B12 Seminar

Presented by: Janelle Morris

NFSC 642: Topics in Vitamins and Minerals

8/30/2011

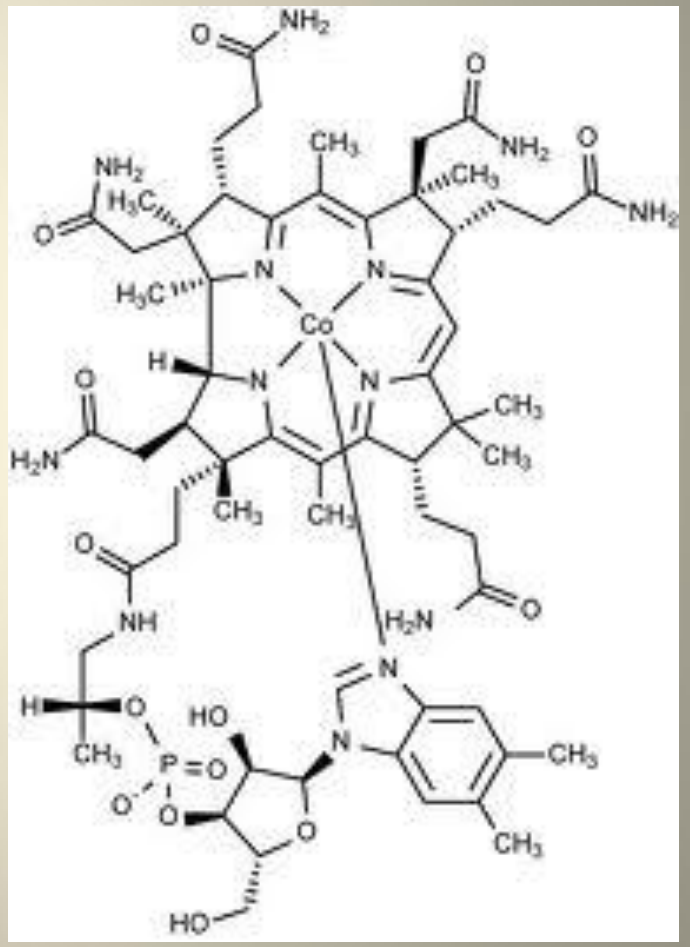
History

- 1824: 1st case pernicious anemia hypothesized to be linked to digestive system
- 1934 Whipple, Minot and Murphey win Nobel Peace Prize for work on pernicious anemia treatment
- 1948 Rickes and associates (USA) and Smith and Parker (England) independently isolate crystalline red pigment deemed B12
- 1973 Chemical synthesis of B12

[1]

Chemical Structure

- Water soluble
- 4 reduced pyrrole rings
- Cobalt Containing
  - "Cobalamins"
- Active forms/ Coenzyme
  - Methylcobalamin
  - 5-deoxyadenosylcobalamin



[2]

Forms

- Supplements:
  - Cyanocobalamin
  - Hydroxycobalamin

- Food:
  - Deoxyadenosyl
  - Methylcobalamin+ polypeptides

– Silliman NFSC 440 Lecture

Food Sources

- Eggs
- Meat
- Milk/ milk products
- Organ meats(liver/kidney)
- Poultry
- Shellfish

What about Vegans/Vegetarians?

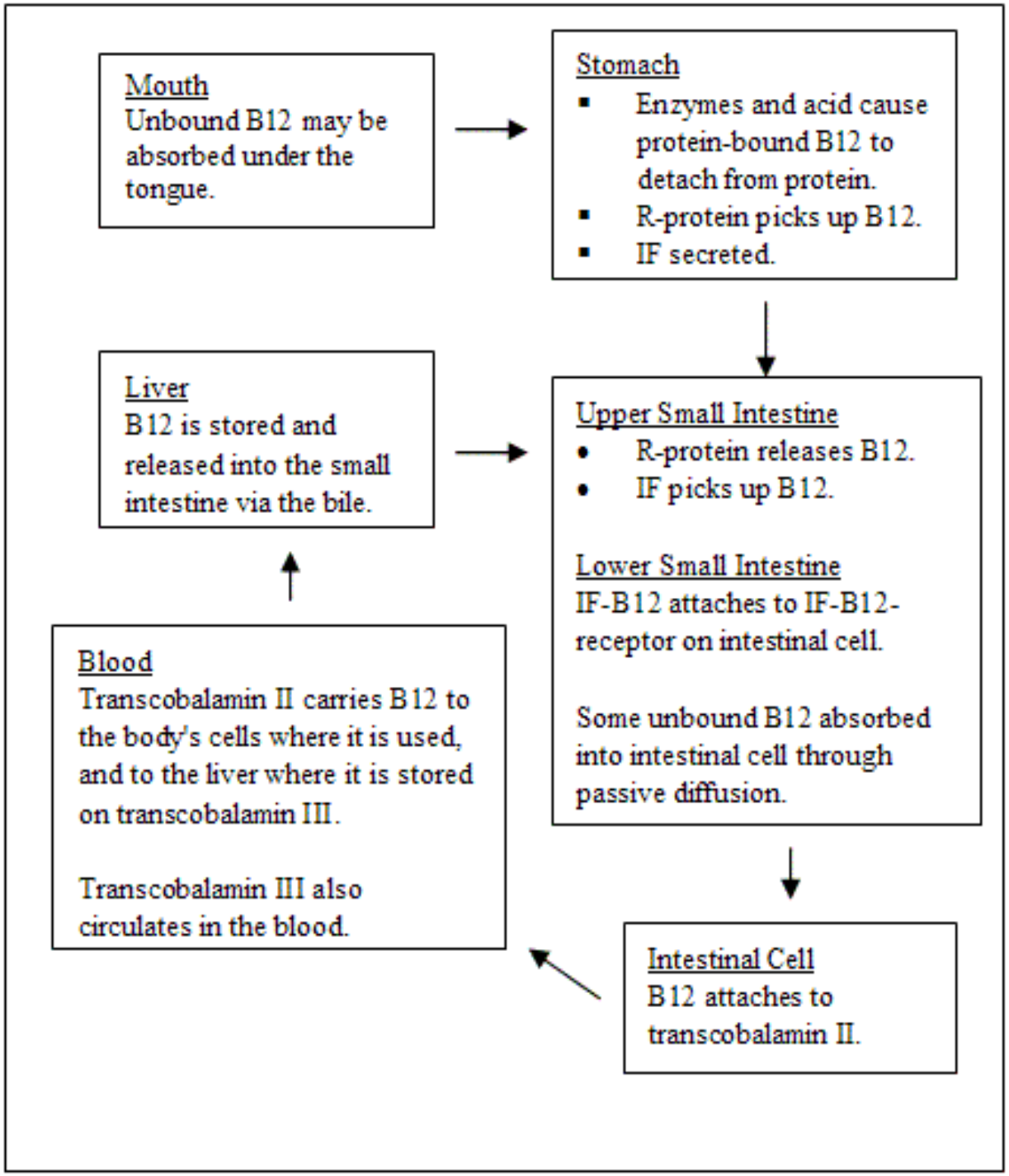
- Deficiency takes years
  - <0.1% stores lost daily
- Supplements need to be taken

DRI

- EAR:
  - 1.5-2.0μg/d
- RDA:
  - 1.8-2.4μg/d
- No UL

DIGESTION

ABSORPTION



What happens in a Deficiency?

- Megabolastic Anemia
- Loss of balance
- Numbness in the extremities
- Weakness
- Neurological disorders
  - Neuropathy
  - Memory impairment
  - Dementia
  - Depression
  - Brain atropy
  - Cerebrovascular disease

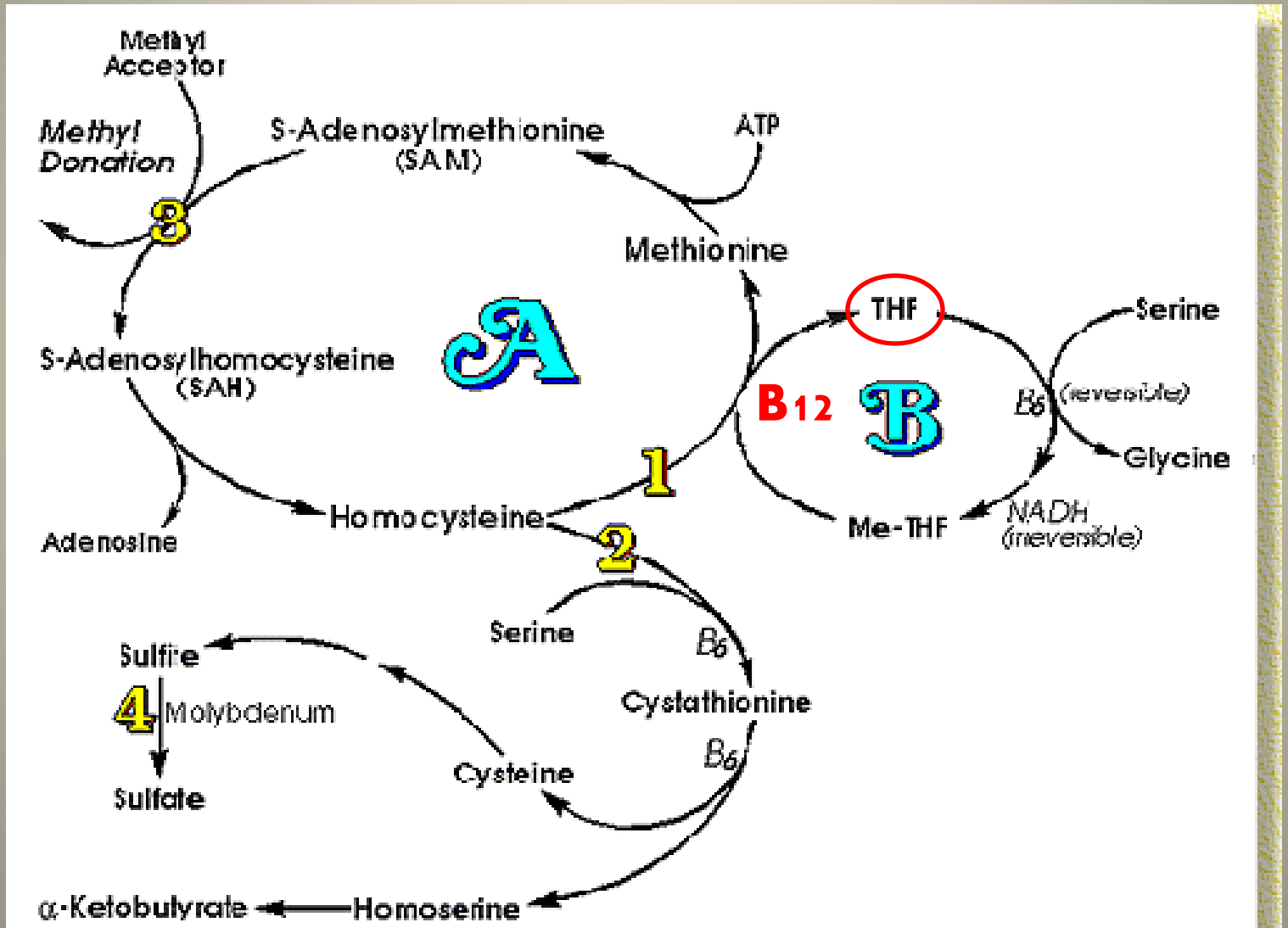
Who is at risk

1. The elderly
   - Most prevalent
   - Decreased absorption capability
2. Infants
3. People who have had gastrointestinal surgery
4. People with digestive disorders
   - Celiacs
   - Crohn's

Functions

- Red blood cell formation
- Neurological
- DNA synthesis
- Cofactor for
  - Homocysteine -------→ Methionine (methylcobalamin)
  - L-methylmalonyl CoA mutase (deoxyadenosylcobalamin)

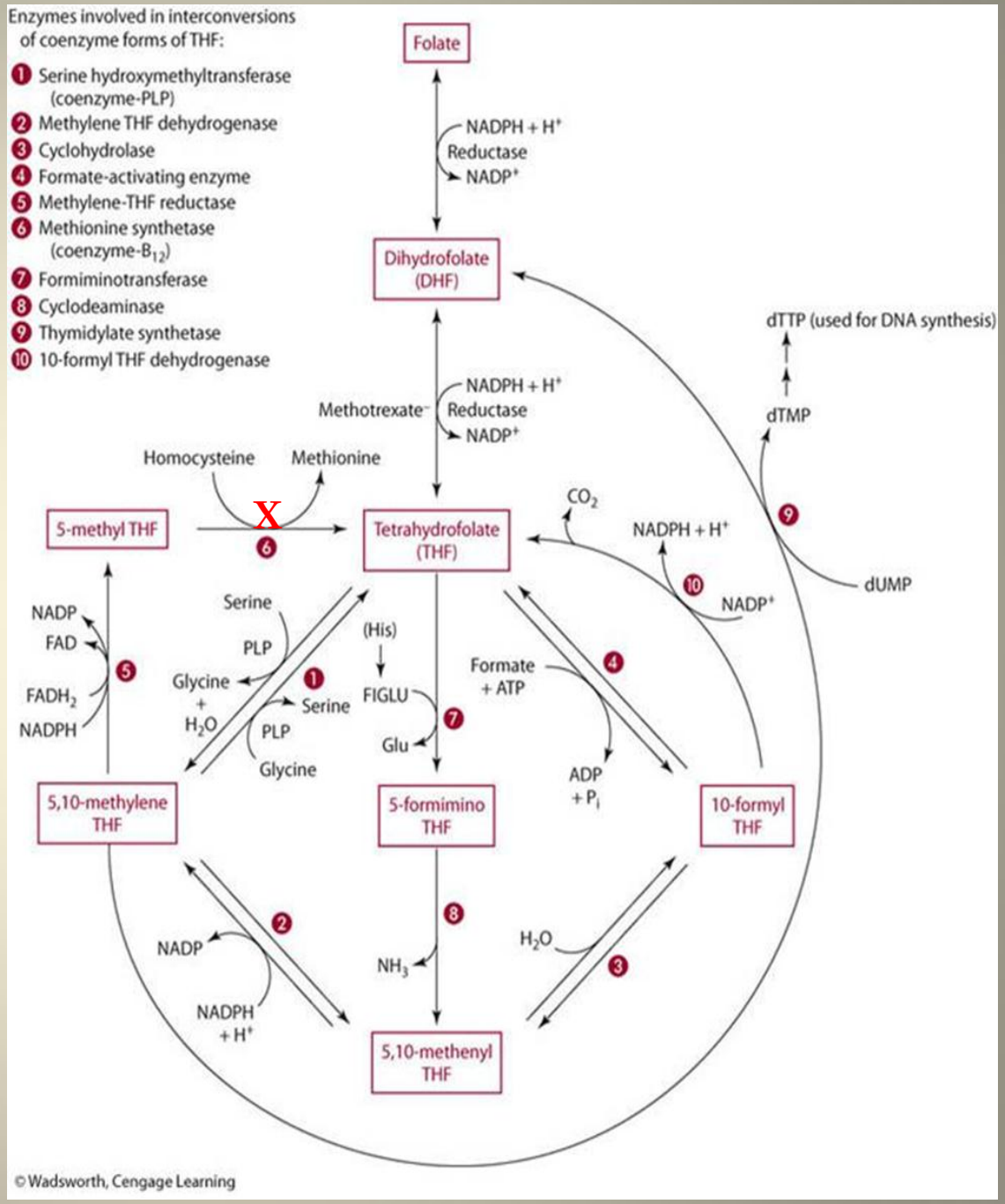
Functions


Methyl Acceptor
Methyl Donation
S-Adenosylmethionine (SAM)
ATP
Methionine
S-Adenosylhomocysteine (SAH)
A
THF
B12
B
Serine
B6 (reversible)
Glycine
Adenosine
Homocysteine
1
Me-THF
NADH (irreversible)
2
Serine
B6
Cystathionine
Sulfite
4
Molybdenum
Sulfate
Cysteine
B6
α-Ketobulyrate
Homoserine

2 Hypotheses to think about

1. The methyl-folate trap

- B-12 deficiency causes hematologic and neuropsychiatric damage
  - Homocysteine X → methionine
  - ↓ SAM
    - (CNS methyl donor)
  - ↓ DNA synth
  - ↓ RBC maturation



2. The "masking effect"

- Normally:
  - Folate circulates as 5-MTHF (converts while passing through intestinal mucosa
- High dose Folate:
  - Unable to convert all THF → 5-MTHF
  - "Cures" B-12 deficiency anemia
  - Causes rapid deterioration of CNS fn
- Unmetabolized folic acid may have stimulatory effect on DNA synth

Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification

Martha Savaria Morris, aul F Jacques, Irwin H Roenburg, and Jacob Selhub

The American Journal of Clinical Nutrition

2007;85:193-200

Folate Fortification Controversy

- USDA requires fortification of all enriched cereal-grain products
  1. Neural Tube Defect prevention
  2. Possible delay in diagnosis or worsen neurologic and neuropsychiatric effects of B12 deficiency
  3. Declined absorption of B12 in elderly and increased autoimmunity against IF or IF-producing parietal cells

- But….. Case reports show folic acid alleviates pernicious anemia while precipitating or worsening neurologic or neuropsychiatric symptoms in seniors
  - Based on mistaken hypothesis that folic acid deficiency causes anemia
  - No proof of fortification having effect on CNS related symptoms
- Does it cause harm?

Hypothesis

- Does high serum folate worsen the effects of vitamin B-12 deficiency?

Objective

- Examine the relationship between vitamin B-12 status relative to anemia, macrocytosis, and cognitive impairment

- Design
  - Cross-sectional design
  - Continuous data taken from NHANES study (1999-2002)

Variables

- Independent: Serum folate and Vitamin B12 status
- Dependent:
  1. Anemia
  2. Macrocytosis
  3. Cognitive impairment in seniors
- Confounders
  - Age
  - Sex
  - Race/ethnicity
  - Education
  - Current smoking status
  - Alcohol intake
  - Self-reported history of cancer
  - Serum concentrations: creatinine and ferritin
- Others?
  - Family history Alzheimer's/Dementia
  - Other blood disorders Hemophilia/Thrombosis

Subjects

- N=1458 anemia
- N=1302 cognitive function
- Seniors ≥60 yrs

- Exclusion
  - Renal dysfunction
  - Recent anemia therapy
  - History of stroke
  - Heavy alcohol consumption
  - Liver/kidney/coronary artery disease

Methods

- NHANES compiled from 2 years
  - Continuous compilation data taken annually
  - In-home interviews on computer system
  - Mobile examination for interview data
  - Blood collection
    - NHANES Phlebotomy Manual

Methods

- Assessments
  - Criteria defined by WHO
    - Anemia
      - Hgb: <12 g/dL (f) and <13g/dL (m)
      - Macrocytosis: ≥99fL Mean cell volume:
    - Cognitive function
      - Wechsler Adult Intelligence Scale III (more sensitive than Mini-Mental State Exam)

B12 and Folate Status Classification

- Low B12 Status
  - Low serum B12: 148pmol/L or
  - High MMA concentration: ≥210nmol/L
- Serum folate
  - Continuous variable
  - High serum folate status: >59nmol/L

- Biochemical Measurements
  - Serum **folate** and **B12**
    - Quantaphase II Radioassay Kit
  - Serum **methylmalonic acid** (MMA)
    - Gas chrommatography
  - Serum **homocysteine**
    - >13µmol/L
    - Florescence polarization immunoassay kit
  - Serum **ferritin**
    - QuantaImmune Ferritin IRMA Kit
  - Serum **creatinine**
    - Based on Jaffe reaction
  - Serum **glucose**
    - Hexokinase enzymatic method

Statistics

- Data analysis: SUDAAN 9.0
- 4y sampling weights
  - Account for complex design
- $p<0.05$ statistically significant
- Multivariate adjustment (unless otherwise stated)
- SUDAAN PROC REGRESS, SUDAAN PROC CROSSTAB, and SUDAAN PROC RLOGIST
  - Examine subjects
  - Least-square means and proportions
  - Compare high vs. low Vit. B-12 status

TABLE 1

Characteristics of eligible senior participants in the National Health and Nutrition Examination Survey (1999–2002) by vitamin B-12 status[1]

Characteristic	Vitamin B-12 status: Normal (n = 1113)	Vitamin B-12 status: Low[2] (n = 346)	OR (95% CI)	P
Age (y)	70 ± 0.30[3]	72 ± 0.39[3]	1.05 (1.03, 1.07)	<0.001
Female (%)	62	66	1.6 (1.1, 2.5)	0.022
Non-Hispanic white (%)	81	85	1.0	Referent
Non-Hispanic black (%)	8.0	4.4	0.4 (0.3, 0.6)	<0.001
Mexican American (%)	3.1	2.1	0.9 (0.6, 1.3)	0.532
<High school diploma (%)	25	32	1.5 (1.01, 2.3)	0.046
Cigarette smoker (%)	12	17	2.1 (1.4, 3.3)	0.002
Supplement user (%)	71	54	0.4 (0.3, 0.7)	<0.001
Serum folate (nmol/L)	39 ± 0.7[4]	34 ± 1.4[4]	—	0.019
Serum creatinine (μmol/L)	67 ± 0.9[4]	74 ± 1.3[4]	—	<0.001
Serum ferritin (μg/L)	102 ± 2.3[4]	94 ± 5.6[4]	—	0.146
Cancer diagnosis (%)	13	14	1.1 (0.7, 1.7)	0.76
Macrocytosis (%)[5]	2.7	6.5	1.8 (1.02, 3.1)	0.041
Anemia (%)[6]	3.2	8.3	2.7 (1.7, 4.4)	<0.001
Cognitive impairment (%)[7]	15	32	2.5 (1.6, 3.8)	<0.001

[1] Subjects with high serum creatinine concentrations and those who reported stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries were excluded. Means, odds ratios (ORs), and P values were generated from a multivariate model that included terms for age, sex, race-ethnicity, educational status, cancer history, and serum concentrations of ferritin and creatinine; percentages are sample-weighted.

[2] Defined as a serum vitamin B-12 concentration <148 pmol/L or a serum methylmalonic acid concentration above the reference range (ie, 60–210 nmol/L) for serum vitamin B-12–replete participants with normal serum creatinine.

[3] $\bar{x}$ ± SEM.

[4] Geometric least-squares $\bar{x}$ ± SEM.

[5] Defined as a mean cell volume ≥99 fL.

[6] Defined as a hemoglobin concentration <12 g/dL (women) or <13 g/dL (men).

[7] Defined as a Digit Symbol-Coding Score <34; test results were available for 1302 nonexcluded seniors.

TABLE 2
Interaction between vitamin B-12 status and serum folate in relation to anemia and cognitive impairment in eligible senior participants in the National Health and Nutrition Examination Survey (1999–2002)[1]

Outcome	Vitamin status		No. of subjects	Percentage with outcome[4]	Percentage with high homocysteine[4–6]	OR (95% CI)		
	B-12[2]	Folate[3]				Basic model[7]	Full model 1[8]	Full model 2[9]
				%	%			
Anemia[10]	Normal	Normal	913	3.5	12	1.0	1.0	1.0
Anemia	Normal	High	198	2.5	7.8	0.6 (0.2, 2.2)	0.6 (0.2, 2.4)	0.6 (0.2, 2.4)
Anemia	Low	Normal	297	6.9	31	2.0 (1.1, 3.5)	2.1 (1.1, 3.7)	1.9 (1.01, 3.6)
Anemia	Low	High[11]	49	15	23	5.2 (2.5, 10.6)	4.9 (2.3, 10.6)	4.8 (2.3, 10.4)
Cognitive impairment[12]	Normal	Normal	826	18	11	1.0	1.0	1.0
Cognitive impairment	Normal	High	180	11	7.8	0.5 (0.2, 0.9)	0.4 (0.2, 0.9)	0.5 (0.2, 0.96)
Cognitive impairment	Low	Normal	253	25	31	1.9 (1.1, 3.1)	1.7 (1.01, 2.9)	1.6 (0.95, 2.8)
Cognitive impairment	Low	High[13]	42	45	25	4.9 (2.6, 9.2)	5.0 (2.7, 9.5)	4.9 (2.6, 9.2)

[1] Subjects with high serum creatinine concentrations and those who reported stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries were excluded.

[2] Low serum vitamin B-12 status defined as a concentration <148 pmol/L or a serum methylmalonic acid concentration above the reference range (ie, 60–210 nmol/L) for serum vitamin B-12–replete participants with normal serum creatinine.

[3] High status defined as a serum folate concentration >59 nmol/L (80th percentile).

[4] Adjusted for age, sex, race-ethnicity, educational status, cancer history, diabetes status, and serum concentrations of ferritin, creatinine, and glucose.

[5] High homocysteine concentration defined as >13 nmol/L.

[6] All percentages within an outcome group are significantly different from each other.

[7] Adjusted for age, sex, and race-ethnicity.

[8] Adjusted for age, sex, race-ethnicity, educational status, cancer history, diabetes status, and serum concentrations of ferritin, creatinine, and glucose.

[9] Adjusted for age; sex; race-ethnicity; educational status; cancer history; diabetes status; serum concentrations of ferritin, creatinine, and glucose; and hyperhomocysteinemia.

[10] Defined as a hemoglobin concentration <12 g/dL (women) or <13 g/dL (men).

[11] In a comparison of this group with the group with low vitamin B-12 status and normal serum folate, $P = 0.01$ with the basic model, $P = 0.028$ with full model 1, and $P = 0.025$ with full model 2. In comparisons between this group and all other groups, regardless of the model used, $P \leq 0.001$.

[12] Defined as a Digit Symbol-Coding Score <34.

[13] In a comparison of this group with the group with low vitamin B-12 status and high serum folate, $P = 0.013$ with the basic model, $P = 0.008$ with full model 1, and $P = 0.005$ with full model 2. In comparisons between this group and all other groups, regardless of the model, $P < 0.001$.

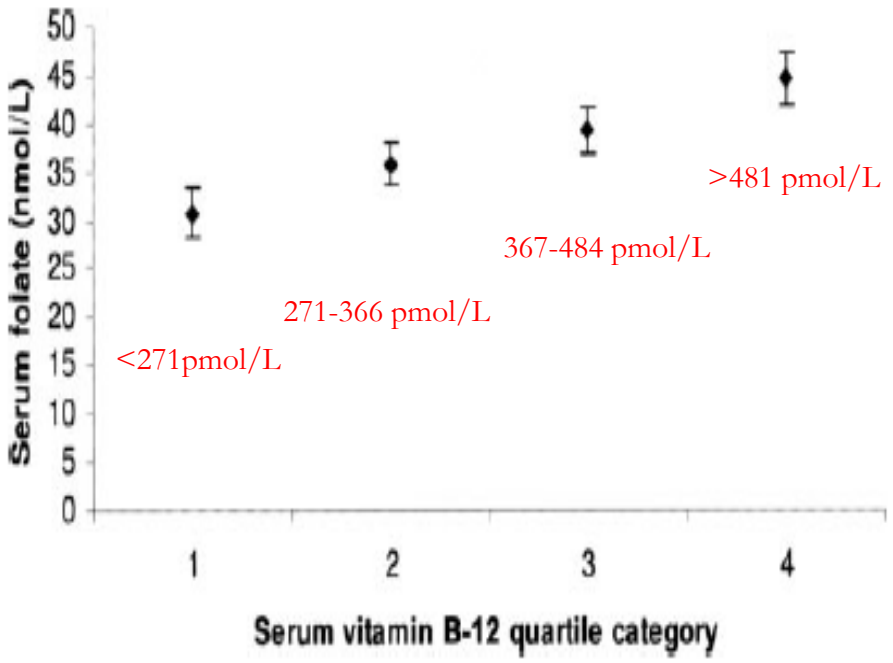


FIGURE 1. Association between serum vitamin B-12 and serum folate in senior participants in the National Health and Nutrition Examination Survey (1999–2002) who had no evidence of renal dysfunction or history of stroke, alcoholism, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries ($n = 1457$). Points represent least-squares geometric means adjusted for age, sex, race-ethnicity, education, cancer, smoking, alcohol intake, and serum concentrations of ferritin and creatinine for supplement users and nonusers combined. Error bars represent 95% CIs. Quartile categories 1, 2, 3, and 4 are <271, 271–366, 367–484, and >484 pmol/L for supplement users and <208, 208–281, 282–350, and >350 pmol/L for nonusers, respectively. The direct association between serum vitamin B-12 and serum folate ($P_{trend} < 0.001$) did not vary with supplement use ($P_{interaction} < 0.751$).

Limitations

- Study design
  - More rigorous, placebo-controlled trial may be considered to be unethical
- Self-reported data
- Actual sample with low/normal B12 (Table 1)
- No "gold standard" indicator of Low B-12 status
- Cognitive Function test administered was nonspecific to cognitive impairment
  - Unable to compare folate status with other neuropsychiatric effects
- Only serum total folate was measured
  - Associations cannot be definitively linked to unmetabolized folic acid

Conclusion

- Folate supplementation in older Americans with low B-12 status increased anemia and cognitive impairment
  - Macrocytosis failed to meet significance

Study Implications

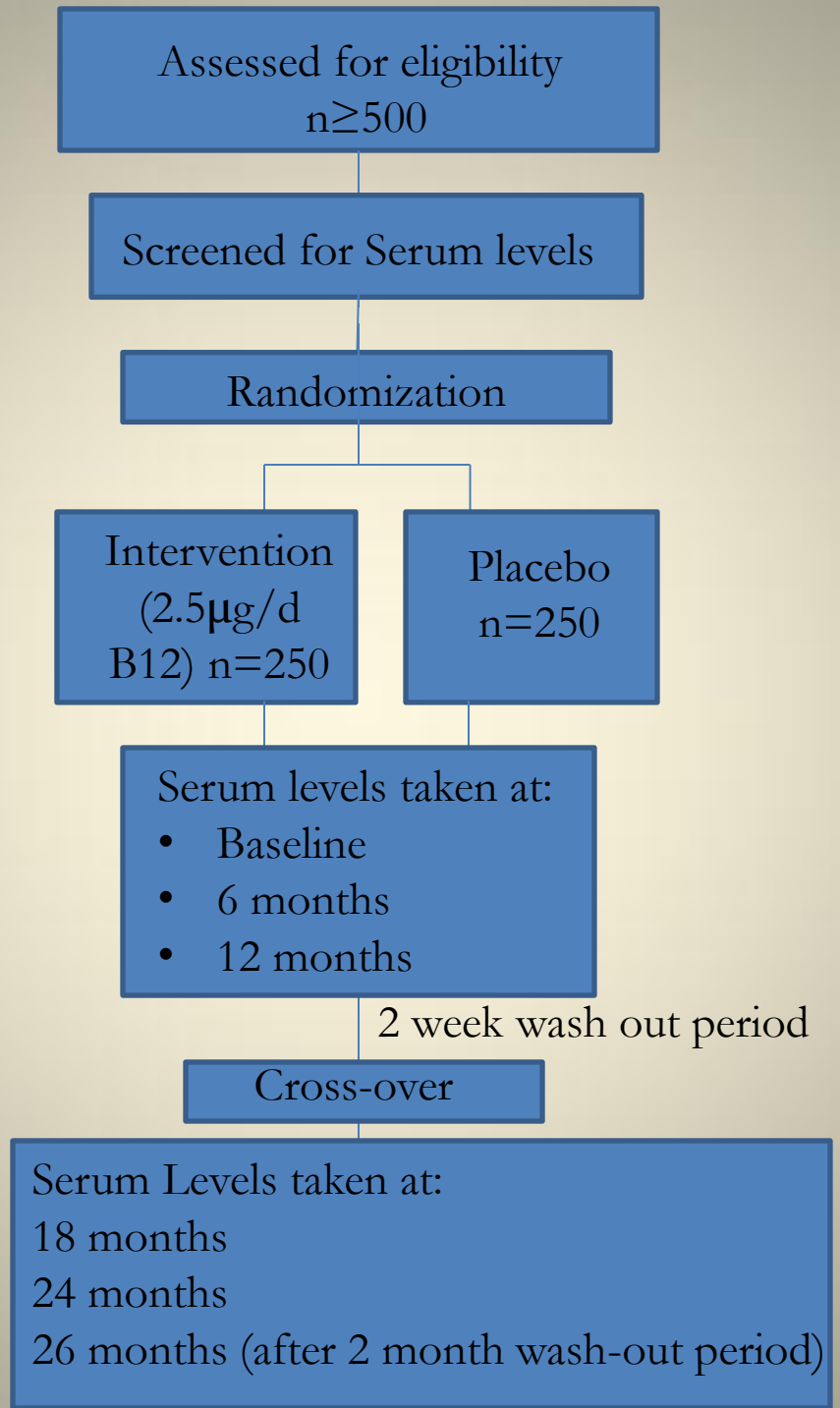
- Seniors who are taking folic acid supplements should be regularly tested for Vitamin B-12 deficiency. Signs of decreased mental cognition and anemia of these individuals should be monitored closely.

Follow-up Study

- **Objective**: To examine the effectiveness of Vitamin B-12 supplementation to cure anemia and reverse cognitive impairment
- **Design**: 2 year, randomized, double-blind, placebo controlled, cross-over trial
- **Subjects:**
  - Seniors recruited from long-term care facilities and hospitals and using advertisement in newspapers and on fliers
  - $n \geq 500$

Methods

- Baseline:
  - Dietary intake from trained nutritionists
  - Serum sample
    - B12
    - Folate
    - Homocystein
    - Methylmalonic acid
    - Creatinine
    - Glucose


Assessed for eligibility
n≥500
Screened for Serum levels
Randomization
Intervention
(2.5µg/d
B12) n=250
Placebo
n=250
Serum levels taken at:
• Baseline
• 6 months
• 12 months
2 week wash out period
Cross-over
Serum Levels taken at:
18 months
24 months
26 months (after 2 month wash-out period)

References

1. *The Facts about Vitamins in Nutrition.* DSM Nutritional Products. 10/29/2009. http://www.vitamin-basics.com/index.php?id=57. Accessed 9/22/11
2. *Vitamin B12.* MedlinePlus A.D.A.M. Inc. http://www.nlm.nih.gov/medlineplus/ency/article/002403.htm. Accessed 9/22/11
3. *Dietary Supplement Fact Sheet: Vitamin B12.* Office of Dietary Supplements. National Institutes of Health. http://ods.od.nih.gov/factsheets/vitaminb12. Accessed 9/22/11
4. *Morris, Martha Savaria et al. Am J Clin Nutr 2007;85: 193-200*
5. *Katie Silliman Lecture notes.* Advanced Human Nutr, NFSC 440. Fall 2010.