High dose vitamin B12 supplements are required to improve status of deficient lactating Guatemalan women and increase B12 in breast milk, but do not improve B12 status of breastfeeding infants.

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Abstract

Vitamin B12 deficiency has potentially irreversible adverse effects on infant development and is prevalent in populations consuming low amounts of animal source foods (ASF). This study was designed to determine the oral B12 dose needed to replete marginally deficient lactating Guatemalan women and their infants. Eligibility criteria were 2 mo ± 2 wk postpartum, exclusive breastfeeding, age ≥18 y. Blood was taken from 40 eligible participants to screen for serum B12 100-221 pmol/L. After stratifying into quartiles based on serum B12, 18 women were randomly assigned to oral supplements of 3 (the RDA), 100, 250, 500, 750 or 1000 ug/d B12, for 2 mo. At baseline and post-supplementation 50 mL breast milk, and maternal and infant blood and urine were collected. Maternal B12 intake was estimated at 0 and 2 mo. Serum B12 was measured by SimulTRAC radioassay, and urinary MMA (UMMA) by GC-MS. ≥90% of the apo-haptocorrin (HC) in breast milk, present in amounts 200-fold those in serum, was adsorbed onto cobinamide-coated EAH sepharose (Lildballe et al.) to prevent interference with the B12 assay.
At baseline, values (median, range) were: milk B12, 67 (23-162) pmol/L; maternal serum B12, 110 (53 to 274) pmol/L; infant serum B12, 122 (69 to 190) pmol/L; maternal urinary MMA 1.54 (0.39 to 8.76) and infant urinary MMA 7.46 (1.22-50.29) mmol/mol creatinine. 88% of the infants were B12 depleted (<221 pmol/L) at baseline. Infant serum B12 was strongly correlated with several maternal parameters; breast milk B12 (r=0.74, P<0.13), UMMA (r=-0.57, P<0.09; and B12 intake (r=0.64, P<0.06). Serum B12 and UMMA were strongly correlated in infants (r=-0.86, P<0.01). Median maternal B12 intake was 1.6 (1.0-6.0) ug/d, with 62% of women failing to meet the Estimated Average Requirement for lactation, and intake correlating strongly with infant serum B12 (r=0.64, P<0.06) and maternal UMMA (r=-0.38, P<0.07), but not with milk B12.
Dose was correlated with breast milk B12 (r=0.48, P<0.05) and maternal serum B12 (r=0.61, P<0.02) but not infant B12 status which remained sig. related to baseline status in spite of maternal supplements. The 3 ug and 100 ug doses had no effect on status of mother or milk. Based on change in breast milk B12 from baseline to post-supplementation infants obtained 1% of the 3 ug dose, but only 0.01-0.05% of doses ≥100 ug/d. At baseline B12 in milk was only 22% of the value assumed to be normal in the US and Canada and after 250-750 ug/d supplementation only increased to 180 pmol/L, ≈50% of the concentration in well-nourished women.

Introduction & Objectives

❑ Vitamin B12 is found only in animal source foods (ASF) which are often consumed in low amounts by women and infants in developing countries.

❑ Lactating women and infants have high B12 requirements; B12 deficiency in infants causes failure to thrive, psychomotor development disorders and megaloblastic anemia.

❑ In 2 previous studies in Guatemala City we found ≈30% of lactating women were B12 deficient (serum B12<148pmol/L) (Casterline, 1997; Jones, 2007).

❑ Serum B12 was positively correlated with breast milk B12 in most but not all previous studies.

❑ Accurate measurement of B12 in breast milk is difficult due to its strong binding to haptocorrin.

❑ One strategy to improve B12 status of lactating women and prevent deficiency in their infants could be B12 supplementation of the lactating mother. The dose required has not been tested. The AI for infants (0-6 mo) is 0.4 µg/d and the RDA for lactating women is 2.8 µg/d, but these doses may be inadequate for repletion.

❑ **This pilot study was designed to establish the dose of B12 supplement needed for B12 depleted, exclusively breastfeeding mothers to achieve normal serum and milk B12 concentrations, and normal serum B12 in the infant. An improved method of measuring B12 in breast milk was used.**

Methods

Location: La Communidad de Mixco, Guatemala.

Subjects: 18 EBF, B12 depleted women (serum B12<225 pmol/L) and their infants, 2 mo postpartum.

Intervention: Mothers randomly assigned to one of six vitamin B12 supplement groups - 3, 100, 250, 500, 750,1000 µg/d for 2 mo.

Indices measured: At baseline, month 1 & 2: milk B12, maternal and infant serum B12, maternal and infant urinary methylmalonic acid (UMMA), maternal B12 intake from food frequency, anthropometry, breastfeeding frequency.

Assessment of vitamin B12 status:

Serum B12 measured by competitive protein-binding radioassay using hog intrinsic factor as binder (Folate/B12 SNB SimulTRAC RIA kits, BioMedicals).

➢Cut-offs: marginal B12 deficiency, mothers <220 pmol/L; infants <148 pmol/L

UMMA analyzed by HPLC/MS/MS using a modified Kushnir's method.

➢Cut-offs: adults >3.6 mmol/mol creatinine; infants >20-23 mmol/mol creatinine

Milk B12 measured using new method involving initial removal of unbound haptocorrin by cobinamide-coated EAH sepharose gel followed by chemiluminesence (Lildballe et al.., Clin Chem Lab Med 47:2009).

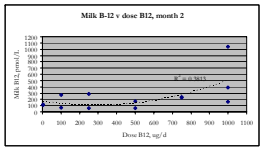
Results

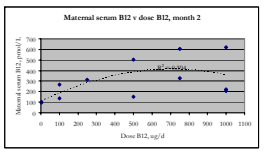
Baseline characteristics of subjects[1] (N=16)[2]

Mothers		Infants	
Age, *y*	26 (19, 39)	Age, *d*	63 (51, 75)
Milk B-12, *pmol/L*	67 (23, 162)	Gender, *% male*	37
Serum B-12, *pmol/L*	110 (53, 274)	Serum B-12, *pmol/L*	122 (69, 190)
Urinary MMA, 8.76) *mmol/mol creatinine*	1.54 (0.39,	Urinary MMA, 50.29) *mmol/mol creatinine*	7.46 (1.22,
B-12 intake, *µg/d*	1.6 (0.5, 5.8)	% Exclusive breastfeeding	10
Pregnancies	2.5 (1, 6)	% Predominant breastfeeding	6

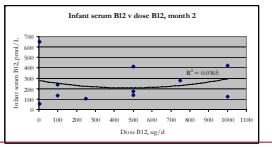
[1]Data are medians (min, max)
[2]No significant differences in baseline characteristics among the 6 supplementation groups

Dose response in milk B12 and maternal and infant serum B12 concentrations after 2 months of B12 supplementation





≥500µg B12/d is needed to increase B12 in maternal serum and milk.



No amount of B12 given to the mother was enough to bring all infants to normal B12 status

Significant correlations between B12 variables in all 6 groups after 1 and 2 months of B12 supplementation

	Dose B12 vs maternal serum B12	Dose B12 vs milk B12	Diet B12 vs Infant serum B12	Maternal serum B12 vs milk B12	Milk B12 vs infant serum B12	Milk B12 vs infant UMMA	Infant serum B12 vs infant UMMA
Mo. 1	0.77***	0.78***	0.58*	0.66**	0.73**	-0.62**	-0.83**
Mo. 2	0.61*	0.49*	0.63*	0.56*			-0.54*

B12 dose was strongly correlated with maternal B12 indices but not with infant B12 status indicators. B12 in maternal diet remained strongly correlated with infant serum B12 after 1 and 2 mo supplementation.

Models to predict infant vitamin B-12 status controlling for baseline values; linear regression

.	Dose	Dose + baseline infant serum B-12	Maternal UMMA at baseline
Maternal serum B-12, mo. 1	r=0.75**		
Maternal serum B-12, mo. 2	r=0.56*		
Milk B-12, mo. 1		r=0.95***	
Milk B-12, mo. 2		r=0.83**	
Infant UMMA, mo 1			r=0.55*

Infant serum B12 at baseline was the best predictor of infant B12 status even after 1 and 2 mo of maternal B12 supplementation.

Conclusions

❑ This was a pilot study with small sample size and limited power; a larger study is needed to more closely determine best dose.

❑ A dose-response was observed in milk B12 and maternal serum, but not in infant serum.

❑ Maternal doses of B12 up to 300 X the RDA were insufficient to increase serum B12 of deficient infants to normal values.

❑ 21% of infants were B12 deficient at baseline and 15% were still deficient after 2 mo of maternal B12 supplementation.

❑ Maternal dietary B12 intake was more strongly associated with infant B12 status than with milk B12.

❑ It needs to be determined if maternal B12 supplementation in pregnancy, rather than in lactation, is more effective for improving infant B12 status.

Implications

B12 deficiency has known adverse effects on infant development and maternal health. The high prevalence of maternal and infant deficiency in such areas, caused by low intake of animal source foods, needs maternal interventions before and during pregnancy. Also, since B12 deficiency is prevalent at all ages in both genders, the efficacy of vitamin B12 fortification of flour and other products should be explored.