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The U.S. Government's Global Hunger & Food Security Initiative

Vitamin B-12 status in infancy is positively associated with development and cognitive functioning 5 y later in Nepalese children.

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VITAMIN B12, ASF AND COGNITION

- **Sources:** Naturally found in animal source food products: (shell)fish, meat, poultry, eggs, milk. Supplements or fortified foods.
- **Roles:** Cell division, myelination, deficiency can lead to impairments in nervous system
- **Epidemiology:**
 - For infants B12 deficiency usually secondary to maternal deficiency.
 - In South Asia, risk of deficiency is high due to low consumption of ASF
- **Hypothesis:** that early vitamin B12 status and maternal intake would be associated with gross, fine, sensorimotor and visuospatial functioning.

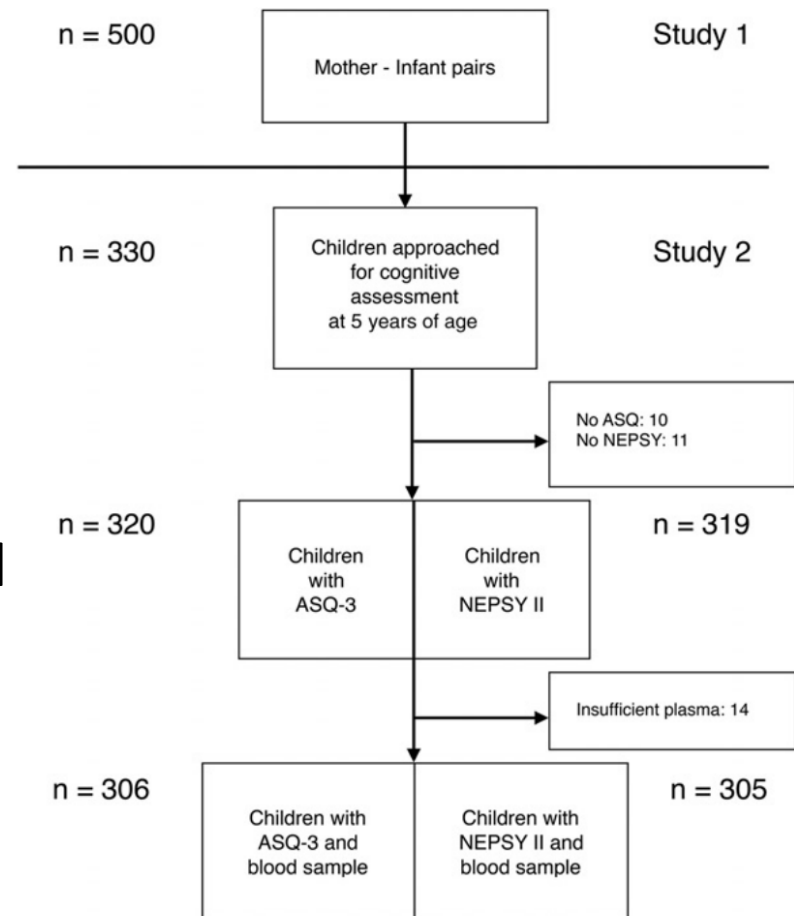


MULTIPLE INDICATORS OF B12 STATUS

Biomarker/Indicator	Indication	Interpretation
Serum total vitamin B12 (log transformed)	Global vitamin B12 status	↓ in vitamin B12 deficiency
Methylmalonic acid (MMA)	Functional marker of B12 deficiency. More sensitive to mild deficiency.	↑ in vitamin B12 deficiency
Total homocysteine (tHcy)	Functional marker of B12 deficiency. More sensitive to mild deficiency	↑ in vitamin B12 deficiency
3cB12	Combined indicator of B12 status using the 3 above, accounting for age+folate status	↓ in vitamin B12 deficiency
3x 24 hour dietary recalls with mothers over 1 year	Dietary intake of B12	

STUDY DESIGN

- **Setting:** Bhaktapur, Nepal
- **Participants:** 500 mother-infant pairs enrolled in 2008 in a cross sectional survey of nutritional status/intake.
- **Design:** Random 2 stage-sample, 330 reidentified 5 years later and reconsented to developmental and neuropsychological assessments.
- Multivariable logistic and linear regression models to examine relationships





AGES AND STAGES-3 QUESTIONNAIRE

- ASQ-3 is a 30 item tool that evaluates 5 areas of child development via direct observation or parental recall based on timing of developmental milestones.
 - Communication
 - Gross motor
 - Fine motor
 - Problem solving
 - Personal social
- Children in the bottom 25% of the ASQ-3 total and subscale scores defined as having poorer development outcomes.



NEPSY-II SUBTESTS AND FUNCTIONS MEASURED

Domain	NEPSY II subtests	Functions measured
Attention and executive functioning	Inhibition	Ability to inhibit automatic responses in favor of a new one
	Statue	Assesses inhibitory control and motor persistence (not being disturbed by distractions)
Sensorimotor	Visuomotor precision	Fine motor ability (timed task, drawing between lines)
Social perception	Affect recognition	Recognizing emotions in matching task
Visuospatial processing	Block construction	Visuomotor and visuospatial abilities
	Geometric puzzles	Ability to recognize, mental rotation of geometric shapes



COVARIATES IN REGRESSION ANALYSES

	Continuous	Categorical
Sex	—	Male or female
Age at enrollment	mo	—
Exclusively breastfed at enrollment	—	Yes or no
Iron status at enrollment	$\mu\text{g/L}$	—
Height for age at enrollment	z score	—
Weight for height at enrollment	z score	—
Weight for age at enrollment	z score	—
Parity	—	1–2 or >2
Energy intake of mother at enrollment	kcal	—
Folic acid supplementation in pregnancy	—	Yes or no
Living in joint family	—	Yes or no
Family owns land	—	Yes or no
Rooms in the home	<i>n</i>	—
Mother's age	<i>y</i>	—
Parents' educational status	—	<10th grade or \geq 10th grade
Parents' occupation	—	No work/agricultural or other work

¹ ASQ-3, Ages and Stages Questionnaire, 3rd edition; NEPSY II, Developmental Neuropsychological Assessment, 2nd edition.



PREVALENCE OF DEFICIENCY

Biomarker/Indicator	Prevalence
Serum total vitamin B12	14.7% <148 pmol/L
Methylmalonic acid (MMA)	77.1% elevated (>0.28 μ mol)
Homocysteine (tHcy)	56.5% elevated (>10 μ mol)
3cB12	64.7% <-0.5 (low status)

TABLE 6Associations between log₂-transformed markers of cobalamin status and the total and subscale scores of the ASQ-3 in Nepali preschoolers¹

Variables	n	Cobalamin ²		MMA ²		tHcy ²		3cB12 ³	
		Value ⁴	P	Value	P	Value	P	Value	P
Linear regression									
Total ASQ-3									
Crude	306	2.44 (-0.24, 5.12)	0.074	-2.87 (-5.01, -0.74)	0.009	-8.13 (-13.07, -3.19)	0.002	4.94 (2.33, 7.55)	0.001
Adjusted ⁵	305	2.43 (-0.12, 4.97)	0.062	-3.12 (-5.35, -0.90)	0.007	-6.78 (-11.90, -9.95)	0.007	4.88 (2.09, 7.68)	0.001
Logistic regression									
Total ASQ-3 subscales									
Crude	306	0.82 (0.58, 1.16)	0.260	1.32 (1.07, 1.62)	0.010	2.45 (1.46, 4.10)	0.001	0.62 (0.45, 0.83)	0.002
Adjusted	305	0.79 (0.55, 1.13)	0.198	1.34 (1.06, 1.70)	0.014	2.25 (1.26, 4.03)	0.006	0.61 (0.43, 0.86)	0.005
Communication									
Crude	306	0.93 (0.68, 1.28)	0.662	1.24 (0.96, 1.60)	0.094	2.31 (1.28, 4.17)	0.006	0.69 (0.49, 0.95)	0.025
Adjusted	305	0.89 (0.63, 1.26)	0.508	1.25 (0.96, 1.64)	0.096	1.88 (0.92, 3.83)	0.083	0.70 (0.49, 1.01)	0.053
Gross motor									
Crude	306	0.82 (0.54, 1.23)	0.328	0.97 (0.74, 1.26)	0.799	1.57 (0.80, 3.06)	0.188	0.87 (0.57, 1.33)	0.519
Adjusted	305	0.79 (0.53, 1.21)	0.284	1.00 (0.76, 1.322)	0.977	1.59 (0.80, 3.18)	0.187	0.83 (0.54, 1.28)	0.406
Fine motor									
Crude	306	0.80 (0.61, 1.05)	0.112	1.23 (0.98, 1.55)	0.073	1.55 (0.86, 2.80)	0.144	0.71 (0.50, 1.01)	0.047
Adjusted	305	0.81 (0.62, 1.06)	0.118	1.23 (0.98, 1.58)	0.092	1.56 (0.81, 3.02)	0.187	0.71 (0.49, 1.03)	0.068
Problem solving									
Crude	306	0.96 (0.67, 1.39)	0.817	1.32 (1.05, 1.66)	0.018	2.32 (1.38, 3.89)	0.001	0.66 (0.47, 0.93)	0.017
Adjusted	305	0.93 (0.65, 1.33)	0.692	1.36 (1.08, 1.71)	0.009	2.33 (1.33, 4.08)	0.003	0.64 (0.47, 0.88)	0.006
Personal-social									
Crude	306	0.81 (0.50, 1.32)	0.404	1.09 (0.82, 1.46)	0.554	0.64 (0.30, 1.35)	0.245	0.92 (0.59, 1.42)	0.707
Adjusted	305	0.79 (0.49, 1.28)	0.331	1.14 (0.87, 1.51)	0.344	0.57 (1.17, 1.27)	0.127	0.89 (0.57, 1.39)	0.613

¹ Values are coefficients (95% CIs) for linear regression models and ORs (95% CIs) for logistic regression models, adjusted for clustering. ASQ-3, Ages and Stages Questionnaire, 3rd edition; MMA, methylmalonic acid; tHcy, total homocysteine; 3cB12, combined indicator of vitamin B-12 status.

² Log-transformed base 2.

³ Calculated based on cobalamin, MMA, and tHcy concentrations.

⁴ An OR >1 indicates the increased odds of being in the lowest 25th percentile for each increment of 1 biomarker unit; e.g., for each doubling of the tHcy units there was an increased odds of 2.25 of being in the lowest quartile of the total ASQ-3 score.

⁵ Adjusted for sex, age at baseline, and weight-for-age z scores at baseline.

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TABLE 7
Associations between log₂-transformed markers of cobalamin status and the NEPSY II subtests in Nepali preschoolers¹

Variables	n	Cobalamin ²		MMA ²		tHcy ²		3cB12 ³	
		Coeff. (95% CI)	P	Coeff. (95% CI)	P	Coeff. (95% CI)	P	Coeff. (95% CI)	P
Attention and executive functioning									
Inhibition-naming completion time total									
Crude	305	2.93 (-0.59, 6.44)	0.101	-0.71 (-3.33, 1.90)	0.586	-5.71 (-11.52, 0.10)	0.054	2.87 (-1.10, 6.85)	0.153
Adjusted ⁴	304	2.79 (-0.83, 6.40)	0.128	-0.68 (-3.39, 2.04)	0.619	-5.22 (-11.03, 0.59)	0.077	2.71 (-1.48, 6.90)	0.200
Inhibition-inhibition completion time total									
Crude	305	5.35 (0.24, 10.45)	0.040	0.36 (-4.84, 5.57)	0.889	-7.95 (-16.05, 0.14)	0.054	3.42 (-3.27, 10.11)	0.310
Adjusted	304	5.30 (0.33, 10.26)	0.037	0.48 (-4.88, 5.83)	0.859	-7.6 (-16.05, 0.94)	0.080	3.26 (-3.66, 10.17)	0.346
Inhibition total errors									
Crude	305	-0.63 (-2.56, 1.30)	0.515	1.81 (-0.8, 3.70)	0.060	-0.22 (-3.31, 2.87)	0.887	-1.75 (-3.91, 0.40)	0.109
Adjusted	304	-0.68 (-2.59, 1.24)	0.483	1.87 (0.02, 3.71)	0.048	-0.42 (-3.59, 2.74)	0.789	-1.77 (-3.91, 0.36)	0.102
Statue total score									
Crude	305	0.16 (-0.17, 0.49)	0.334	-0.19 (-0.47, 0.10)	0.189	-0.31 (-1.11, 0.49)	0.440	0.28 (-0.15, 0.71)	0.203
Adjusted	304	0.19 (-0.14, 0.52)	0.247	-0.23 (-0.53, 0.08)	0.140	-0.39 (-1.15, 0.38)	0.313	0.34 (-0.10, 0.77)	0.131
Sensorimotor functioning									
Visuomotor precision total completion time									
Crude	305	-3.37 (-11.72, 4.98)	0.422	-3.41 (-8.80, 1.97)	0.209	-1.76 (-13.45, 9.93)	0.764	1.75 (-6.76, 10.26)	0.682
Adjusted	304	-3.49 (-11.54, 4.57)	0.389	-3.60 (-8.81, 1.62)	0.172	-0.26 (-12.97, 12.46)	0.968	1.50 (-6.62, 9.62)	0.712
Social perception									
Affect recognition total score									
Crude	305	0.50 (0.20, 0.80)	0.002	-0.52 (-0.87, -0.18)	0.004	-1.33 (-1.92, -0.75)	0.001	0.90 (0.55, 1.25)	0.001
Adjusted	304	0.46 (0.15, 0.77)	0.005	-0.50 (-0.83, -0.18)	0.003	-1.10 (-1.68, -0.52)	0.001	0.82 (0.49, 1.14)	0.001
Visuospatial processing									
Geometric puzzles									
Crude	305	0.60 (0.16, 1.04)	0.009	-0.30 (0.06, 0.01)	0.054	-0.78 (-1.39, -0.18)	0.013	0.65 (0.15, 1.15)	0.012
Adjusted	304	0.57 (0.13, 1.02)	0.013	-0.27 (-0.56, 0.01)	0.061	-0.66 (-1.28, -0.5)	0.036	0.59 (0.10, 1.09)	0.020
Block construction									
Crude	305	0.21 (-0.03, 0.46)	0.085	-0.18 (-0.32, -0.04)	0.015	-0.39 (-0.72, -0.06)	0.020	0.31 (0.10, 0.53)	0.006
Adjusted	304	0.17 (-0.06, 0.41)	0.147	-0.13 (-0.27, 0.01)	0.064	-0.31 (-0.67, 0.06)	0.095	0.24 (0.02, 0.47)	0.035

¹ Linear regression models adjusted for clustering. Coeff., coefficient; MMA, methylmalonic acid; NEPSY II, Developmental Neuropsychological Assessment, 2nd edition; tHcy, total homocysteine; 3cB12, combined indicator of vitamin B-12 status.

² Log-transformed base 2.

³ Calculated based on cobalamin, MMA, and tHcy concentrations.

⁴ Adjusted for sex, age at baseline, and weight-for-age z score at baseline.

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FINDINGS

- All B12 markers except plasma cobalamin significantly associated with total asq-3 scores
- ↑ plasma total homocysteine and MMA associated with lower scores on NEPSY II affect recognition and geometric puzzle subtests.
- 3cB12 associated with affect recognition, geometric puzzle, and block construction scores.
- Contrary to hypothesis, no associations between executive function, attention or sensorimotor domains.
- Adjusting for socioeconomic status, iron status, growth, breastfeeding, maternal energy intake, folate supplementation did not change estimates



STRENGTHS AND LIMITATIONS

- First longitudinal study to investigate early B-12 and neurodevelopment in population at risk of low B-12 status
 - Prospective design, large SS, multiple assessments of status and outcomes.
-
- High dropout (330/500 mother/child pairs dropped out).
 - ASQ-3 and NEPSY-II not validated for a Nepalese population
 - Cannot rule out residual confounding

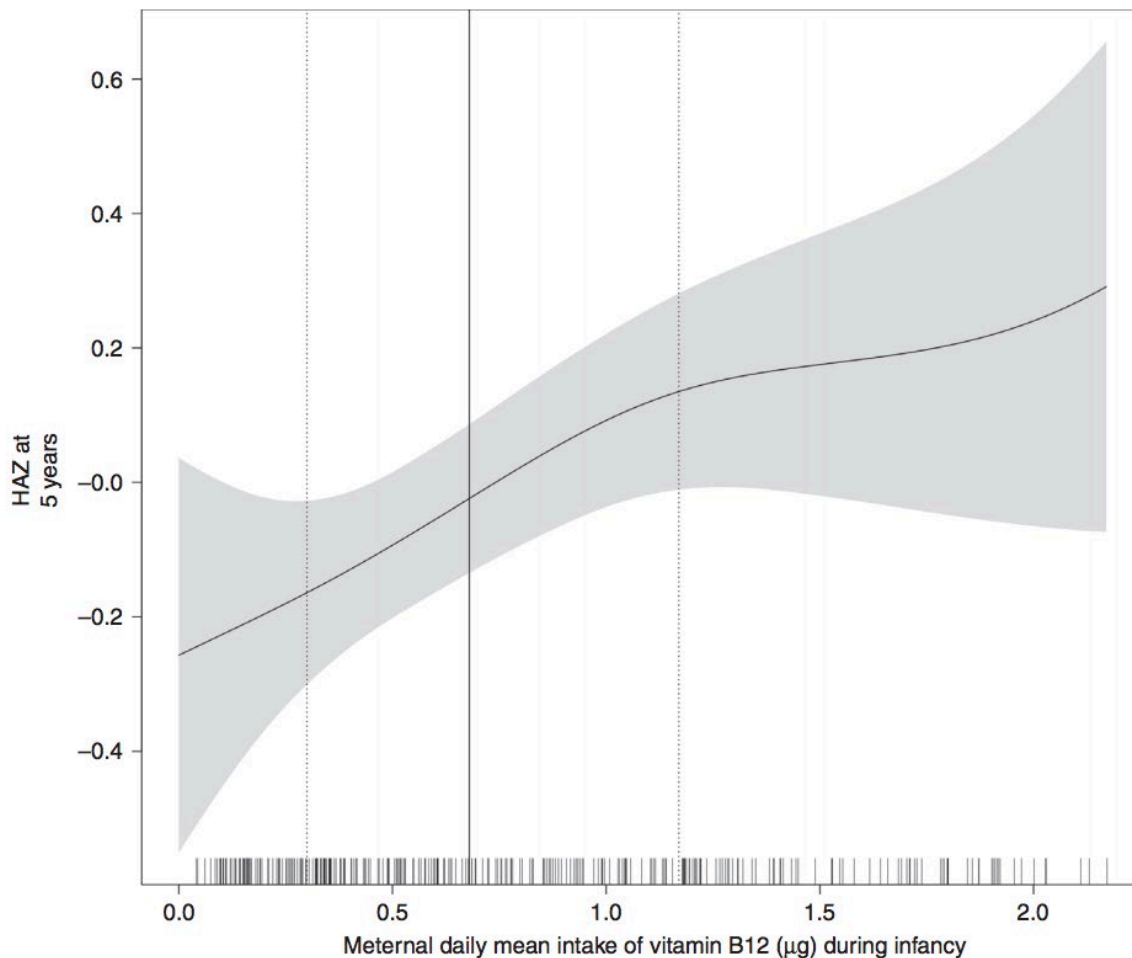


CONCLUSIONS AND RECOMMENDATIONS

- Vitamin B-12 deficiency is a potential cause of adverse developmental outcomes in this population
- Longer term effects need more study
- Trials already underway to explore effects of supplements, but trials of food sources of B12 in pregnancy and early childhood should be conducted with early childhood development and growth outcomes



RELATIONSHIP BETWEEN MATERNAL B12 INTAKE AND CHILD HAZ AT 5Y



Adjusted for age, sex, maternal education, intake of energy, intake of fiber, place of birth, and maternal length.

Strand TA, Ulak M, Kvestad I, Henjum S, Ulvik A, Shrestha M, Thorne-Lyman AL, Ueland PM, Shrestha PS, Chandyo RK. Maternal and infant vitamin B12 status during infancy predict linear growth at 5 years. *Pediatric research*. 2018 Nov;84(5):611.



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