Maternal vitamin B12 in pregnancy and risk of preterm birth and low birth weight: A systematic review and individual participant data meta-analysis

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Abstract

Vitamin B12 (B12)-deficiency in pregnancy is prevalent, and has been associated with 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 an individual participant data meta-analysis to evaluate the associations between maternal serum or plasma B12 concentration in pregnancy and 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 an increased risk of newborn low birth weight (adjusted risk ratio (RR) 1.15 (95% confidence interval (CI) 1.01, 1.31)). There was a linear association between maternal levels of B12 and preterm birth (adjusted RR for preterm birth was 0.89 (95% CI 0.82, 0.97) per one standard deviation increase in B12). Accordingly, B12-deficiency was associated with increased risk of preterm birth (adjusted RR 1.21 (95% CI 0.99, 1.49). Lower maternal B12 in pregnancy increased the risk of preterm birth. This finding supports the conduct of randomized controlled trials of vitamin B12 supplementation in pregnancy.

Keywords

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

Globally, preterm birth and low birth weight (LBW) cause over a third of the 2.9 million neonatal deaths each year, and prevention of these events is important to reduce under-five year mortality (1,2). The etiology of preterm birth, however, is complex, and few interventions have been successful in preventing it (3).

Vitamin B12 (B12) is a vitamin with metabolic roles closely related to folate and homocysteine, and is found in animal-derived foods only (4). It is important for the synthesis (5) and methylation (6) of DNA, and 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 over three quarters of some pregnant populations (9).

Few supplementation-studies of B12 in pregnancy have been undertaken to assess possible effects on birth weight and length of gestation. However, a recent meta-analysis concluded that multiple-micronutrient supplementation may reduce the risk of LBW and the number of

stillbirths, but not preterm birth or neonatal mortality (10). Thus, a more targeted micronutrient supplementation practice may be warranted.

The aim of this systematic review and individual participant data (IPD) meta-analysis was to study whether maternal serum or plasma B12 levels in pregnancy may be associated with birth weight and length of gestation. Individual studies have reported conflicting results. A recent systematic review that included traditional meta-analyses was 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 their 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 PRISMA and MOOSE guidelines (11,12), and the protocol was registered at PROSPERO (13).

Study inclusion criteria

We included studies that assessed the association between maternal B12 in serum or plasma during pregnancy and birth weight or gestational age at delivery.

Only studies of longitudinal cohort design were eligible for this review. To be eligible, information on birth weight had to be registered at birth and could not be retrospectively reported and length of gestation, in completed days or weeks, had to be estimated by either ultrasound or last menstrual period, or a combination of the two. Studies where B12 was measured after conception and prior to 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 (TR) and a librarian trained in medical database searches, and conducted in PubMed, Scopus, Web of Knowledge, EBSCO-host (CINAHL) and OvidSP (MEDLINE, EMBASE and GLOBAL HEALTH); last accessed August 2015. No language restriction was applied. The reference lists of all studies read in full-text were hand searched to find additional eligible studies. Web Appendix 1 provides complete information on the electronic searches.

Data collection

Electronic literature searches were carried out by the first author (TR). Duplicates were removed and eligibility of all references evaluated by screening of the titles and abstracts by

the first author (TR). All potentially eligible studies were read in full-text and assessed for inclusion independently by two authors (TR and KRR). A handsearch of reference lists was done independently by two authors (TR and KRR or MJT). When multiple reports from the same study were found, we used the most complete report.

Risk of bias was independently assessed by two authors (TR and MJT) based on a modified version of the Newcastle-Ottawa Scale (range 0-7) (14). Disagreements were resolved by consulting a third reviewer (KRR). We defined high risk of bias as a score of four or less, and moderate to low risk was defined as scores five through seven.

Authors from all eligible studies were contacted to obtain IPD, each research group being approached at least three times. IPD was received without personal identification. For studies where IPD could not be shared, authors were asked to provide results from pre-specified reanalyzes of their data. When neither IPD nor reanalyzes could be retrieved, relevant estimates were extracted from the publications.

Variables

The main exposure of interest was vitamin B12 levels in maternal serum or plasma. We calculated trimester-specific standard deviation (SD) scores based on studies providing IPD and reanalyzed aggregate data. Analyses were performed for B12-deficiency pre-defined as <148 pmol/L (15), and B12 tertiles constructed on the basis of included individual data; <148 pmol/L (tertile 1), 148-216 pmol/L (tertile 2), and >216 pmol/L (tertile 3).

The three pre-defined main outcomes were: birth weight as a continuous measure in grams, LBW (birth weight <2,500 g) and small-for-gestational-age (SGA; birth weight SD score <10th centile) (1). Birth weight SD score was calculated using gestational age at delivery and sex-specific reference standards published by the INTERGROWTH 21st Project (16). We assumed birth weight SD score to serve as a proxy of fetal growth, and defined SGA 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 effect of covariates on outcome and exposure: maternal age (continuous), pre-pregnancy or pregnancy body mass index (BMI, continuous) and parity (nulliparous versus primiparous or multiparous). Maternal weight was used when information on BMI was unavailable. Also, we considered smoking habits (smoking versus not smoking during pregnancy) and highest completed education (completed high school, equal to 13 years of education, versus not completed high school).

Statistical analysis

We applied a two-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 by 95% confidence intervals (CI).

Mean difference of the continuous outcomes birth weight (g), gestational age at delivery (days) and birth weight SD score (SD) were analyzed by linear regression. To estimate risk ratios (RR), Poisson regression with robust error variance (17) was used to analyze the dichotomous outcomes LBW, SGA, and preterm birth.

We conducted a meta-analysis that 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 (Stata Corporation, College Station, TX, USA). The statistical analyses, including sensitivity analyses, are described in more detail in Web Appendix 2.

Results

Availability of data

The electronic literature search and hand search of reference lists identified 606 unique references (Figure 1). Twenty-two studies met eligibility criteria (11,993 observations) of which 18 studies were included in the meta-analyses (11,216 observations), representing 94% of all eligible observations (18–35). Four eligible studies (777 observations) were not included as they neither reported on the association between maternal B12 and birth weight or length of gestation, nor provided the necessary IPD or results from requested reanalyzes (36–39). Fourteen of the included studies reported estimates for the association between B12 in pregnancy and 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, ten studies provided IPD (8,928 observations) (18,19,21–23,26–29,32), two studies provided results from reanalyzes (973 observations) (20,35), and relevant information and estimates were extracted from the published reports of six studies (1,315 observations) where IPD or reanalyzes of 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 not included are presented in Web Table 1 (36–39). Of the included studies, one was conducted in North America (34), nine in Europe (18,19,22,25–28,31,32), one in Africa (30), one in Oceania (24), and six 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 seven 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 (SD) B12 concentrations in the first, second and third trimester were 219.8 (128.2), 187.8 (91.3) and 188.7 (82.5) pmol/L, respectively. Preterm deliveries were excluded from four studies (25,26,31,33).

Key maternal and newborn characteristics of the included studies are presented in Table 2. B12-deficiency was identified in 0% to 69% of pregnancies (median 33%). The incidence of LBW ranged from 0% to 33% (median 6%), preterm births from 4% to 14% (median 8%),

and SGA from 5% to 32% (median 11%). Higher maternal weight was associated with lower maternal B12; one SD higher maternal BMI or weight was associated with an 11 pmol/L decrease in B12 (95% CI -15, -7).

Systematic review

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

Length of gestation—Only two published reports reported on the association between B12 and length of gestation or preterm birth. The first study observed that higher B12 was associated with a longer length of gestation and a reduced risk of preterm birth, but the small sample size yielded low precision of the estimates (21). The second study 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 between three and seven, and that two studies were classified with 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 5.1 g increase in birth weight per SD increase in B12 (95% CI -10.9, 21.0; I^2=30%).

Subgroup and sensitivity analyses are presented in Web Table 3. Stratification by country income showed that there was an association between B12 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 pre-pregnancy or early pregnancy BMI/ weight in the other studies, reduced the heterogeneity from I^2=30% to I^2=13% (results not presented). One study reported an association between B12 and birth weight that greatly deviated from the other studies (33). Excluding this study did not notably change the effect estimate, but resulted in a modest reduction in heterogeneity (from I^2=30% to I^2=21%; results not presented). Sensitivity analyses excluding each of the included studies one by one, and excluding studies only evaluating newborns born at term, did not meaningfully alter the association between B12 and birth weight (results not presented).

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

B12-deficiency was associated with a 15% (95% CI 1%, 31%; I^2=5%) increased risk of LBW (Figure 3A).

The funnel plot of B12 and birth weight indicated low risk of publication bias (see Web Figure 1).

Since 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 with length of gestation in days (0.1 days (95% CI -0.2, 0.3; I^2=0%) per SD increase of B12). However, *increasing* levels of B12 were associated with a reduced risk of preterm birth (RR 0.89 (95% CI 0.82, 0.97; I^2=0%) per SD increase in B12; Web Figure 2). Accordingly, B12-deficiency in pregnancy was associated with a 21% increased risk of preterm birth (95% CI -1%, 49%; I^2=20%); Figure 3B).

The association between B12 and preterm birth was similar within all subgroup and sensitivity analyses, although there was a loss of precision in these subgroup analyses due to smaller sample sizes (see Web Table 5).

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

B12 was not associated with birth weight SD scores in the main analysis (see Web Figure 3). However, B12 was associated with birth weight SD score in low- and middle-income countries (0.08 SD per 1 SD increase in B12 (95% CI 0.03, 0.14; I^2=0%)), but not in high-income countries (-0.02 SD (95% CI -0.05, 0.02; I^2=23%)).

Women with B12-deficiency were not at higher risk of SGA births than non-deficient women (Figure 3C), and B12 levels were similar in SGA and non-SGA pregnancies (see Web Table 6).

Discussion

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

Strengths and limitations

A strength of this study is the use of IPD and reanalyzed data. Due to substantial heterogeneity in the published analyses, a traditional meta-analysis could not 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. with traditional meta-analysis on the association between B12 and birth weight (9). That study reported an odds ratio of 1.70 (95% CI 1.16, 2.50; I^2=84%) of the association between "low B12" and "adverse birth weight". A more moderate association was found in the present study in a comparable analysis of B12-deficiency in relation to LBW (RR 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 reported as being "insignificant"; for instance, from the largest individual study in the present review (19). The comparable meta-analysis in the present review included roughly ten times as many pregnancies as the meta-analysis in Sukumar's review. Additionally, of eight individual results included in Sukumar's meta-analysis, five evaluated most of the same women from a single original study, exaggerating the influence of a single, outlying study (8,23). By collecting IPD and requesting reanalyzes from 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. that 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, the present study enabled conduction of subgroup and sensitivity analyses, along with more complete adjustment for important confounders (e.g. maternal weight).

We included 94% of all eligible participants, permitting an unbiased summary of the published literature. Given the relative large number of included subjects, we had increased 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 could potentially skew estimates. However, a funnel plot did not suggest publication bias. We were unable to include four eligible studies (777 observations, 6% of all observations). 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 by use of gestational age and sex specific birth weight charts is suboptimal, as these outcomes are ideally estimated using serial ultrasound measurements during pregnancy (41). Furthermore, we did not have sufficient data at hand 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 association 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, not specifically to B12. A predominantly plant-based diet is low in B12, but also other nutrients, such as vitamin D and zinc, which 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 our study report associations, and that causal effects must be explored through trials (see below). Reassuringly, we found little discrepancy in the pooled results of adjusted main models as compared to extended adjusted models (i.e. additionally adjusting for maternal education and smoking habits).

Possible explanation of findings

Low birth weight is a result of preterm birth, of being born small at term, or a combination of the two (45). While we found an increased risk of preterm birth and LBW among B12-deficient women, there was little evidence that maternal B12 levels influenced offspring birth weight SD 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 was associated with higher birth weight in low- and middle-income countries, but not in high-income countries. Four of the five studies included in the low- and middle-income group were performed in an Indian population. Therefore, generalization of these results to low- or middle-income countries outside India should be treated with caution. Indian women generally have lower dietary intake of B12, due to a 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 greatest risk of giving birth to small newborns were the ones 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 is hypothesized that this association is due to altered fat distribution and metabolism in the overweight compared with normal weight (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 etiologies (50). Unfortunately, information on spontaneous versus medically indicated preterm births were not available to us. Medically indicated preterm birth are most commonly caused by severe preeclampsia or severely restricted fetal growth (51). Our findings do not support maternal level of B12 to be associated with fetal growth. Maternal B12 may, however, be associated with risk of preeclampsia, potentially through homocysteine, but 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, a Cochrane review concluded that supplementation of folic acid during pregnancy did not reduce risk of either preterm birth or LBW (60). Two small (256 pregnancies and 68 pregnancies) randomized controlled trials of B12 supplementation during pregnancy reported on birth weight and length of gestation (61,62). Both observed higher B12 plasma levels in the supplemented group compared with the control group, but no reduction in homocysteine levels. 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) (61,62). 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 low birth weight births globally each year (1). The greatest burden of LBW is found in South Asia, while 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), post-neonatal under-five mortality rate was reduced by 58% (2). Reduction in neonatal mortality was less pronounced (47%) (2). Prevention of preterm birth is thus a key strategy to reduce neonatal deaths and reach the new target of under-five year mortality 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 comparable to 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

Vitamin B12-deficiency during pregnancy is common. Results of this systematic review with IPD meta-analyses provides robust evidence that lower B12 levels during pregnancy are associated with increased risk of preterm birth, particularly in B12-deficient women. Our findings support conducting randomized controlled trials to evaluate whether maternal B12 supplementation in pregnancy reduces the risk of preterm birth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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Abbreviations

B12	vitamin B12
BMI	body mass index
CI	confidence interval
IPD	individual participant data
LBW	low birth weight
RR	risk ratio
SD	standard deviation
SGA	small-for-gestational-age

References

1. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. Lancet. 2014; 384(9938):189–205. [PubMed: 24853593]

2. UNICEF, WHO, World Bank. Levels and trends in child mortality 2015. 2015:1–36.

3. Barros FC, Bhutta Za, Batra M, et al. Global report on preterm birth and stillbirth (3 of 7): evidence for effectiveness of interventions. BMC Pregnancy Childbirth. 2010; 10(Suppl 1):S3. [PubMed: 20233384]

4. Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. Adv Exp Med Biol. 1994; 352:173–186. [PubMed: 7832046]

5. Allen RH, Stabler SP, Savage DG, et al. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. FASEB J. 1993; 7(14):1344–1353. [PubMed: 7901104]

6. Chiang PK, Gordon RK, Tal J, et al. S-Adenosylmethionine and methylation. FASEB J. 1996; 10(4): 471–480. [PubMed: 8647346]

7. Halarnkar PP, Blomquist GJ. Comparative aspects of propionate metabolism. Comp Biochem Physiol B. 1989; 92(2):227–231. [PubMed: 2647392]

8. Muthayya S, Kurpad A, Duggan C, et al. Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. Eur J Clin Nutr. 2006; 60(6):791–801. [PubMed: 16404414]

9. Sukumar N, Rafnsson S, Kandala N, et al. Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis. Am J Clin Nutr. 2016; 103(5):1232–1251. [PubMed: 27076577]

10. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane database Syst Rev. 2015; 11:CD004905.

11. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009; 339:b2700. [PubMed: 19622552]

12. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000; 283(15):2008–2012. [PubMed: 10789670]

13. PROSPERO. University of York; York, UK: 2011. (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015025141) [Accessed January 15, 2016]

14. Wells, G., Shea, B., O'Connell, D., et al. The Newcastle-Ottawa Scale for assessing the quality of nonrandomised studies in meta-analyses. 2013. (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

15. Stabler SP. Vitamin B12 Deficiency. N Engl J Med. 2013; 368(2):149–160. [PubMed: 23301732]

16. Villar J, Ismail LC, Victora CG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet. 2014; 384:857–868. [PubMed: 25209487]

17. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004; 159(7):702–706. [PubMed: 15033648]

18. Baker PN, Wheeler SJ, Sanders TA, et al. A prospective study of micronutrient status in adolescent pregnancy. Am J Clin Nutr. 2009; 89(4):1114–1124. [PubMed: 19244368]

19. Bergen NE, Jaddoe VWV, Timmermans S, et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: The Generation R Study. BJOG. 2012; 119(6):739–751. [PubMed: 22489763]

20. Bhate VK, Joshi SM, Ladkat RS, et al. Vitamin B12 and folate during pregnancy and offspring motor, mental and social development at 2 years of age. J Dev Orig Health Dis. 2012; 3(2):123–130. [PubMed: 25101923]

21. Chen LW, Lim AL, Colega M, et al. Maternal folate status, but not that of vitamins B-12 or B-6, is associated with gestational age and preterm birth risk in a multiethnic Asian population. J Nutr. 2015; 145(1):113–120. [PubMed: 25527665]

22. Dayaldasani A, Ruiz-Escalera J, Rodriguez-Espinosa M, et al. Serum vitamin B12 levels during the first trimester of pregnancy correlate with newborn screening markers of vitamin B12 deficiency. Int J Vitam Nutr Res. 2014; 84(1–2):92–97. [PubMed: 25835239]

23. Dwarkanath P, Barzilay JR, Thomas T, et al. High folate and low vitamin B-12 intakes during pregnancy are associated with small-for-gestational age infants in South Indian women: a prospective observational cohort study. Am J Clin Nutr. 2013; 98(6):1450–1458. [PubMed: 24108785]

24. Furness D, Fenech M, Dekker G, et al. Folate, vitamin B12, vitamin B6 and homocysteine: impact on pregnancy outcome. Matern Child Nutr. 2013; 9(2):155–166. [PubMed: 22023381]

25. Halicioglu O, Sutcuoglu S, Koc F, et al. Vitamin B12 and folate statuses are associated with diet in pregnant women, but not with anthropometric measurements in term newborns. J Matern Fetal Neonatal Med. 2012; 25(9):1618–1621. [PubMed: 22185230]

26. Hay G, Clausen T, Whitelaw A, et al. Maternal folate and cobalamin status predicts vitamin status in newborns and 6-month-old infants. J Nutr. 2010; 140(3):557–564. [PubMed: 20071650]

27. Hogeveen M, Blom HJ, van der Heijden EH, et al. Maternal homocysteine and related B vitamins as risk factors for low birthweight. Am J Obstet Gynecol. 2010; 202(6):572, e1–6. [PubMed: 20400059]

28. Kaymaz C, Demir A, Bige O, et al. Analysis of perinatal outcome by combination of first trimester maternal plasma homocysteine with uterine artery Doppler velocimetry. Prenat Diagn. 2011; 31(13):1246–1250. [PubMed: 22120509]

29. Krishnaveni GV, Veena SR, Karat SC, et al. Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. Diabetologia. 2014; 57(1):110–121. [PubMed: 24162586]

30. Mamabolo RL, Alberts M, Steyn NP, et al. The effect of maternal glucose metabolism, iron, vitamin B12 and folate status on pregnancy outcomes. South African J Clin Nutr. 2006; 19(3):120–130.

31. Relton CL, Pearce MS, Parker L. The influence of erythrocyte folate and serum vitamin B12 status on birth weight. Br J Nutr. 2005; 93(05):593. [PubMed: 15975157]

32. Sukumar N, Bawazeer N, Patel V, et al. Low B12 Level Is Associated with Maternal Obesity and Higher Birthweight in Gestational Diabetes. Journal of Developmental Origins of Health and Disease. 2011:128–129.

33. Takimoto H, Mito N, Umegaki K, et al. Relationship between dietary folate intakes, maternal plasma total homocysteine and B-vitamins during pregnancy and fetal growth in Japan. Eur J Nutr. 2007; 46(5):300–306. [PubMed: 17623226]

34. Wu BTF, Innis SM, Mulder Ka, et al. Low plasma vitamin B-12 is associated with a lower pregnancy-associated rise in plasma free choline in Canadian pregnant women and lower postnatal growth rates in their male infants. Am J Clin Nutr. 2013; 98(5):1209–1217. [PubMed: 24004892]

35. Yajnik CS, Deshpande SS, Jackson aa, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008; 51(1):29–38. [PubMed: 17851649]

36. Karakantza M, Androutsopoulos G, Mougiou A, et al. Inheritance and perinatal consequences of inherited thrombophilia in Greece. Int J Gynecol Obstet. 2008; 100(2):124–129.

37. López-Quesada E, Vilaseca MA, Vela A, et al. Perinatal outcome prediction by maternal homocysteine and uterine artery Doppler velocimetry. Eur J Obstet Gynecol Reprod Biol. 2004; 113(1):61–66. [PubMed: 15036713]

38. Lee S, Guillet R, Cooper EM, et al. Maternal Inflammation at Delivery Affects Assessment of Maternal Iron Status. J Nutr. 2014; 144(10):1524–1532. [PubMed: 25080540]

39. Neumann CG, Oace SM, Chaparro MP, et al. Low vitamin B12 intake during pregnancy and lactation and low breastmilk vitamin 12 content in rural Kenyan women consuming predominantly maize diets. Food Nutr Bull. 2013; 34(2):151–159. [PubMed: 23964388]

40. Glasziou P, Altman DG, Bossuyt P, et al. Reducing waste from incomplete or unusable reports of biomedical research. Lancet. 2014; 383(9913):267–276. [PubMed: 24411647]

41. Nyberg, Da, Abuhamad, A., Ville, Y. Ultrasound assessment of abnormal fetal growth. Semin Perinatol. 2004; 28(1):3–22. [PubMed: 15058898]

42. Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. J Am Diet Assoc. 2003; 103(6):748–765. [PubMed: 12778049]

43. Ota E, Mori R, Middleton P, et al. Zinc supplementation for improving pregnancy and infant outcome. Cochrane database Syst Rev. 2015; 2:CD000230.

44. De-Regil LM, Palacios C, Lombardo LK, et al. Vitamin D supplementation for women during pregnancy. Cochrane database Syst Rev. 2016; 1:CD008873.

45. United Nations Children's Fund and World Health Organization. Low Birthweight: Country, regional and global estimates. 2004:1–31.

46. Yajnik CS, Chandak GR, Joglekar C, et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. Int J Epidemiol. 2014; 43(5):1487–1497. [PubMed: 25052622]

47. Knight BA, Shields BM, Brook A, et al. Lower Circulating B12 Is Associated with Higher Obesity and Insulin Resistance during Pregnancy in a Non-Diabetic White British Population. PLoS One. 2015; 10(8):e0135268. [PubMed: 26288227]

48. Krishnaveni GV, Hill JC, Veena SR, et al. Low plasma vitamin B12 in pregnancy is associated with gestational "diabesity" and later diabetes. Diabetologia. 2009; 52(11):2350–2358. [PubMed: 19707742]

49. Romundstad PR, Smith GD, Nilsen TIL, et al. Associations of prepregnancy cardiovascular risk factors with the offspring's birth weight. Am J Epidemiol. 2007; 166(12):1359–1364. [PubMed: 17978410]

50. Goldenberg RL, Culhane JF, Iams JD, et al. Epidemiology and causes of preterm birth. Lancet. 2008; 371(9606):75–84. [PubMed: 18177778]

51. Gyamfi-Bannerman C, Fuchs KM, Young OM, et al. Nonspontaneous late preterm birth: Etiology and outcomes. Am J Obstet Gynecol. 2011; 205(5):456.e1–456.e6. [PubMed: 22035950]

52. Wadhwani NS, Patil V V, Mehendale SS, et al. Increased homocysteine levels exist in women with preeclampsia from early pregnancy. J Matern Fetal Neonatal Med. 2016; 29(16):2719–2725. [PubMed: 26552939]

53. Makedos G, Papanicolaou A, Hitoglou A, et al. Homocysteine, folic acid and B12 serum levels in pregnancy complicated with preeclampsia. Arch Gynecol Obstet. 2007; 275(2):121–124. [PubMed: 16941105]

54. Sanchez SE, Zhang C, Malinow MR, et al. Plasma folate, vitamin B12, and homocyst(e)ine concentrations in preeclamptic and normotensive Peruvian women. Am J Epidemiol. 2001; 153(5): 474–480. [PubMed: 11226979]

55. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832):2162–2172. [PubMed: 22682464]

56. Hogeveen M, Blom HJ, den Heijer M. Maternal homocysteine and small-for-gestational-age offspring: systematic review and meta-analysis. Am J Clin Nutr. 2012; 95(1):130–136. [PubMed: 22170376]

57. Miner SES, Evrovski J, Cole DEC. Clinical chemistry and molecular biology of homocysteine metabolism: An update. Clin Biochem. 1997; 30(3):189–201. [PubMed: 9167894]

58. Refsum H, Ueland PM, Nygård O, et al. Homocysteine and cardiovascular disease. Annu Rev Med. 1998; 49(1):31–62. [PubMed: 9509248]

59. Dodds L, Fell DB, Dooley KC, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem. 2008; 54(2):326–334. [PubMed: 18070815]

60. Lassi ZS, Salam RA, Haider BA, et al. Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. Cochrane database Syst Rev. 2013; 3:CD006896.

61. Duggan C, Srinivasan K, Thomas T, et al. Vitamin B-12 supplementation during pregnancy and early lactation increases maternal, breast milk, and infant measures of vitamin B-12 status. J Nutr. 2014; 144(5):758–64. [PubMed: 24598885]

62. Siddiqua TJ, Ahmad SM, Ahsan KB, et al. Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but vaccine response in mothers only: a randomized clinical trial in Bangladesh. Eur J Nutr. 2016; 55(1):281–293. [PubMed: 25648738]
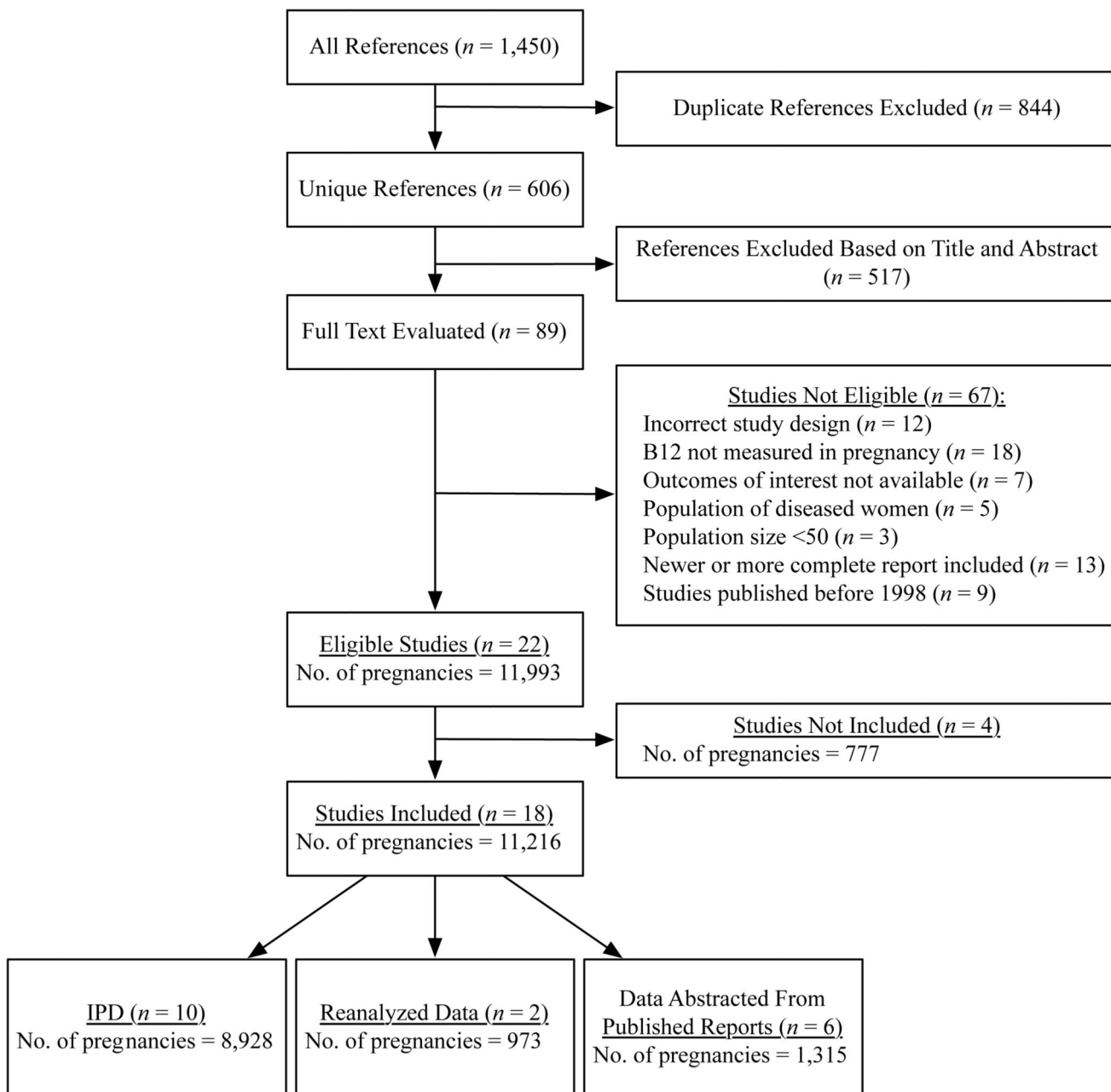
Figure 1. Flow chart of studies included in at least one of the meta-analyses of the association between B12 and birth weight or length of gestation

B12, vitamin B12; IPD, individual participant data.

Four studies were not included because IPD or reanalyses were not provided, and results could not be abstracted from the published reports.

First Author, Year (Reference No.)	No.		Mean Difference (95% CI)
Baker, 2009 (18)	290		-50.4 (-109.5, 8.7)
Bergen, 2012 (19)	5,577		-2.9 (-18.2, 12.5)
Bhate, 2012 (20)	194		-1.9 (-69.6, 65.9)
Chen, 2015 (21)	972		-4.3 (-37.0, 28.3)
Dayaldasani, 2014 (22)	182		-16.6 (-88.2, 55.0)
Dwarkanath, 2014 (23)	344		47.2 (-3.5, 97.9)
Hay, 2010 (26)	143		48.8 (-30.4, 128.0)
Kaymaz, 2011 (28)	103		-15.4 (-183.7, 152.9)
Krishnaveni, 2014 (29)	654		33.4 (3.0, 63.9)
Sukumar, 2011 (32)	196		20.5 (-38.2, 79.1)
Takimoto, 2007 (33)	51		-134.6 (-285.9, 16.7)
Yajnik, 2008 (35)	700		1.9 (-34.7, 38.5)
Overall	9,406		5.1 (-10.9, 21.0)

Test for heterogeneity: $I^2 = 30\%$

-150   0   150

Mean Difference

Figure 2. Forest plot presenting the association between B12 and birth weight
Meta-analysis of studies of the association between vitamin B12 and birth weight after adjustment for maternal age, parity and body mass index or weight. Effect estimates are expressed as change in birth weight per one standard deviation increase of vitamin B12 (i.e. mean difference). CI, confidence interval; No, number pregnancies.

A)
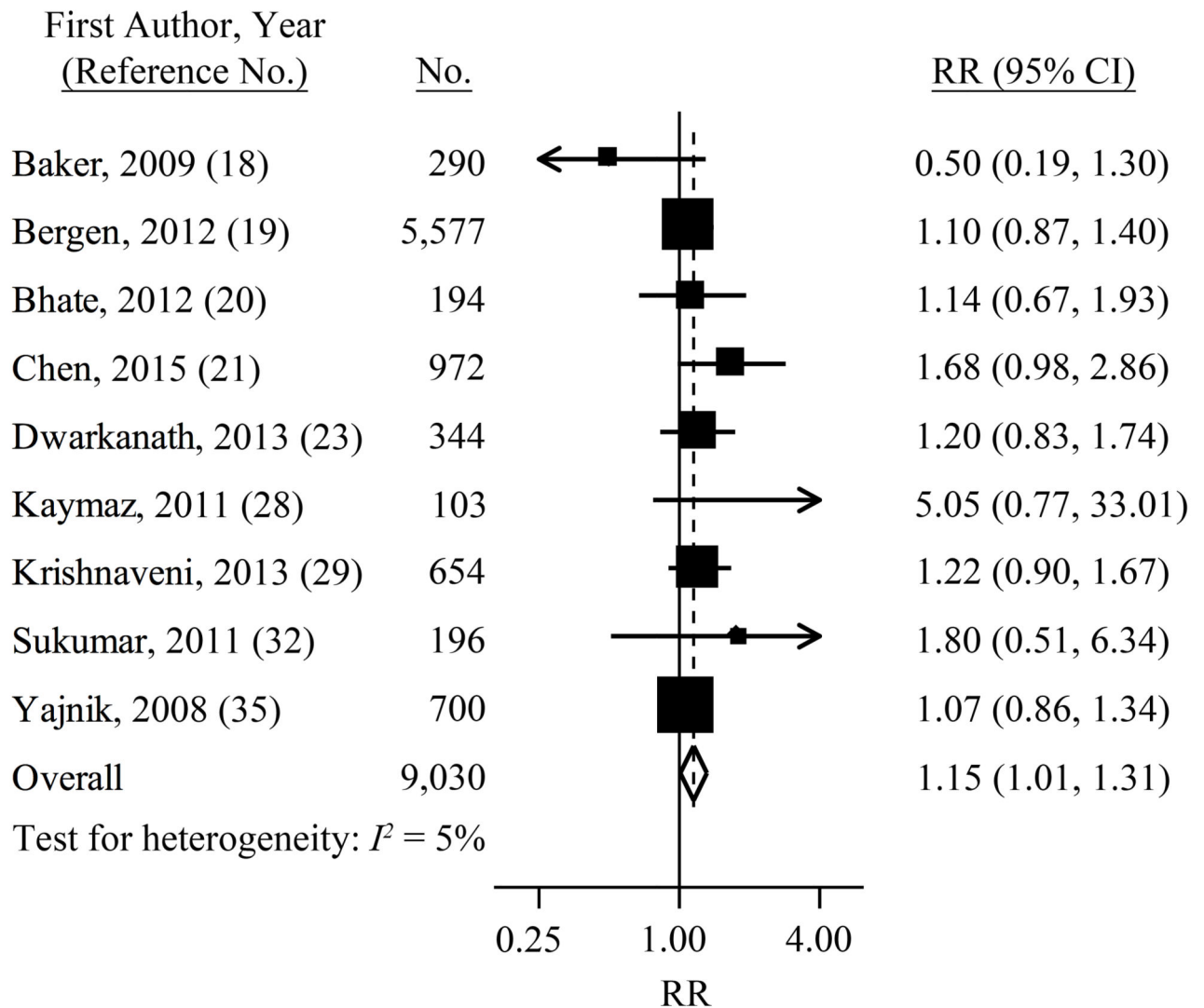
Figure 3A. Forest plot presenting the association between B12-deficiency and the risk of low birth weight

Meta-analysis of studies of the association between vitamin B12-deficiency and the risk of low birth weight after adjustment for maternal age, parity and body mass index or weight. Effect estimate expressed as risk ratio of the outcome comparing B12 deficient to non-deficient. CI, confidence interval; No, number pregnancies.

B)

First Author, Year (Reference No.)	No.		RR (95% CI)
Baker, 2009 (18)	290		1.74 (0.77, 3.91)
Bergen, 2012 (19)	5,576		1.01 (0.79, 1.29)
Bhate, 2012 (20)	214		0.56 (0.20, 1.54)
Chen, 2015 (21)	972		1.69 (1.02, 2.79)
Dwarkanath, 2013 (23)	344		0.99 (0.54, 1.81)
Kaymaz, 2011 (28)	103		0.95 (0.25, 3.55)
Krishnaveni, 2013 (29)	654		1.42 (0.89, 2.26)
Sukumar, 2011 (32)	196		5.60 (0.71, 43.87)
Yajnik, 2008 (35)	753		1.31 (0.87, 1.97)
Overall	9,102		1.21 (0.99, 1.49)

Test for heterogeneity: $I^2 = 20\%$

0.25   1.00   4.00

RR

Figure 3B. Forest plot presenting the association between B12-deficiency and the risk of preterm birth
Meta-analysis of studies of the association between vitamin B12-deficiency and the risk of preterm birth after adjustment for maternal age, parity and body mass index or weight. Effect estimate expressed as risk ratio of the outcome comparing B12 deficient to non-deficient. CI, confidence interval; No, number pregnancies.

C)

First Author, Year (Reference No.)	No.		RR (95% CI)
Baker, 2009 (18)	286		0.82 (0.40, 1.70)
Bergen, 2012 (19)	5,508		0.97 (0.80, 1.18)
Bhate, 2012 (20)	(no data)		(no data)
Chen, 2015 (21)	965		1.03 (0.57, 1.86)
Dwarkanath, 2013 (23)	340		1.32 (0.95, 1.84)
Kaymaz, 2011 (28)	(no data)		(no data)
Krishnaveni, 2013 (29)	640		1.07 (0.85, 1.35)
Sukumar, 2011 (32)	195		1.12 (0.42, 2.94)
Yajnik, 2008 (35)	(no data)		(no data)
Overall	7,934		1.05 (0.92, 1.19)
Test for heterogeneity:	$I^2 = 0\%$		

0.33   1.00   3.00

RR

Figure 3C. Forest plot presenting the association between B12-deficiency and the risk of small-for-gestational-age birth

Meta-analysis of studies of the association between vitamin B12-deficiency and the risk of small-for-gestational-age birth after adjustment for maternal age, parity and body mass index or weight. Effect estimate expressed as risk ratio of the outcome comparing B12 deficient to non-deficient. CI, confidence interval; No, number pregnancies.
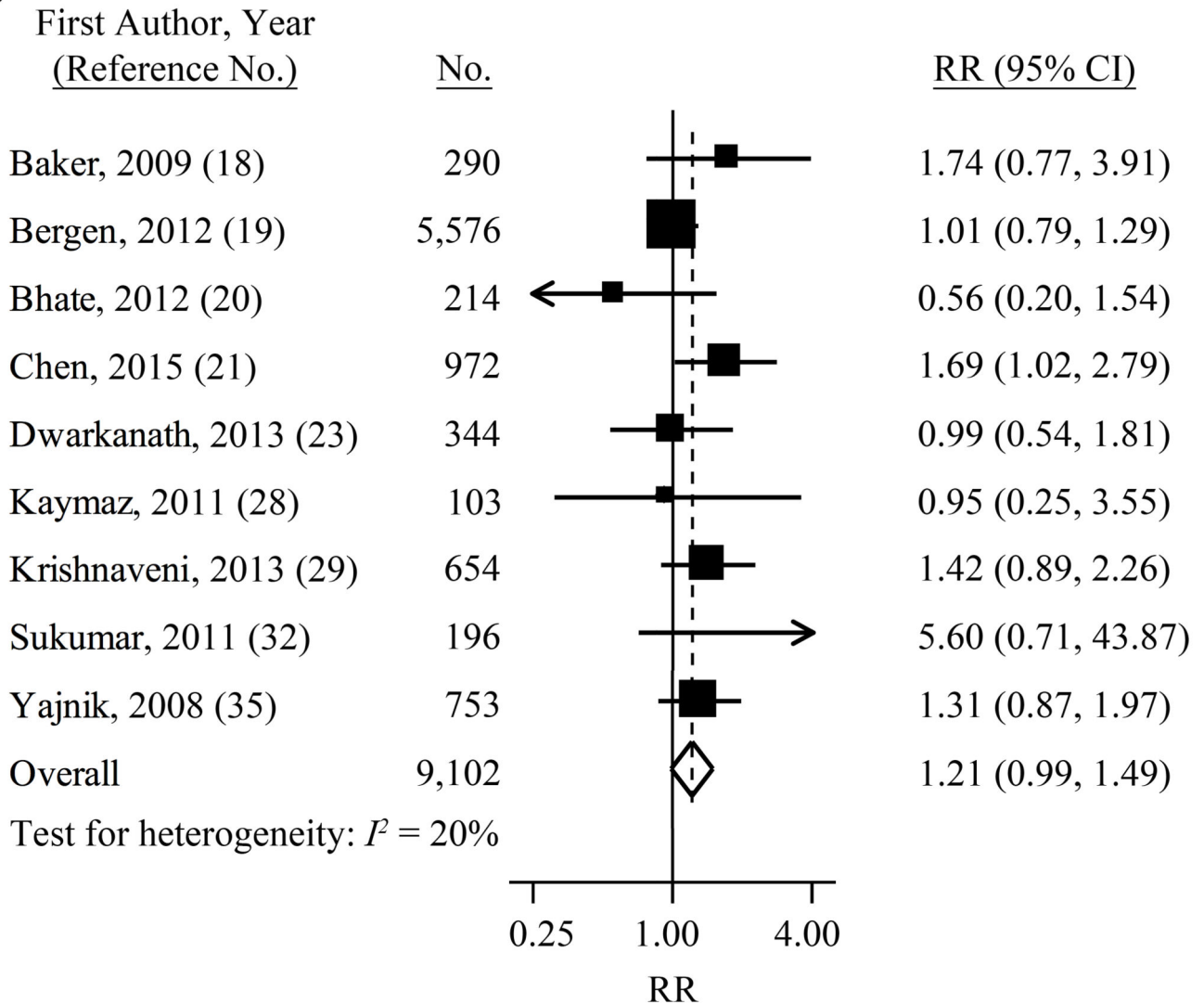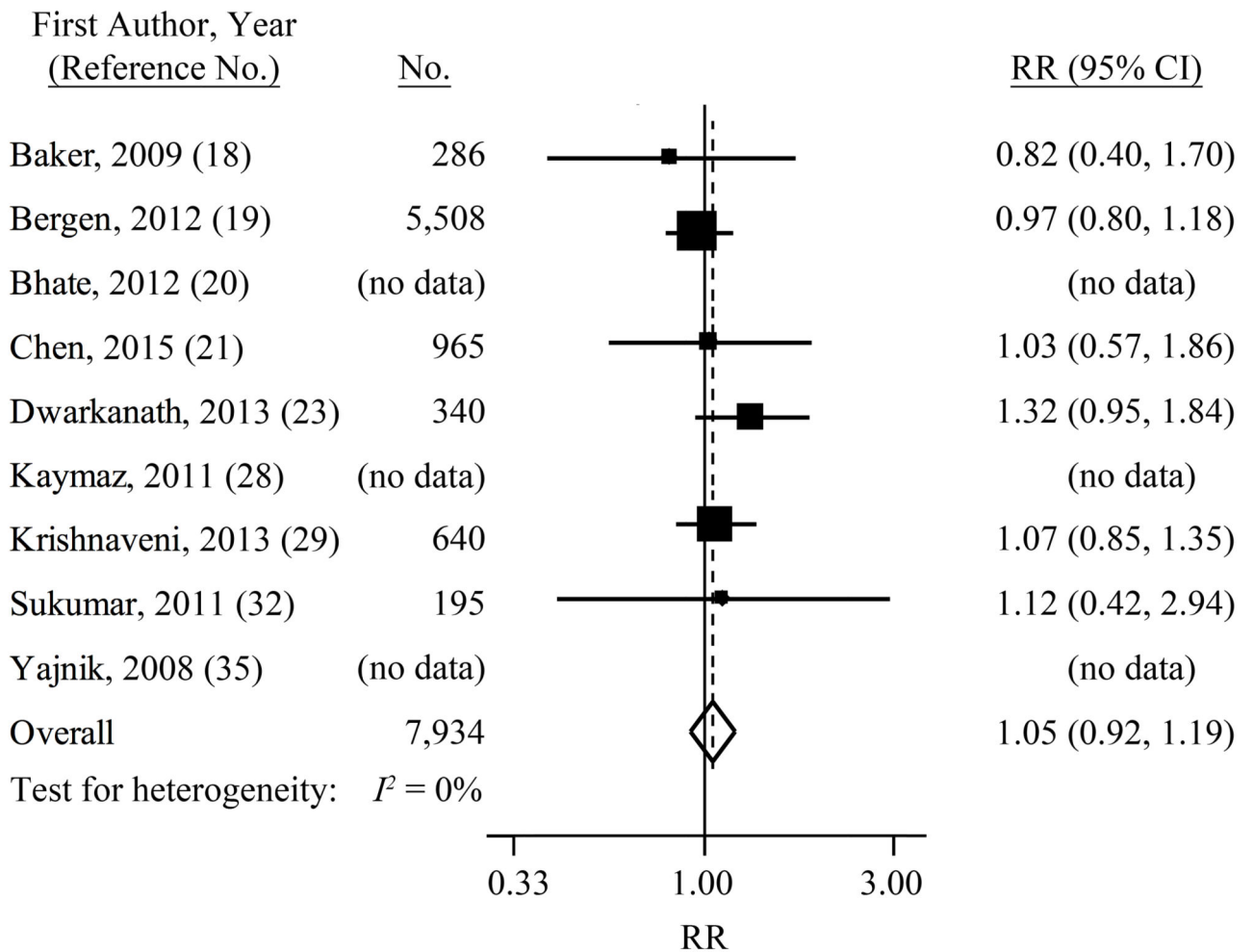
Table 1

Characteristics of Studies Included in the Meta-Analysis

Study	Data	n	Country	Study years	B12 analysis method	Week of B12 measurement		Birth weight	Included in specific meta-analyses[a]				
						Range	Median		LBW	SGA	Birth weight SD score	Length of gestation	Preterm birth
Baker, 2009 (18)	IPD	290	The United Kingdom	2004-2007	RIA	27-43	30	x	x	x	x	x	x
Bergen, 2012 (19)	IPD	5,641	The Netherlands	2002-2006	ECL	5-18	13	x	x	x	x	x	x
Bhate, 2012 (20)	Reanalysed data	214	India	2004-2006	Micro-biological	24-30	28	x	x			x	x
Chen, 2015 (21)	IPD	988	Singapore	2009-2010	ECL	26-29	27	x	x	x	x	x	x
Dayaldasani, 2014 (22)	IPD	187	Spain	2011	ECL	3-23	10	x^b	x^b	x^b	x^b	x^b	x^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	x	x	x	x	x	x
Furness, 2013 (24)	Data from publication	84	Australia	NA	ECL	18-20	NA			x^c			
Halicioglu, 2012 (25)	Data from publication	208	Turkey	2008	ECL	>37	NA	x^d					
Hay, 2010 (26)	IPD	149	Norway	1997	Micro-biological	17-19	NA	x					
Hogeveen, 2010 (27)	IPD	363	The Netherlands	2002-2004	Micro-biological	27-38	31	x^e	x^e	x^e	x^e	x^e	x^e
Kaymaz, 2011 (28)	IPD	103	Turkey	2007	ECL	11-14	13	x	x			x	x
Krishnaveni, 2014 (29)	IPD	654	India	1997-1998	Micro-biological	22-35	26	x	x	x	x	x	x
Mamabolo, 2006 (30)	Data from publication	219	South Africa	1999-2000	RIA	28-36	NA			x^c			
Relton, 2005 (31)	Data from publication	500	The United Kingdom	2000-2002	RIA	NA	11.5 (5.8)^f				x^e		
Sukumar, 2011 (32)	IPD	209	The United Kingdom	2005-2010	RIA (n=182), ECL (n=27)	0-37	24	x	x	x	x	x	x
Takimoto, 2007 (33)	Data from publication	88	Japan	2001-2003	ECL	T1: 7-14 T3: 34-36	T1: NA T3: NA	x^g					
Wu, 2013 (34)	Data from publication	216	Canada	NA	RIA	NA	36	x^d					
Yajnik, 2008 (35)	Reanalysed data	759	India	1994-1996	Micro-biological	NA	T2: 18 (2)^f	x	x			x	x

Studies are referred to according to their citation number in the text. ECL, electroluminescence; IPD, individual participant data; n, number of pregnancies; NA, not available; RIA, radioimmunoassay; SD, standard deviation; SGA, small-for-gestational-age; T1, 1st trimester; T2, 2nd trimester; T3, 3rd trimester.

[a]
included in the analyses of the exposures B12 SD score and B12-deficiency, both crude and adjusted (maternal age, body mass index or weight, and parity), if not otherwise specified;

[b]
does not contribute in the analyses of B12-deficiency (none of the participants were deficient);

[c]
level of B12 in SGA versus non-SGA, crude analysis;

[d] birth weight among B12-deficient versus non-deficient, crude analysis;

[e] crude analysis;

[f] 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

Study	Maternal age (years), mean (SD)	Maternal BMI (kg/m²), mean (SD)	Para 0		Vitamin B12 (pmol/L), mean (SD)	B12-deficient[a]		Birth weight (g), mean (SD)	LBW[b]		SGA[c]		Length of gestation (weeks), mean (SD)	Preterm birth[d]	
			n	%		n	%		n	%	n	%		n	%
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	NA	NA	38.6 (2.6)	18	8
Chen, 2015 (21)	31 (5)	66 (12)[e]	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)[e]	203	59	205 (115)[f]	100	29[f]	2,771 (498)	95	28	102	30	38.3 (1.7)	47	14
Furness, 2013[g] (24)	33 (7)	27 (5)	NA	NA	234 (129)	NA	NA	3,390 (789)	NA	NA	21	25[h]	38.8 (2.9)	NA	NA
Halicioglu, 2012[g] (25)	28 (5)	NA	NA	NA	120[i]	99	48[j]	3,357 (476)	NA	NA	NA	NA	NA	NA	NA
Hay, 2010 (26)	30 (4)	65 (10)[e]	67	45	294 (87)	2	1	3,727 (476)	0	0	NA	NA	NA	NA	NA
Hogeveen, 2010 (27)	33 (4)	NA	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	NA	NA	38.4 (1.9)	9	9
Krishnaveni, 2013 (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[g] (30)	25 (7)	27 (4)	NA	NA	175 (77)	36	16[k]	3,120 (550)	NA	NA	66	30[l]	NA	NA	NA
Relton, 2005[g] (31)	28 (6)[m]	NA	NA	43[m]	239 (97)	NA	NA	3,430 (470)[m]	NA	NA	NA	NA	NA	NA	NA
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[g] (33)	29 (5)	21 (3)	NA	NA	405 (146)[f]	13	16[n]	3,120 (411)	5	5	NA	NA	39.6 (1.0)	NA	NA
Wu, 2013[g] (34)	33 (4)	NA	NA	NA	224 (96)	51	24	3,486 (452)	NA	NA	NA	NA	NA	NA	NA
Yajnik, 2008 (35)	21 (4)	18 (2)	252	31	151 (78)	447	59	2,612 (392)	230	33	NA	NA	38.8 (2.1)	87	11

Studies are referred to according to their citation number in the text. BMI, body mass index; LBW, low birth weight; NA, not available; SD, standard deviation; SGA, small-for-gestational-age.

[a] B12 <148 pmol/L;

[b] birth weight <2,500 g;

[c] birth weight SD score (i.e. accounting for length of gestation and sex) below 10th centile;

[d] length of gestation <37 weeks;

[e] kg (BMI not available);

[f] first measurement;

[g] data extracted from publication;

[h] serial tapering of growth in abdominal circumference and of estimated fetal weight below the 10th centile of an Australian growth chart;

[i] median (range not available);

[j] B12 118 pmol/L;

[k] B12-deficiency not defined;

[l] lowest birth weight tertile (mean birth weight 2,940 g) used as approximation of SGA for the purpose of this review;

[m] based on a larger study population than the subgroup with available B12 data included in this review (n=974-997);

[n] third trimester

[o] weight (kg)/height (m)^2.