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Systematic Reviews and Meta- and Pooled Analyses

Associations of Maternal Vitamin B12 Concentration in Pregnancy With the Risks of Preterm Birth and Low Birth Weight: A Systematic Review and Meta-Analysis of Individual Participant Data

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Vitamin B12 (hereafter referred to as B12) deficiency in pregnancy is prevalent and has been associated with both lower birth weight (birth weight <2,500 g) and preterm birth (length of gestation <37 weeks). Nevertheless, current evidence is contradictory. We performed a systematic review and a meta-analysis of individual participant data to evaluate the associations of maternal serum or plasma B12 concentrations in pregnancy with offspring birth weight and length of gestation. Twenty-two eligible studies were identified (11,993 observations). Eighteen studies were included in the meta-analysis (11,216 observations). No linear association was observed between maternal B12 levels in pregnancy and birth weight, but B12 deficiency (<148 pmol/L) was associated with a higher risk of low birth weight in newborns (adjusted risk ratio = 1.15, 95% confidence interval (CI): 1.01, 1.31). There was a linear association between maternal levels of B12 and preterm birth (per each 1-standard-deviation increase in B12, adjusted risk ratio = 0.89, 95% CI: 0.82, 0.97). Accordingly, B12 deficiency was associated with a higher risk of preterm birth (adjusted risk ratio = 1.21, 95% CI: 0.99, 1.49). This finding supports the need for randomized controlled trials of vitamin B12 supplementation in pregnancy.

low birth weight; pregnancy; preterm birth; systematic review; vitamin B12

Abbreviations: B12, vitamin B12; BMI, body mass index; CI, confidence interval; IPD, individual participant data; LBW, low birth weight; SGA, small for gestational age.

Globally, preterm birth and low birth weight (LBW) cause more than a third of the 2.9 million neonatal deaths each year, and prevention of these events is an important component of reducing the mortality rate among children younger than 5 years of age (1, 2). The causes of preterm birth, however, are complex, and few interventions have been successful in preventing it (3).

Vitamin B12 (hereafter referred to as B12) is a vitamin with metabolic roles closely related to those of folate and homocysteine, and it is found in animal-derived foods only (4). It is important for the synthesis (5) and methylation (6) of DNA, and it plays a role in the energy production of the cell (7). It has been hypothesized that B12 may affect placentation and fetal growth (8). B12 deficiency may affect more than three-quarters of some pregnant populations (9).

Few studies of B12 supplementation during pregnancy have been undertaken to assess possible effects on birth weight and length of gestation. However, in a recent meta-analysis, Haider and Bhutta (10) concluded that multiple-micronutrient supplementation may reduce the risk of LBW and the number of stillbirths but not the risk of preterm birth or neonatal mortality. Thus, a more targeted micronutrient supplementation practice may be warranted.

Our aim in this systematic review and individual participant data (IPD) meta-analysis was to study whether maternal serum or plasma B12 levels in pregnancy were associated with birth weight and length of gestation. Results from individual studies have conflicted. In a recent systematic review that included traditional meta-analyses, the authors were unable to conclude whether maternal B12 levels were associated with offspring birth weight (9). However, high heterogeneity in the meta-analyses, dependence among some of the included studies, and reporting bias may have biased the results. We collected IPD and single-study estimates from eligible studies in order to pool effects across all studies in a meta-analysis. This approach allowed for exploration of confounding factors and evaluation of preplanned subgroup effects.

METHODS

The systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines (11, 12), and the protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) (13). This study was approved by the Regional Committee for Medical and Health Research Ethics of Norway. The studies included in this review were approved by their respective regional ethics committees.

Study inclusion criteria

We included studies in which the associations of maternal B12 in serum or plasma during pregnancy with birth weight or gestational age at delivery were assessed. Only studies of a longitudinal cohort design were eligible for this review. In order for a study to be eligible to be included, information on birth weight had to be registered at birth (it could not be retrospectively reported) and length of gestation, in completed days or weeks, had to be estimated by either ultrasound, date of last menstrual period, or a combination of the two. Studies in which B12 was measured after conception and before delivery were eligible. If a study was designed to evaluate women or offspring with a specific condition (e.g., preeclampsia or congenital malformations) and there was a marked overrepresentation of participants with such a condition, that study was excluded. Studies with fewer than 50 participants were not considered. Given the need to collaborate with authors of the original studies, we included only those studies published in 1998 or later.

Search methods

The electronic literature search was constructed by the first author (T.R.) and a librarian trained in medical database searches and was conducted in PubMed, Scopus, Web of Knowledge, EBSCO-host (CINAHL), and OvidSP (MEDLINE, EMBASE, and GLOBAL HEALTH), with the date of the last access being August 2015. No language restriction was applied. The reference lists of all studies for which the full text was read were hand searched to find additional eligible studies. Web Appendix 1 (available at http://aje.oxfordjournals.org/) provides complete information on the electronic searches.

Data collection

Electronic literature searches were carried out by the first author (T.R.). Duplicates were removed and the eligibility of all references were evaluated by screening of the titles and abstracts by the first author (T.R.). The full texts of all potentially eligible studies were read and independently assessed for inclusion by 2 authors (T.R. and K.R.R.). A hand search of reference lists was done independently by 2 authors (T.R. and K.R.R. or M.J.T.). When multiple reports from the same study were found, we used the most complete report.

Risk of bias was independently assessed by 2 authors (T.R. and M.J.T.) based on a modified version of the Newcastle-Ottawa Scale (range, 0–7) (14). Disagreements were resolved by consulting a third reviewer (K.R.R.). We defined high risk of bias as a score of 4 or less and moderate to low risk as a score of 5–7.

Authors from all eligible studies were contacted to obtain IPD, with each research group being approached at least 3 times. IPD was received without personal identification. For studies in which IPD could not be shared, authors were asked to provide results from prespecified re-analyses of their data. When neither IPD nor re-analyses could be retrieved, relevant estimates were extracted from the publications.

Variables

The main exposure of interest was B12 levels in maternal serum or plasma samples. We calculated trimester-specific standard-deviation scores based on studies that provided IPD and re-analyzed aggregate data. Analyses were performed for B12 deficiency, which was predefined as a level less than 148 pmol/L (15), and B12 tertiles, which were constructed based on included individual data (tertile 1, <148 pmol/L; tertile 2, 148–216 pmol/L; and tertile 3, >216 pmol/L).

The 3 predefined main outcomes were birth weight as a continuous measure in grams, LBW (birth weight <2,500 g), and small-for-gestational-age (SGA) birth (birth weight standard-deviation score <10th percentile) (1). Birth weight standard-deviation score was calculated using gestational age at delivery and sex-specific reference standards published by the INTERGROWTH 21st Project (16). We used birth weight standard-deviation score as a proxy for fetal growth and defined SGA birth as a proxy of restricted fetal growth. Outcomes related to length of gestation were gestational age at delivery (days) and preterm birth (gestational age at delivery <37 weeks).

Three main confounders were identified based on a priori assumptions of confounding factors, availability of data, and exploration of the potential effects of covariates on outcome and exposure: maternal age (continuous), prepregnancy or pregnancy body mass index (BMI; continuous), and parity (nulliparous vs. primiparous or multiparous). Maternal weight was used when information on BMI was unavailable. We also considered smoking habits (smoking during pregnancy

vs. not smoking) and highest completed educational level (completed high school, which was equal to 13 years of education, vs. did not complete high school).

Statistical analysis

We applied a 2-step IPD meta-analysis with random effects to pool the results across studies, including aggregate data from individual studies when IPD was not available. All presented results are adjusted for maternal age, BMI/weight, and parity (the "main model"), unless otherwise specified. Precision was assessed using 95% confidence intervals.

Mean differences in the continuous outcomes birth weight (grams), gestational age at delivery (days), and birth weight standard-deviation score were analyzed using linear regression. To estimate risk ratios, Poisson regression with robust error variance (17) was used to analyze the dichotomous outcomes LBW, SGA birth, and preterm birth.

We conducted a meta-analysis in which we evaluated how B12 was associated with maternal weight. Publication bias was explored using funnel plots. Heterogeneity between the studies was explored by computing the I^2 statistic and was considered to be present when I^2 was greater than 30%. All statistical analyses were carried out using Stata SE, version 13.1 (StataCorp LP, College Station, Texas). The statistical analyses, including sensitivity analyses, are described in more detail in Web Appendix 2.

RESULTS

Availability of data

Via the electronic literature search and hand search of reference lists, we identified 606 unique references (Figure 1). Twenty-two studies met the eligibility criteria (11,993 observations), 18 of which were included in the meta-analyses (11,216 observations). This represented 94% of all eligible

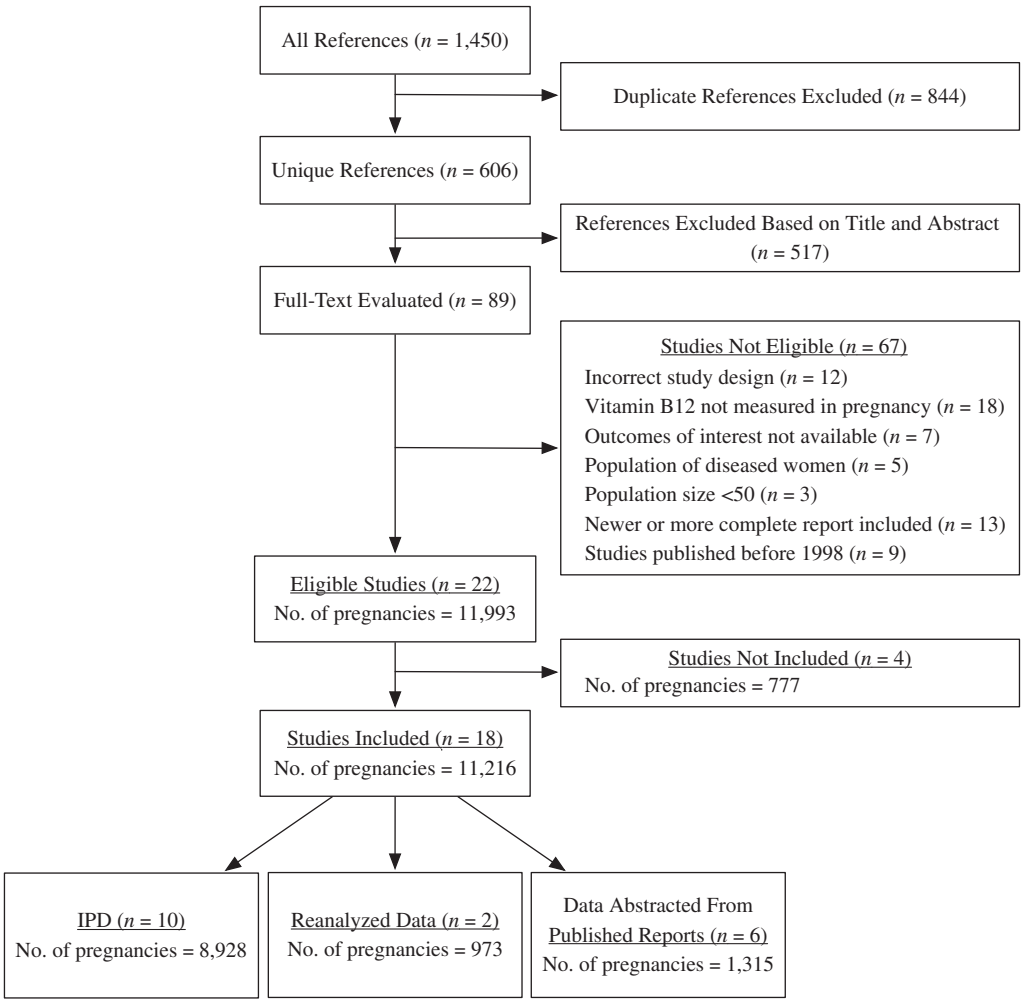

Figure 1. Flow chart of studies included in at least 1 of the meta-analyses of the association between vitamin B12 and birth weight or length of gestation. Four studies were not included because individual participant data (IPD) or reanalyses were not provided and results could not be abstracted from the published reports.

observations (18–35). Four eligible studies (777 observations) were not included because they neither reported on the association of maternal B12 with either birth weight or length of gestation nor provided the necessary IPD or results from requested re-analyses (36–39). Fourteen of the included studies reported estimates of the association of B12 in pregnancy with either birth weight or length of gestation and were qualitatively appraised in the systematic review section (10,563 observations) (18, 19, 21, 23–25, 27, 29–35).

For the meta-analyses, we used IPD from 10 studies (8,928 observations) (18, 19, 21–23, 26–29, 32), results from re-analyses of 2 studies (973 observations) (20, 35), and relevant information and estimates extracted from the published reports of 6 studies (1,315 observations) for which IPD or re-analyses of the original data were not provided (24, 25, 30, 31, 33, 34).

Details of eligible studies

Studies included in the meta-analyses are described in Table 1; details of the eligible studies that were not included are presented in Web Table 1 (36–39). Of the included studies, 1 was conducted in North America (34), 9 in Europe (18, 19, 22, 25–28, 31, 32), 1 in Africa (30), 1 in Oceania (24), and 6 in Asia (20, 21, 23, 29, 33, 35). The number of pregnancies studied ranged from 84 to 5,641. B12 was measured during the first trimester in 7 studies (19, 22, 23, 28, 31–33), during the second trimester in 15 studies (18–24, 26–29, 31–33, 35), and during the third trimester in 12 studies (18, 20, 21, 23, 25, 27, 29, 30, 32–35). Mean B12 concentrations in the first, second, and third trimester were 219.8 (standard deviation, 128.2) pmol/L, 187.8 (standard deviation, 91.3) pmol/L, and 188.7 (standard deviation, 82.5) pmol/L, respectively. Preterm births were excluded from 4 studies (25, 26, 31, 33).

Key maternal and newborn characteristics listed the included studies are presented in Table 2. B12 deficiency was identified in 0%–69% of pregnancies (median, 33%). The incidence of LBW ranged from 0% to 33% (median, 6%), the incidence of preterm births ranged from 4% to 14% (median, 8%), and the incidence SGA birth ranged from 5% to 32% (median, 11%). Higher maternal weight was associated with lower maternal B12 level; a 1-standard-deviation higher maternal BMI or weight was associated with an 11-pmol/L decrease in B12 (95% confidence interval (CI): −15, −7).

Systematic review

Birth weight and SGA birth. The association between B12 and birth weight or risk of SGA birth was reported in 14 of 22 eligible studies. In 3 studies, a clear association was reported: in one, birth weight was higher among B12-deficient women than among nondeficient women (34); in another, lower B12 was associated with higher birth weight only among women with gestational diabetes mellitus (32). Conversely, investigators in a third study reported that lower values of B12 significantly increased the risk of SGA birth (23). In the remaining 11 studies, there was weak evidence of an inverse association in 3 studies (25, 27, 33) and no association in 8 studies (18, 19, 21, 24, 29–31, 35).

Length of gestation. There were only 2 published reports in which the authors reported on the association of B12 with length of gestation or preterm birth. In the first study, researchers observed that a higher B12 level was associated with a longer length of gestation and a lower risk of preterm birth, but the small sample size yielded low precision of the estimates (21). In the second study, investigators did not find evidence of an association between B12 and length of gestation (19). Evaluation of the risk of bias showed that the scores ranged from 3 and 7 and that 2 studies were classified as having a high risk of bias (see Web Table 2).

Meta-analysis of maternal B12 in relation to birth weight and LBW

In the meta-analysis, we found no evidence of a linear association between B12 and birth weight (Figure 2). The adjusted estimate was a 5.1-g increase in birth weight per each standard-deviation increase in B12 level (95% CI: −10.9, 21.0; $I^2 = 30\%$).

Results of subgroup and sensitivity analyses are presented in Web Table 3. Stratification by country income showed that there was an association between B12 level and birth weight in low- and middle-income countries but not in high-income countries. Heterogeneity among the studies was explained largely by country income level and maternal BMI or weight. Excluding a study that used late-pregnancy BMI (29) instead of prepregnancy or early pregnancy BMI/weight, which were used in the other studies, reduced the heterogeneity from $I^2 = 30\%$ to $I^2 = 13\%$ (data not shown). In 1 study, investigators reported an association between B12 and birth weight that deviated greatly from those in the other studies (33). Excluding that study did not notably change the effect estimate, but it did result in a modest reduction in heterogeneity (from $I^2 = 30\%$ to $I^2 = 21\%$; data not shown). Sensitivity analyses in which we excluded each of the included studies 1 by 1 and those in which we excluded studies that only evaluated newborns born at term did not meaningfully alter the association between B12 and birth weight (data not shown).

Results for categories of B12 supported our main results. Neither B12 deficiency nor B12 tertile was associated with birth weight (see Web Table 4).

B12 deficiency was associated with a 15% (95% CI: 1, 31; $I^2 = 5\%$) higher risk of LBW (Figure 3A). The funnel plot for B12 and birth weight indicated a low risk of publication bias (see Web Figure 1). Because birth weight may be regarded as a summary measure of fetal growth and gestational age, we further performed analyses to assess a possible influence of B12 on these factors.

Meta-analysis of maternal B12 in relation to length of gestation and preterm birth

The analyses did not support a linear association between maternal B12 levels and length of gestation in days; the length of gestation increased by 0.1 days (95% CI: −0.2, 0.3; $I^2 = 0\%$) per each 1-standard-deviation

Table 1. Characteristics of Studies Included in the Meta-Analysis

First Author, Year (Reference No.)	Type of Data	No.	Country	Study Years	Vitamin B12 Analysis Method	Week of B12 Measurement		Included in Specific Meta-Analyses[a]					
						Range	Median	Birth Weight	LBW	SGA Birth	Birth Weight SD Score	Length of Gestation	Preterm Birth
Baker, 2009 (18)	IPD	290	United Kingdom	2004–2007	RIA	27–43	30	Yes	Yes	Yes	Yes	Yes	Yes
Bergen, 2012 (19)	IPD	5,641	The Netherlands	2002–2006	ECL	5–18	13	Yes	Yes	Yes	Yes	Yes	Yes
Bhate, 2012 (20)	Reanalysed data	214	India	2004–2006	Microbiological	24–30	28	Yes	Yes			Yes	Yes
Chen, 2015 (21)	IPD	988	Singapore	2009–2010	ECL	26–29	27	Yes	Yes	Yes	Yes	Yes	Yes
Dayaldasani, 2014 (22)	IPD	187	Spain	2011	ECL	3–23	10	Yes[b]	Yes[b]	Yes[b]	Yes[b]	Yes[b]	Yes[b]
Dwarkanath, 2013 (23)	IPD	344	India	2001–2003	ECL	T1, 5–19; T2, 20–29; T3, 30–39	T1, 12; T2, 24; T3, 34	Yes	Yes	Yes	Yes	Yes	Yes
Furness, 2013 (24)	Data from publication	84	Australia	N/A	ECL	18–20	N/A			Yes[c]			
Halicioglu, 2012 (25)	Data from publication	208	Turkey	2008	ECL	>37	N/A	Yes[d]					
Hay, 2010 (26)	IPD	149	Norway	1997	Microbiological	17–19	N/A	Yes					
Hogeveen, 2010 (27)	IPD	363	The Netherlands	2002–2004	Microbiological	27–38	31	Yes[e]	Yes[e]	Yes[e]	Yes[e]	Yes[e]	Yes[e]
Kaymaz, 2011 (28)	IPD	103	Turkey	2007	ECL	11–14	13	Yes	Yes			Yes	Yes
Krishnaveni, 2014 (29)	IPD	654	India	1997–1998	Microbiological	22–35	26	Yes	Yes	Yes	Yes	Yes	Yes
Mamabolo, 2006 (30)	Data from publication	219	South Africa	1999–2000	RIA	28–36	N/A			Yes[c]			
Relton, 2005 (31)	Data from publication	500	United Kingdom	2000–2002	RIA	N/A	11.5 (5.8)[f]				Yes[e]		
Sukumar, 2011 (32)	IPD	209	United Kingdom	2005–2010	RIA ($n = 182$), ECL ($n = 27$)	0–37	24	Yes	Yes	Yes	Yes	Yes	Yes
Takimoto, 2007 (33)	Data from publication	88	Japan	2001–2003	ECL	T1, 7–14; T3, 34–36	N/A	Yes[g]					
Wu, 2013 (34)	Data from publication	216	Canada	N/A	RIA	N/A	36	Yes[d]					
Yajnik, 2008 (35)	Reanalysed data	759	India	1994–1996	Microbiological	N/A	T2, 18 (2)[f]	Yes	Yes			Yes	Yes

Abbreviations: ECL, electroluminescence; IPD, individual participant data; N/A, not available; RIA, radioimmunoassay; SD, standard deviation; SGA, small-for-gestational-age; T1, first trimester; T2, second trimester; T3, third trimester.

[a] Included in the analyses of the exposures vitamin B12 SD score and B12 deficiency, both crude and adjusted (maternal age, body mass index or weight, and parity), unless otherwise specified.

[b] Does not contribute to the analyses of vitamin B12 deficiency (none of the participants were deficient).

[c] Level of vitamin B12 in a crude analysis among those who were born SGA versus those who were not.

[d] Birth weight in a crude analysis among those who were vitamin B12–deficient versus those who were not.

[e] Crude analysis.

[f] Values are expressed as mean (SD).

[g] Adjusted analysis (maternal age, body mass index or weight, and parity).

Table 2. Maternal and Newborn Characteristics of Studies Included in the Meta-Analysis

First Author, Year (Reference No.)	Maternal Age, years, mean (SD)	Maternal BMI[a], mean (SD)	Para 0		Vitamin B12, pmol/L, mean (SD)	Vitamin B12 Deficient[b]		Birth Weight, g, mean (SD)	LBW[c]		SGA Birth[d]		Length of Gestation, weeks, mean (SD)	Preterm Birth[e]	
			No.	%		No.	%		No.	%	No.	%		No.	%
Baker, 2009 (18)	18 (1)	65 (14)[e]	277	96	192 (84)	93	32	3,232 (534)	26	9	33	12	39.7 (1.8)	22	8
Bergen, 2012 (19)	30 (5)	25 (5)	3,208	57	188 (93)	2,098	37	3,418 (563)	280	5	412	7	39.9 (1.8)	268	5
Bhate, 2012 (20)	23 (3)	20 (3)	165	71	145 (84)	148	69	2,707 (411)	49	25	N/A	N/A	38.6 (2.6)	18	8
Chen, 2015 (21)	31 (5)	66 (12)[f]	420	43	220 (79)	161	16	3,101 (449)	76	8	86	9	38.6 (1.4)	85	9
Dayaldasani, 2014 (22)	30 (6)	26 (5)	96	51	387 (123)	0	0	3,267 (526)	11	6	12	7	38.8 (1.9)	14	8
Dwarkanath, 2013 (23)	24 (4)	53 (10)[f]	203	59	205 (115)[g]	100	29[g]	2,771 (498)	95	28	102	30	38.3 (1.7)	47	14
Furness, 2013[h] (24)	33 (7)	27 (5)	N/A	N/A	234 (129)	N/A	N/A	3,390 (789)	N/A	N/A	21	25[i]	38.8 (2.9)	N/A	N/A
Halicioglu, 2012[h] (25)	28 (5)	N/A	N/A	N/A	120[j]	99	48[k]	3,357 (466)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hay, 2010 (26)	30 (4)	65 (10)[f]	67	45	294 (87)	2	1	3,727 (476)	0	0	N/A	N/A	N/A	N/A	N/A
Hogeveen, 2010 (27)	33 (4)	N/A	109	30	186 (69)	120	34	3,436 (545)	18	5	19	5	39.5 (1.6)	21	6
Kaymaz, 2011 (28)	27 (3)	24 (4)	45	44	152 (59)	54	52	3,241 (553)	5	5	N/A	N/A	38.4 (1.9)	9	9
Krishnaveni, 2014 (29)	24 (4)	24 (4)	331	51	187 (100)	264	40	2,857 (475)	126	19	202	32	39.0 (1.8)	63	10
Mamabolo, 2006[h] (30)	25 (7)	27 (4)	N/A	N/A	175 (77)	36	16[l]	3,120 (550)	N/A	N/A	66	30[m]	N/A	N/A	N/A
Relton, 2005[h] (31)	28 (6)[n]	N/A	N/A	43[n]	239 (97)	N/A	N/A	3,430 (470)[n]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sukumar, 2011 (32)	31 (6)	27 (6)	68	33	168 (126)	114	55	3,381 (558)	10	5	16	8	39.3 (1.7)	9	4
Takimoto, 2007[h] (33)	29 (5)	21 (3)	N/A	N/A	405 (146)[g]	13	16[o]	3,120 (411)	5	5	N/A	N/A	39.6 (1.0)	N/A	N/A
Wu, 2013[h] (34)	33 (4)	N/A	N/A	N/A	224 (96)	51	24	3,486 (452)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Yajnik, 2008 (35)	21 (4)	18 (2)	252	31	151 (78)	447	59	2,612 (392)	230	33	N/A	N/A	38.8 (2.1)	87	11

Abbreviations: BMI, body mass index; LBW, low birth weight; N/A, not available; SD, standard deviaton; SGA, small-for-gestational-age.
[a] Weight (kg)/height $(m)^2$.
[b] Vitamin B12 level <148 pmol/L.
[c] Birth weight <2,500 g.
[d] Birth weight SD score (i.e., accounting for length of gestation and sex) below the 10th percentile.
[e] Length of gestation <37 weeks.
[f] Values are expressed in kilograms because BMI was not available.
[g] First measurement.
[h] Data extracted from publication.
[i] Serial tapering of growth in abdominal circumference and of estimated fetal weight below the 10th percentile of an Australian growth chart.
[j] Values are expressed as median (range not available).
[k] Vitamin B12 level ≤118 pmol/L.
[l] B12 deficiency not defined.
[m] Lowest birth weight tertile (mean birth weight = 2,940 g) used as an approximation of SGA birth for the purpose of this review.
[n] Based on a larger study population than the subgroup with available vitamin B12 data included in this review (n = 974–997).
[o] Third trimester.

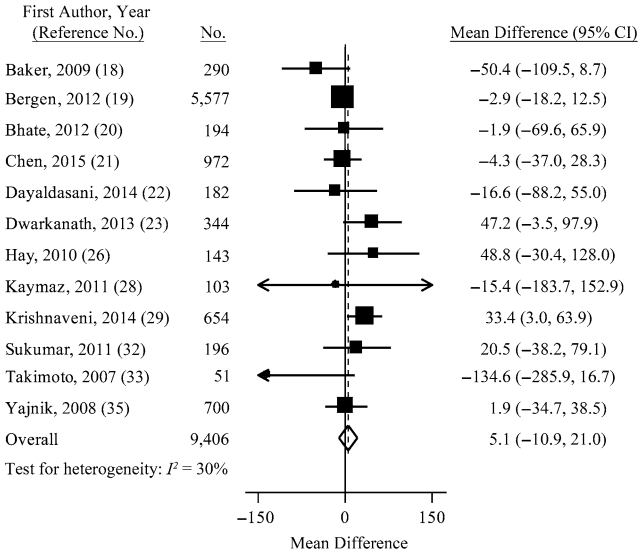

Figure 2. Forest plot presenting the association between maternal vitamin B12 level and birth weight. Results are from a meta-analysis that was adjusted for maternal age, parity, and body mass index or weight. Effect estimates are expressed as change in birth weight per a 1-standard-deviation increase in vitamin B12 (i.e., mean difference). CI, confidence interval.

increase of B12. However, increasing levels of B12 were associated with decreasing risk of preterm birth (per 1-standard-deviation increase in B12, risk ratio = 0.89, 95% CI: 0.82, 0.97; $I^2 = 0\%$) (Web Figure 2). Accordingly, B12 deficiency in pregnancy was associated with a 21% higher risk of preterm birth (95% CI: −1, 49; $I^2 = 20\%$) (Figure 3B). The associations between B12 and preterm birth were similar within all subgroup and sensitivity analyses, although there was a loss of precision in these subgroup analyses because of smaller sample sizes (see Web Table 5).

Meta-analysis of maternal B12 in relation to birth weight standard-deviation score and SGA birth

B12 was not associated with birth weight standard-deviation scores in the main analysis (see Web Figure 3). However, B12 was associated with birth weight standard-deviation score in low- and middle-income countries (per 1-standard-deviation increase in B12, standard deviation, 0.08, 95% CI: 0.03, 0.14; $I^2 = 0\%$) but not in high-income countries (standard deviation, −0.02, 95% CI: −0.05, 0.02; $I^2 = 23\%$). Women with a B12 deficiency were not at higher risk of SGA births than nondeficient women (Figure 3C), and B12 levels were similar in SGA and non-SGA pregnancies (see Web Table 6).

DISCUSSION

The results from the present systematic review and meta-analysis do not support any linear association between maternal B12 levels in pregnancy and offspring birth weight. However, our findings provide evidence that lower maternal B12 levels are associated with a higher risk of preterm birth and that the risk of preterm birth is particularly high in the presence of B12 deficiency during pregnancy.

Strengths and limitations

A strength of this study is the use of IPD and re-analyzed data. Because there was substantial heterogeneity in the published analyses, we could not use a traditional meta-analysis to answer our research questions. Incomplete or selective reporting may reduce the replicability of studies and distort the literature (40). This is illustrated by comparing the findings of this review with those of a recently published systematic review by Sukumar et al. (9) that included traditional meta-analysis of the association between B12 and birth weight. In that study, the authors reported an odds ratio of 1.70 (95% CI: 1.16, 2.50; $I^2 = 84\%$) for the association between low B12 level and adverse birth weight. In the present study, we found a more moderate association in a comparable analysis of B12 deficiency in relation to LBW (risk ratio = 1.15, 95% CI: 1.01, 1.31; $I^2 = 5\%$). One reason for the discrepant results may be that Sukumar et al. depended solely on data presented in the published reports and were unable to include results that were reported as being insignificant, as in the largest individual study in the present review (19). The comparable meta-analysis in the present review included roughly 10 times as many pregnancies as the meta-analysis in the review by Sukumar et al. Additionally, of the 8 individual results included in the meta-analysis by Sukumar et al., 5 evaluated mostly the same women from a single original study, which exaggerated the influence of a single outlying study (8, 23). By collecting IPD and requesting re-analyses of contributing studies, we were able to standardize the analyses across most of the included studies, thereby reducing heterogeneity and facilitating interpretation of results. Compared with the review by Sukumar et al. in which they presented meta-analyses with high levels of heterogeneity (I^2 scores from 74% to 98% in the primary analyses), the present study had I^2 scores between 0% and 30% in the primary analyses. Additionally, in the present study, we were able to conduct subgroup and sensitivity analyses that included more complete adjustment for important confounders (e.g., maternal weight).

We included 94% of all eligible participants, which permitted an unbiased summary of the published literature. Given the relatively large number of included subjects, we had higher power to evaluate findings reported with low precision in individual studies. We tested the stability of our findings with a broad range of sensitivity analyses.

Another strength was that our analyses were not post hoc but followed a detailed protocol. We performed a thorough literature search without language restrictions and systematically reviewed all eligible studies.

There are several limitations. Unpublished studies were not considered for this review, which potentially could have skewed the estimates. However, a funnel plot did not suggest publication bias. We were unable to include 4 eligible studies (777 observations; 6% of all observations).

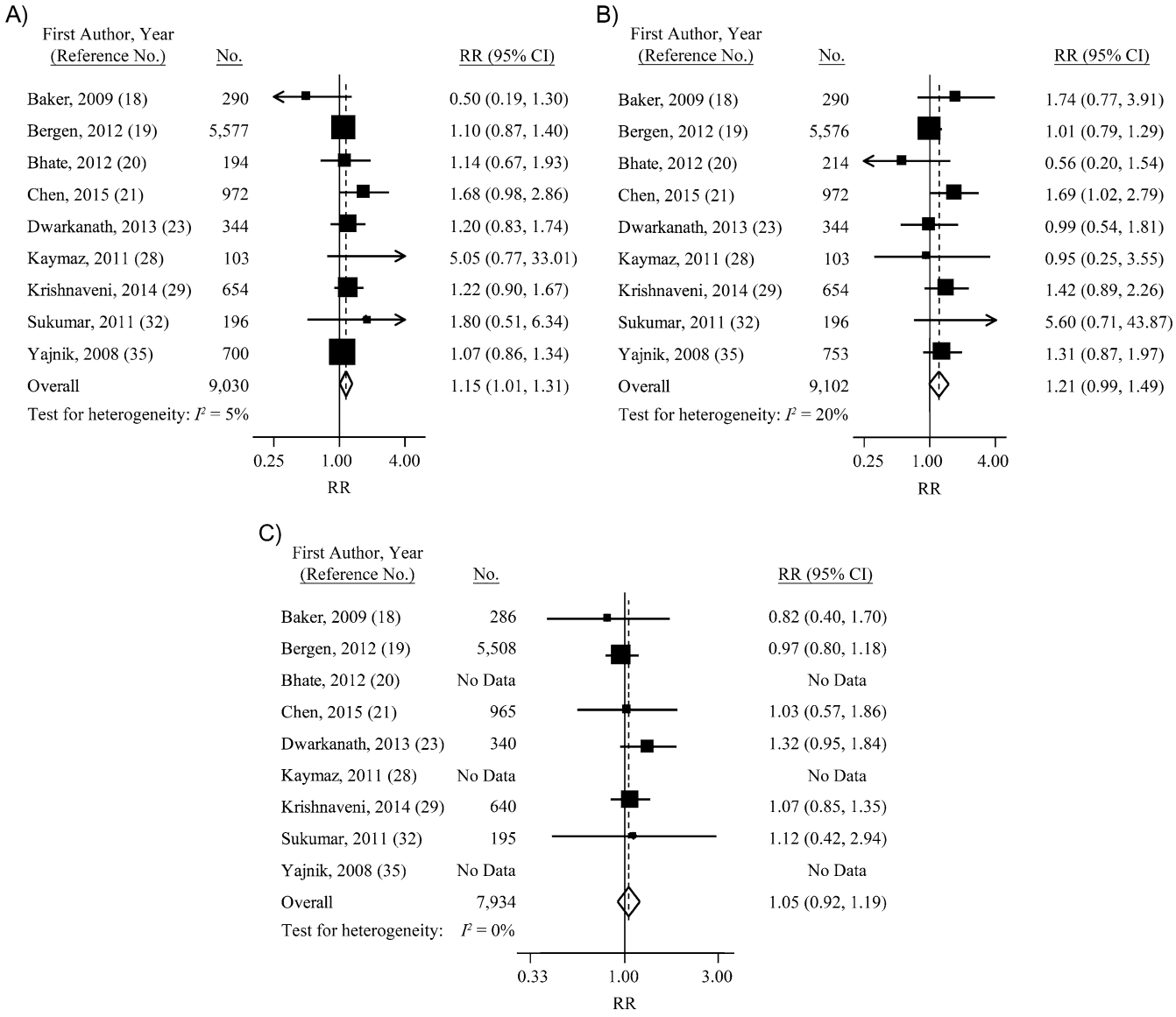

Figure 3 Forest plots presenting the association between maternal vitamin B12 deficiency and the risk of low birth weight (A), preterm birth (B), and small-for-gestational-age birth (C). Results are from meta-analyses that were adjusted for maternal age, parity, and body mass index or weight. Effect estimate expressed as risk ratio (RR) of the outcome when comparing B12-deficient women with nondeficient women. CI, confidence interval.

Given the small number of observations, it is unlikely that inclusion of these remaining studies would have importantly influenced our main results.

Our approximations of fetal growth and restricted fetal growth that were created using gestational age- and sex-specific birth weight charts are suboptimal because these outcomes are ideally estimated using serial ultrasound measurements during pregnancy (41). Furthermore, we did not have sufficient data available to evaluate the possible implications of low levels of B12 during different periods in pregnancy in the same woman. Sensitivity analyses stratified by trimester of B12 measurement across studies, however, did not reveal important variation in the associations between B12 and the outcomes of interest.

Importantly, B12 deficiency may be a proxy for inadequate nutritional status, and it is possible that some of our findings are related to nutritional status and not specifically to B12. A predominantly plant-based diet is low in B12 but also other nutrients, such as vitamin D and zinc, that to some degree may be associated with preterm birth (42–44). We did not have information on dietary intake or blood levels of these nutrients. Nutritional status could explain the present finding of an association between B12 and birth weight in low- and middle-income countries but not high-income countries. However, lower vitamin B12 levels were associated with higher risk of preterm birth irrespective of country income. It seems less likely that nutritional status can fully explain this finding.

Mixing of effects is inherent in observational studies, and residual confounding cannot be ruled out. We emphasize that we are reporting associations and that causal effects must be explored through trials (see below). Reassuringly, we found little discrepancy in the pooled results of the adjusted main models as compared with extended adjusted models (i.e., additional adjustment for maternal educational level and smoking habits).

Possible explanation of findings

Low birth weight is a result of preterm birth, of being born SGA at term, or a combination of the two (45). Although we found a higher risk of preterm birth and LBW among infants born to B12-deficient women, there was little evidence that maternal B12 levels influenced offspring birth weight standard-deviation score or SGA status. It seems more likely that the observed higher risk for LBW in B12-deficient women can be explained by preterm birth rather than by reduced fetal growth.

Higher B12 level was associated with higher birth weight in low- and middle-income countries but not in high-income countries. Four of the 5 studies included in the low- and middle-income group were conducted in an Indian population. Therefore, generalization of these results to low- or middle-income countries outside of India should be treated with caution. Indian women generally have lower dietary intakes of B12 because of their mainly vegetarian diet, making them susceptible to B12 deficiency (46). Additionally, Indian newborns are among the smallest in the world (45). Our findings suggest that pregnancies already at the greatest risk of resulting in small newborns were the ones that were most vulnerable to low levels of B12. The association between B12 and the risk of preterm birth was consistent across studies in both high-income and low- and middle-income countries, and generalization to countries not studied may be feasible.

In line with our findings, maternal obesity has been associated with B12 deficiency in several populations (47, 48). It has been hypothesized that this association is due to altered fat distribution and metabolism in overweight women compared with normal-weight women (47). Maternal weight is positively correlated with newborn weight (49), and failure to adjust for maternal weight may underestimate a positive association between B12 and birth weight.

Potential mechanism of action

Preterm birth may be categorized into spontaneous and medically indicated, with varying causes (50). Unfortunately, information on spontaneous versus medically indicated preterm births were not available to us. Medically indicated preterm births are most commonly caused by severe preeclampsia or severely restricted fetal growth (51). Our findings do not support an association between maternal level of B12 and fetal growth. Maternal B12 level might be associated with risk of preeclampsia, potentially through elevated homocysteine levels; however, the results from reports are discrepant (52–54). The rate of medically indicated preterm births is higher in high-income countries than in low- and middle-income countries (55). In analysis stratified by country income, we found similar associations between B12 and risk of preterm birth in low-, middle-, and high-income countries. Still, this finding does not link B12 to specific etiologies of preterm birth, which is a topic that deserves further studies.

It is possible that supplementation of B12 or folic acid, with a subsequent reduction of homocysteine, increases birth weight and length of gestation. However, in a Cochrane review, Lassi et al. (56) concluded that supplementation with folic acid during pregnancy did not reduce the risk of either preterm birth or LBW. In 2 small (68 pregnancies and 256 pregnancies, respectively) randomized controlled trials of B12 supplementation during pregnancy, investigators reported on birth weight and length of gestation (57, 58). In both, B12 plasma levels were higher in the supplemented group than in the control group, but no reduction in homocysteine levels was seen. No differences were observed in birth weight, length of gestation, or frequency of LBW births or preterm births in the supplemented group compared with the control group in either study (C. Duggan, Harvard University, personal communication, 2015) (57, 58). However, the studies were not powered to detect small but meaningful differences in preterm birth.

Context

There are 15 million preterm births and 20 million infants born with LBWs globally each year (1). The greatest burden of LBW is found in South Asia, whereas preterm birth is highest in Africa (1). Preterm birth is the leading cause of neonatal deaths (1). In the era of The Millennium Development Goals (1990–2015), the postneonatal mortality rate for children younger than 5 years of age was reduced by 58% (2). The reduction in neonatal mortality was less pronounced (47%) (2). Prevention of preterm birth is thus a key strategy for reducing neonatal deaths and reaching the new target of a mortality rate in children younger than 5 years of age of 25 per 1,000 live births by 2030, down from 43 per 1,000 in 2015 (2).

Our systematic review was not designed to study the prevalence of B12 deficiency during pregnancy. However, this condition was common in the studies in our review, and the rates were comparable to those in a systematic review of B12 deficiency during pregnancy (9). A large group of women are thus affected by a potential preventable risk of preterm birth.

Conclusion and implications for clinical practice and future research

B12 deficiency during pregnancy is common. Results of the present systematic review in which we included IPD meta-analyses provide robust evidence that lower B12 levels during pregnancy are associated with a higher risk of preterm birth, particularly in B12-deficient women. Our findings support the need to conduct randomized controlled trials to evaluate whether maternal B12 supplementation in pregnancy reduces the risk of preterm birth.

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