Vitamin B Deficiency Impacts in Children

Research Question: How Does Maternal Deficiencies in Vitamin B-12 and Vitamin B-9 (Folate) Influence the Neurological Development (Cognitive, Psychological) of Their Offsprings?

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Abstract

Vitamin B plays an important role in the methylation of DNA to help regulate gene expression, especially in children experiencing rapid cell growth phases, such as pregnant women or young children. In addition, folate deficiency in the periconceptional period can contribute to neural tube defects, while vitamin B12 deficits have been associated with acute megaloblastic anemia. This research examines the relationship between maternal plasma vitamin B levels with their children and the long-term impacts of deficits in vitamin B to the cognitive abilities of pattern reasoning, verbal fluency, and memory retrieval as a few examples of how their cognitive and psychological developments are assessed. Further investigations should focus on the specific genes potentially muted due to vitamin B deficiency and examine how to prevent folate vitamin B12 deficiency in developing/third world countries.

Key Words: Malnutrition, Vitamin B12, Vitamin B12 Deficiency, Folate, Folate Deficiency, Cognitive Development, Maternal Folate, Psychological Development

Introduction

Malnutrition is caused by several economic, geographical, and social challenges families face, especially in Sub-Saharan Africa and small Middle Eastern countries (Roser and Ritchie (1)).malnutrition is closely linked to many micronutrient deficiencies such as vitamin B-12 and B-9 (folate), hindering the child's ability to reach targeted cognitive and psychological milestones.

One of the primary micronutrient deficiencies is vitamin B-12, an essential water-soluble compound obtained from food. For example, vitamin B-12 is commonly found in animal-sourced food such as beef liver, mollusks, clam, veal, and dairy products ("FoodData Central"). This vitamin is vital for the development, myelination and functioning of the central nervous system. In addition, vitamin B-12 acts as a cofactor for synthesizing methionine, an amino acid used with cysteine (a sulphur-containing amino acid) to produce protein tissues, which is important during fetal development to ensure proper gene expression and cell differentiation (Phillips). Another highly deficient micronutrient is vitamin B-9 (folate). Folate regulates the production of genetic material such as DNA and the division of cells. Deficiencies in vitamin B-12 may interrupt metabolic processes and lead to megaloblastic anemia. At the same time, inadequate folate supplementation during pregnancy may result in conditions like neural tube defects in newborns ("Spina Bifida"). Therefore, vitamin B-12 and folate are essential for individuals experiencing rapid cell growth phases, such as pregnant women or young children ("Vitamin B-12") ("Folate").

Nowadays, folate deficiency is rare in North America and Asia but highly prevalent in Sub-Saharan Africa, where access to foods and supplements is expansive and scarce. Whereas

US citizens consume folic acid, those in rural regions of Africa may lack access to such supplements. In this research, we conducted a meta-analysis of 9 studies to gain a better understanding of how maternal folate deficiency affects the cognitive development of their offspring in Africa.

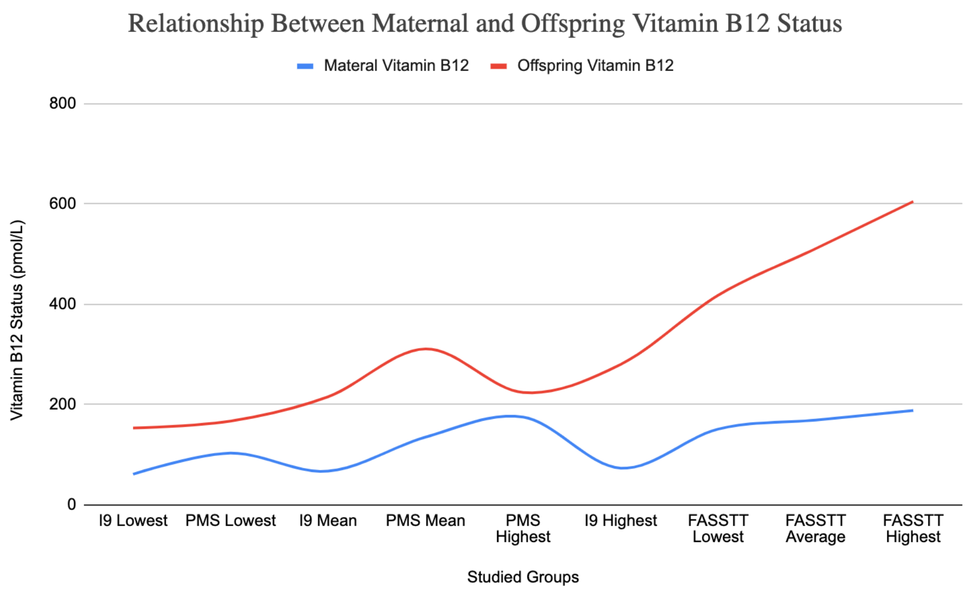
Research Method

Nine research studies that examine the impact of maternal vitamin B-12 and folate deficiencies on their offspring's neurological development were acquired through PubMed. Cognitive and psychological tests were used to assess the child's brain development for the frontal lobe (emotion, cognitive, short-term memory) and the temporal lobe (auditory information, long-term memory).

Here, we specifically analyzed studies from countries with the highest prevalence of micronutrient deficiencies in children: namely, in Africa and South Asia (Roser and Ortiz-Ospina) (Roser and Ritchie (1)) (Ritchie and Roser (2)), with medical reports from these regions within the last 20 years (blood plasma probe for detecting micronutrients was only invented in 2004 (Adámek et al.)). In addition, we restricted the age of pregnant mothers to 20-40 to limit the likelihood of other abnormal diseases in mothers influencing the cognitive scores in children. The essay majorly examines the relationship between vitamin deficiency and cognitive development, but it will also examine the correlation between maternal and children vitamin deficiencies and the evidence in numbers for the consequence of maternal deprivation of vitamin B (12 and 9).

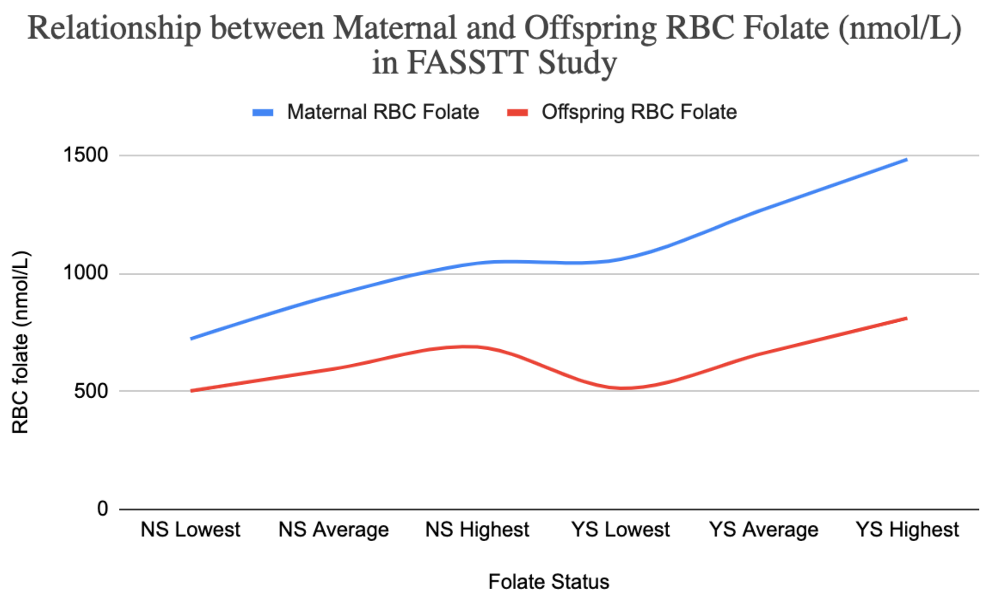
Data

The Relationship between Maternal Plasma Vitamin B-12 (ng/mL) and Offspring Vitamin B-12 (ng/mL)



*I9: Higher Maternal Plasma Folate associated with better cognitive function scores in 9-10 year old children in South India
**PMS: Pune Maternal Study
***FASSTT: Effects of maternal folic acid supplementation during the second and third trimesters of pregnancy on neurocognitive development in the child: an 11-year follow-up from a randomised controlled trial

Effects of maternal folic acid supplementation during the second and third trimesters of pregnancy on neurocognitive development in the child: an 11-year follow-up from a randomised controlled trial (FASSTT)



NS: No supplementation; YS: Yes supplementation (of folic acid)

Group Studied:

This study was a follow-up investigation in 11-year-old children whose mother participated in the FASSTT trial in pregnancy. The FASSTT trial contained women aged 18-35 years who were given supplementations of 400ug folic acid/day or a placebo until the end of their pregnancy.

Methods:

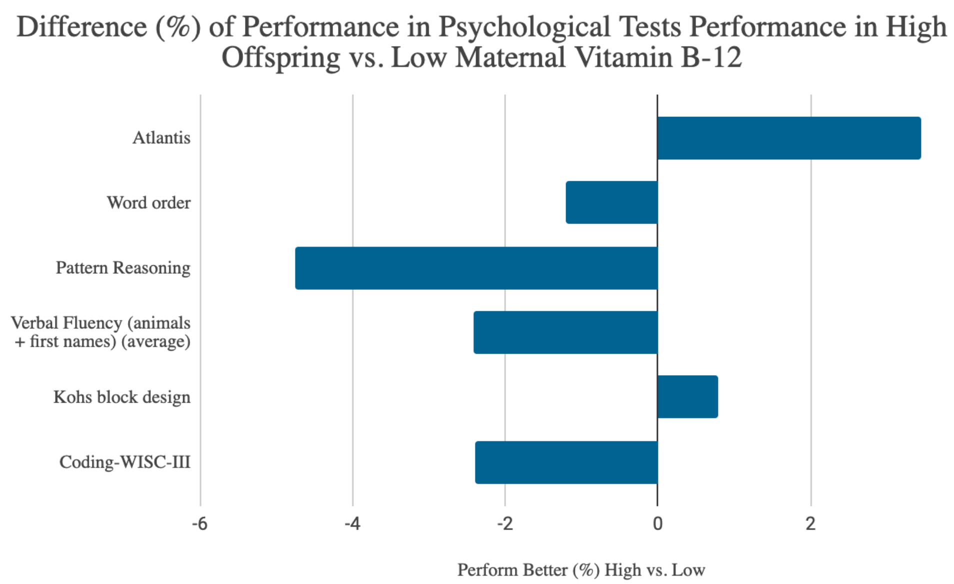
For B-Vitamin measurements in the mothers, non-fasting blood samples were collected at 14th and 36th GW and the umbilical cord blood was collected at delivery. For the children, non-fasting blood samples were collected from a pediatric phlebotomist and processed within 4 hours for serum vitamin B-12.

Limitations:

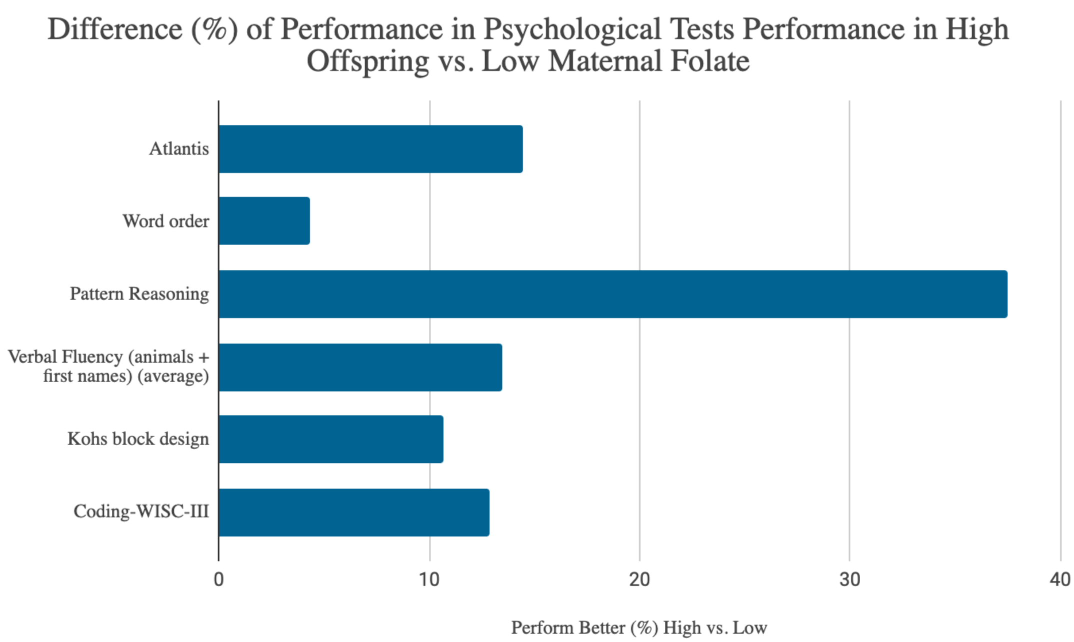
In the FASSTT trial, 296 women were initially recruited, and 119 mothers completed the full FASST trial. In this follow-up trial, only 68 pairs of mother-child were available for further investigation (placebo n=31, folic acid n=37). The relatively small sample size of mother-child pairs may have limited the ability to detect more differences in the biomarkers of mothers and children for each nutrient collected.

Higher Maternal Plasma Folate associated with better cognitive function scores in 9 to 10 year old children in South India

[Graph 1]



[Graph 2]



<u>Group Studied:</u>

This experiment examined the children aged 9-10 (n=536, boys=259, girls=277) years from the Mysore Partenon study conducted in 1997-1998, which focused on the incidence and effects of short-term and long-term gestational diabetes mellitus impacts on newborns.

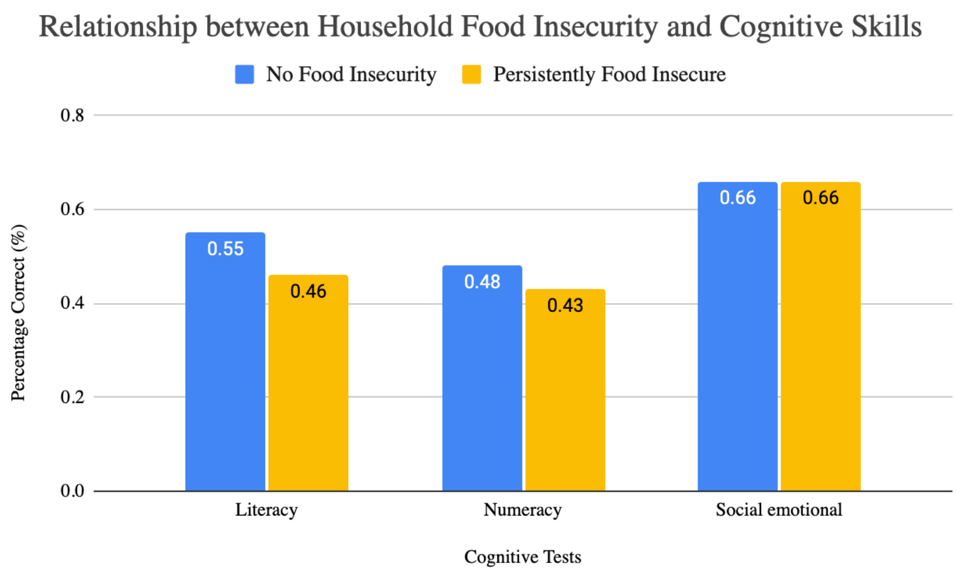
<u>Methods:</u>
The child's maternal vitamin B-12 and folate status biomarkers were obtained using maternal blood samples collected at 30 ± 2 wk of gestation.
The cognitive assessments tested specific domains in the Carroll model, three core tests from the Kaufman Assessment Battery for children, and other tests adapted to the local cultural context. Specifically, it tests domains of long-term memory and retrieval ability (Atlantis), short-term memory (word order), reasoning ability (pattern reasoning), language production (verbal fluency), visuospatial ability (Kohs' block design), and visuomotor processing and coordination with the WISC-III (coding-Wechsler Intelligence Scale for Children-III. (see appendix B for details)

<u>Findings:</u>
According to Graph 1, the children with higher plasma vitamin b-12 performed better on long-term memory and retrieval (Atlantis) but were weaker in areas of word order, pattern reasoning, verbal fluency, coding-WISC-III. Thus, there is no clear association between the level of plasma v12 concentration to the child's cognitive abilities. Looking at Graph 2, an increase in maternal folate led to higher cognitive scores in children, as children with the highest maternal folate consistently performed better than children with low maternal folate in all tests of cognitive domains, with the most evidence in the increased score performance score of 37.5% concluded by the comparison of scores from higher maternal folate versus low maternal folate (Veena, Sargoor R et al).

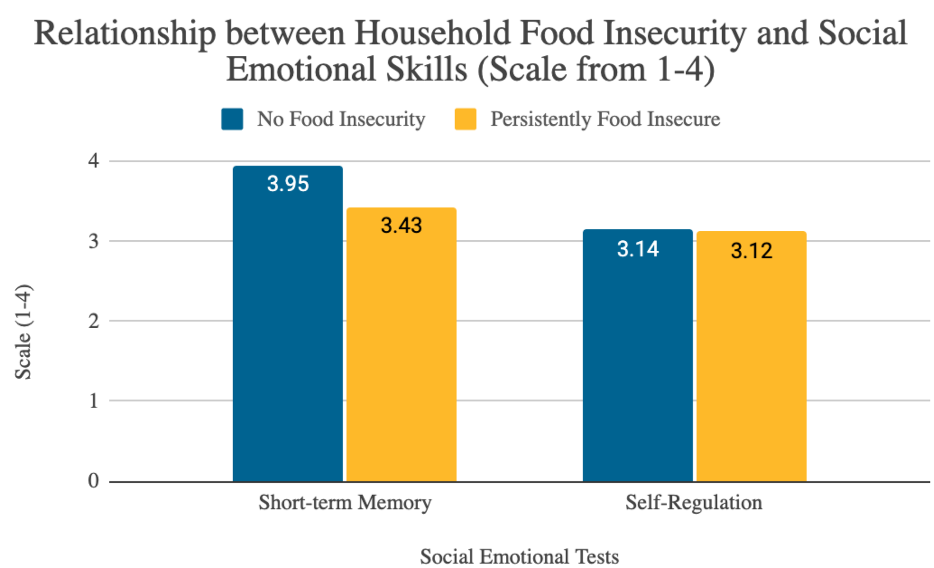
<u>Limitations:</u>
Although higher maternal folate led to higher scores from their children, other factors may have contributed. Folate is most commonly found in animal products, which is relatively more expensive compared to vegetables. Thus, the disadvantaged families may have less access to higher-quality foods and meats and a generally lower standard of living, impacting the quality of education that their children receive, contributing to lower cognitive scores.

Household food insecurity and early childhood development: Longitudinal evidence from Ghana

[Graph 1]



[Graph 2]



<u>Group Studied:</u>

In this study, over 1300 Ghanian children were tracked over three years to investigate the effects of household food insecurity and deficiencies in nutrients with early childhood development in lower primary schools. The group studied takes place in the most disadvantaged regions of the Greater Accra Region, based on the 2014 UNICEF District League Table. This table indicates the regions in Ghana according to their development of essential services such as education, health, sanitation, and governance.

<u>Methods:</u>

The experiment was conducted at three different time waves: wave 1 (September 2015), wave 2 (May 2017), and wave 3 (May 2018). These children and their caregivers were interviewed via a survey to collect baseline characteristics and data. They assessed the cognitive abilities of children by literacy, numeracy, short-term memory, social-emotional development and self-regulation tests through the Early Grade Reading Assessment (Literacy), the Early Grade Math Assessment (Numeracy), forward digit span tests (short-term memory), subtests from IDELA, and with an adapted version of Preschool Self-Regulation Assessment–Assessor Report (PSRA-AS) (Self-regulation), respectively. Please refer to appendix D for more details.

Findings:

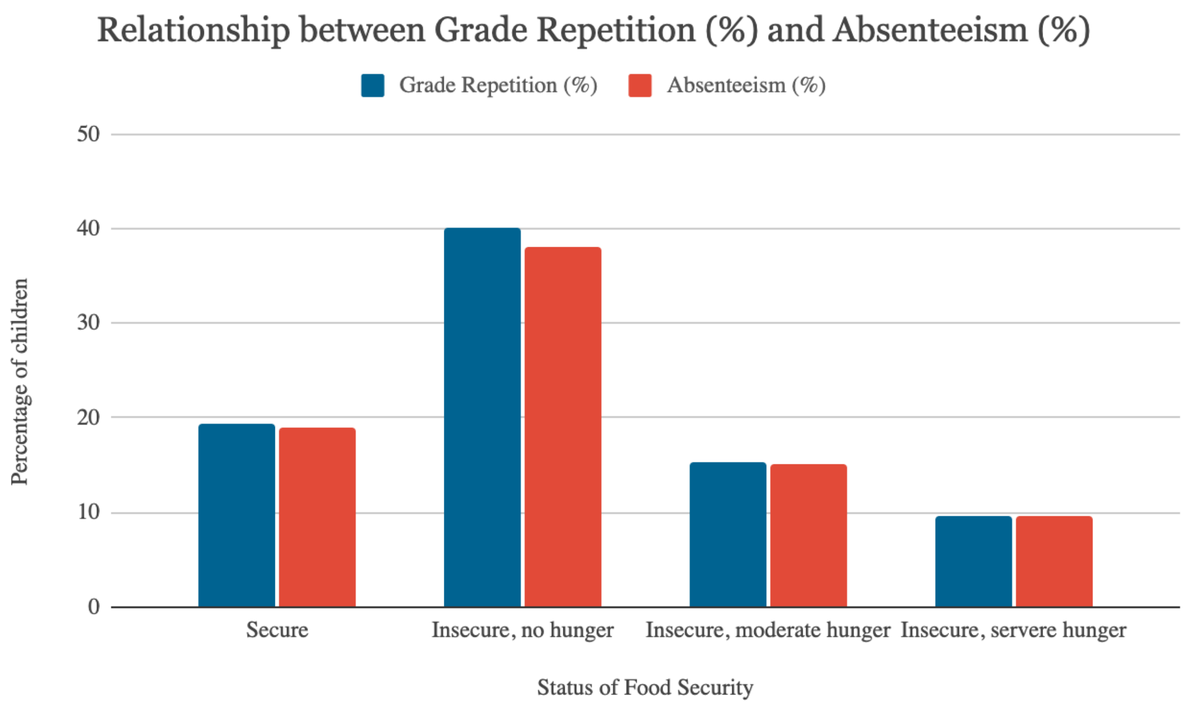
It is seen that most of the children in this study had no food insecurity (n=1116) (84%), and only 44 (3%) children were persistently food insecure. As seen in table 1, children with no food insecurity persistently performed better than those with persistent food insecurity in domains of literacy, numeracy, and short-term memory. However, social-emotional skills and self-regulation skills scored no significant changes or relationship to the child's food security level.

Limitations:

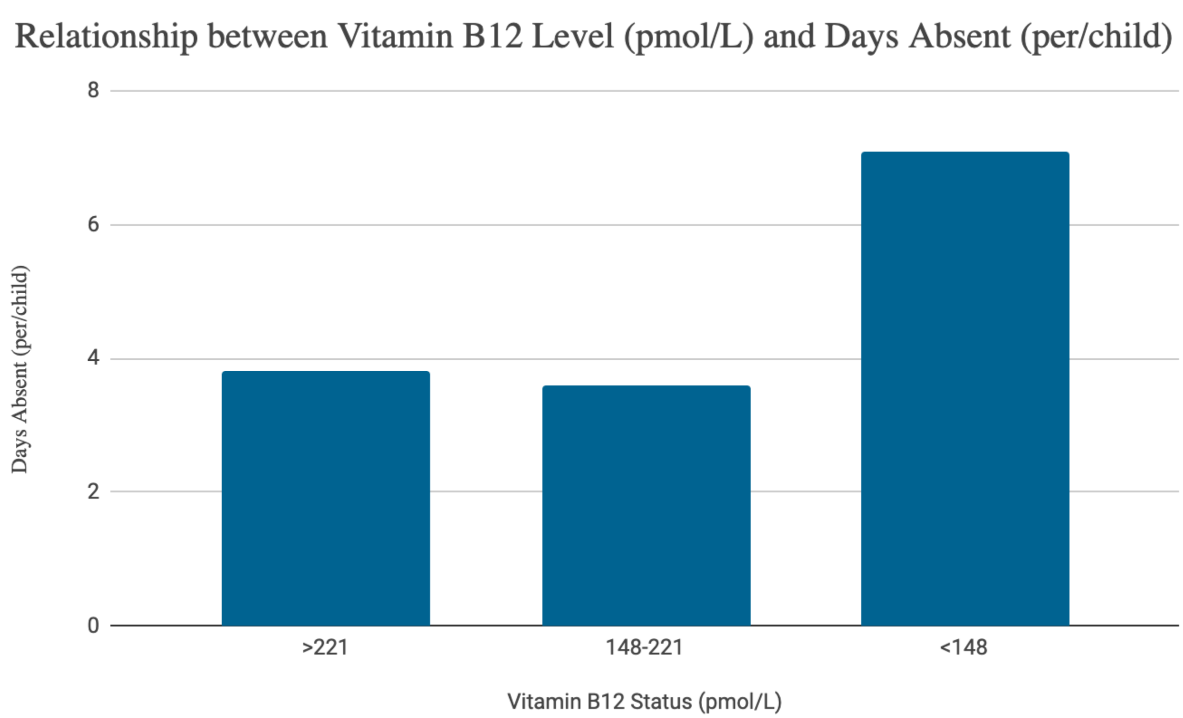
As this study was conducted in the most urbanized city of Greater Accra, the sample group and results may be limited to the sampling frame and selection bias. Additionally, these tests were conducted with children in schools, meaning that children out of school were not assessed, limiting the reliability of the data as not all Ghanian children have the advantage of attending schools.

Vitamin B-12 Deficiency in Children Is Associated with Grade Repetition and School Absenteeism, Independent of Folate, Iron, Zinc, or Vitamin A Status Biomarkers

[Graph 1]



[Graph 2]



Group Studied:
This study was conducted on 2834 children aged 5-12 years of age who were a part of the Bogota School Children Cohort, an investigation on the nutrition and health in public schooled children in Bogota, Columbia. The investigation was carried out in 2006.

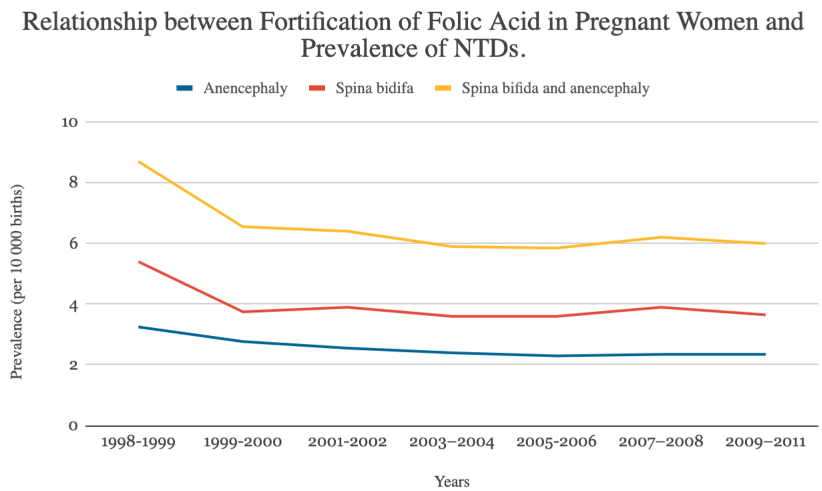
Methods:
Information on the baseline characteristics of children was obtained through parental self-administered questionnaires, which included ranking the level of food security by using a scale adapted from the Spanish-language version of the USDA Household Food Security Survey Module and the Community Childhood Hunger Identification project. Fasting blood samples were also obtained at the beginning of the study and processed to identify plasma erythrocyte folate concentrations and plasma vitamin B-12 concentrations.

Parents or caregivers filled records of days children were absent from school during the academic year after enrollment using a diary returned to the researchers weekly. In addition, the number of children who repeated grades was collected after one full year after enrollment. In Bogota's academic system, a child was considered to fail a grade if they failed three subjects, 2 of which were math and language.

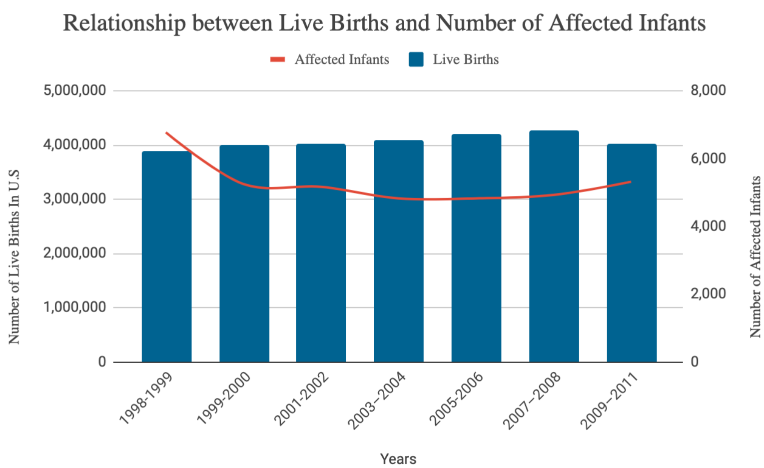
Findings:
The majority of children studied in this cohort had no food insecurity. Still, a large portion of children was, to some degree, food insecure. According to graph 1, the percentage of children who repeated grades or were absent increased from "no food insecurity" to "food insecure, no hunger" but decreased again as the scale progressed to severe food insecurity.
There was also an increase in the days of absence of children with lower vitamin B-12, though no trends were found with the different erythrocyte folate levels.

Limitations:
The intake of vitamin B-12 may not directly correlate and accurately represent the amount absorbed within the body. There is also a possibility of social-economical factors contributing to the higher incidences of grade repetition and absenteeism. Children with poor economic factors may have difficulties obtaining sufficient nutrients for their development and growth, thus impacting their ability to pass academic tests.

Updated Estimates of Neural Tube Defects Prevented by Mandatory Folic Acid Fortification — United States, 1995–2011
[Graph 1]



[Graph 2]



<u>Group Studied:</u>
In August 2014, 19 population-based birth defects surveillance programs in the United States reported to CDC for the number of cases of spina bifida and anencephaly, which are common forms of birth defects. In addition, the spina bifida and anencephaly cases were further recorded after the fortification of foods from 1999-2011 for programs with and without prenatal ascertainment for birth defects.

<u>Methods:</u>
All data obtained from CDC Wonder Database (Centers for Disease Control and Prevention).

<u>Findings:</u>
Comparing the number and percentage of birth defects pre and after folic acid fortification, the number has decreased and remained relatively stable across ten years.

Evaluation

Many studies examined showed a positive relationship between maternal and offspring's folate and vitamin B-12 status. Though vitamin B-12 and folate are both essential micronutrients for the development of the brain, children whose mothers had a high folate measurement performed consistently better in both cognitive and psychological tests, as seen in studies 5 and 6. Similarly, a decrease in folate concentration led to lower test scores and higher grade repetition (seen in studies 5 and 8). On the other hand, children whose mothers had high vitamin B-12 did not necessarily show an increase in cognitive test scores, meaning that vitamin B-12 likely did not influence the neurological development in children as much as folate did (shown in study 4). Also, while maternal vitamin B-12 deficiency is shown to be closely linked to adverse conditions such as anemia in their offspring, folate deficiency may lead to more life-threatening conditions such as open spina bifida or anencephaly. Folate is involved in DNA methylation. Downstream consequences include psychomotor development and red blood cell (RBC) production (Phillips). Higher maternal folate is associated with beneficial effects on infants brain and motor development parents who were vegan or vegetarian during pregnancy can result in improper methylation in offspring, which reduces the child's gene expression and reduces the RBC production resulting in anemia and thus hindering their cognitive functions (Kalyan G et al.)

Likewise, as shown by study 5, the group deficient in vitamin B-12 and folate were likely influenced by their mother's nutrient deficiency during pregnancy. In this study, the children whose mothers had low folate status performed 16% lower on psychological tests such as word order and pattern reasoning. Their scores support the conclusion by indicating that the maternal folate level hinders the children's cognitive development.

While folate deficiency is dangerous, an excess intake of folate can be harmful. The recommended amount of folic acid for pregnant women usually is between 0.4mg and 0.9mg (Kennedy and Koren). For example, a study conducted by Paniz et al. showed that mothers who consumed 5mg of folic acid each day during pregnancy were associated with lower neuropsychological outcomes scores in children. This is because the body can only absorb folic acid to a certain extent. Thus, the excess nutrients will accumulate in the bloodstream, forcing other organs such as bone marrow to increase red blood cells' production to absorb the folic acid, resulting in lower numbers and natural killer (NK) cells (Paniz et al.). This change in the number of NK cells reduces the immunological defence system and increases the offspring's susceptibility to oncogenic viruses (Moon and Powis) (Orange).

To lower the prevalence of neural tube defects in children, many countries such as the US, Canada, UK, and Chile began fortifying commonly consumed foods with folic acid (Bailey et al.). For example, in the US, folic acid was directly fortified into flour, cornmeal, rice and other grain products for 140 mcg of folic acid per 100g in 1998. Fortification significantly decreased the number of NTDs in the US (as shown in study 9). However, pregnant mothers would need to supplement with folic acid well before their expected pregnancy as the neural tube closing occurs after 17-30 days after conception (CDC), and any supplements taken after this phase would not be effective.

Conversely, most of the studies were conducted in rural regions in India, Ghana, and Kenya. The living conditions in these countries are different from the United States, thus impacting mothers' ability to obtain adequate micronutrients during pregnancy. For example, study number 8 (Food Insecurity study) shows that many families struggle to be fed every day; hence, mothers cannot consume adequate folic acid. To counteract this issue, the government began fortifying wheat flour with 2.08mg/kg of folic acid and 0.01mg/kg of B-12. However, upon investigation, only 23% of wheat flour was adequately fortified (Nyumuah et al.) Likewise, in India, the government issued flours and grain manufactures to include at least 1.3mg/kg of folic acid and 0.01mg/kg of B-12 (Food Safety and Standards Authority of India and Bakshi). Yet, in reality, less than 20% of foods produced with a standard for fortification meets national requirements, meaning that it is impossible to consume the required folate levels without supplements (Debadrita Sinclair).

Another factor influencing the mother's micronutrients and the offspring's neurological development is their diet. For example, many regions in India predominantly follow vegetarian or vegan diets due to the limited availability of animal products in average-income families. In a report conducted by the Food Foundation in Pune, almost ¼ children in Pune are stunted, ¼ are wasted, ¼ are underweight (The Food Foundation and Scott). Another study in Pune showed that more than 37% of 210 children that looked healthy between 6 and 23 months of age were deficient in vitamin B-12, thus indicating a strong connection between the offsprings' low vitamin B levels with their culture's predominantly plant-based diet (Kalyan G et al.).

Results in Study 7 further supports the positive correlation between children's cognitive tests scores and their nutritional status from food insecurity. As identified earlier, the maternal vitamin is similar to their offspring's; hence, the food insecure children would signal that their mother was likely deficient in B vitamins. Two factors may contribute to deficiency in mothers: diet and supplement inadequacy. In a study conducted by the University of Ghana, over 53.8 % of women (non-childbearing) are deficient in folate (<10 nmol/L) hough Ghanian dishes usually are vegetarian and include vegetable stews with naturally occurring folate found in leafy greens, less than half of the folate content is retained after heating, thereby unchanging the total blood plasma folate concentration (McKillop et al.) (Owusu et al.) (University of Ghana). Furthermore, more than 12% of people in Ghana live below the International Poverty Line with an average GDP per capita of approximately $1743 ("GDP per Capita (Current US$) - Ghana") (Roser and Ortiz-Ospina). Thus, the low income of each household limits the mother's ability to take folic acid supplements as each 100 tablets accounts for 4.6% of their annual income.

Hence, it is unrealistic for households with food insecurity to continuously supplement folic acid during her 40 weeks of pregnancy, preventing the mother from obtaining optimal concentrations of folic acid. Furthermore, education may be hard to enforce when food security is a prominent issue. Together, these factors also explain the positive relationship between grade repetition and absenteeism with increasing hunger from the Botoga study (shown in study 8).

With minimal access to fortified foods and a plant-based diet, it's challenging for pregnant mothers to consume adequate vitamin B-12 and folate. In comparing Pune and US study groups, the difference in vitamin intake differed by 34ng/mL (135ng/mL compared to 169ng/mL, respectively). Hence, the people's food, along with high levels of school absteensim (shown in study 8) and food insecurities (shown in study 7), may have dramatically impacted the offspring's ability to develop critical thinking skills and perform well in the cognitive and psychological tests.

In this study, we analyze the effects of folate with cognitive function and show how vitamin B-12 and folate (B9) can dramatically impact the performance of their offspring in tests, including information retention, pattern recognition, and language proficiency. Thus, pregnant mothers should be monitoring their consumption of folate and other micronutrients to ensure that their offspring’s blood nutrient is not deprived of avoiding conditions such as spina bifida or anencephaly. As we analyzed the experiments, we found a limitation in the relatively small sample sizes, making them inevitable to sampling bias and, hence, increasing the conclusions’ uncertainty. However, there was a clear trend with low vitamin B consumption and a lower cognitive score. This conclusion prompts the research for finding the specific genes that may have been muted due to vitamin B deficiency to examine how to prevent folate vitamin B12 deficiency in developing/third world countries.

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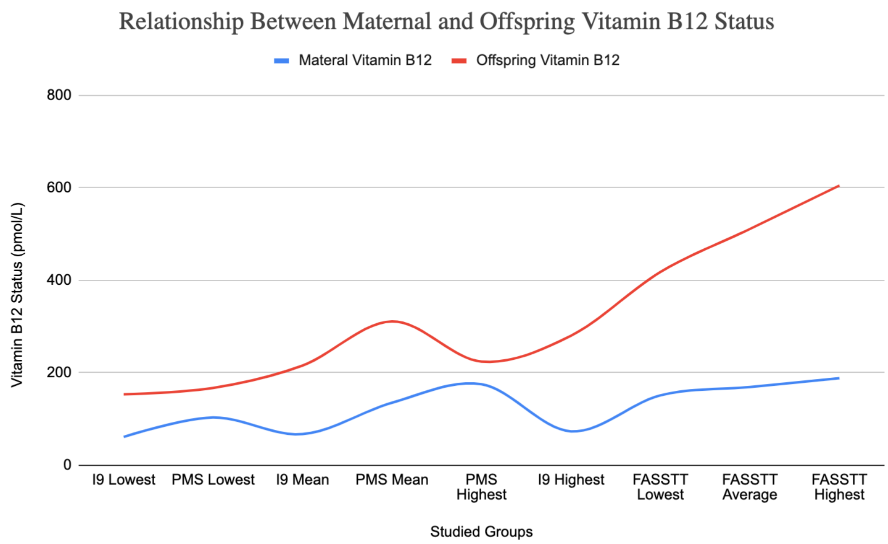
Appendixes:

All Data

[1]: The Relationship between Maternal Plasma Vitamin B-12 (ng/mL) and Offspring Vitamin B-12 (ng/mL)

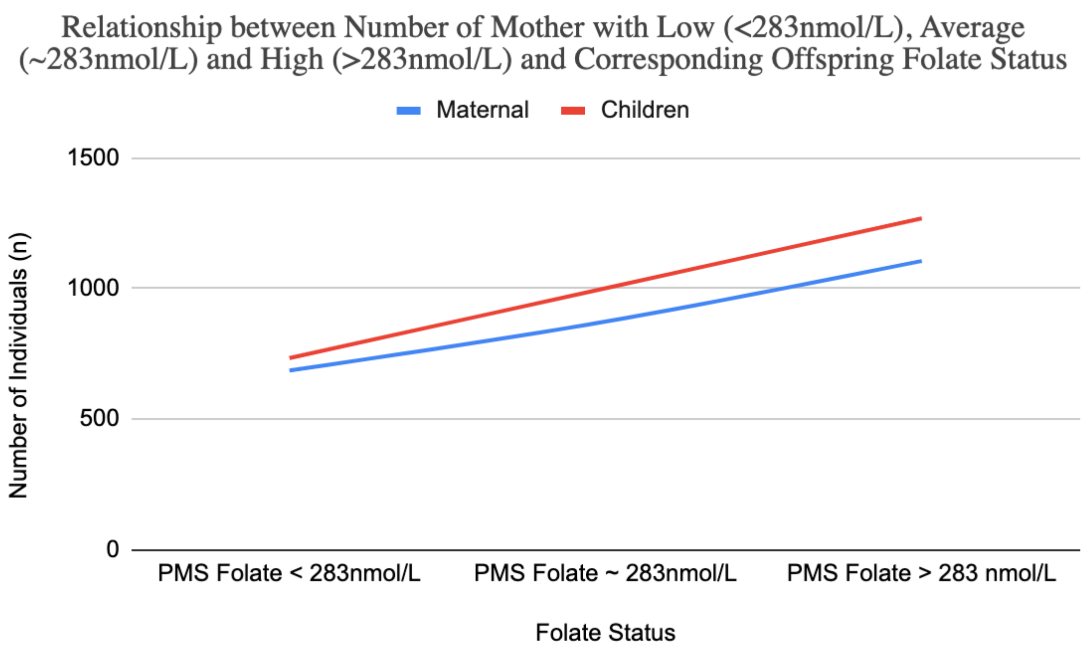
	Maternal Vitamin B-12	Offspring Vitamin B-12
I9* Lowest	61	153
PMS** Lowest	103	167
I9 Mean	67	215.5
PMS Mean	135	311
PMS Highest	175	224
I9 Highest	73	280
FASSTT*** Lowest	151	418
FASSTT Average	169	511
FASSTT Highest	188	605

*I9: Higher Maternal Plasma Folate associated with better cognitive function scores in 9-10 year old children in South India
**PMS: Pune Maternal Study
***FASSTT: Effects of maternal folic acid supplementation during the second and third trimesters of pregnancy on neurocognitive development in the child: an 11-year follow-up from a randomised controlled trial



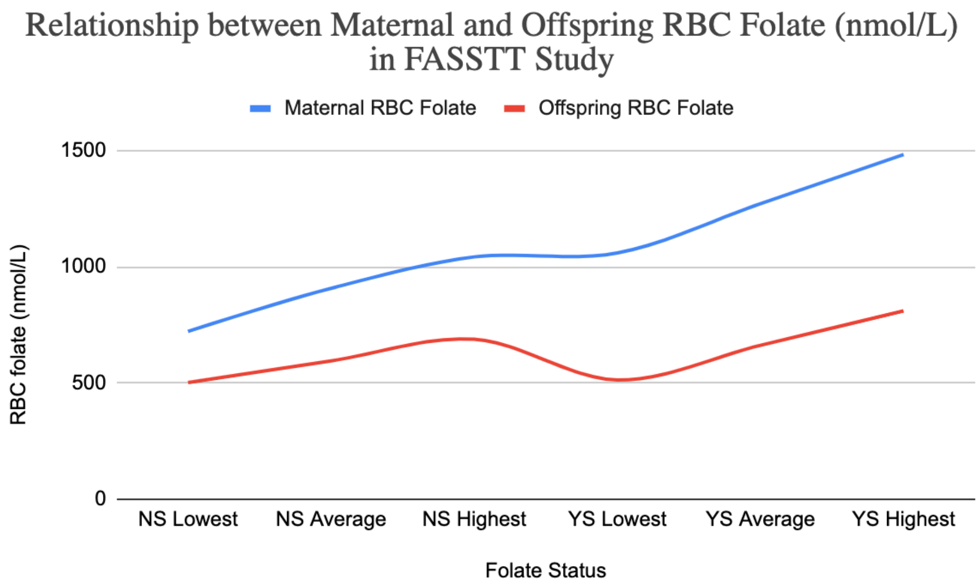
[2]: Relationship between Maternal Folate and Offspring Folate Status from Pune Maternal Study

Relationship between Maternal Erythrocyte, Total Homocysteine, and Methylmalonic Acid (μmol/l)			
	Erythrocyte folate (nmol/L)	plasma total homocysteine (μmol/l) (tHcy)	plasma methylmalonic acid (μmol/l)
PMS Folate < 283nmol/l	687	6.8	0.5
PMS Folate ~ 283nmol/l	874	8.1	0.8
MS Folate > 283	1106	10.3	1.34



[3]: Effects of maternal folic acid supplementation during the second and third trimesters of pregnancy on neurocognitive development in the child: an 11-year follow-up from a randomised controlled trial (FASSTT)

Relationship between Maternal and Offspring RBC Folate (nmol/L)		
	Maternal RBC Folate (nmol/L)	Offspring RBC Folate (nmol/L)
NS Lowest	722	502
NS Average	907	595
NS Highest	1042	688
YS Lowest	1059	513
YS Average	1271	662
YS Highest	1482	810



NS: No supplementation; YS: Yes supplementation (of folic acid)

<u>Group Studied:</u>
This study was a follow-up investigation in 11-year-old children whose mother participated in the FASSTT trial in pregnancy. The FASSTT trial contained women aged 18-35 years who were given supplementations of 400ug folic acid/day or a placebo until the end of their pregnancy.

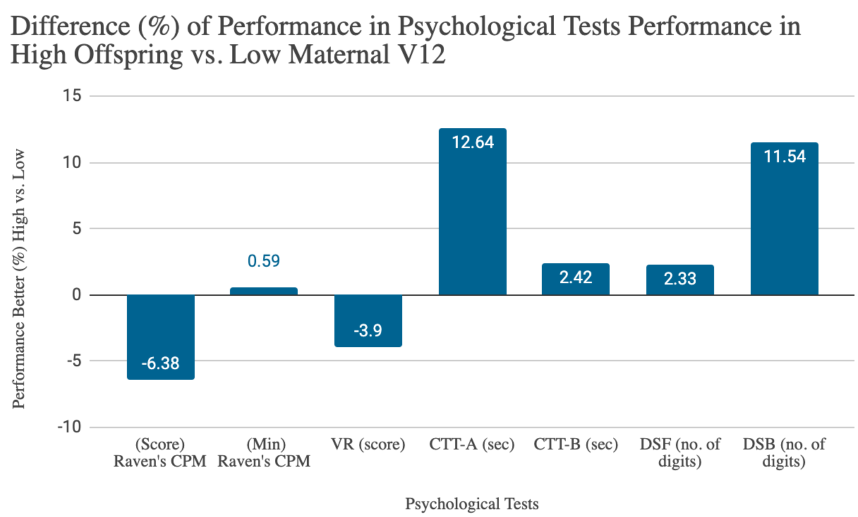
<u>Methods:</u>
For B-Vitamin measurements in the mothers, non-fasting blood samples were collected at 14th and 36th GW and the umbilical cord blood was collected at delivery. For the children, non-fasting blood samples were collected from a pediatric phlebotomist and processed within 4 hours for serum vitamin B-12.

<u>Limitations:</u>
In the FASSTT trial, 296 women were initially recruited, and 119 mothers completed the full FASST trial. In this follow-up trial, only 68 pairs of mother-child were available for further investigation (placebo n=31, folic acid n=37). The relatively small sample size of mother-child pairs may have limited the ability to detect more differences in the biomarkers of mothers and children for each nutrient collected.

[4]: Vitamin B-12 status of pregnant Indian women and cognitive function in their 9-year-old children

	Raven's CPM (score)	Raven's CPM (min)	Visual Recognition (score)	CTT-A (sec)	CTT-B (sec)	Digit Span Forwards (no. of digits)	Digit Span Backwards (no. of digits)
Children with Low V12 Mother (< 77 pM) (n=49, 19 boys)	23.5	16.9	7.7	182	289	4.3	2.6
Children with High V12 Mother (>224 pM) (n=59, 31 boys)	22	16.8	7.4	159	282	4.4	2.9
Perform Better (%) High vs. Low	-6.38	0.59	-3.90	12.64	2.42	2.33	11.54

Example Calculation: CTT (less is better) (159-182) / 182 x 100 = -12.64% Faster



<u>Group Studied:</u>

This trial investigated the children born in the Pune Maternal Nutrition Study. The research was conducted from six villages near Pune between 1993 and 1997, which recorded the plasma vitamin B-12 concentrations at 28 wells of gestation. The children tested in this experiment were investigated at nine years of age in 2005. The children were grouped according to their mother's low plasma vitamin B-12: Low plasma vitamin B-12 = < 77 pM (n=49), high plasma vitamin B-12 > 224 pM (n=59).

<u>Methods:</u>

Four cognitive tests were carried out and scored according to Indian norms. Please see appendix A for more details.

<u>Findings:</u>

As seen in table 2, children with low maternal concentrations performed worse than children in group 2 with high maternal vitamin B-12. For example, in the CCT-A test, group 1 children on average took 182 seconds while group 2 children only took 159 seconds to complete the task of connecting numbers serially, roughly 13% faster. Additionally, group 2 children performed better on digit-span backwards, where group 2 recalled on average 2.9 digits compared to 2.6 digits in group 1, correlating to a roughly 12% increase in performance. However, there were no significant differences for visual recognition or intelligence tests as Raven's CMP.

Limitations:

Though the trials conducted tested many different domains of cognitive functions, the relatively small sample size of children may limit the extent to which the data of their plasma vitamin B-12 concentration and cognitive abilities are reliable and consistent with other children of the same conditions.

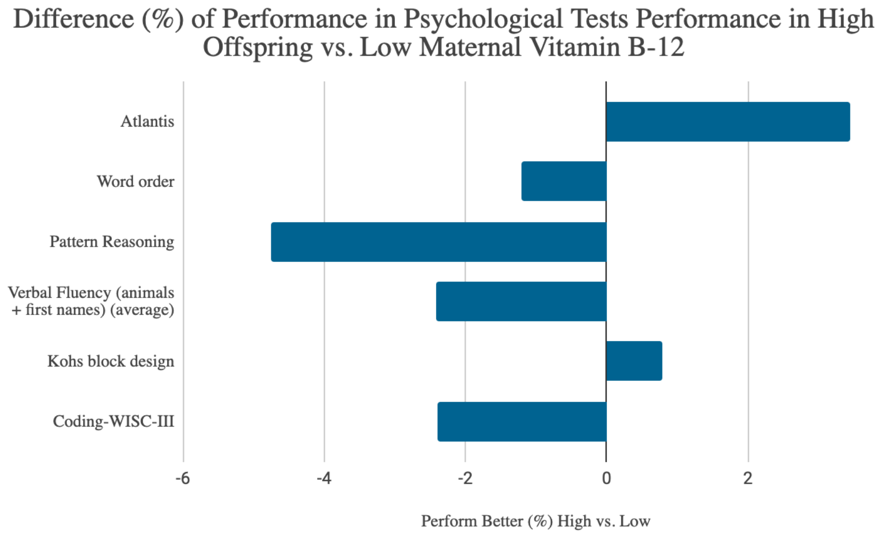
[5]: Higher Maternal Plasma Folate associated with better cognitive function scores in 9 to 10 year old children in South India

Relationship between Vitamin B-12 Status and Psychological Tests						
	Atlantis	Word order	Pattern Reasonin g	Verbal Fluency (average)	Kohs block design	Coding-WISC-III
South India Children, Maternal Low Vitamin B-12 (≤124 pmol/L)	69.6	16.5	10.5	14.5	76.6	33.2
South India Children, Maternal Normal Vitamin B-12 (125-162 pmol/L)	69	16.5	10	14.25	73.4	33.3
South India Children, Maternal Higher Vitamin B-12 (163-220 pmol/L)	64.6	16.3	9	13.45	77.2	31.8
South India Children, Highest Maternal Vitamin B-12 (>220 pmol/L)	67.2	16.3	10	14.15	77.2	32.4
Perform Better (%) High vs. Low	3.45	-1.21	-4.76	-2.41	0.78	-2.4

Average Value Calculation: South India Children, Low Maternal V12: (12.4 + 16.6)/2 = 14.5

Better performance calculation: Atlantis: 67.2 - 69.6 = 2.4/69.6 x 100 = -3.45 % Faster

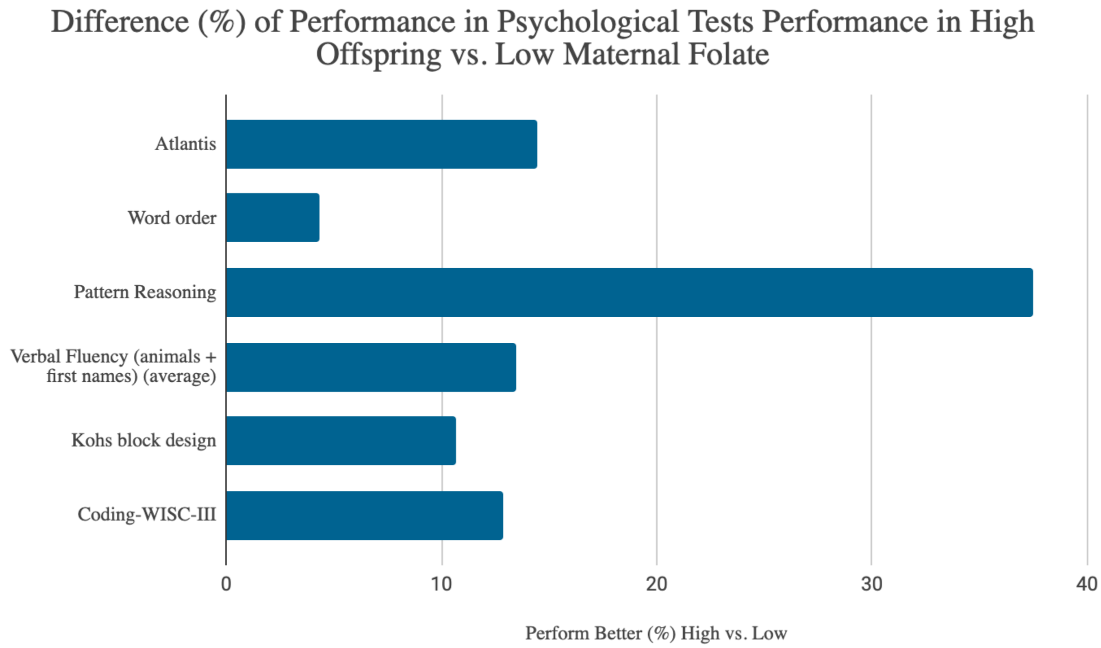
[Graph 1]



Relationship between Vitamin B-12 Status and Psychological Tests						
Folate (nmol/L)	Atlantis	Word order	Pattern Reasonin g	Verbal Fluency (average)	Kohs block design	Coding-WISC-III
South India Children, Maternal Low Folate (≤17.4)	63.5	16.1	8	13	74	30.4
South India Children, Maternal Normal Folate (17.5-33.9)	67.3	16.2	9	14	73.1	32.6
South India Children, Maternal Higher Folate (34-50.6)	66.9	16.7	10	14.45	77.2	33.4
South India Children, Maternal Highest Folate (≥50.7)	72.7	16.8	11	14.75	81.9	34.3
Perform Better (%) High vs. Low	14.49	4.35	37.50	13.46	10.66	12.83

Calculation Better Performance: High vs. Low Atlantis: 63.5 - 72.7 = 9.2/63.5 x 100 = -14.49% faster

[Graph 2]



<u>Group Studied:</u>
This experiment examined the children aged 9-10 (n=536, boys=259, girls=277) years from the Mysore Partenon study conducted in 1997-1998, which focused on the incidence and effects of short-term and long-term gestational diabetes mellitus impacts on newborns.

<u>Methods:</u>
The child's maternal vitamin B-12 and folate status biomarkers were obtained using maternal blood samples collected at 30 ± 2 wk of gestation.
The cognitive assessments tested specific domains in the Carroll model, three core tests from the Kaufman Assessment Battery for children, and other tests adapted to the local cultural context. Specifically, it tests domains of long-term memory and retrieval ability (Atlantis), short-term memory (word order), reasoning ability (pattern reasoning), language production (verbal fluency), visuospatial ability (Kohs' block design), and visuomotor processing and coordination with the WISC-III (coding-Wechsler Intelligence Scale for Children-III. (see appendix B for details)

<u>Findings:</u>
According to Graph 1, the children with higher plasma vitamin b-12 performed better on long-term memory and retrieval (Atlantis) but were weaker in areas of word order, pattern reasoning, verbal fluency, coding-WISC-III. Thus, there is no clear association between the level of plasma v12 concentration to the child's cognitive abilities. Looking at Graph 2, an increase in maternal folate led to higher cognitive scores in children, as children with the highest maternal folate consistently performed better than children with low maternal folate in all tests of cognitive domains, with the most evidence in the increased score performance score of 37.5% concluded by the comparison of scores from higher maternal folate versus low maternal folate (Veena, Sargoor R et al).

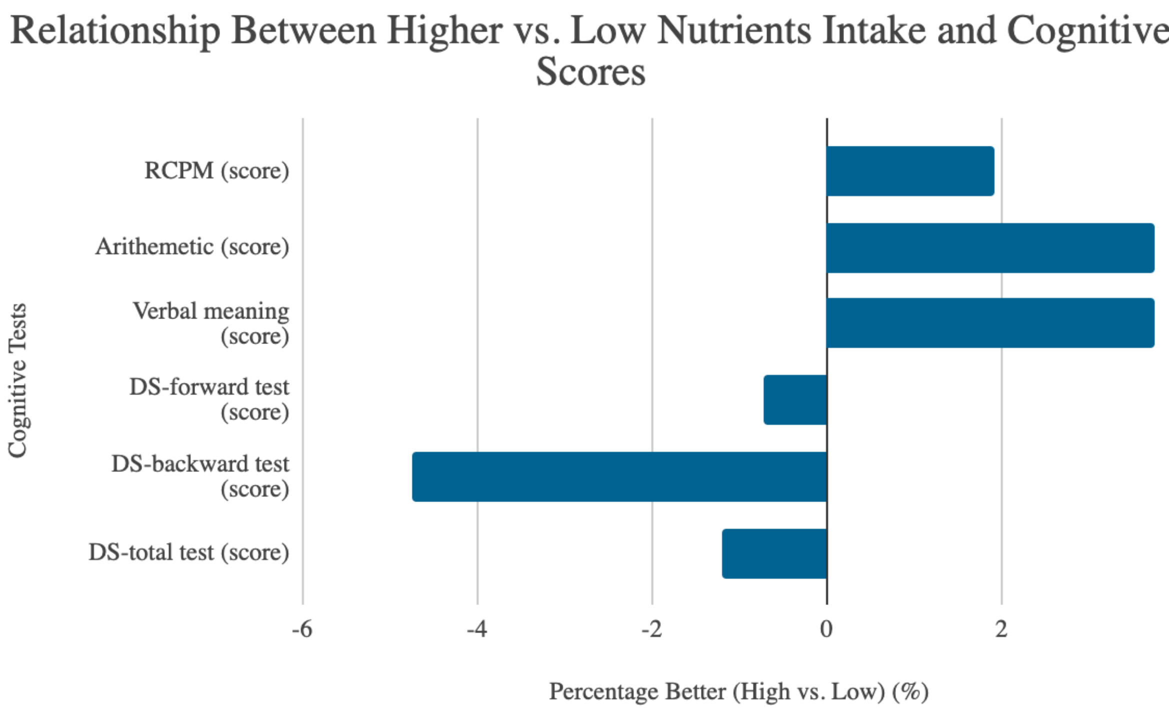
Limitations:
Although higher maternal folate led to higher scores from their children, other factors may have contributed. Folate is most commonly found in animal products, which is relatively more expensive compared to vegetables. Thus, the disadvantaged families may have less access to higher-quality foods and meats and a generally lower standard of living, impacting the quality of education that their children receive, contributing to lower cognitive scores.

[6]: Dietary micronutrients are associated with higher cognitive function gains among primary school children in rural Kenya (Boys Average Age: 7.8, Girls Average Age: 7.4)

Average Daily Nutrient Intakes of Boys and Girls in Study			
	All	Boys	Girls
Energy (kcal)	1792	1857	1724
Protein	53.95	56	51.6
Vitamin B-12 (ug)	0.64	0.65	0.62
Folate (ug)	514	541	484

Cognitive Assessments Scores in Children				
	All	Boys	Girls	Differences (boys vs. girls)
RCPM (score)	17.37	17.53	17.2	1.92
Arithmetic (score)	7.07	7.19	6.93	3.75
Verbal meaning (score)	27.1	27.6	26.6	3.76
DS-forward test (score)	2.79	2.78	2.8	-0.71
DS-backward test (score)	2.26	2.21	2.32	-4.74
DS-total test (score)	5.05	4.99	5.11	-1.19

Calculation: DS-total test score: (4.99-5.05) / 5.05 x 100 = -1.19%
**Boys had overall high folate and vitamin nutrient intake, thus the chart below



**Note that boys are "higher" nutrient group as indicated by the nutrients table above

Group Studied: This study was carried out in Kyeni South Division of Embu District in Eastern Province, Kenya, from 1998 to 2000. Five hundred fifty-four children enrolled in grade 1 (average 7.4 years) from twelve selected primary schools participated.

Methods: The dietary information for each child was collected based on monthly and bi-monthly 24h recall data in the duration of the study from an interview with the child's mother or caretaker and a CNP snack consumption provided by the child's school. For the cognitive tests, please refer to appendix C.

Findings: The results shown indicate that boys had an overall higher intake of energy and protein compared to girls. As folate is mainly found in animal products, a higher overall folate concentration may be inferred from the nutrient markers. According to the graph, boys with higher nutrition intakes performed better on RCPM, arithmetic, and verbal meaning scores. On the other hand, girls performed better on both digit-span tests, which tests short-term memory, long-term memory and retrieval. Thus, there are clear positive associations between any of the nutrients and the cognitive abilities of children.

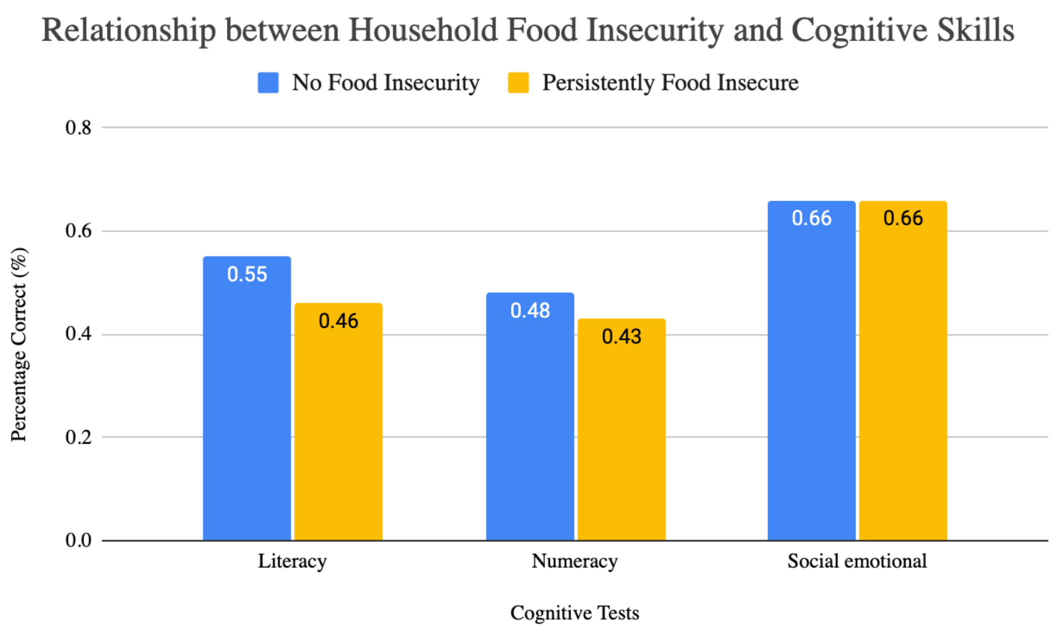
Limitations: A 24h recall method to record dietary intakes may only estimate the total nutrients obtained by each individual. In addition, as each family may have different recipes for traditional dishes, the nutrient intakes may be relatively hard to measure accurately.

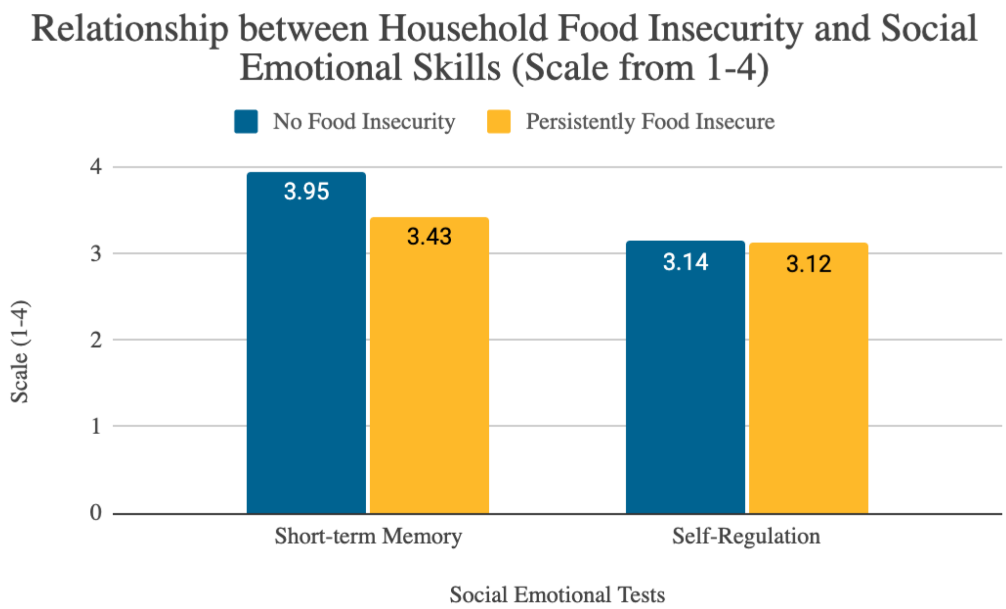
[7]: Household food insecurity and early childhood development: Longitudinal evidence from Ghana

[Table 1]

Relationship between Household Food Insecurity and Cognitive Skills					
	Literacy	Numeracy	Social emotional	Short-term Memory	Self-Regulation
Full Sample	0.53	0.47	0.66	3.5	3.12
No Food Insecurity (n=1116)	0.55	0.48	0.66	3.95	3.14
Transitory Food Insecure (n=173) (13%)	0.51	0.45	0.66	3.66	3.09
Persistently food insecure (n=44) (3%)	0.46	0.43	0.66	3.43	3.12
Perform Better (%) No vs. Persistent	19.57	11.63	0	15.16	0.64

Sample Calculation: (0.46-0.5) / 0.46 x 100= -19.57% Better
A score based on the percent correct for each domain was computed
Items were scored on a 1–4 scale and coded such that higher scores indicated better self-regulation





Group Studied:

In this study, over 1300 Ghanian children were tracked over three years to investigate the effects of household food insecurity and deficiencies in nutrients with early childhood development in lower primary schools. The group studied takes place in the most disadvantaged regions of the Greater Accra Region, based on the 2014 UNICEF District League Table. This table indicates the regions in Ghana according to their development of essential services such as education, health, sanitation, and governance.

Methods:

The experiment was conducted at three different time waves: wave 1 (September 2015), wave 2 (May 2017), and wave 3 (May 2018). These children and their caregivers were interviewed via a survey to collect baseline characteristics and data.

They assessed the cognitive abilities of children by literacy, numeracy, short-term memory, social-emotional development and self-regulation tests through the Early Grade Reading Assessment (Literacy), the Early Grade Math Assessment (Numeracy), forward digit span tests (short-term memory), subtests from IDELA, and with an adapted version of Preschool Self-Regulation Assessment–Assessor Report (PSRA-AS) (Self-regulation), respectively. Please refer to appendix D for more details.

Findings:

It is seen that most of the children in this study had no food insecurity (n=1116) (84%), and only 44 (3%) children were persistently food insecure. As seen in table 1, children with no food insecurity persistently performed better than those with persistent food insecurity in domains of literacy, numeracy, and short-term memory. However, social-emotional skills and self-regulation skills scored no significant changes or relationship to the child's food security level.

Limitations:

As this study was conducted in the most urbanized city of Greater Accra, the sample group and results may be limited to the sampling frame and selection bias. Additionally, these tests were conducted with children in schools, meaning that children out of school were not assessed, limiting the reliability of the data as not all Ghanian children have the advantage of attending schools.

[8]: Vitamin B-12 Deficiency in Children Is Associated with Grade Repetition and School Absenteeism, Independent of Folate, Iron, Zinc, or Vitamin A Status Biomarkers

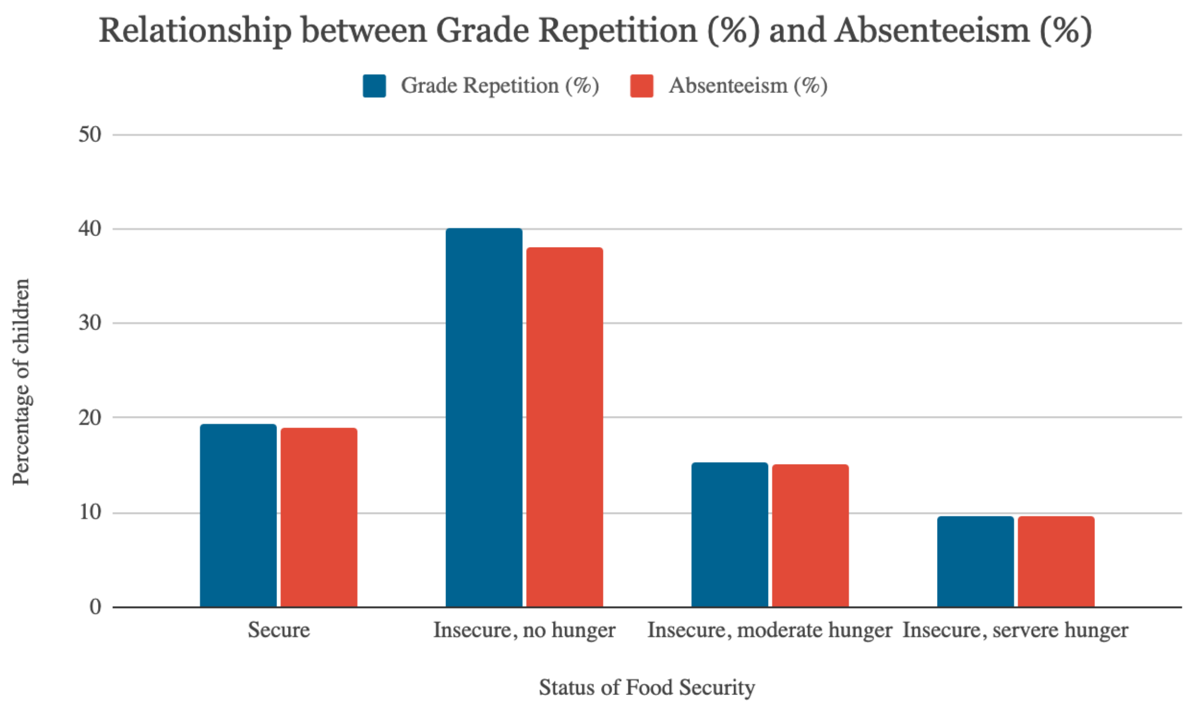
Relationship between Food Insecurity and Grade Repetition and School Absenteeism					
	Grade Repetition (n)	Grade Repetition (%)	Absenteeism (n)	Absenteeism (%)	Days Absent per/Child
Food Insecurity					
Secure	551	19.44	587	19.03	3.5
Insecure, no hunger	1105	40.00	1172	38	3.4
Insecure, moderate hunger	433	15.28	466	15.11	4.8
Insecure, servere hunger	271	9.56	294	9.53	3.8
Erythrocyte folate, nmol/L					
Q1 (median=620)	623	21.98	667	21.63	3.7
Q2 (median=671)	623	21.98	669	21.70	3.8
Q3 (median=893)	622	21.95	668	21.66	3.8
Q4 (median=1101)	625	22.05	667	2163	3.5
Plasma vitamin B-12, pmol/L					
<148	37	1.31	43	1.39	7.1
148-221	383	13.51	401	13.00	3.6

>221	2074	73.18	2231	72.34	3.8

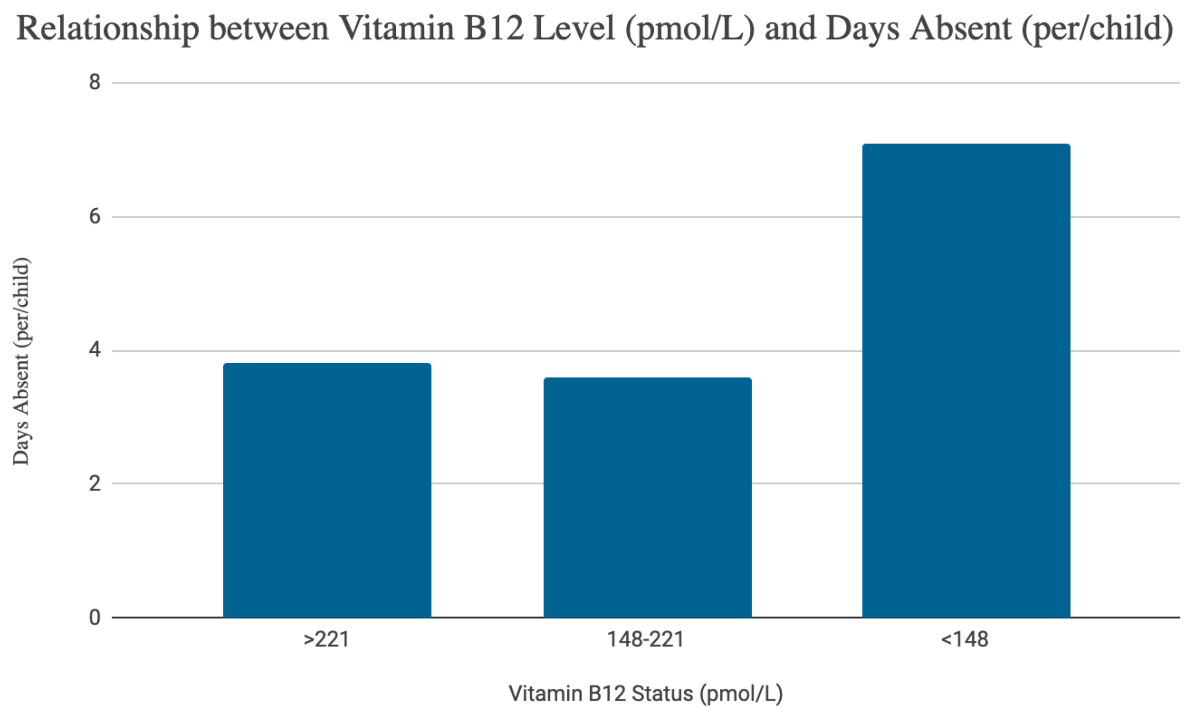
n=2834 for grade repetition and n=3084

Calculation for percentage of children grade repetition: Secure and grade repetition: (551/2834) x 100 = 19.44%

[Graph 1]



[Graph 2]



<u>Group Studied:</u>
This study was conducted on 2834 children aged 5-12 years of age who were a part of the Bogota School Children Cohort, an investigation on the nutrition and health in public schooled children in Bogota, Columbia. The investigation was carried out in 2006.

<u>Methods:</u>
Information on the baseline characteristics of children was obtained through parental self-administered questionnaires, which included ranking the level of food security by using a scale adapted from the Spanish-language version of the USDA Household Food Security Survey Module and the Community Childhood Hunger Identification project. Fasting blood samples were also obtained at the beginning of the study and processed to identify plasma erythrocyte folate concentrations and plasma vitamin B-12 concentrations.

Parents or caregivers filled records of days children were absent from school during the academic year after enrollment using a diary returned to the researchers weekly. In addition, the number of children who repeated grades was collected after one full year after enrollment. In Bogota's academic system, a child was considered to fail a grade if they failed three subjects, 2 of which were math and language.

<u>Findings:</u>
The majority of children studied in this cohort had no food insecurity. Still, a large portion of children was, to some degree, food insecure. According to graph 1, the percentage of children who repeated grades or were absent increased from "no food insecurity" to "food insecure, no hunger" but decreased again as the scale progressed to severe food insecurity.

There was also an increase in the days of absence of children with lower vitamin B-12, though no trends were found with the different erythrocyte folate levels.

Limitations:
The intake of vitamin B-12 may not directly correlate and accurately represent the amount absorbed within the body. There is also a possibility of social-economical factors contributing to the higher incidences of grade repetition and absenteeism. Children with poor economic factors may have difficulties obtaining sufficient nutrients for their development and growth, thus impacting their ability to pass academic tests.

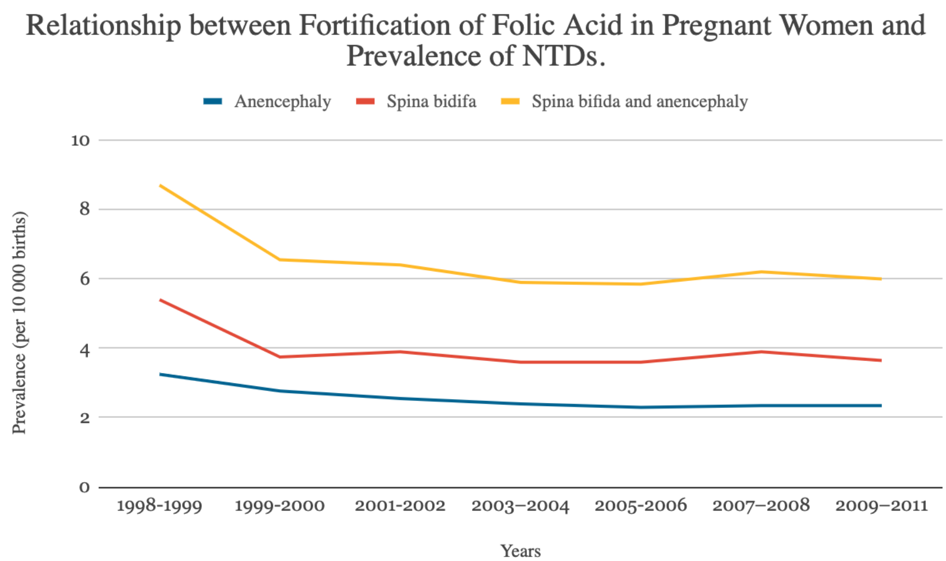
[9]: Updated Estimates of Neural Tube Defects Prevented by Mandatory Folic Acid Fortification — United States, 1995–2011

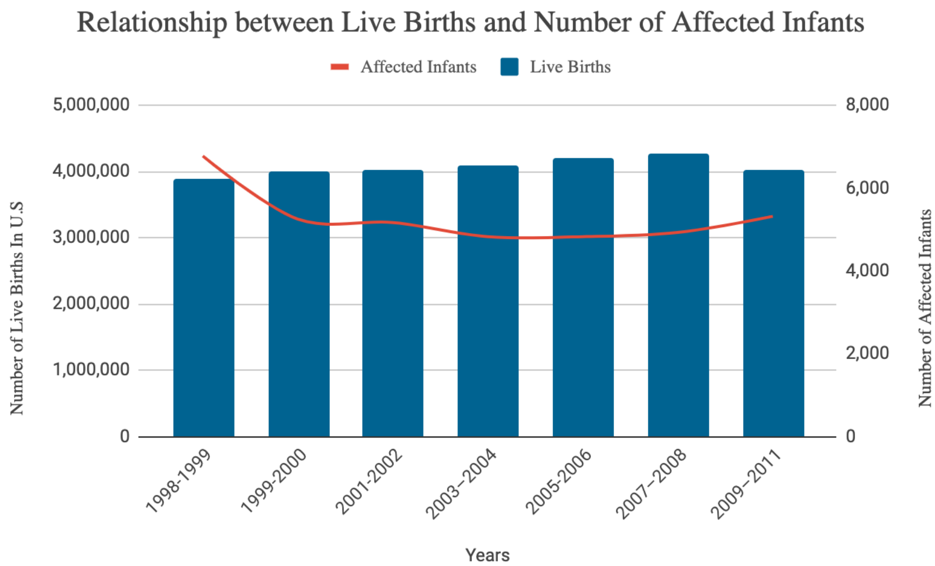
Relationship between Fortification of Folic Acid and Prevalence of NTDs in 10 000 Infants							
	Pre-fortification (average)	Post 1999-2000 (average)	Post 2001-2002 (average)	Post 2003–2004 (average)	Post 2005-2006 (average)	Post 2007–2008 (average)	Post 2009–2011 (average)
Anencephaly							
Prevalence	3.25	2.77	2.55	2.4	2.3	2.35	2.35
Estimated annual cases (95% Cl)	1270.5	1114.5	1025.5	941.5	961.5	997	943.5
Spina bifida							
Prevalence	5.4	3.75	3.9	3.6	3.6	3.9	3.65
Estimated annual cases (95% Cl)	2117	1511	1559	1471.5	1503	1662	1459.5
Anencephaly and Spina bifida							
Prevalence	8.7	6.55	6.4	5.9	5.85	6.2	6
Estimated annual cases (95% Cl)	3388	2625.5	2584.5	2413	2464	2659	2403.5
Sum of all cases	6775.5	5251	5169	4826	4928.5	5318	4806.5

Percentage Decrease From Pre-Fortification (Base year) (%)	0	22.5	23.71	28.77	27.26	21.51	29.06

Calculation of Average Prevalence and Pre-fortification number of cases from "Programs without" and "with prenatal ascertainment": (4.2 + 2.3)/2 = 3.2
Percentage decrease: (2625.5+1511+1114.5)-(3388+2217+1270.5)/(3388+2217+1270.5) x100 = -37.36%
Prevalence (per 10,000 live births)
Sum of all cases: 3388 + 2117+ 1270.5 = 6775.5

Live Births Affected by NTDs in Comparison with the Entire Population							
	1998-1999	1999-2000	2001-2002	2003–2004	2005-2006	2007–2008	2009–2011
Affected	6775.5	5251	5169	4826	4928.5	5318	4806.5
Live Births	3,895,542	4,009,116	4,023,830	4,101,001	4,201,952	4,281,964	4,027,880





Group Studied:

In August 2014, 19 population-based birth defects surveillance programs in the United States reported to CDC for the number of cases of spina bifida and anencephaly, which are common forms of birth defects. In addition, the spina bifida and anencephaly cases were further recorded after the fortification of foods from 1999-2011 for programs with and without prenatal ascertainment for birth defects.

Methods:

All data obtained from CDC Wonder Database (Centers for Disease Control and Prevention).

Findings:

Comparing the number and percentage of birth defects pre and after folic acid fortification, the number has decreased and remained relatively stable across ten years.

Cognitive Test Criteria:

[A] Vitamin B-12 status of pregnant Indian women and cognitive function in their 9-year-old children

Raven's CPM:
"Raven's Colored Progressive Matrices (CPM) is a group-administered test to measure intelligence. A series of patterns from which one piece is missing is shown to the child, who is asked to choose the correct piece from six options. The number of correct responses and the time taken to complete the tests are recorded."

Visual Recognition
Visual Recognition is a test of visual agnosia. The child is shown 10 pictures of different objects and asked to name them. The number of correct responses is recorded.

CTT: Colour Trail Test
"The Color Trail test (CTT) measures sustained attention and executive function. Colored circles (yellow and pink) are numbered 1 to 25, and the child is required to connect numbers serially, at two levels of complexity (A and B).
CTT-A is a sustained-attention task, whereas **CTT-B** involves complex thinking and is a test of executive function. The time taken to complete each part of the test and the number of errors are recorded"

(a) CTT-A: a test of sustained visual attention involving perceptual tracking and simple sequencing which requires the use of the frontal lobe. The frontal lobe controls cognitive skills such as emotions, memory, problem solving, and impulse control.
(b) CTT-B: assesses higher-order functioning of the frontal systems and is more complex than part A. The digit span test will assess the ability of the temporal lobe, which is associated with long-term memory and is crucial for object recognition and language recognition (Johnson).

The Digit Span test measures short-term or working memory. A series of numbers or digit strings of increasing length is read aloud, and the child has to repeat the numbers in a forward and backward sequence. The longest digit string recalled is considered the span

[B]: Higher Maternal Plasma Folate associated with better cognitive function scores in 9 to 10 year old children in South India

Supplemental Table 1. Description of the cognitive tests used in the study

Tests from KABC[1]-II		
Name of the test	Description	Cognitive abilities
Atlantis	The child is taught nonsense names for fish, plants and shells and is asked to point to the named object among an array of pictures	Learning ability/long-term storage and retrieval, associative memory
Word order	The child points to a series of silhouettes of common objects in the same order as mentioned by the examiner; an interference task (color naming) is added between the stimulus and the response for the more difficult items	Memory span, short term memory, working memory
Pattern Reasoning	The child completes a pattern by selecting the correct image from a set of 4 to 6 options shown; most stimuli are abstract, geometric shapes and the difficulty of the task increases as the test progresses.	Reasoning abilities such as induction and deduction and fluid reasoning
Additional tests		
Verbal fluency a) Animals b) First names	The child is asked to name as many animals as possible in 1 minute and then asked to name as many first names as possible in 1 minute.	Broad retrieval ability; speed and flexibility of verbal thought process; neuropsychological test of language production
Kohs block Design	A psychometric test in which the child arranges groups of 4, 9, or 16 multi-colored blocks to copy picture designs presented on test cards.	Visuo-spatial problem solving, visual perception and organization
Coding-WISC[2]-III	The child has to substitute specific symbols for numbers presented in boxes, and complete as many items as possible in 2 minutes.	Visual-motor processing speed and coordination, short term memory, visual perception, visual scanning, cognitive flexibility, attention

[1] Kaufman assessment battery for children-2nd edition (36)
[2] Wechsler Intelligence Scale for Children-3rd edition (40)

[C]: Dietary micronutrients are associated with higher cognitive function gains among primary school children in rural Kenya

"**RCPM** was used to assess problem-solving abilities and requires observation and understanding of abstract patterns and sequences(33, 36). Each child was presented with a matrix-like arrangement of symbols and asked to select the correct symbol to complete the pattern. No time limit was set to solve each problem. A total of thirty-six items were administered to each child."

"**The verbal meaning** test is similar to the Peabody picture vocabulary test but with pictures, and tests for expressive language abilities and verbal skills obtained over time. The test was designed in East Africa and has been previously used in Kenya, Uganda and Tanzania. The child was presented with four pictures and asked to point to the one named by the tester. A total of thirty-six items were administered to children, with simpler items requiring recognition of simple nouns, whereas the most advanced items were more abstract concepts. No time limit was set to identify each picture. The pictures and items used in the verbal meaning and arithmetic tests were modified to be more relevant to the Embu context and have been extensively used in a previous study in Embu."

"**The arithmetic test** was adapted from the Wechsler Intelligence Scales for Children – Revised (Psychological Corporation, New York, NY, USA)(41) and was used to assess basic knowledge of arithmetic. The child was asked to add and subtract simple numbers and then proceed to more difficult items involving division, multiplication and decimals. The test consisted of nineteen orally presented arithmetic word problems and a time limit of 30 s to reply verbally with the answer to each problem."

"**The DS tests** for short-term memory and concentration, and measures the child's ability to remember and repeat aloud a sequence of numbers. The test consisted of fourteen items for the forward and backward recall. The tester read increasingly longer strings of numbers, which the child was asked to recall and repeat in forward order for the DS-forward test and in backward order for the DS-backward test."

Food Intake Information:
"Twenty-four-hour recall interviews were conducted for a total of 19 d (at least 1 d per month) over a 2-year period. CNP snacks were served in the classrooms every school day by project-trained feeding assistants. The project feeding assistants recorded absences, types and amount of snacks served and leftovers for each child."

[D]: Food Insecurity in relationship to cognitive abilities in Ghana Study

Household Food Insecurity Trajectories:

The Household Hunger Scale (HHS) was administered at each survey round to the child's caregiver. The scale includes the following three items:

(a) "In the past [4 weeks/30 days], was there ever no food to eat of any kind in your house because of lack of resources to get food?"
(b) "In the past [4 weeks/30 days], did you or any household member go to sleep at night hungry because there was not enough food?"
(c) "In the past [4 weeks/30 days], did you or any household member go a whole day and night without eating anything at all because there was not enough food?".

For each item, if households indicated yes, the frequency of occurrence in the past four weeks of the specific condition was also asked (1 = rarely (1–2 times), 2 = sometimes (3–10 times), and 3 = often (more than 10 times)). Based on this score generated using HHS (0-6), households were categorised with no or little hunger if the HHS score is 0–1; with moderate hunger for HHS scores is 2–3; and with severe hunger for HHS scores equal to 4–6.

Literacy skills were assessed through five domains of skills which were based primarily on the Early Grade Reading Assessment. These included expressive vocabulary, listening comprehension (in both English and the child's mother tongue), letter-sound identification, and non-word decoding. A measure of phonological awareness from the International Development and Early Learning Assessment (IDELA) was also included.

Numeracy skills were assessed through five domains using the Early Grade Math Assessment. These included number identification, quantity discrimination, addition, subtraction, word problems, and missing number pattern identification. A score based on the percent correct for each domain was computed.

Short-term memory, a common measure of cognition, was measured using the forward digit span, where children were presented seven iterations of a string of numbers (two to six digits) and asked to repeat them back; children received a score of 0–7 based on the number of items answered correctly ($\alpha = 0.66$). We also examined two non-academic skills.

Social-emotional development was assessed using five subtasks from the IDELA measured with 14 items grouped into self-awareness, emotion identification, perspective taking and empathy, friendship, and conflict and problem solving. A score based on the percent correct for each domain was computed.

Self-regulation was measured using an adapted version of the Preschool Self-Regulation Assessment–Assessor Report (PSRA-AS). PSRA-AR was designed to assess self-regulation of emotions, attention, and behaviour of children. The instrument was shortened and adapted for the Zambian context and used in other LMIC samples. This version consisted of 13 items focused on the child's attention and behaviour. Sample items include "pays attention during instructions and demonstrations", "remains in seat appropriately during test", "modulates and regulates arousal level in self," and "child shows intense angry/irritable feelings and/or behaviours." Items were scored on a 1–4 scale and coded such that higher scores indicated better self-regulation.