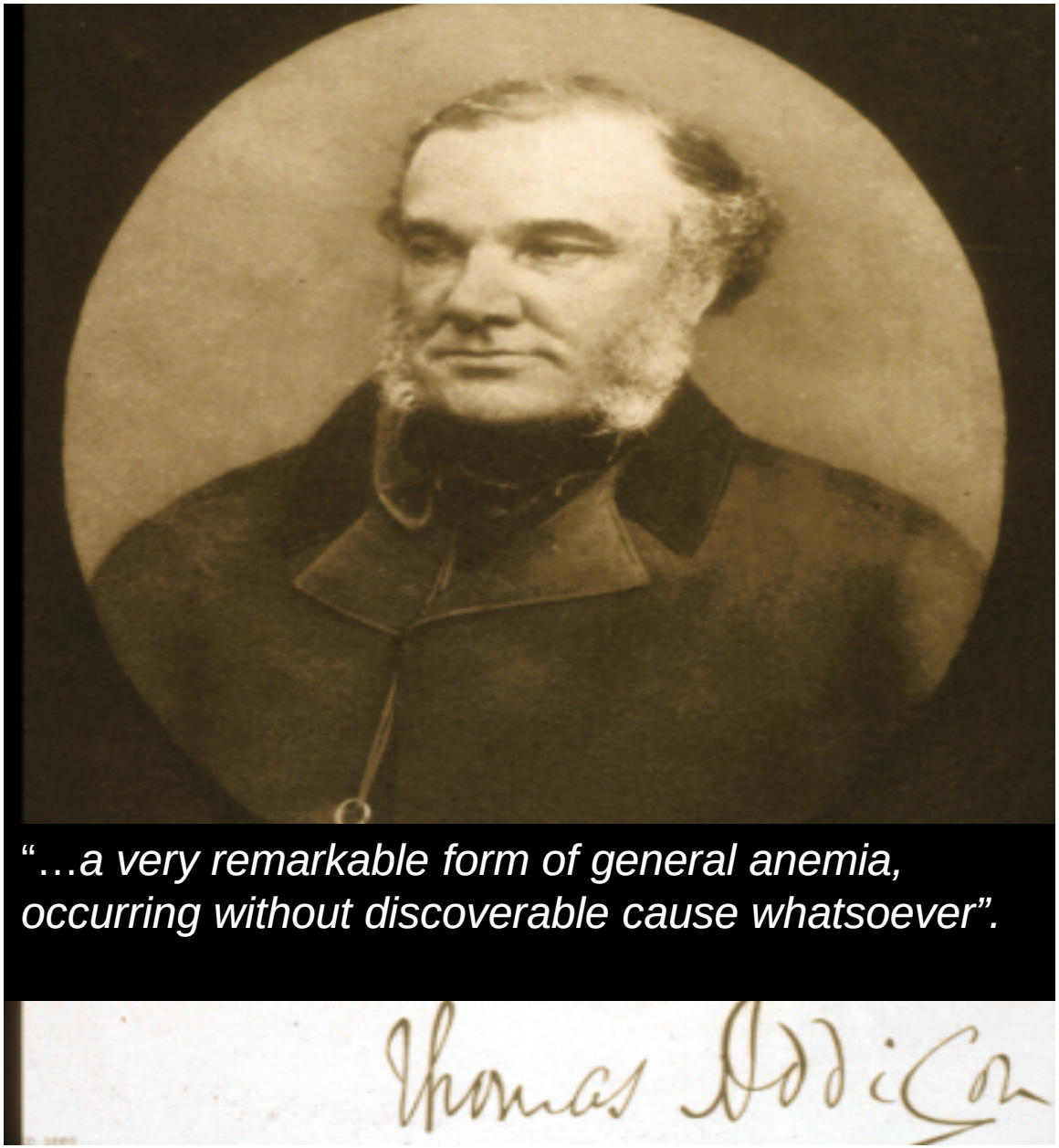
Focus on Vitamin B12: Present Knowledge and Future Opportunities

Lindsay H. Allen

Center Director

USDA, ARS Western Human Nutrition Research Center, UC Davis, CA

The first description of Pernicious Anemia



"…a very remarkable form of general anemia, occurring without discoverable cause whatsoever".

Sir Thomas Addison – Guys Hospital London

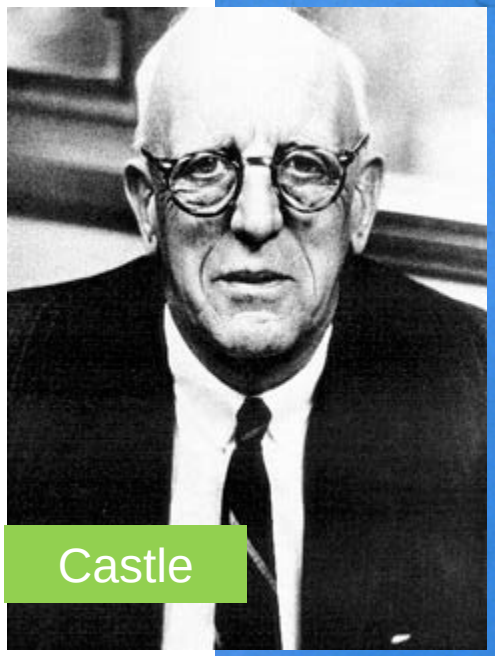
The conquest of Pernicious Anemia & the characterization of vitamin B12

1934: Minot, Whipple & Murphy Nobel Prize for Physiology & Medicine – “Cure of PA”

1948: Folkers & Smith crystallized Anti-pernicious anemia principle from liver; named B12

1965: Robert B. Woodward Nobel Prize for Chemistry. First chemical synthesis of B12

1964: Dorothy Hodgkin – Nobel Prize for Chemistry for studies on X-Ray crystallographic structure of B12



Castle

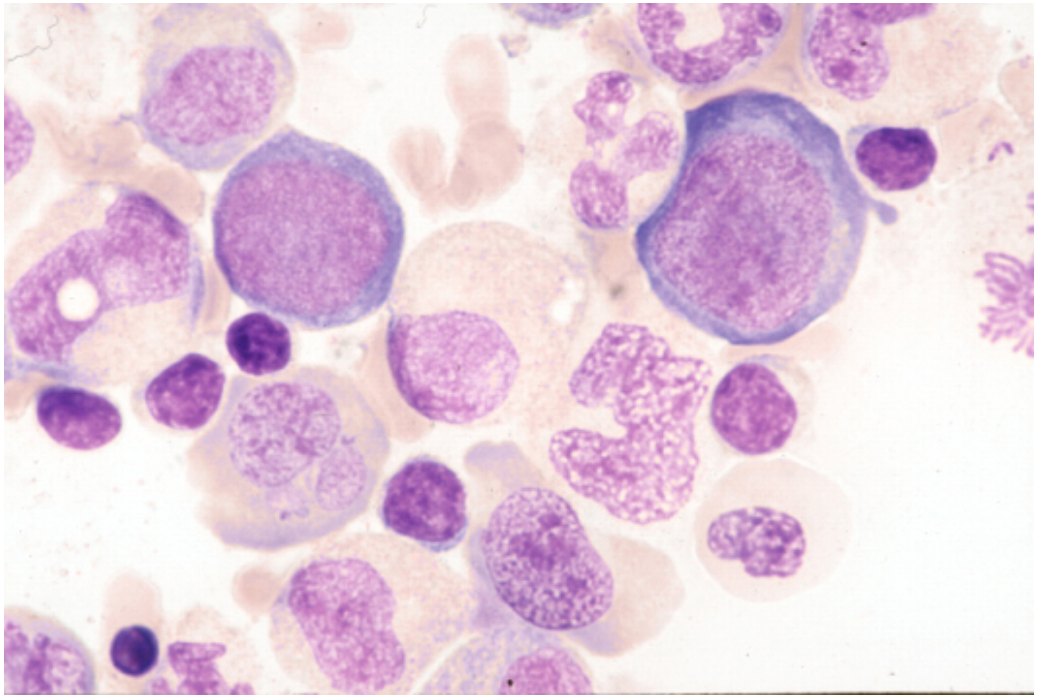
1930s

Stomach extract for treatment of pernicious anemia

Vitamin B12: Pernicious Anemia and beyond

"The [pernicious anemia] patient, formerly a pleasant individual, is now a cantankerous curmudgeon with prematurely gray hair, light complexion, blue eyes, large ears, broad cheekbones, lemon-yellow complexion with moderate scleral icterus, and vitiligo melanotic pigmentation."

Harris, JW, and Kellermeyer RW. 1970. *The Red Cell*



Bone marrow – megaloblastic anemia

B_{12} is attached to protein when ingested

Digested

B_{12} Complexed to HC to form HoloHC and transported to the small intestine

Degraded

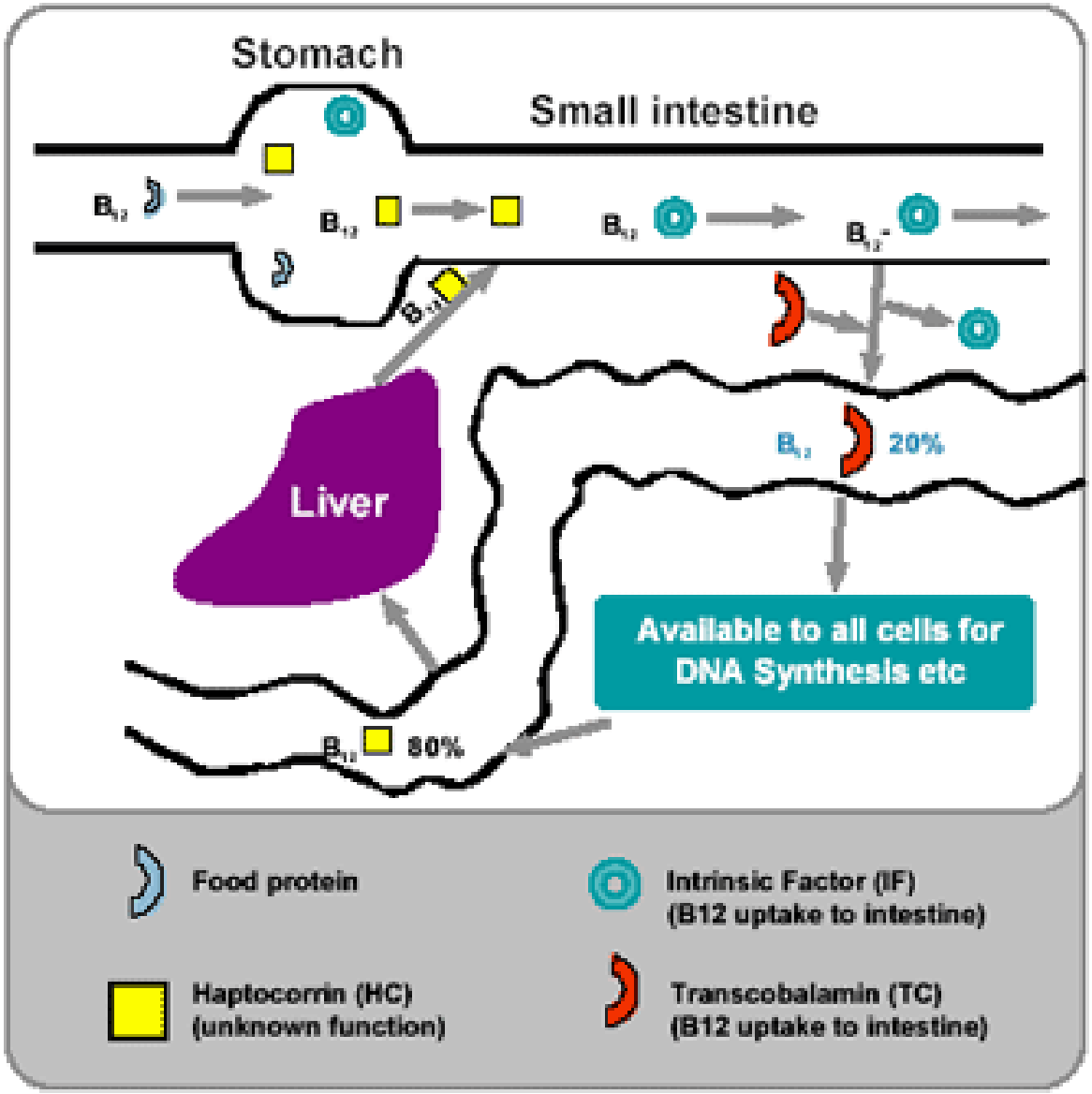
B_{12} Complexed to IF and absorbed in the intestine

Degraded

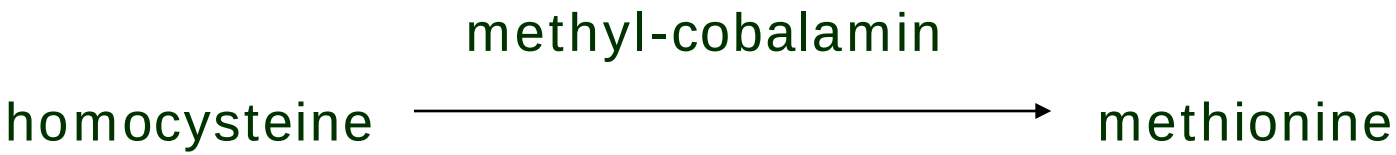
B_{12} Complexed to TC, to form Active B12 (holotranscobalamin)

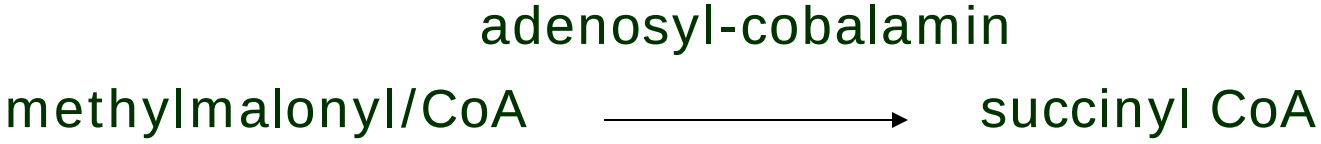
Released in Blood

Taken up by specific cell receptors



Vitamin B-12 (cobalamin) metabolism





RDA 0.9-2.4 ug/d

(to maintain plasma B12 and prevent anemia)

1 µg/d excreted in bile and ≅ 50% reabsorbed

NIH's Biomarkers for Nutrition & Development (BOND): B12 Expert Panel

- Serum or plasma B12
  - Most common, rel. specific, marks stores. Cheap.
  - Changes slowly with change in intake
- Serum holotranscobalamin (holoTC)
  - Affected by recent intake and absorption
  - Sensitivity and specificity ≥ serum B12
  - Deficiency = <35-40 pmol/L
  - Gives similar deficiency prevalence to serum B12 in surveys

NIH's Biomarkers for Nutrition & Development (BOND): B12 Expert Panel: serum B12 cut-points

Cut-point (pmol/L)	Rationale
<75, severe deficiency	Anemia, neurological symptoms
75-<150, likely deficient	50% have clinical symptoms
150-221, depleted	↑ risk of clinical and metabolic dysfunction (98% elevated MMA)
>221 adequate	But, 250-350 assoc. ↑ risk of poor cognition & white matter loss in elderly

- Levels naturally higher in infants and lower in late pregnancy
- Does not change much in elderly until ≈70 years
- Reflects usual intake (but responds slowly)

NIH's Biomarkers for Nutrition & Development (BOND): the 3 other markers of B12 status

- ❑ Serum holotranscobalamin (holoTC) <35-40 pmol/L
  - Form carried through blood and tissues
  - Reflects recent intake and absorption
- ❑ Serum methylmalonic acid (MMA) >271 nmol/L
  - Most sensitive and specific
  - Reflects stores, not recent intake
  - ↑ by impaired renal function (measure creatinine), age
- ❑ Plasma total homocysteine (tHcy)
  - Not specific for B12

Causes of B12 deficiency

Low intake

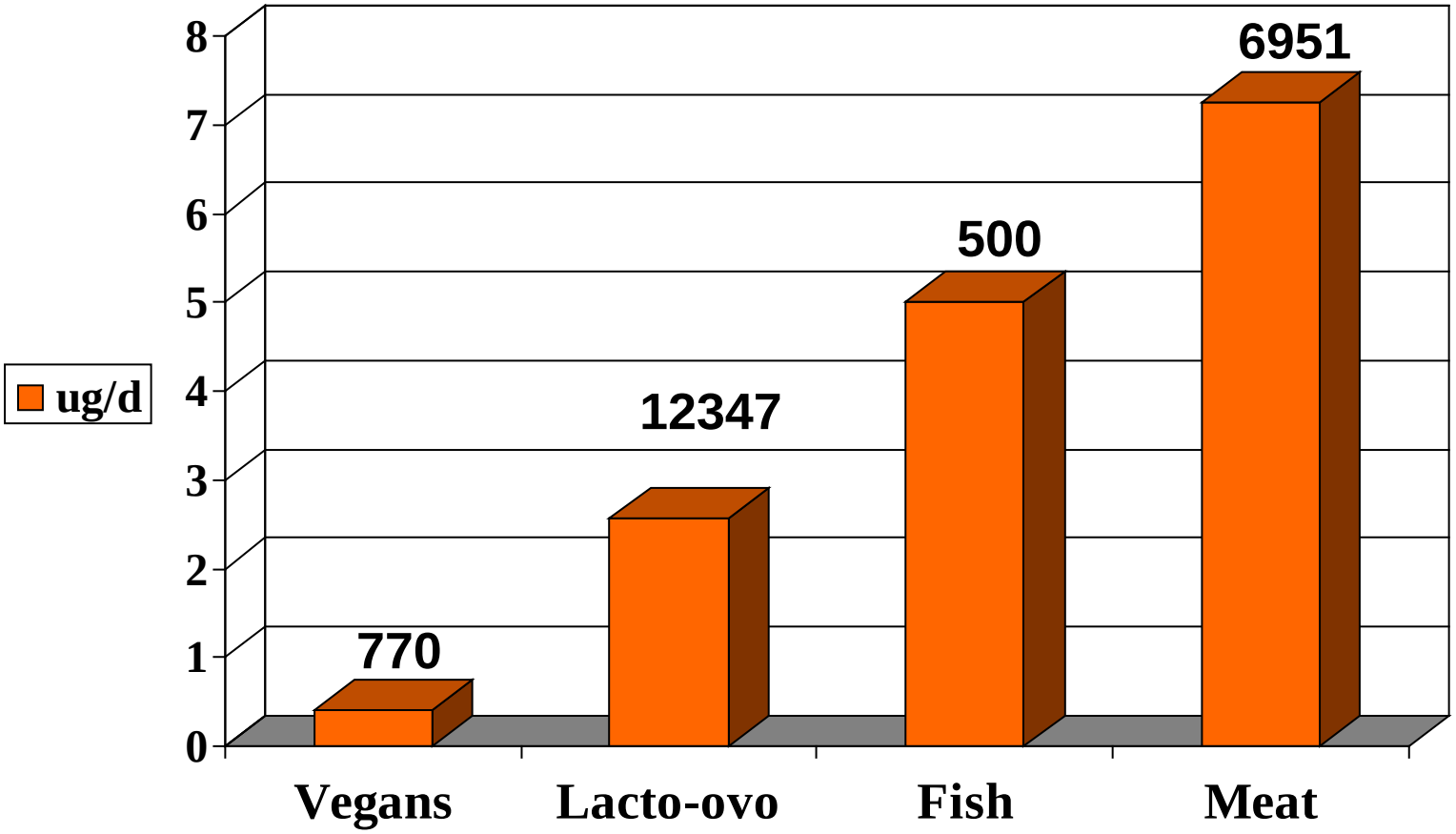
Malabsorption

Aging

Dietary sources of B12 (RDA = 2.4 ug/d)
(NONE in any plant source foods)

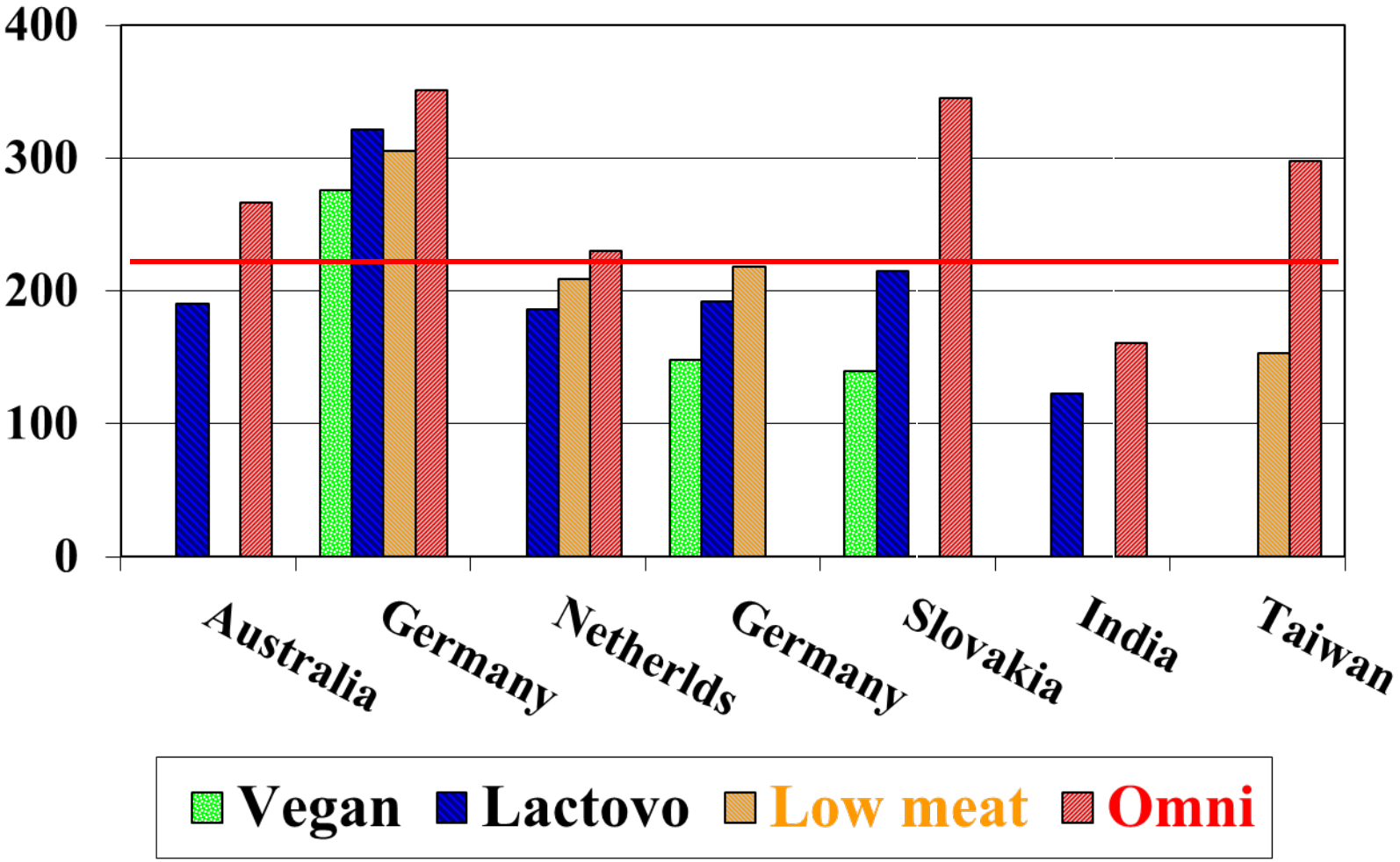
Food	Amount	ug
Liver (cooked)(low % absorbed)	100 g	84
Fish	100 g	2.5-5
Meat	100 g	1.4
Milk	1 cup	1.2
Yogurt	1 cup	1.1
Cheese	100 g	0.9
Egg	1 large	0.6
Chicken	100 g	0.3

Mean B12 intakes of men by diet groups, EPIC (Davey, 2002)

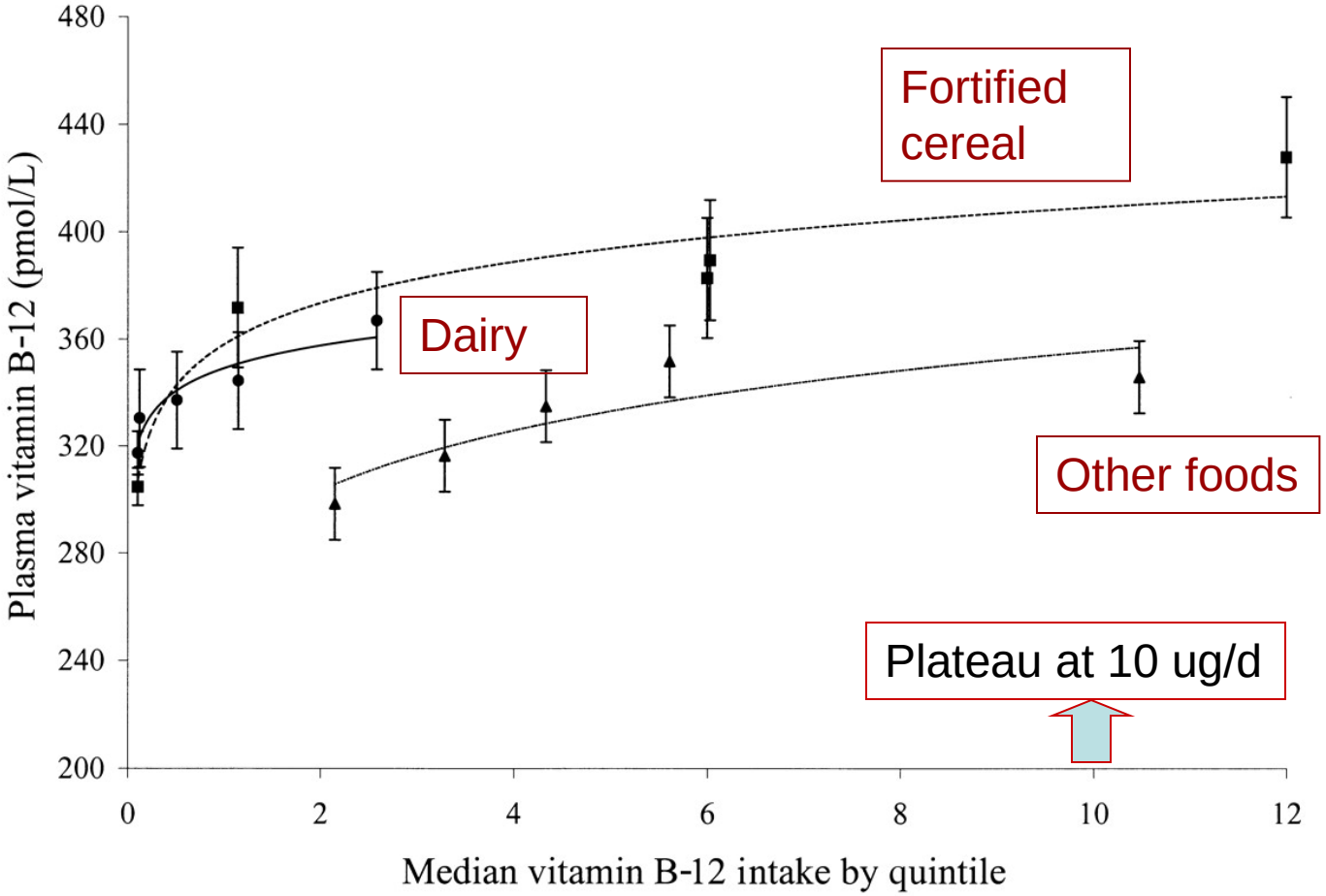


Intakes assessed by FFQ

Serum B-12 (pmol/L) vs. usual diet

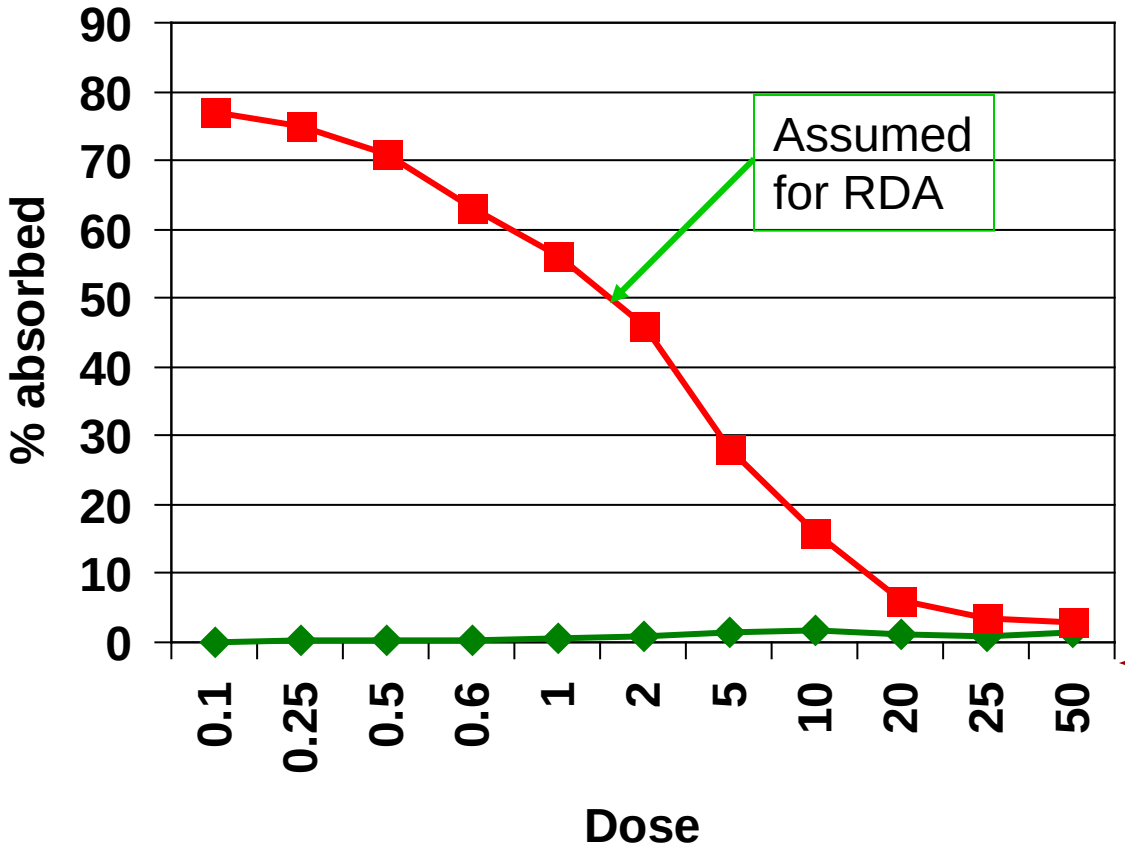


B12 intake vs. plasma B12, by dietary source



Tucker, K. L et al. Am J Clin Nutr 2000;71:514-522

% B12 absorbed is inversely proportional to dose (Chanarin)



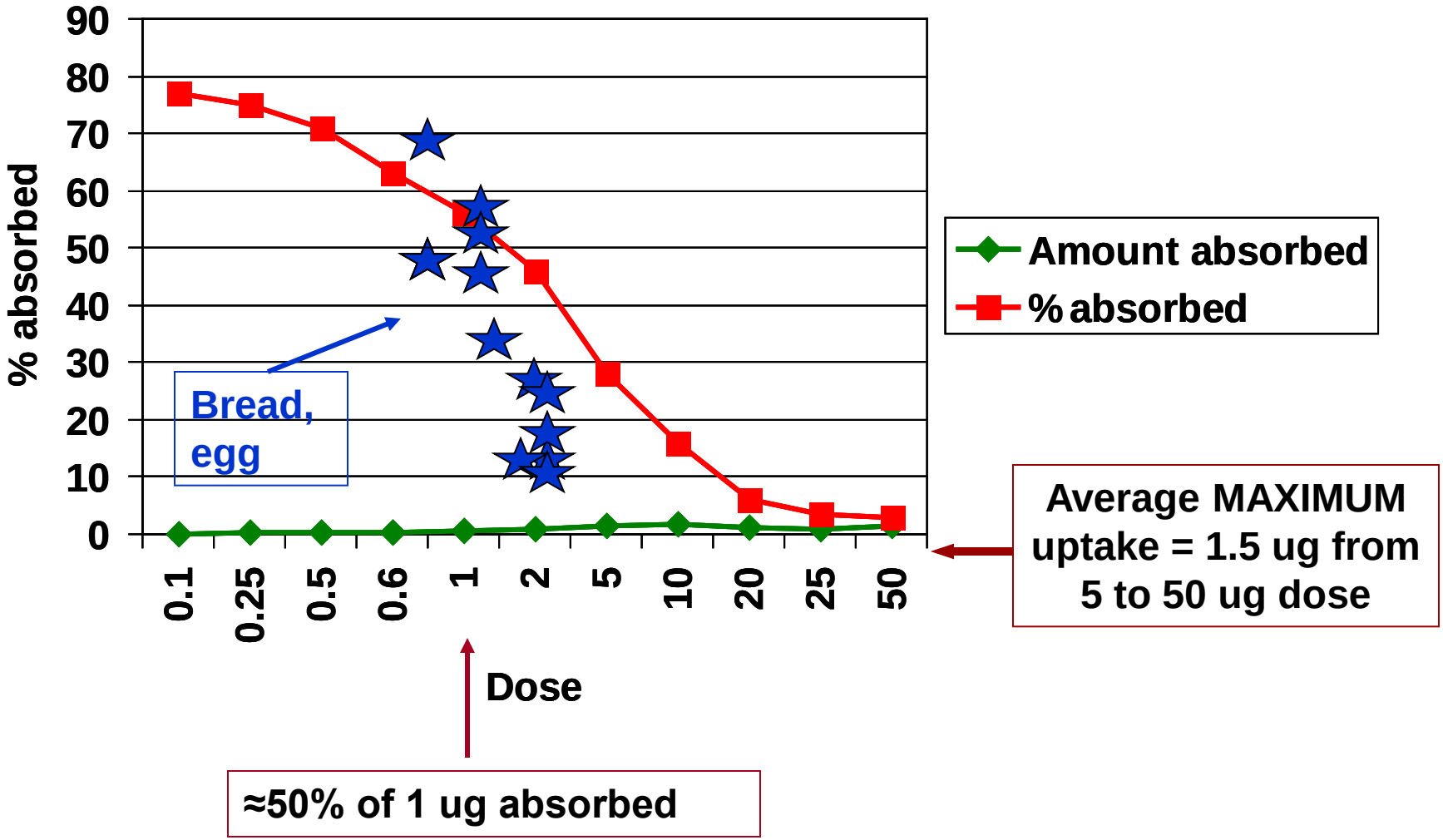
% Absorbed similar from meat vs. pills

(but 20% lower from liver)

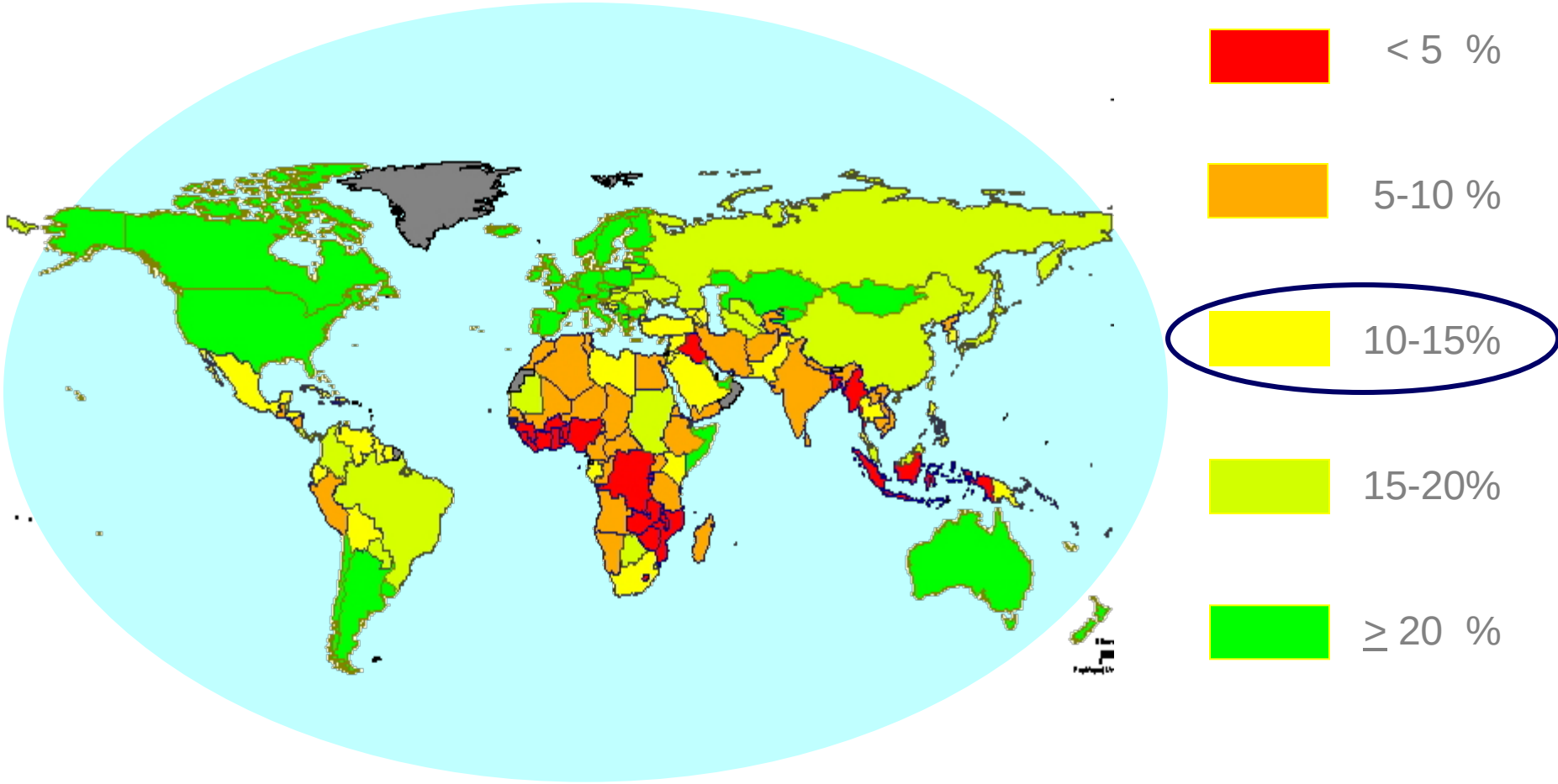
Average MAXIMUM uptake = 1.5 ug from 5 to 50 ug dose

≈50% of 1 ug absorbed

% B12 absorbed is inversely proportional to dose (Chanarin)



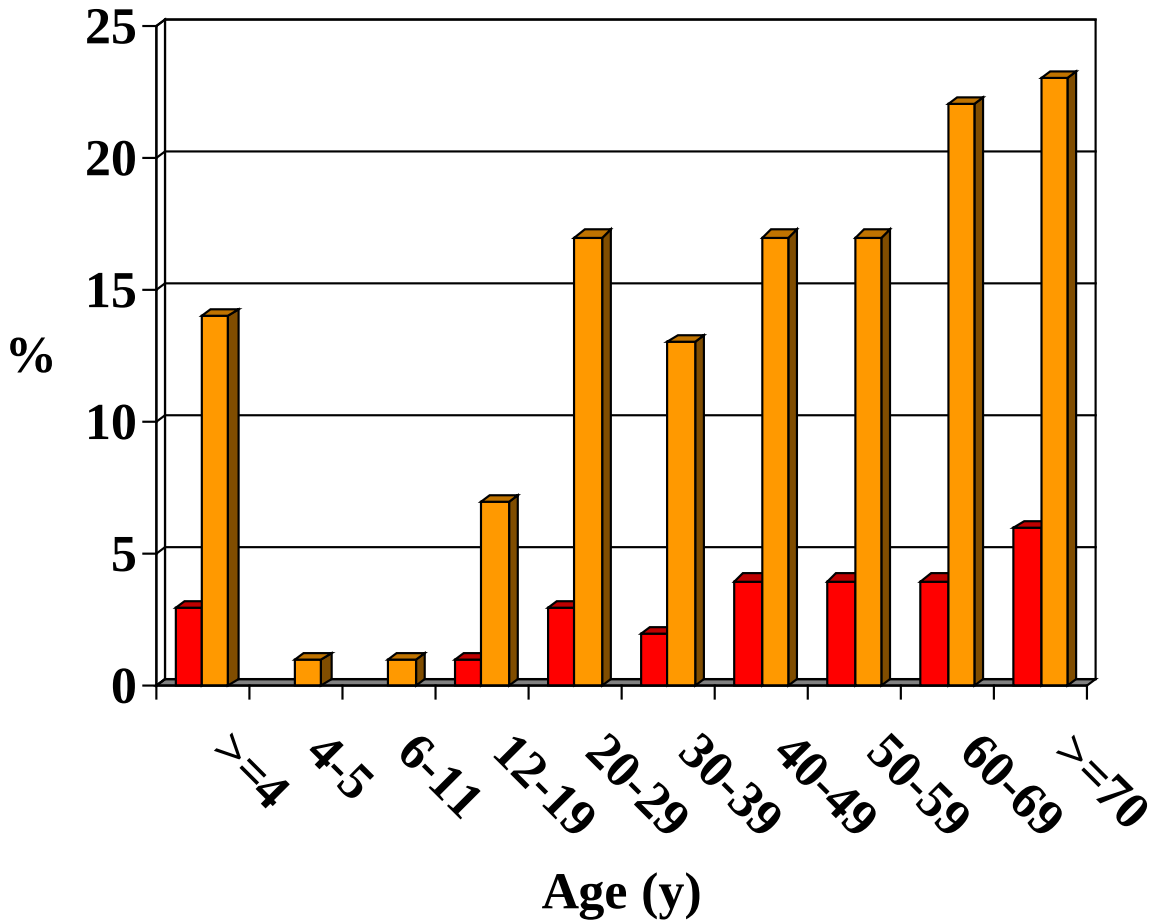
Percent of energy in food supply from animal source foods (ASF) predicts B12 status



Malabsorption

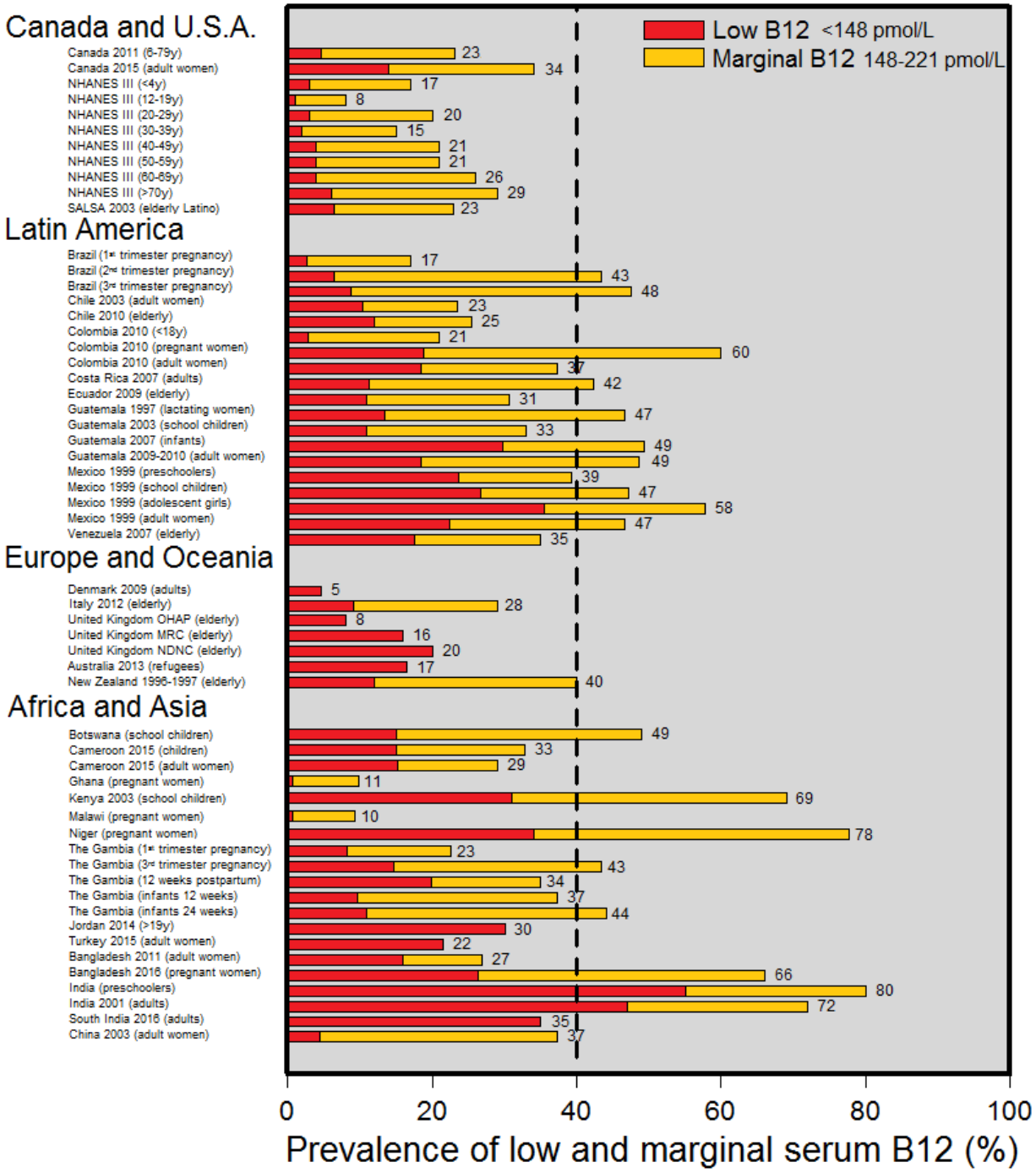
- Pernicious anemia – autoimmune disease where ↓ IF; only 2-4% cases deficiency, mostly >60 y.
- Need i.m. injections (1/month) or high doses oral B12 (≥500 ug/d).
- Food cobalamin malabsorption – mostly in elderly. Crystalline B12 absorbed OK.
- IBD, ileal resection (↓ absorption in ileum).
- Gastrectomy (↓ acid and IF).
- PPIs (↓ acid).

NHANES III: Prevalence of deficient and marginal serum B12 (Pfeiffer et al. 2007)



Prevalence of B12 deficiency

Probably the most common nutrient deficiency in the world



High prevalence of deficiency

In wealthy and poor countries

Across all ages and physiological groups

>40% in many countries

Brito and Allen

BOND report

Consequences of B12 deficiency – does it matter?

Stages of B12 deficiency

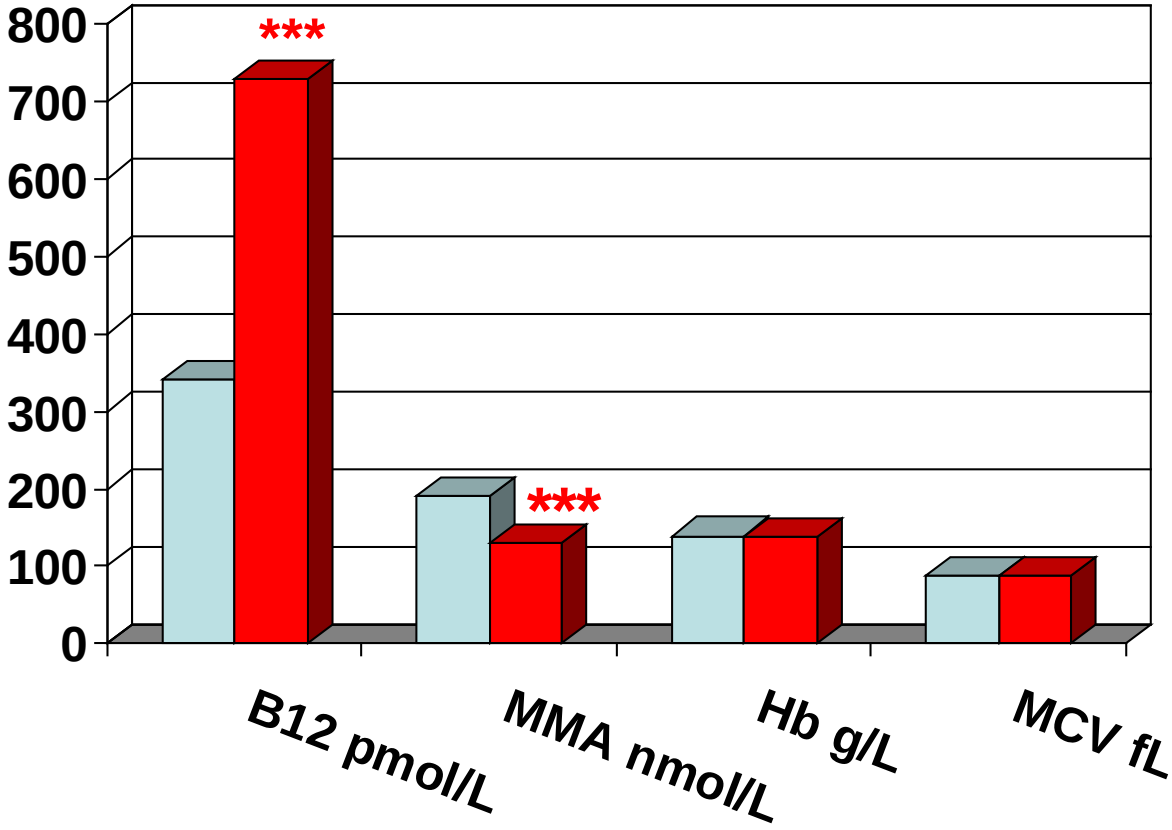
- Changes in biomarkers
  - ↑ plasma and urine MMA
  - ↓ serum holoTC and B12
  - ↑ plasma tHcy
  - ↓ RBC B12
  - Megaloblastic anemia
- Weakness and fatigue
- Demyelination of neurons
- Reduced neuron conductivity
- Peripheral neuropathy, abnormal gait and position sense
- Subacute combined degeneration (myelopathy)
- Dementia, depression, memory loss, psychosis

Serum B12 links with poor function

	V. deficient <100 pmol/L	Deficient <150 pmol/L	Marginal <221 pmol/L
Anemia	+ +	+ / -	No
Neuropathy	+ +	+	No
↑ Hcy, MMA	+ +	+ +	+ +
Breast milk	+ +	+ +	+
Child devpt	+ +	Assoc.	Assoc.
Cognition	+ +	+ / -	Assoc.
Depression	Assoc.	Assoc.	Assoc.
Bone mineral	+	Assoc.	Assoc.
Vaccine resp.	→	→	+ +
NTD	?	?	Assoc.

Only severe B12 deficiency causes anemia:

B12 status of depleted Mexican women was normalized by 1 mg i.m. + 500 ug/d for 3 mo, but no effect on any CBC measure



54% deficient or marginal at baseline

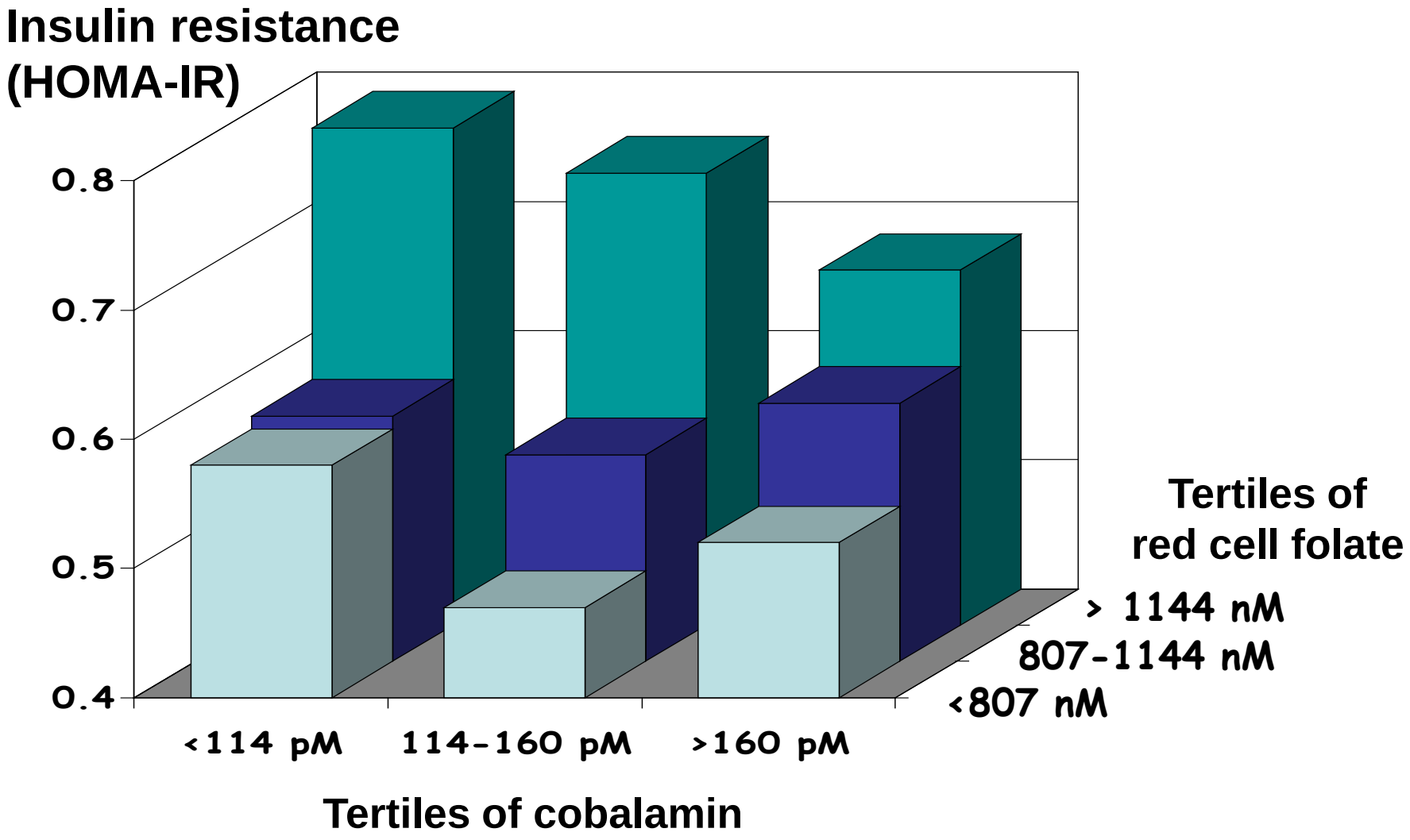
Systematic review: probable perinatal effects of maternal B12 depletion in pregnancy

- NTDs (risk ↑2-3x if serum B12 <190 pmol/L in Ireland)
- Pre-eclampsia, birth defects
- Lower birthweight
- Epigenetic effects - postnatal insulin resistance
- Poor infant development

Rush et al. Eur J Clin Nutr 2014

High folate and lowB12 in pregnancy, and insulin resistance at 6 y

(Yajnik et al., 2008)



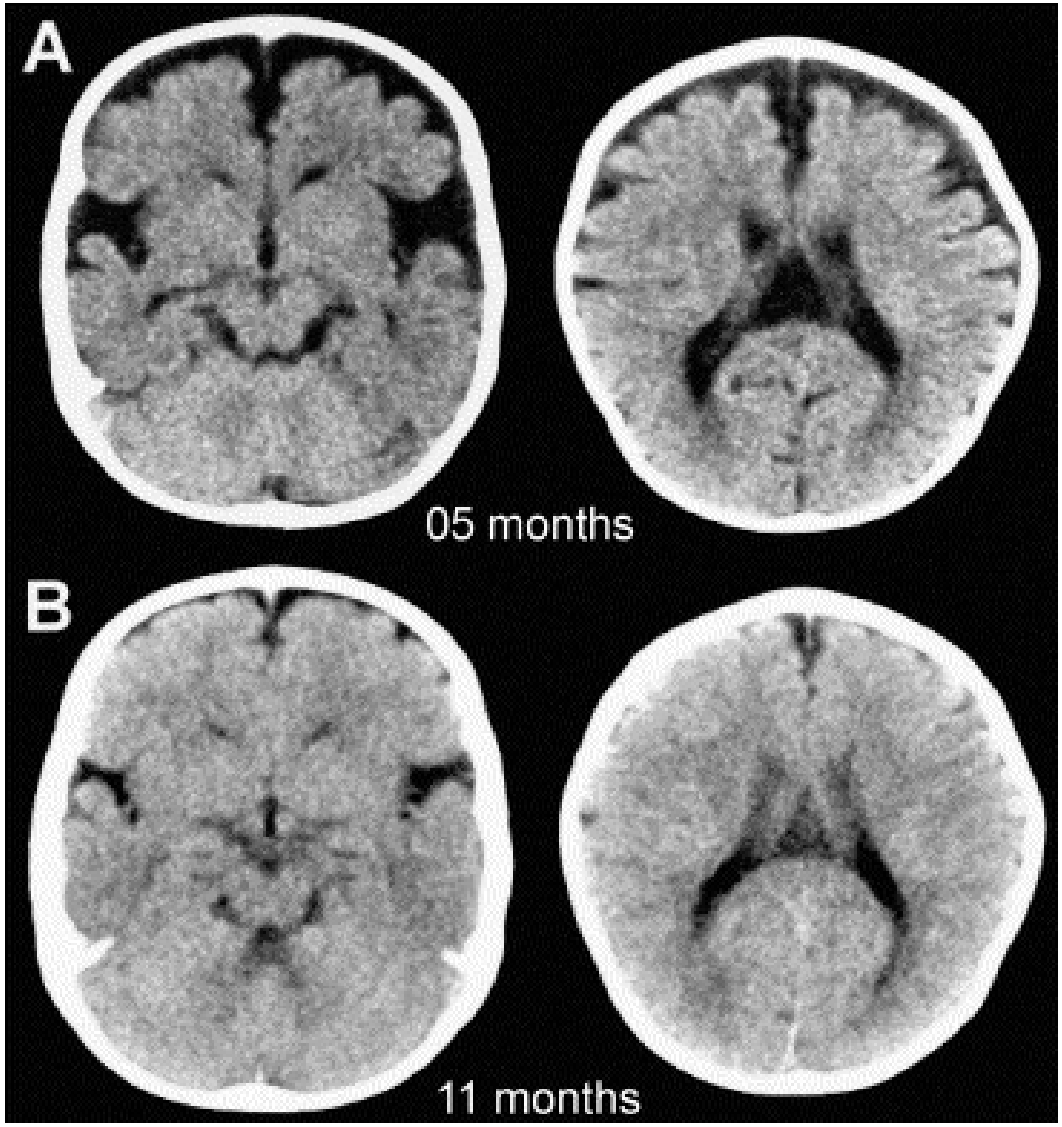
Child development

% 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

Cerebral atrophy (MRI or CT) reported in ≈ 30%

Cassell, 2005



Diffuse cerebral atrophy at 5 mo;

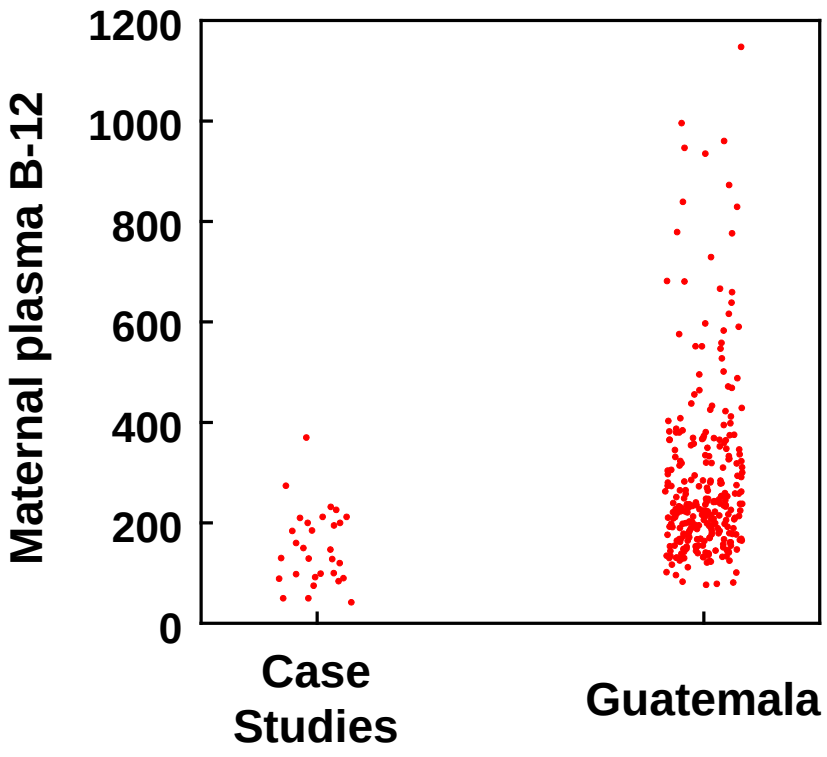
after supplementation, normal by 11 mo.

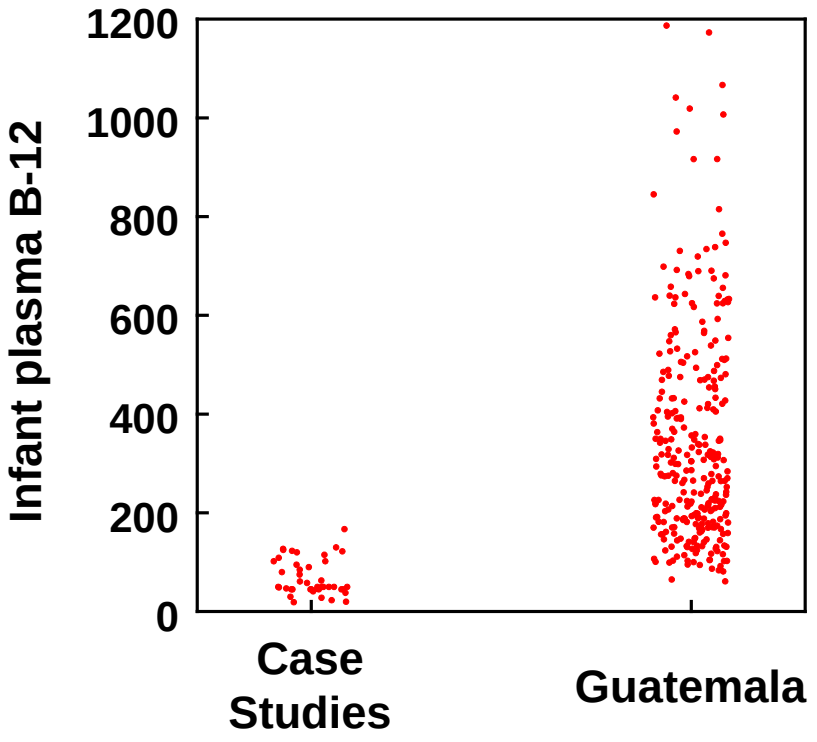
In some cases atrophy persists for years.

Only 30% recover full cognitive function.

Peri-urban Guatemala City

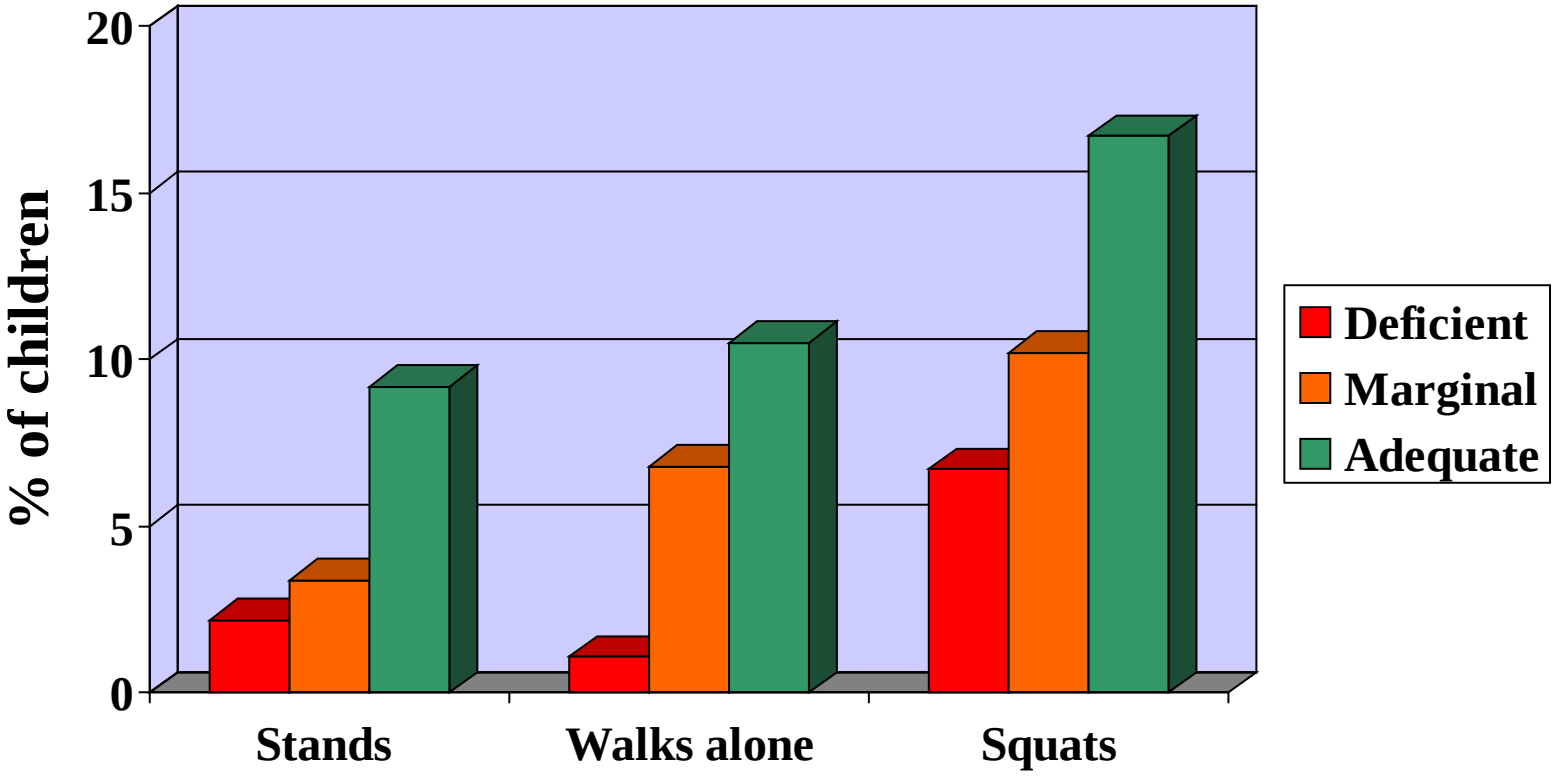
Plasma B12 in clinical case studies overlaps with values in Guatemala (12 mo. postpartum)



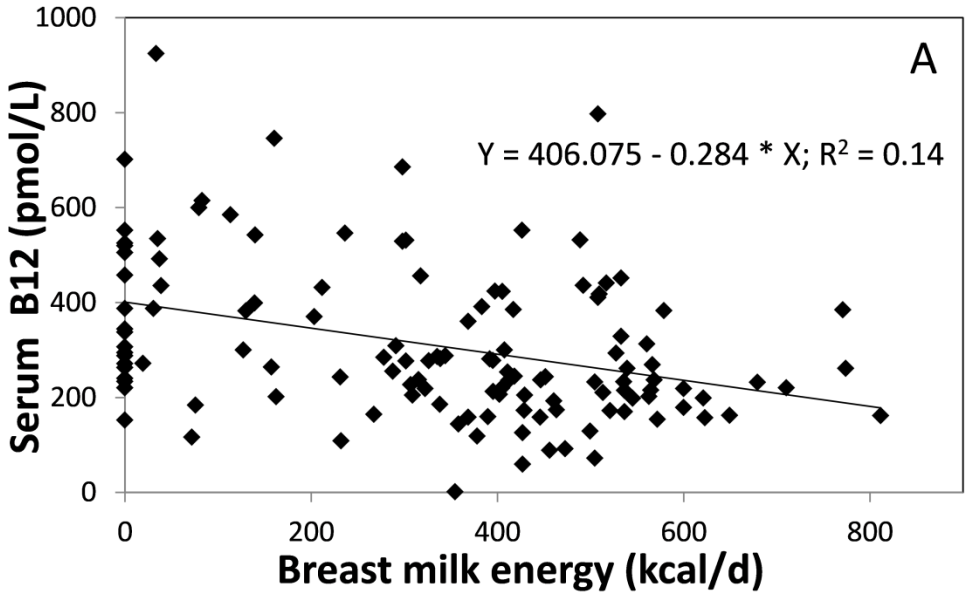


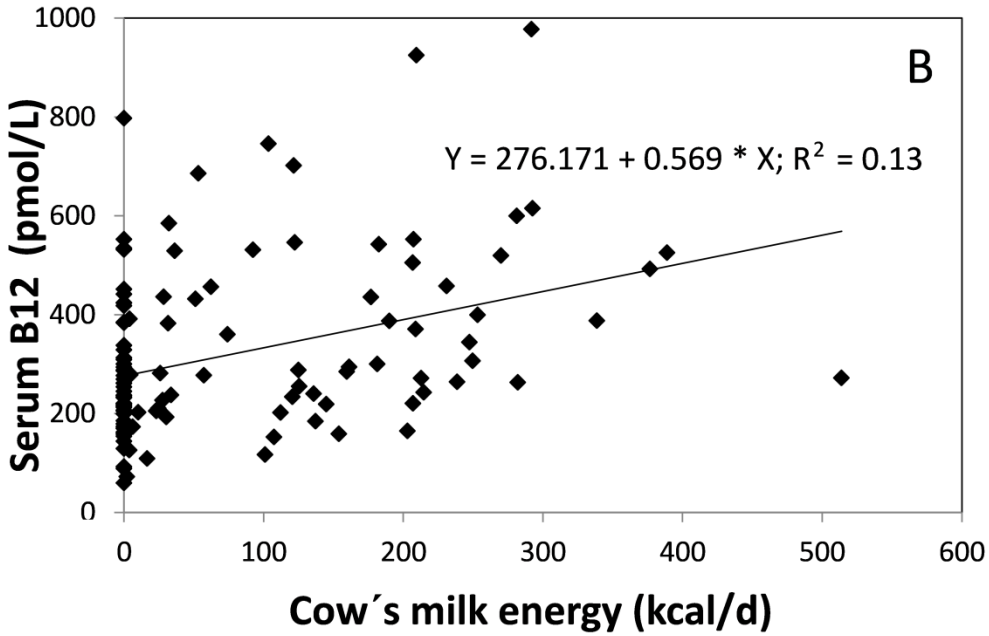
Guatemala: infant B12 status predicts motor development at 12 months

n=220: 30% deficient, 20% marginal



Factor score: Deficient lower than Adequate ($P<0.01$) adjusted for SES, environment etc.

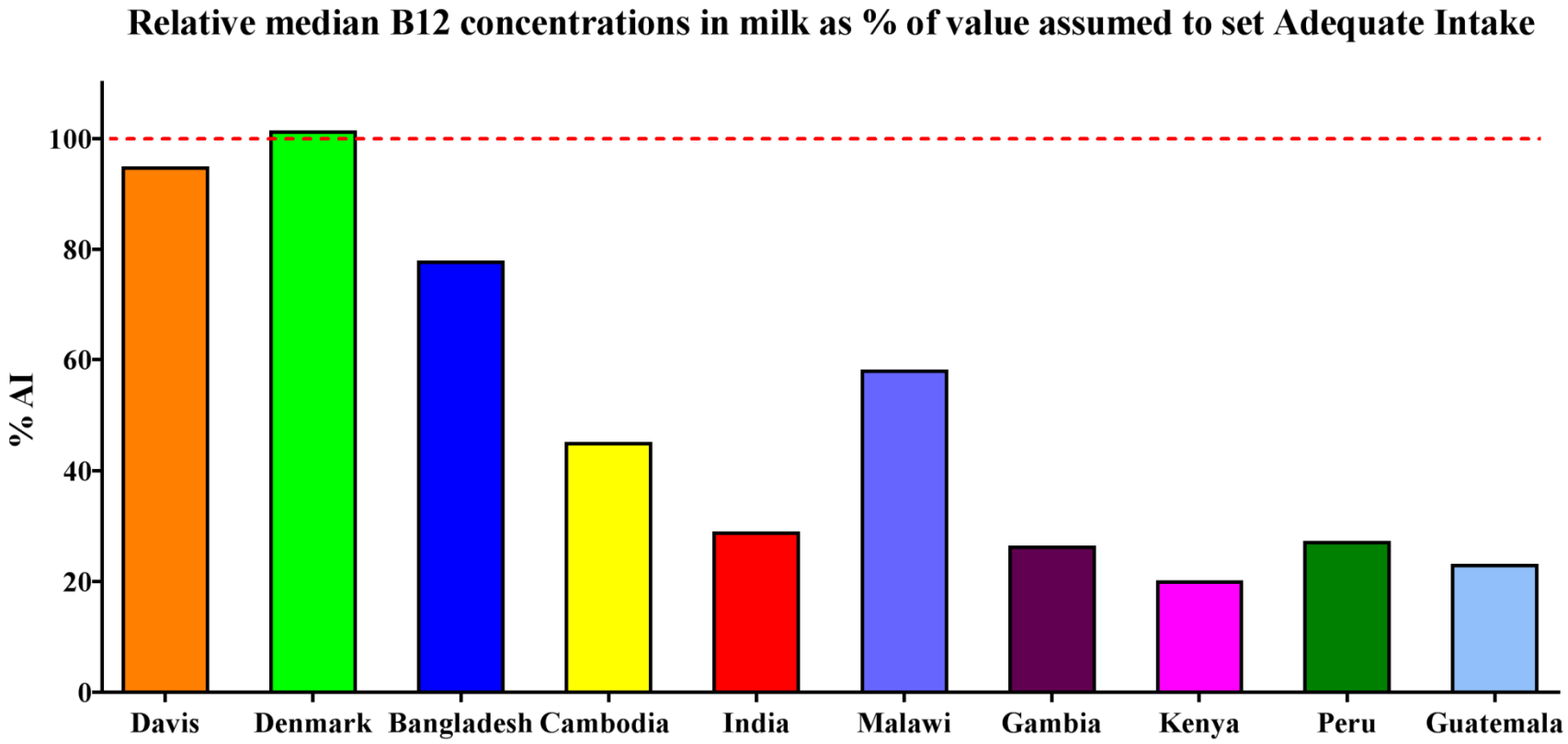




B12 status often better in non-breastfed infants:

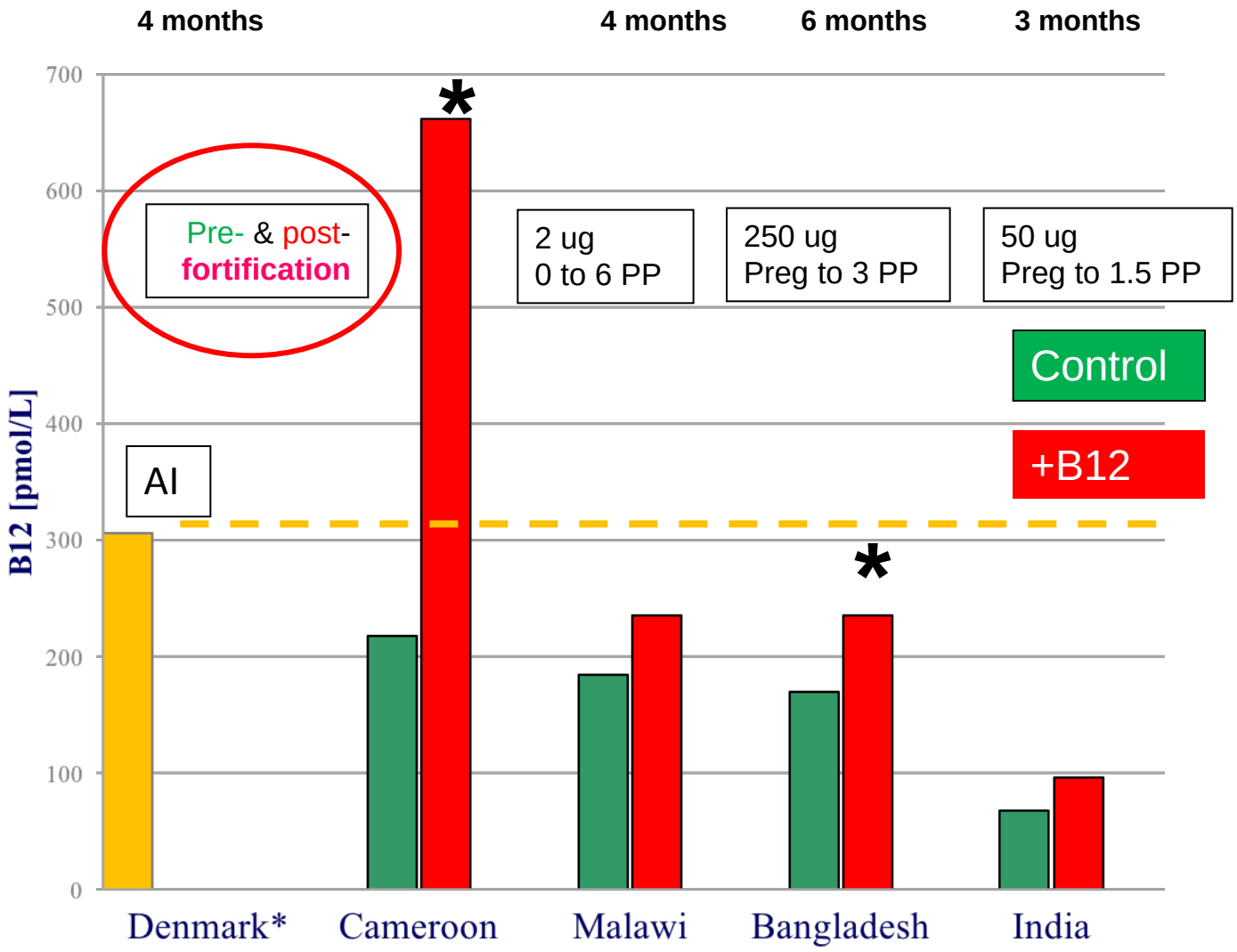
In Guatemala, breast milk provides only 10% of the AI.

Global values for milk B12: analyses from the Allen lab
Median values as % of Adequate Intake value



In Cameroon flour fortification increased milk B12 to ≈300% of AI

Dose vs. B-12 in breast milk, by country



Milk B-12 reflects maternal stores more than recent intake

Cameroon: flour fortification improves status and milk most – frequent small doses?

Allen Lab

Mother-child B12 depletion is a continuum

Maternal depletion in pregnancy

↓

Low B12 stores in infant at birth
& in colostrum, breast milk

↓

Infant depletion

↓

Depletion at 7, and 12 months

Breastfed freq (-)

Cows milk (+)

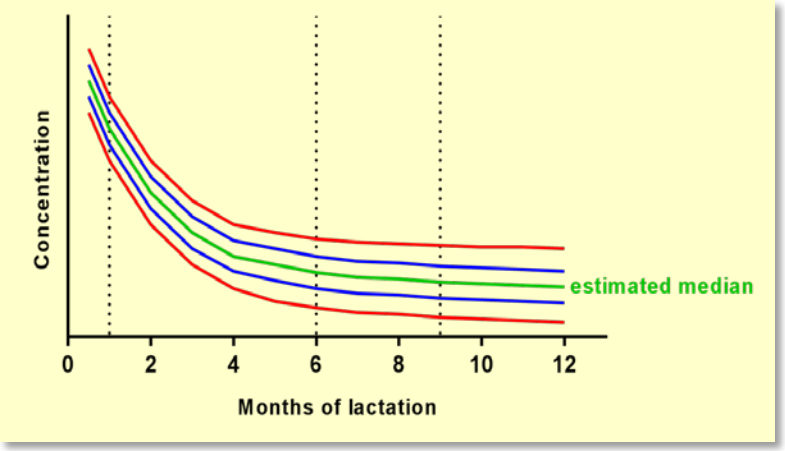
↓

Depletion at 21 months (still correlated with early maternal B12 status)

↓ weight, length, motor development

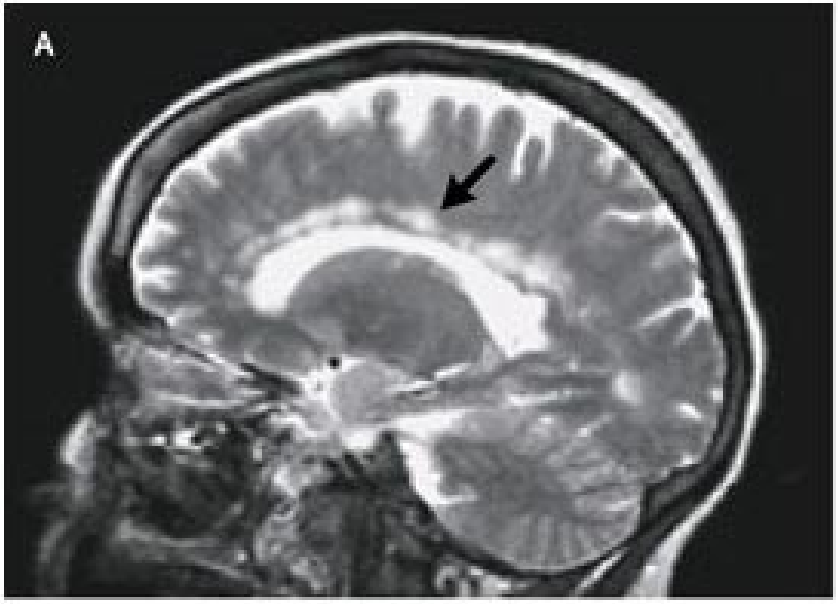
What is “normal” milk B12? MILQ study

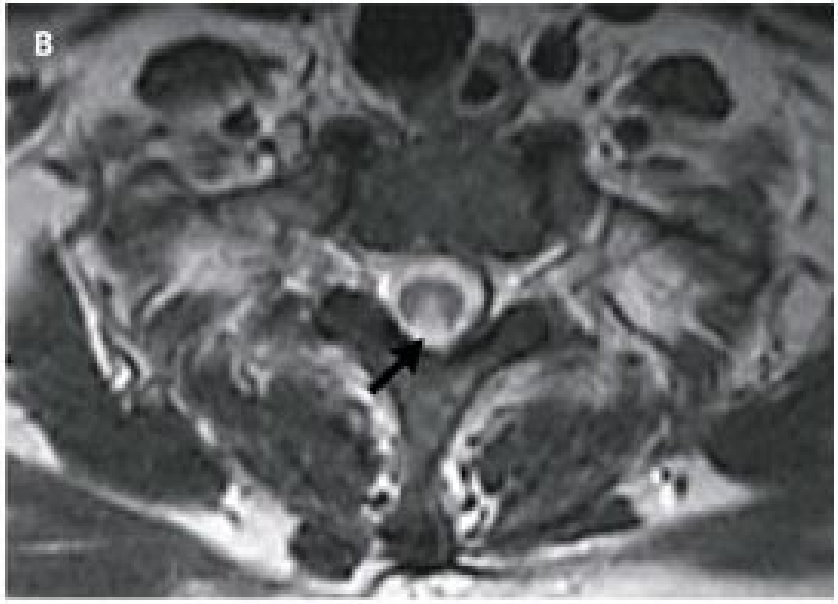
- Funded by BMGF to establish Reference Values for each nutrient across first 9 months lactation, which will
- help interpret values (Need for ASF, supplements, fortification? Impact of interventions?).
- To improve DRIs for infants, young children, lactation.
- Well-nourished (but not supplemented) mothers.
- 4 countries, same methods.
- Supported by data on diets, status, milk volume, other factors.



Cognitive loss with aging, neuropathy

Neurological manifestations of B12 deficiency: brain changes seen with MRI





Hyperintense signal in the periventricular white matter

Hyperintense signal in the posterior column of the lower cervical spinal cord

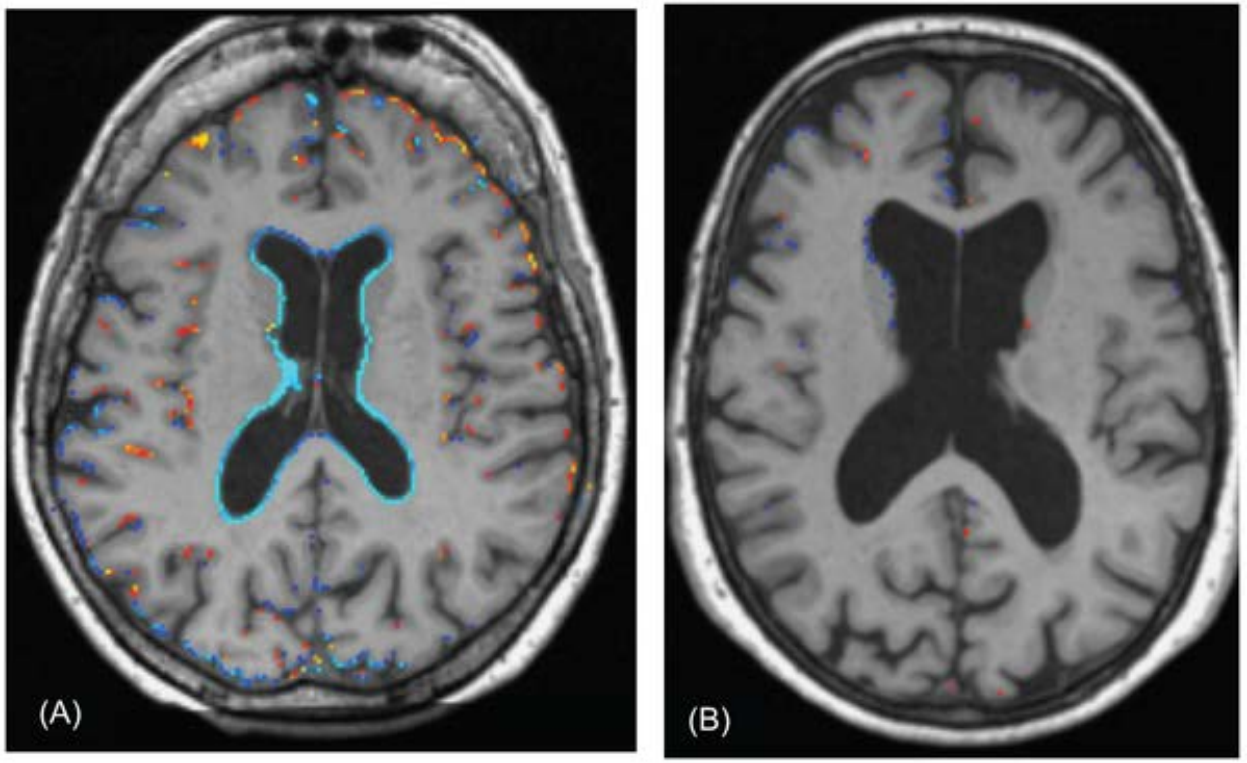
Scherer K, *New Engl J Med 348; 2208: 2003*

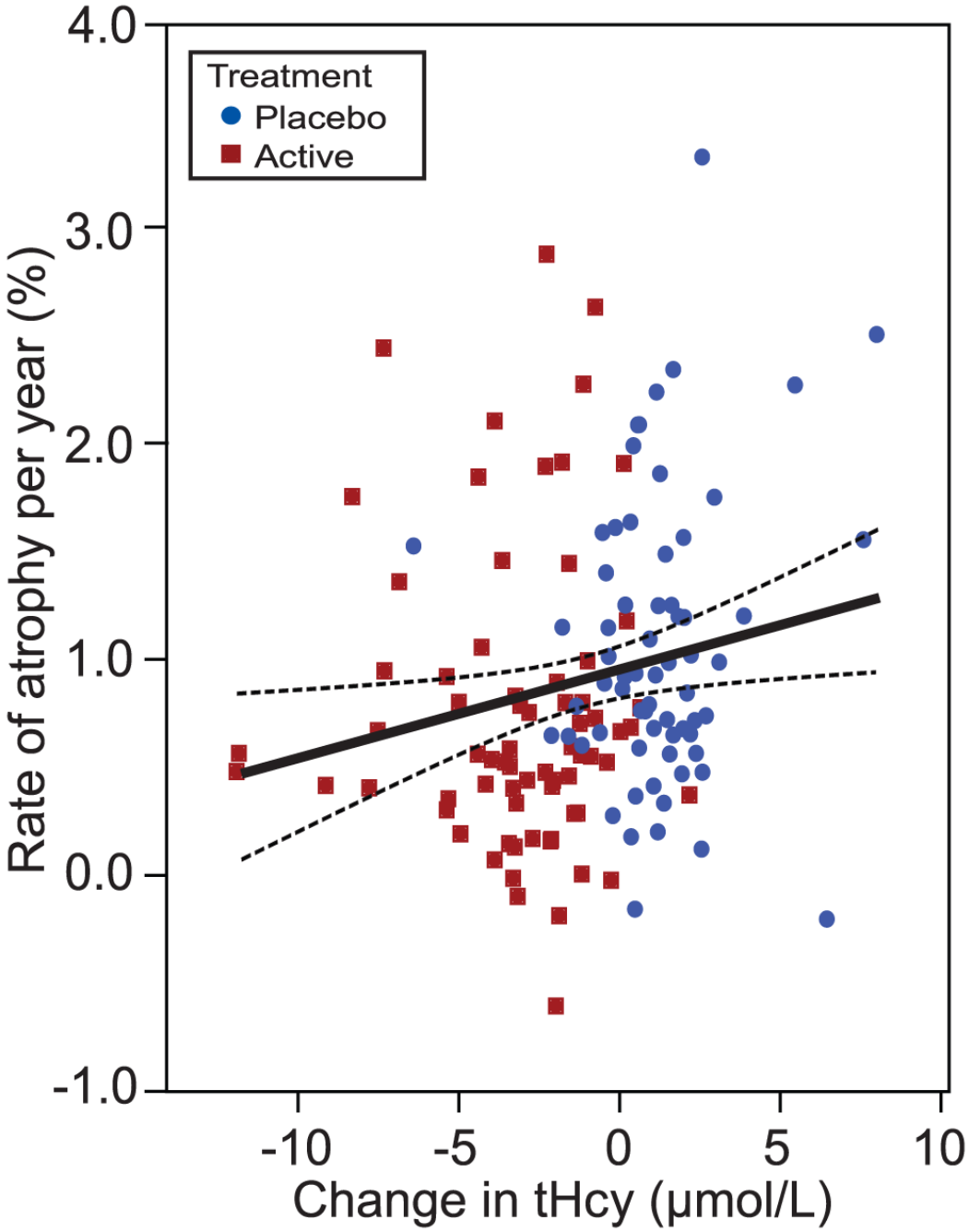
Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: A Randomized Controlled Trial (Smith et al., PLOS One 2007)

Atrophy (blue) over 2 years

Placebo, -2.5%/y

B vitamins (FA+B6+B12)





Rate of atrophy was faster with greater ↑ in Hcy.

Atrophy rate most strongly predicted by lower holoTC.

Looking to the future – evolving opportunities

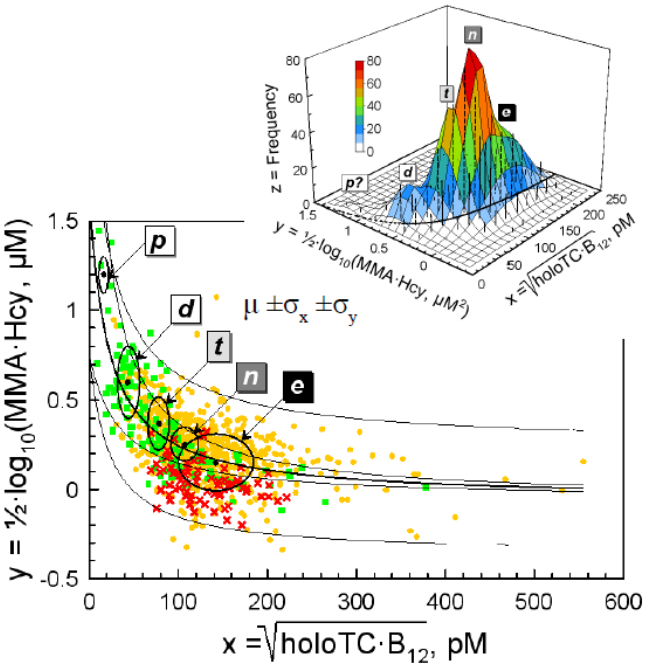
- Better detection and diagnosis
- Functional effects of marginal depletion identified
- Large-scale B12 fortification (with folic acid)

Sergey N. Fedosov*, Alex Brito, Joshua W. Miller, Ralph Green and Lindsay H. Allen

Combined indicator of vitamin B_{12} status: modification for missing biomarkers and folate status and recommendations for revised cut-points

DE GRUYTER (Clin Chem Lab Med 2015

n= 5,211



Fedosov, Brito, Miller, Green, Allen *CCLM 2015*

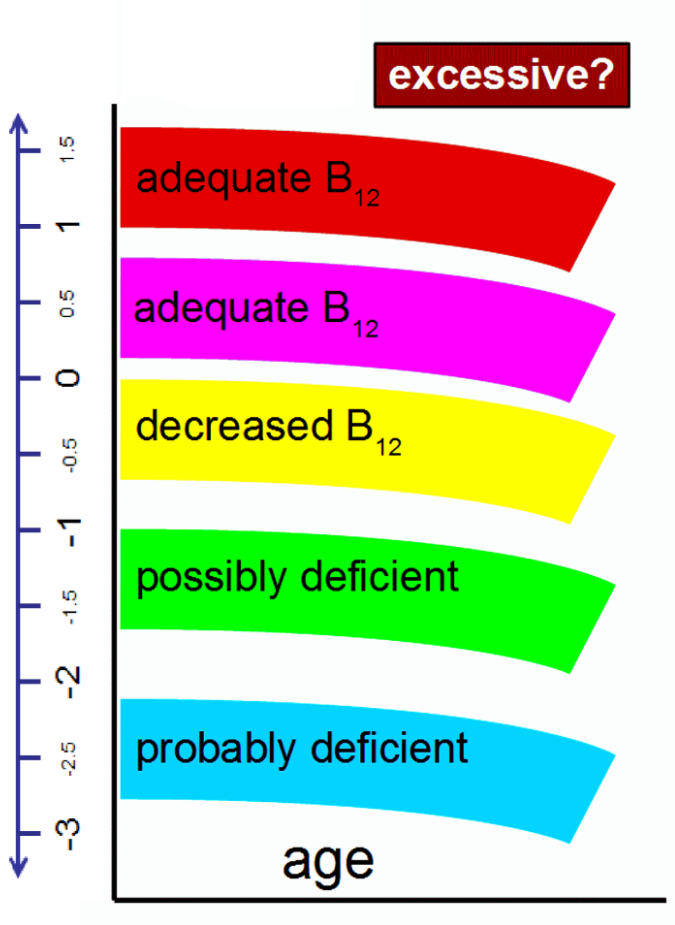
cB12: A combined indicator of B12 status

Fedosov, Brito, Miller, Green and Allen, Clin Chem Lab Med 2015

B12
Holo-TC
MMA
tHcy

Combine 4 biomarkers into one parameter

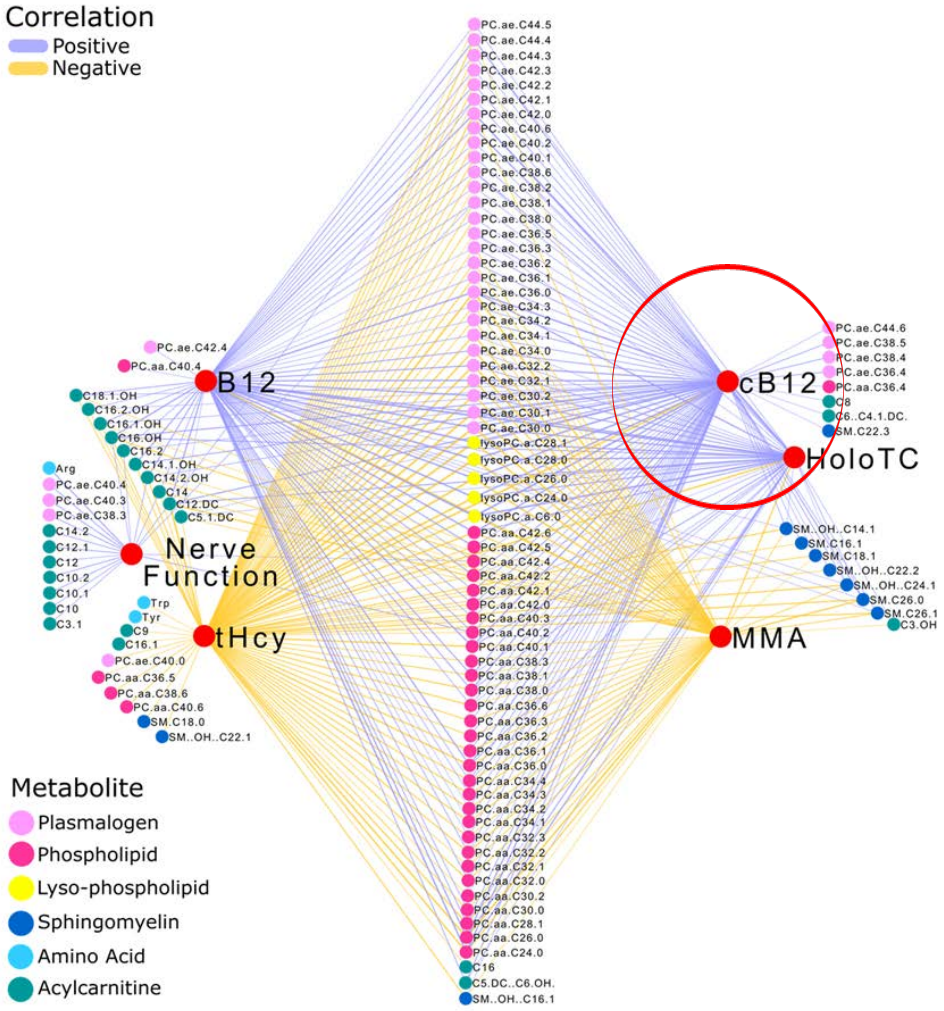
$$cB_{12} = \log_{10}\left(\frac{holoTC \cdot B_{12}}{MMA \cdot Hcy}\right)_{Test} - \log_{10}\left(\frac{holoTC \cdot B_{12}}{MMA \cdot Hcy}\right)_{Ref} = Test - \frac{3.79}{1+\left(\frac{age}{230}\right)^{2.6}}$$

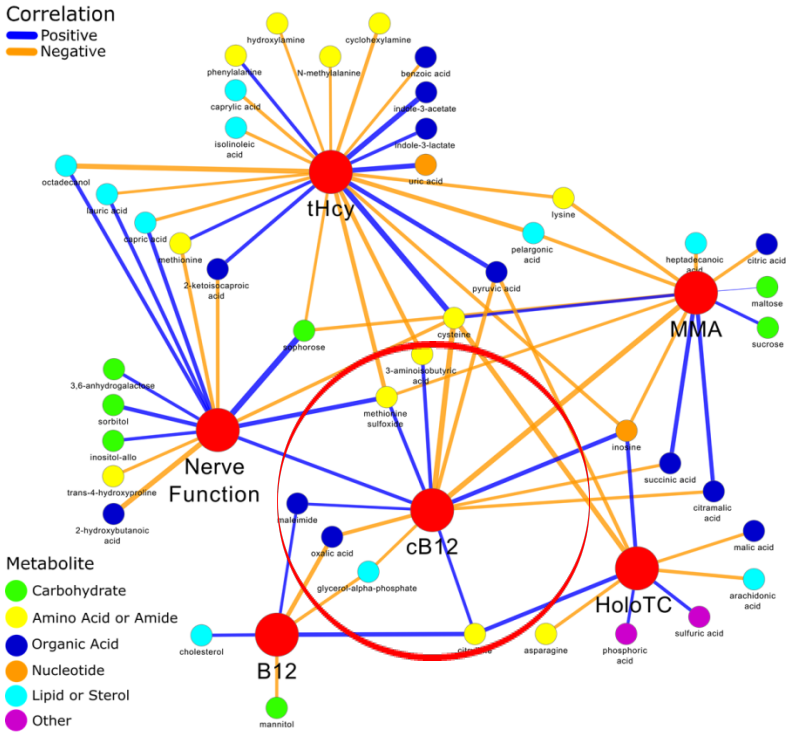


- Can estimate with 2, 3 or 4 biomarkers
- Can correct for low sfolate (↑tHcy)
- Best predictor of Hb, MMSE, and now, peripheral nerve function

Fedosov, Brito, Miller, Green, Allen *CCLM 2015*

cB-12 key in first characterization of the human serum metabolome in vitamin B-12 deficiency





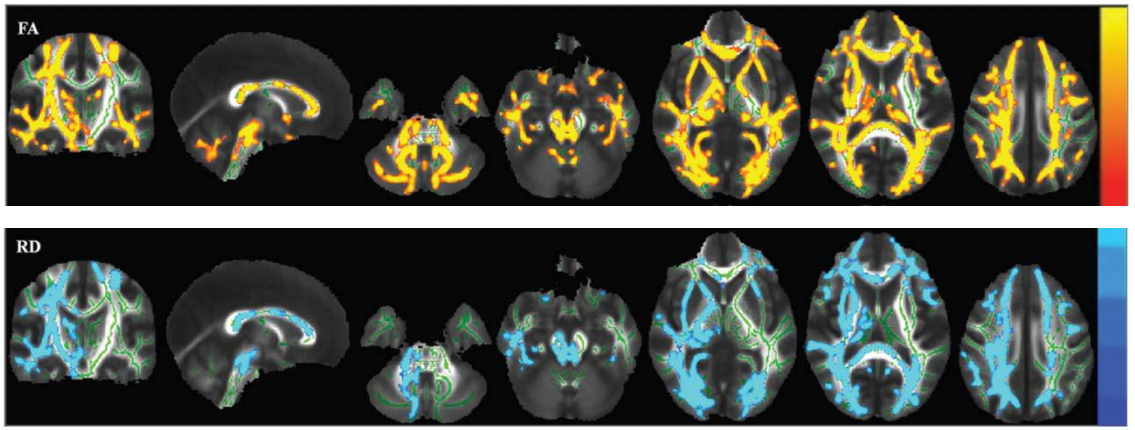
Brito et al *(J Nutr Sept. 2017)*

DTI Correlates of Cognition in Conventional MRI of Normal-Appearing Brain in Patients with Clinical Features of Subacute Combined Degeneration and Biochemically Proven Vitamin B_{12} Deficiency

P.K. Gupta, R.K. Gupta, R.K. Garg, Y. Rai, B. Roy, C.M. Pandey, H.S. Malhotra, and P.A. Narayana

51 patients
B12 deficient
Age-sex matched controls

Differences in MRI-DTI



No differences in conventional MRI, no difference in white matter volume, but significant reductions in myelination (microstructural changes)

Gupta et al AJNR 2013

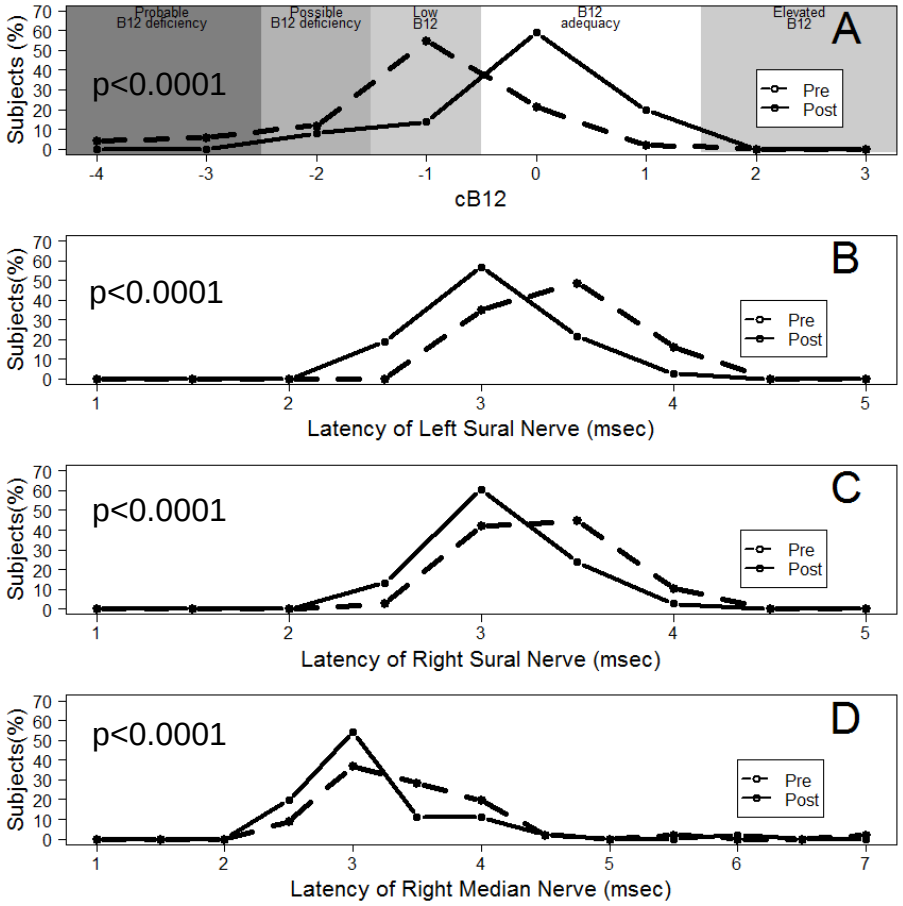
The American Journal of Clinical Nutrition

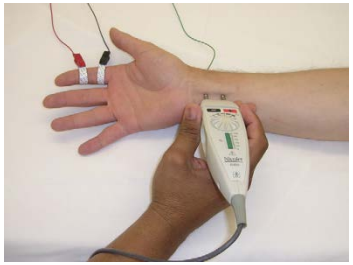
Vitamin B-12 treatment of asymptomatic, deficient, elderly Chileans improves conductivity in myelinated peripheral nerves, but high serum folate impairs vitamin B-12 status response assessed by the combined indicator of vitamin B-12 status[1,2]

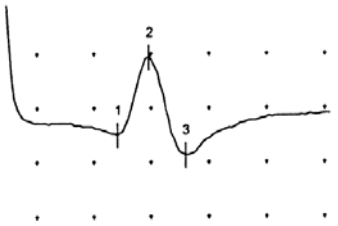
*Alex Brito,[3] Renato Verdugo,[4] Eva Hertrampf,[5] Joshua W Miller,[6,7] Ralph Green,[7] Sergey N Fedosov,[8] Setareh Shahab-Ferdows,[3] Hugo Sanchez,[5] Cecilia Albala,[5] Jose L Castillo,[4] Jose M Matamala,[4] Ricardo Uauy,[5] and Lindsay H Allen[3]**

51 adults >70 y
B12 deficient
10 mg i.m. B12
Assessment at baseline and after 4 mo

Improvement in large myelinated fibers



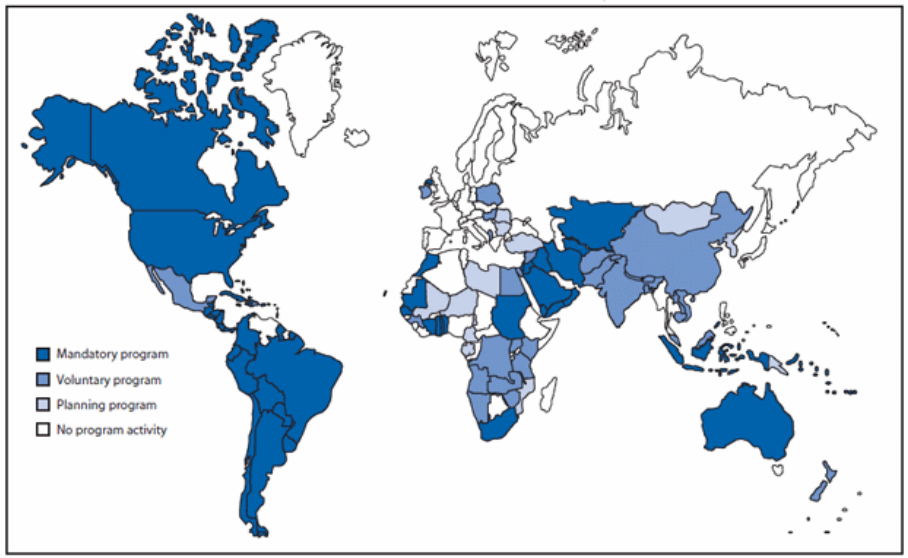




Brito et al *AJCN 2016*

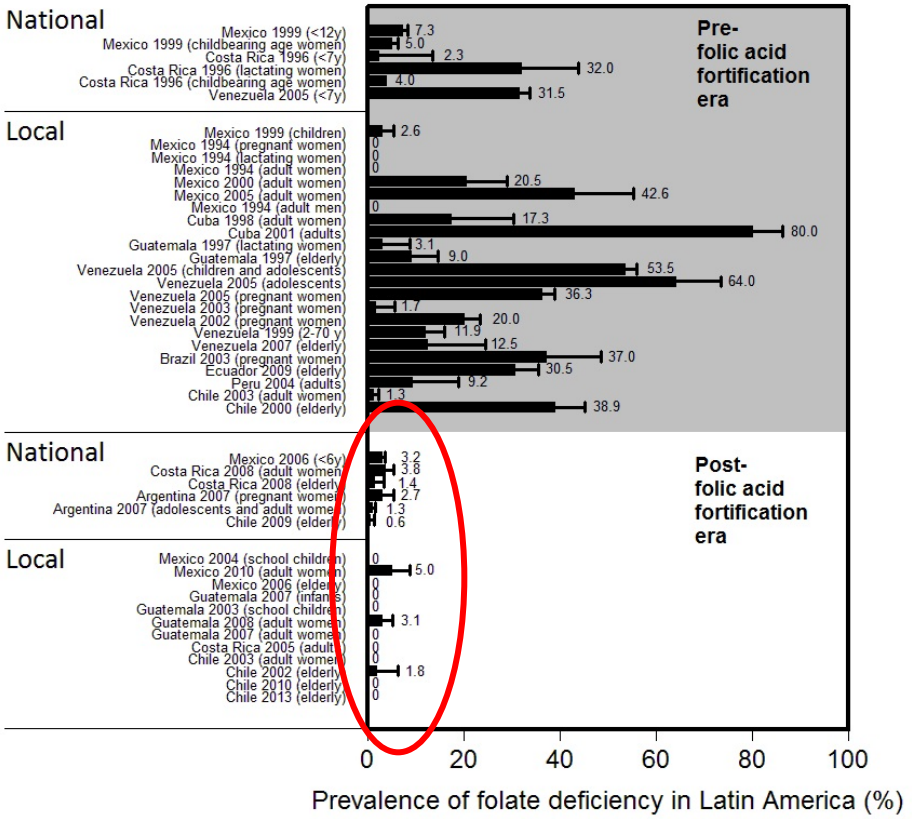
Folate repletion after folic acid fortification of flour

Fortification with folic acid



CDC 2010

Latin America as an example

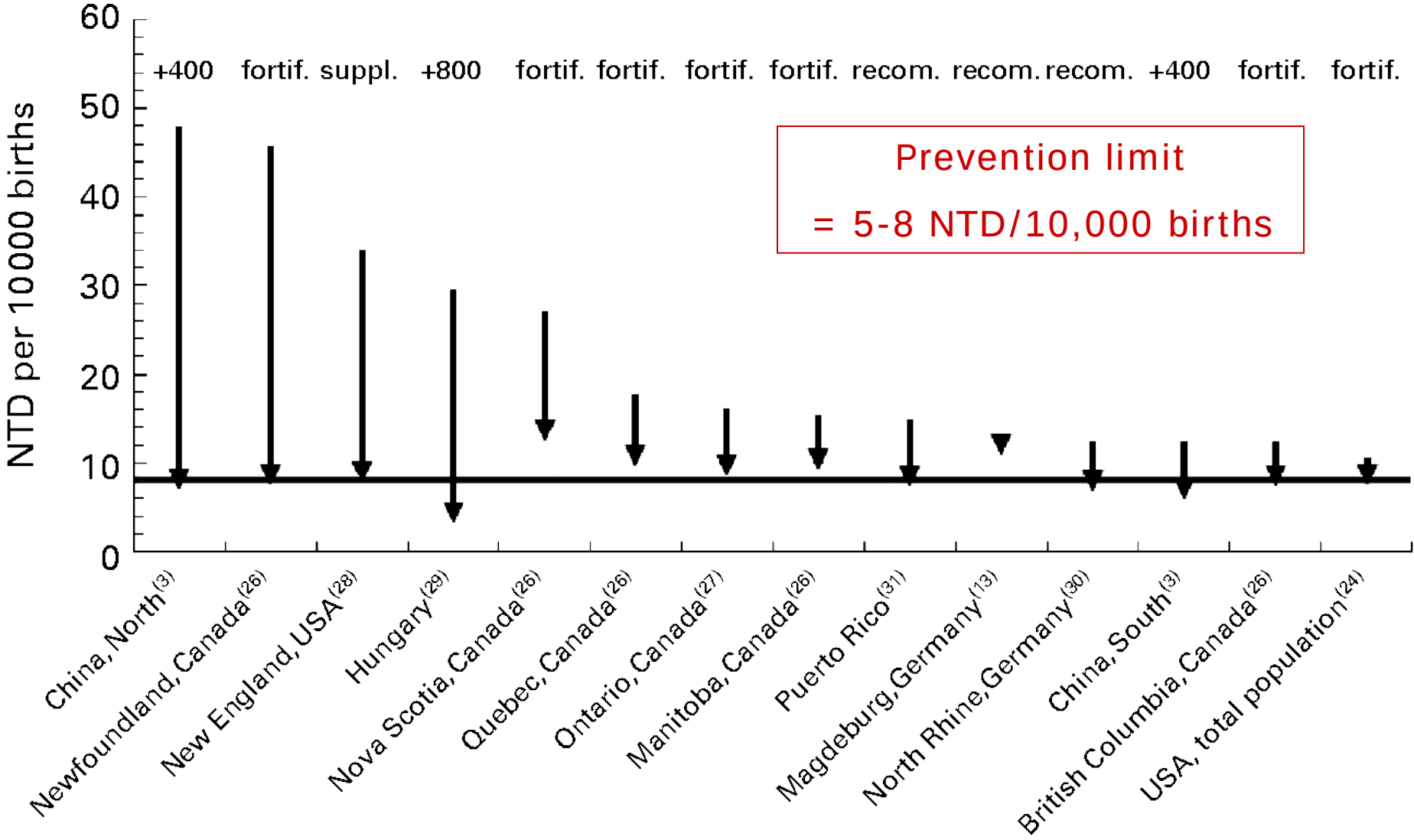


Brito et al FNB 2015

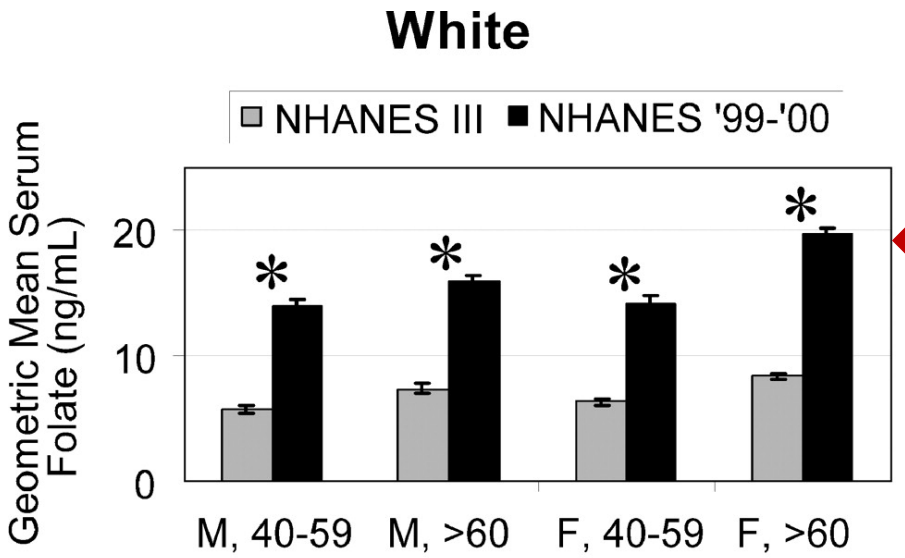
Low folate prevalence dropped to <5%
in most countries after fortification

Folic acid reduces NTDs

(Heseker et al. Br J Nutr 2009)



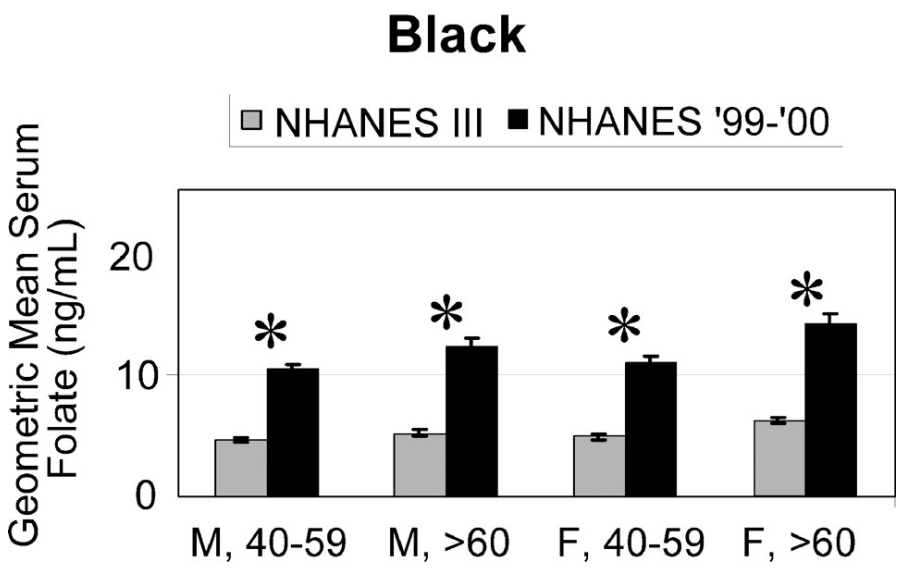
Serum folate; ≥40 years of age in NHANES III 1991-1994 and NHANES 1999-2000, USA (Yang, Circulation 2006)



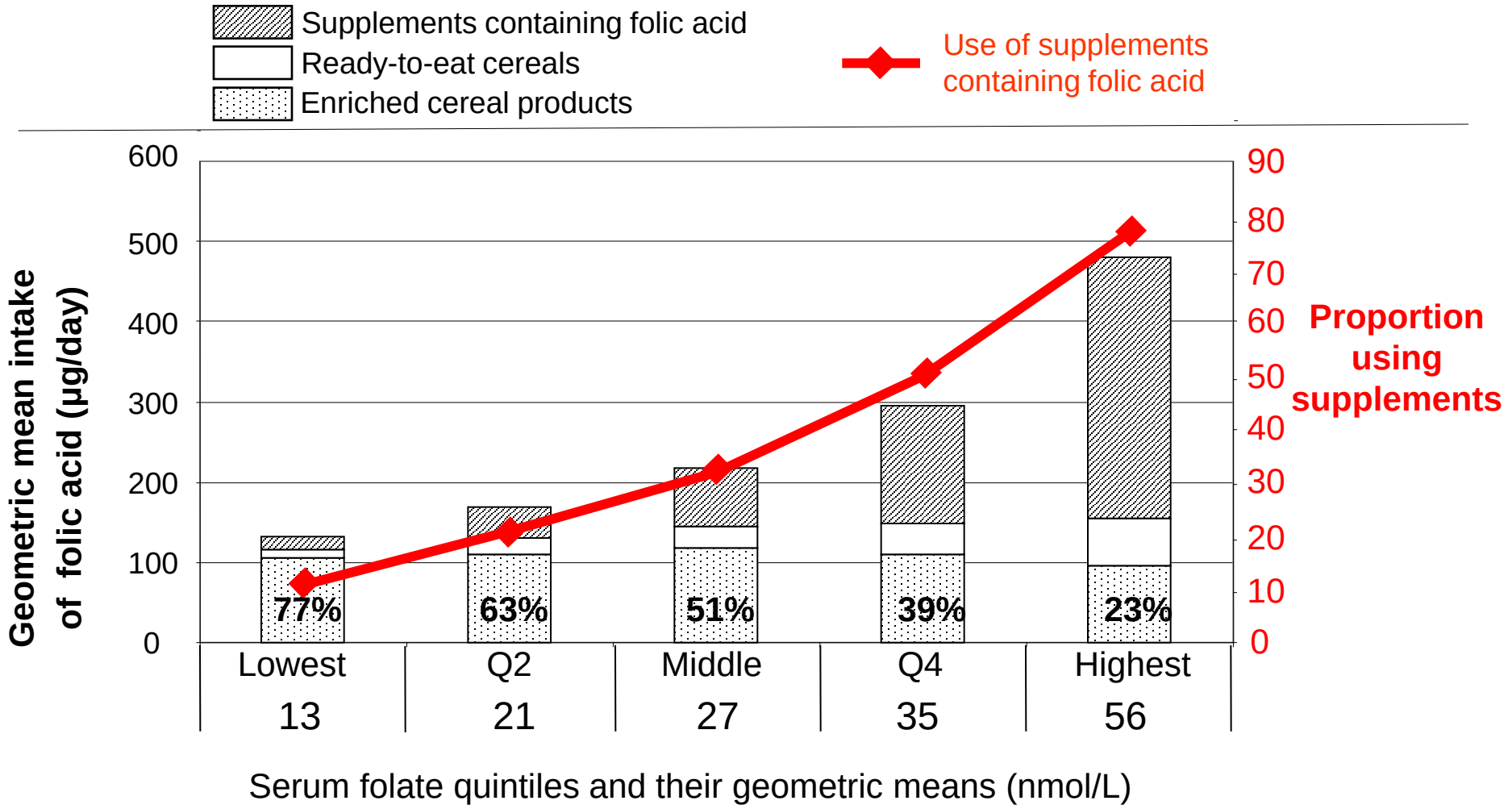
20 ng/mL = 9 nmol/L

Folate deficiency

20-25% → ≈ 1%

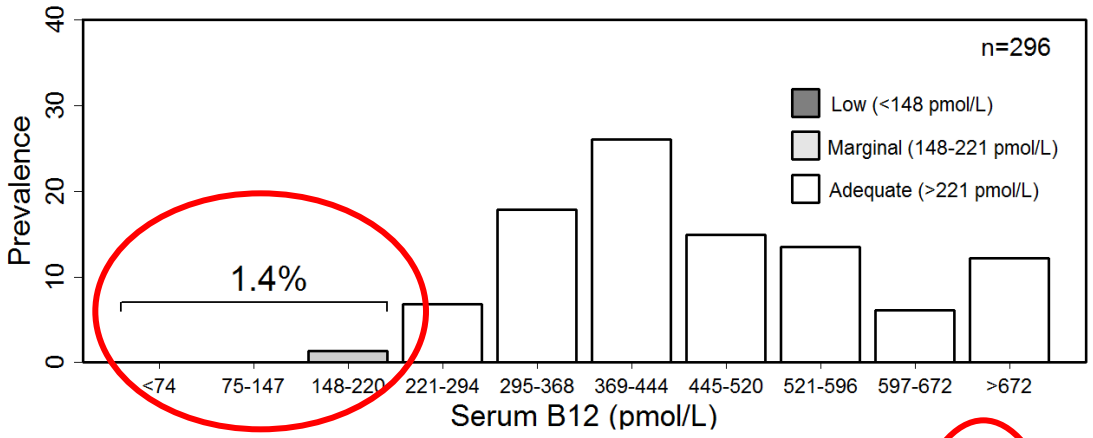


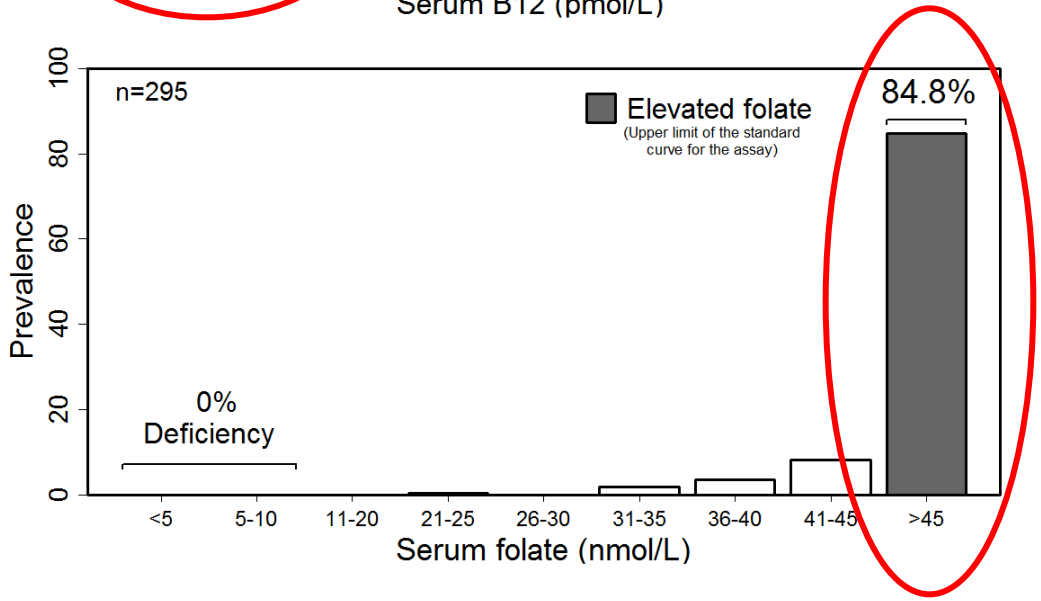
Geometric mean intake- folic acid, from enriched cereal products, ready-to-eat cereals & supplements, and % participants who took supplements containing folic acid – by serum folate quintiles NHANES 2001-2004, ≥19 years, n=8,655



Yeung, LY et al. *JAMA* 2008;300:2486-7

School children (Queretaro, Mexico) n= ~ 300

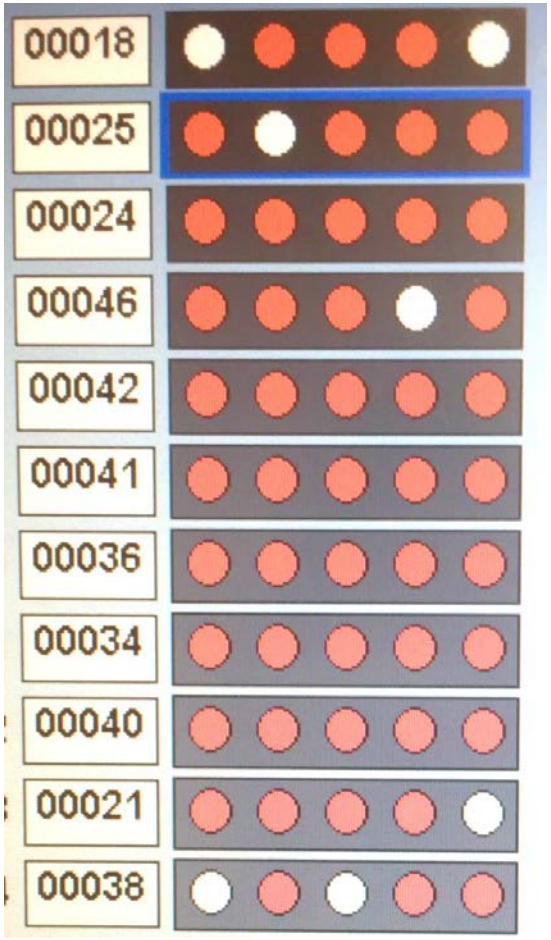




Adequate B-12 & very high folate

Brito A, Garcia O, Allen LH (in preparation)

Are elevated folate and B-12 deficiency a public health problem?



Mexican school children

>45.4 nmol/L serum folate

Need better definition of high folate in context of B12 deficiency

Does excess folic acid exacerbate vitamin B12 deficiency?

- Observations from the 1940s - 1950s
  - Folic acid improved anemia of B12 deficiency
  - Improvement in anemia was sub-optimal and temporary, relapse after 3-4 years.
  - Neurological manifestations not prevented,
  - often aggravated or precipitated (if dose >1000 ug/d).

Reynolds, *J Neurol Neurosurg Psychiatry* 2002

Possible adverse effects of excess folic acid

- ↑ colorectal cancer incidence and progression (U-shaped relationship)?
- ↓ immune function (NK cytotoxicity)?
- Epigenetic alterations?
- Exacerbation of vitamin B12 deficiency?
  - Neurological symptoms
  - Anemia
  - Cognitive performance
  - ↑ Hcy, MMA and ↓ holotranscobalamin

Folate-B12 interaction, cognitive impairment and anemia (Selhub, Am J Clin Nutr 2009)

	B12 status	Folate status	Odds Ratio
Anemia	Normal	Normal	1
	Normal	High	0.5
	Deficient	Normal	1.6
	Deficient	High	5.1
Cog. impair	Normal	Normal	1
	Normal	High	0.4
	Deficient	Normal	1.6
	Deficient	High	4.3

Summary

- B12 deficiency much more common than recognized, due to low intake of animal source foods (not only in vegans).
  - Functional consequences diverse – neurological, cognitive, pregnancy outcome, low breast milk B12, developmental, NTDs
  - For many functions the cut-point between poor status and functional changes is unclear
  - Need further work with RCTs, and use of new indicators e.g. cB12, metabolomics, DTI, etc.
  - These will inform need for adequate B12 intake and strategies to prevent depletion and deficiency
- Future:
  - increased use of cB12, documented adverse effects of moderate deficiency, widespread B12 co-fortification with folic acid.

BOND – BIOMARKERS FOR NUTRITION AND DEVELOPMENT

- Detailed reviews and recommendations for biomarkers of folate & iodine (published), iron, zinc, vitamin A, B12.
- B12 Report is In Press in J. Nutrition

- See BOND website: www.nichd.nih.gov