Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight

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Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis[1,2]

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ABSTRACT

Background: Vitamin B-12 and folate are micronutrients essential for normal embryogenesis. Vitamin B-12 insufficiency in pregnancy is high in certain parts of the world, such as India, and although this has been linked to low birth weight (LBW) in these populations, the relation between vitamin B-12 and birth weight (BW) elsewhere is unknown.

Objectives: We performed a systematic review to assess *1*) the worldwide prevalence of vitamin B-12 insufficiency in pregnancy and *2*) its association with BW.

Design: A search of 5 electronic databases was performed to identify eligible articles. Random-effects meta-analysis was conducted according to geographic regions and pregnancy trimesters for the prevalence subreview and by categorical measures of BW.

Results: A total of 57 and 23 articles were included for the prevalence and BW subreviews, respectively. The pooled estimates of vitamin B-12 insufficiency were 21%, 19%, and 29% in the first, second, and third trimesters, respectively, with high rates for the Indian subcontinent and the Eastern Mediterranean. The large heterogeneity between studies was partially addressed by creating a standardized score for each study (mean vitamin B-12 insufficiency ÷ cutoff value), which internally corrected for geographic region, trimester, and assay type. Twelve of the 13 longitudinal studies included showed a decrease in mean or median vitamin B-12 across trimesters. Pooled analysis showed nonsignificantly lower maternal vitamin B-12 concentrations in LBW than in normal-BW infants and higher odds of LBW with lower vitamin B-12 values (adjusted OR: 1.70; 95% CI: 1.16, 2.50), but studies from India largely contributed to the latter.

Conclusions: Our review indicates that vitamin B-12 insufficiency during pregnancy is common even in nonvegetarian populations and that concentrations of vitamin B-12 decrease from the first to the third trimester. There is no consistent association between vitamin B-12 insufficiency and LBW. However, given the long-term risks of LBW, this observation warrants further cohort studies and randomized controlled trials. *Am J Clin Nutr* doi: 10.3945/ajcn.115.123083.

Keywords: vitamin B-12 insufficiency, pregnancy, low birth weight, geographic variation, systematic review, meta-analysis

INTRODUCTION

Vitamin B-12, also known as cobalamin, is a micronutrient essential for cellular growth, differentiation, and development (1). Together with folic acid, vitamin B-12 is necessary for the synthesis of DNA, RNA, lipids, and protein in the cellular cytoplasm (2, 3). More specifically, vitamin B-12 and folate are necessary cofactors for the conversion of homocysteine to methionine, the latter being an important methyl donor required for the synthesis of neurotransmitters and phospholipids.

Vitamin B-12 insufficiency was previously perceived to be a problem that affected the elderly, due to malnutrition or intrinsic factor–mediated malabsorption (4), and has been related to anemia, dementia, and cognitive dysfunction (5, 6). Although hyperhomocysteinemia (most commonly due to vitamin B-12 or folate deficiency) has been identified as an independent risk factor for atherosclerotic vascular disease (7, 8), a systematic review showed no definite association between vitamin B-12 insufficiency in adults and composite cardiovascular endpoints (9). These 2 B-vitamins are, however, pivotal in normal em-

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[2] Supplemental Tables 1–3 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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bryogenesis, and therefore there is increasing attention on optimizing concentrations in young women in the periconceptional period and pregnancy. Both low vitamin B-12 and folate concentrations have been associated with pregnancy complications such as neural tube defects (NTDs),[10] spontaneous abortion (10), pre-eclampsia (11, 12), and preterm birth (13), with the latter 2 conditions mediated in part by elevated homocysteine. Folic acid supplementation is effective in reducing the risk of NTDs by >40% (14), but because more than half of pregnancies are unplanned, mandatory folic acid fortification of wheat flour and cereal products was introduced in North America in 1997 and many other parts of the world in the early 2000s. This resulted in a halving of NTDs due to folate deficiency over 10 y (15). However, the number of NTDs attributable to vitamin B-12 deficiency has tripled during this time (16).

In addition, suboptimal vitamin B-12 concentrations in pregnancy have been shown to be independently associated with low birth weight (LBW) (17), an adverse lipid profile in neonates (18), and higher insulin resistance in children (19). LBW or small-for-gestational age (SGA) are outcomes of particular interest because they are well-established surrogate markers for metabolic disorders such as obesity, type 2 diabetes, and metabolic syndrome in later life in many populations (20–22).

However, the relation between vitamin B-12 and birth weight (BW) is far from established (23, 24). Most studies showing the link between vitamin B-12 and LBW are from the Indian subcontinent, where rates of both LBW/SGA and vitamin B-12 insufficiency are high (19, 25, 26). The high prevalence of vitamin B-12 insufficiency in India has been attributed to vegetarianism (i.e., no consumption of animal products except for dairy) and infrequent meat consumption in omnivores (i.e., consumption of small quantities of nonvegetarian food less often than alternate days) (19, 27). However, vitamin B-12 insufficiency has also been found in other countries where vegetarianism is rare, such as in Brazil and Turkey (28, 29). The aims of our systematic review and meta-analysis are to evaluate the prevalence of vitamin B-12 insufficiency in pregnancy across a worldwide population and assess whether this is associated with LBW and/or SGA.

METHODS

This systematic review comprises 2 subreviews: *1*) prevalence of vitamin B-12 insufficiency and *2*) vitamin B-12 insufficiency and BW. These subreviews will be divided accordingly where appropriate in the following sections.

Sources of data

Published guidelines on reporting systematic reviews and meta-analysis of observational studies [MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines] were followed (30). A comprehensive literature search in 5 bibliographic databases was conducted for the prevalence subreview: MEDLINE/PubMed (National Library of Medicine and NIH; http://www.ncbi.nlm.nih.gov/pubmed/), EMBASE (the Excerpta Medica database; http://ovidsp.tx.ovid.com/), Global Health (CABI; https://www.ebscohost.com/academic/global-health/), CAB (Commonwealth Agricultural Bureau database; http://www.cabdirect.org/), and CINAHL (Cumulative Index to Nursing and Allied Health Literature; http://www.ebscohost.com/). For the BW subreview, 4 databases were used, namely MEDLINE/PubMed, EMBASE, Global Health, and Scopus (Elsevier; http://www.scopus.com/). All databases were searched from inception until December 2014. We also examined reference lists of key publications for further articles. When needed, the authors were contacted by e-mail for more complete information.

Search criteria

For the prevalence subreview, a search strategy based on the following keywords and medical subject headings (MeSH) was used: "cobalamin," "vitamin B12," "vitamin B12 insufficiency," "vitamin B12 deficiency," "methylmalonic acid," "holotranscobalamin," "homocysteine," "pregnancy," and "pregnant women." Search words were combined by using Boolean operators (AND, OR). A similar search string was used in all bibliographic databases. Studies conducted in pregnant women (aged 18–45 y) at any trimester, including delivery, which reported the prevalence of vitamin B-12 insufficiency with clearly defined cutoffs were included. Only results from the trimesters in which vitamin B-12 values were available from at least 50 women are reported.

The keywords and MeSH used for the BW subreview included the following: "cobalamin," "vitamin B12 insufficiency," "vitamin B12 deficiency," "methylmalonic acid," "holotranscobalamin," "homocysteine," "pregnancy outcome," "birth weight," "intrauterine growth retardation," and "small for gestational age." An identical approach to that described above was used for combining search words.

Eligibility criteria

Both longitudinal and cross-sectional observational studies conducted in the community or hospital setting in pregnant women (aged 18–45 y) without any major comorbidities were included. We restricted the search to studies conducted in human subjects and published in the English language in peer-reviewed journals. If the results of a study were reported in >1 publication, the study with the most complete information pertaining to our review's outcomes was used. If these were identical, the study published earlier was included. The following types of studies were excluded: randomized controlled trials in which vitamin B-12 supplementation was given as part of the study design, case-control studies, and studies conducted exclusively in mothers with comorbidities (e.g., HIV, post–bariatric surgery). In addition, we excluded studies that were designed specifically to look at pregnancies with NTDs, intrauterine growth retardation (IUGR), early pregnancy loss, and anemia; however, if the studies were conducted in healthy pregnant women with no previous medical history and reported rates of anemia in their results, they were included. For the BW subreview, we included studies that reported vitamin B-12 results from maternal or cord blood and offspring BW.

[10] Abbreviations used: BW, birth weight; IUGR, intrauterine growth restriction; LBW, low birth weight; NTD, neural tube defect; SGA, small-for-gestational age.

Data extraction

Level 1 screening of initial database search results (titles and abstracts) was independently performed by at least 2 reviewers (NS and SBR) according to the inclusion and exclusion criteria. Level 2 screening was conducted by reviewing the full manuscripts of the articles. Two reviewers (NS and SBR) independently extracted the study characteristics onto predesigned forms that included information on the study population and methods, vitamin B-12 values, and BW outcomes. Any discrepancy in data extraction was resolved by consensus and by consulting a third reviewer (PS) when necessary.

Data synthesis

For the prevalence subreview, we analyzed and reported the results of the systematic review according to the 3 trimesters of pregnancy to ensure like-for-like comparison. It is well known from longitudinal studies that there is a progressive decline in vitamin B-12 during the course of pregnancy (31, 32), which reaches a nadir toward the end of the third trimester (33). To assess the impact of geography on the worldwide prevalence of vitamin B-12 insufficiency, the broad WHO region classification was used (i.e., Africa, Americas, South East Asia, Europe, Eastern Mediterranean, and Western Pacific) (34). However, we further divided the Americas and South East Asia regions into North America and Central/South America and Indian subcontinent and South East/East Asia, respectively, in an attempt to bring together the populations on the basis of dietary habits, vegetarianism, and consumption of animal products (4).

For the BW subreview, the included studies reported 3 different types of effect sizes, namely the following: *1*) odds of having an adverse-BW outcome below a threshold of maternal/cord vitamin B-12, *2*) comparison of mean/median maternal/cord vitamin B-12 values between adverse and normal BWs, and *3*) the effect of maternal/cord vitamin B-12 as a linear variable on BW (regression coefficient or correlation coefficient). Adverse-BW outcome was defined as LBW (BW $<$2500 g) (26), SGA (BW $<$10th centile for gestational age), IUGR (estimated fetal weight $<$10%) (35), or as the lowest tertile or quartile of BW in the included studies.

Statistical analysis

Prevalence subreview

To estimate the pooled estimates of the vitamin B-12 insufficiency rate per trimester, we obtained an estimate from each study of the proportion of pregnant women with vitamin B-12 concentrations below the cutoff defined in that study. Subgroup analysis was undertaken for the studies from the second and third trimesters to determine whether the prevalence of vitamin B-12 insufficiency in pregnancy varied according to the geographic areas. Statistical heterogeneity was calculated by using the I^2 statistic (36). A random-effects meta-analysis (37) was undertaken by using STATA version 13 software (StataCorp) (38). We assessed publication bias by using a funnel plot and Egger's and Begg's tests to find out whether there was a bias toward publication of studies with positive results among the smaller studies (results not shown).

To correct for differences in the vitamin B-12 measurement assays and cutoffs used by the studies, we calculated a standardized score by dividing the mean vitamin B-12 concentration used in the study by the cutoff used to define insufficiency in that study (mean vitamin B-12 $\div$ insufficiency cutoff value). In the studies in which a median vitamin B-12 value was reported, it was used to estimate the mean when sample sizes were large (39). Stepwise linear regression was then performed to determine the predictors of the percentage of vitamin B-12 insufficiency in a model that included the trimester of sampling, assay type, and geographic region. Log-transformed standardized scores were used for this because the variable was not normally distributed. SPSS version 22 was used for the analysis (40).

BW subreview

The software Review Manager (41) was used to conduct meta-analyses from the included studies. We obtained an estimate from each study of the adjusted ORs with 95% CIs by using a random-effects model. Statistical heterogeneity was calculated by using the I^2 statistic (36). Adjusted outcome measures were tabulated where these were reported. Continuous effect measures data were expressed as mean $\pm$ SD differences of vitamin B-12 between infants of LBW or the equivalent (termed "adverse birth weight outcome cases") and normal-BW outcome. Pooled analyses were not done for the studies that reported vitamin B-12 and BW as linear variables, because there was too much heterogeneity in the reporting of the independent variable and outcome (e.g., some reporting unit values and others SD scores).

Risk of bias and quality of evidence

The methodologic quality assessment of the studies was performed by using a checklist for cohort studies adapted from the Scottish Intercollegiate Guidelines Network (42). The quality assessment focused on evaluating how minimal the risk of bias was in study reporting by using 14 key criteria from the checklist (**Supplemental Table 1**). However, for certain cross-sectional studies that reported only the point prevalence of vitamin B-12 insufficiency, only 12 relevant criteria were used (criteria relating to dropout rate and comparison between full participants and those lost to follow-up were not used, because these were not relevant). The overall study quality grade was calculated as per standard guidelines according to the proportion of total criteria fulfilled (42).

RESULTS

Prevalence of vitamin B-12 insufficiency

Study characteristics

The electronic database search yielded 4742 citations, of which 153 were selected for full-text review (**Figure 1**A). There were 6 studies identified from the full-text review that reported vitamin B-12 insufficiency rates during pregnancy but without specifying a trimester (43–46) or clearly stating the number of women sampled per trimester (47, 48). These studies were not included in further analysis.

A total of 57 studies (19, 28, 29, 31, 49–101) met all of the inclusion criteria and comprised 16 longitudinal studies (n = 34 results) and 41 cross-sectional studies, giving a total of 75 results. Details of these studies, including the country in which the fieldwork was done, the proportion of vitamin B-12 supplement or

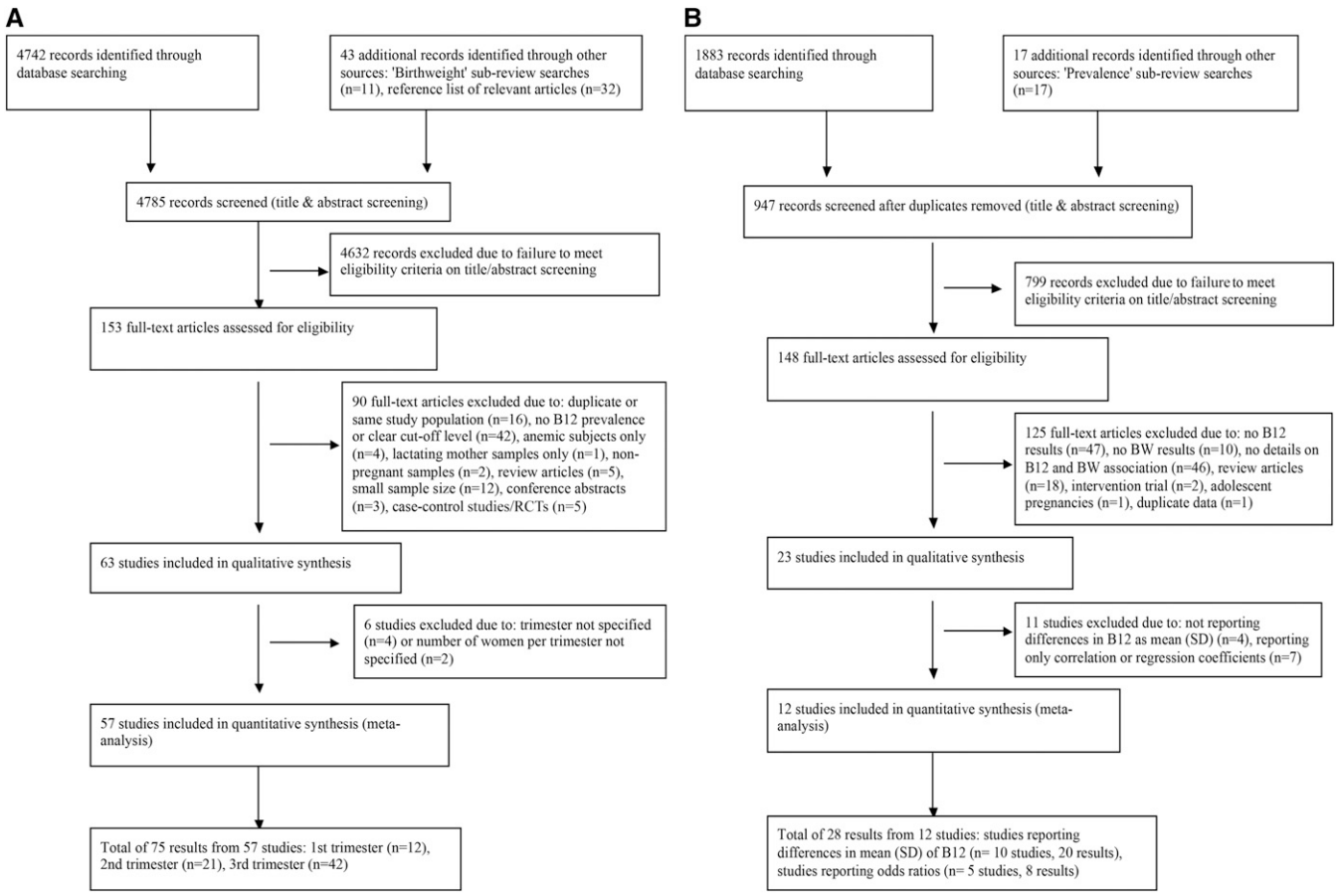

FIGURE 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram showing the study selection process. (A) Prevalence of B-12 insufficiency in pregnancy subreview and (B) B-12 insufficiency and birthweight subreview. B12, vitamin B-12; BW, birth weight; RCT, randomized controlled trial.

multivitamin use, vitamin B-12 assay method, and insufficiency rates, are presented in **Table 1**. For the setting of the study, they were broadly categorized into a community or hospital setting (including health centers) to reflect where the population was sampled. In the first, second, and third trimesters, there were 12, 21, and 42 results, which were obtained by sampling 10,474, 8621, and 11,667 pregnant women, respectively.

Prevalence of vitamin B-12 insufficiency in pregnancy

The overall prevalence of maternal vitamin B-12 insufficiency during pregnancy from all of the studies, across all 3 trimesters, was 25%. When analyzing by trimester, the rates were 21%, 19%, and 29% for the first, second, and third trimesters, respectively (**Figures 2**–**4**).

Of the first-trimester studies, there was insufficient representation from all of the geographic regions to do a comprehensive subgroup analysis. The 4 studies from the Indian subcontinent showed a high insufficiency rate (pooled estimate: 32%; Figure 2). This observation was once again seen in the second trimester, with the pooled insufficiency rate from the Indian subcontinent increasing to 64% (Figure 3). Apart from North America, South East/East Asia, and Europe, the other geographic regions were also poorly represented in the second trimester, but there were notably high insufficiency rates of 59%, 49%, and 46% found from studies carried out in Venezuela (31), Turkey (28), and South Korea (88), respectively.

Of the studies included in the third trimester, the pooled insufficiency rate of the studies from the Indian subcontinent was 60%. An additional striking finding was the pooled insufficiency rate of 65% (I^2 = 95%) (Figure 4) (28, 52, 65, 77, 93) from the 5 studies in the Eastern Mediterranean region. On the contrary, insufficiency rates of <8% were found in 2 studies conducted in Thailand and Sudan, where the authors attributed the low rates of insufficiency to the consumption of fish and animal/fermented products, respectively (although details of dietary intake were not provided) (49, 50).

Mean vitamin B-12 concentrations across trimesters

Eleven studies included in this review reported mean vitamin B-12 results through the course of pregnancy and 2 studies reported median vitamin B-12 results longitudinally. Ten of the 11 studies that reported the mean showed a consistent decrease in vitamin B-12 concentrations from the first to the third (91, 95), from the second to the third (28, 45, 46, 81, 87), and from the first to the second to the third (85, 96, 98) trimesters. The exception was the study by Marzan et al. (83), which showed a marginal increase in mean vitamin B-12 concentrations across the pregnancy (266, 270, and 286 pmol/L in the first, second, and third trimesters, respectively), despite the participants not

TABLE 1
Prevalence of vitamin B-12 insufficiency during pregnancy: key study characteristics and results[1]

First author, publication year (ref), country, year of field study	Study design, number of participants with vitamin B-12 results	Setting of study population, response rate	Vitamin B-12 supplement or multivitamin use, %	Vitamin B-12 cutoff, pmol/L	Vitamin B-12 insufficiency, %	Vitamin B-12, pmol/L
First trimester (n = 12)						
Microbiological assay						
Whiteside, 1968 (a) (98), Australia, N/R	L, 56	Hospital, N/R	N/R	<74	5	217
Roberts, 1973 (a) (91), England, 1971	L, 320	Hospital, N/R	N/R	<118	35	165 ± 84.9[3]
Jiang, 2005 (74), Nepal, 1998–2001	CS, 1158	Community, 89% consented	None	<150	28.3	237.1 ± 138.3
Murphy, 2007 (a) (85), Spain, 1992–1996	L, 88	Hospital, N/R	27[2]	<150	0	267 (144, 449)[4]
Radioimmunoassay						
García-Casal, 2005 (a) (31), Venezuela, 2001–02	CS, 129	Hospital, N/R	N/R	<148	43.4	N/R
Ray, 2008 (90), Canada, 2007	CS, 3734	N/R, N/R	N/R	<125	8.5	249 (244, 255)[5]
Chemiluminescence						
Köşüş, 2012 (78), Turkey, N/R	CS, 228	Hospital, N/R	N/R	<156	12.5	200 (95.6)[6]
Dwarkanath, 2013 (60), India, N/R	L, 1838	Hospital, 73% consented	None	<150	32	N/R
Heppe, 2013 (67), Netherlands, 2002–2006	CS, 2173	Hospital, N/R	N/R	<150	26	175 (100)[6]
Samuel, 2013 (92), India, 2008–2010	CS, 352	Hospital, 88% consented	None	<150	51.1	149 (109, 205)[6]
Shamim, 2013 (94), Bangladesh, 2001–2007	CS, 285	Community, N/R	N/R	<150	19.6	206.3 ± 84.5
Assay method not described						
Shields, 2011 (a) (95), Scotland, 2008–2009	CS, 113	Hospital, N/R	N/R	<156	16	215
Second trimester (n = 21)						
Microbiological assay						
Lowenstein, 1960 (a) (81), Canada, N/R	L, 59	N/R, N/R	N/R	<148	15.2	235 ± 101
Whiteside, 1968 (b) (98), Australia, N/R	L, 50	Hospital, N/R	N/R	<74	25	127
Jacob, 1976 (72), USA, 1972–1974	CS, 182	Hospital, 100% consented	20	<111	4.5	303
Murphy, 2007 (b) (85), Spain, 1992–1996	L, 90	Hospital, N/R	27[2]	<150	0	230 (123, 432)[4]
Yajnik, 2008 (a) (19), India, 1994–1996	L, 638	Community, 92% consented	N/R	<150	60	135 (103, 175)[6]
Katre, 2010 (75), India, 2004–2006	L, 163	Hospital, 97.3% consented	29[2]	<150	73	119 (87, 161)[6]
Radioimmunoassay						
Marzan, 1971 (a) (83), Philippines, N/R	CS, 100	Hospital, N/R	None	<59	1.5	270.1 ± 79
Areekul, 1976 (a) (50), Thailand, N/R	CS, 71	Hospital, N/R	N/R	<111	7	All trimesters: 211 ± 106
Knight, 1991 (a) (76), USA, 1985–1990	L, 108	Hospital, N/R	91[2]	<148	7	N/R
Bruinse, 1995 (a) (56), Netherlands, N/R	L, 70	Hospital, N/R	None	<180	0	N/R

(Continued)

TABLE 1 (*Continued*)

First author, publication year (ref), country, year of field study	Study design, number of participants with vitamin B-12 results	Setting of study population, response rate	Vitamin B-12 supplement or multivitamin use, %	Vitamin B-12 cutoff, pmol/L	Vitamin B-12 insufficiency, %	Vitamin B-12, pmol/L
Açkurt, 1995 (a) (28), Turkey, 1991	L, 129	Hospital, 66% responded to invitation	35	<111	48.8	140.8 ± 105
Pagán, 2002 (a) (87), USA, 1986–1988	L, 285	N/R, N/R	N/R	<148	0.35	357 ± 131
Park, 2004 (88), South Korea, N/R	CS, 89	Hospital, N/R	42	<258	46.1	N/R
García-Casal, 2005 (b) (31), Venezuela, 2001–2002	CS, 430	Hospital, N/R	N/R	<148	58.6	N/R
Li, 2008 (80), Bangladesh, 2002	L, 753	Community, 78% consented	N/R	<185	60	N/R
Chemiluminescence						
Takimoto, 2007 (a) (96), Japan, 2001–2003	L, 77	Hospital, N/R	N/R	<148	8	301 ± 96
Goedhart, 2011 (64), Netherlands, 2003–2004	CS, 2921	Community, 35% consented	N/R	<148	6	N/R
Enzyme immunoassay						
House, 2000 (70), Canada, 1996–1997	CS, 1424	N/R, N/R	N/R	<130	25.3	180 (130, 240)[6]
Milman, 2006 (a) (84), Denmark, 1995–1996	L, 406	N/R, N/R	34[2]	<150	15	225 (118, 381)[7]
Wu, 2013 (a) (99), Canada, N/R	L, 264	Hospital, N/R	N/R	<148	10	287 ± 126
Others						
Hinderaker, 2002 (68), Tanzania, 1995–1996	CS, 312	Hospital, 78% consented	N/R	HPLC: <150	16.7	N/R
Third trimester (n = 42)						
Microbiological assay						
Lowenstein, 1960 (b) (81), Canada, N/R	L, 252	N/R, N/R	N/R	<148	19	221 ± 126
Zachau-Christiansen, 1962 (101), Denmark, N/R	CS, 365	Hospital, N/R	N/R	<111	17	177
Roberts, 1973 (b) (91), England, 1971	L, 119	Hospital, N/R	N/R	<118	48	134 ± 70.9
Yusufji, 1973 (100), India, N/R	CS, 998	Hospital, N/R	N/R	<103	52	117 ± 90
Baker, 1975 (51), USA, N/R	CS, 174	N/R, N/R	76[2]	<59	23	85 ± 832
Osifo, 1976 (86), Nigeria, N/R	CS, 50	N/R, N/R	N/R	<148	40	208 ± 123
Bjørke Monsen, 2001 (54), Norway, 1996–1997	CS, 169	Hospital, N/R	36[2]	<150	15	245 (175, 323)[6]
Murphy, 2007 (c) (85), Spain, 1992–1996	L, 90	Hospital, N/R	27[2]	<150	0	224 (117, 444)[4]
Pathak, 2007 (89), India, N/R	CS, 266	Community, 94% consented	N/R	<148	74.1	N/R
Yajnik, 2008 (b) (19), India, 1994–1996	L, 594	Community, 92% consented	N/R	<150	71	122 (94, 160)[6]
Krishnaveni, 2009 (79), India, 1997–1998	CS, 774	Hospital, N/R	31[2]	<150	43	162 (123, 221)[6]
Radioimmunoassay						
Marzan, 1971 (b) (83), Philippines, N/R	CS, 57	Hospital, N/R	None	<59	0	286.3 ± 86

(Continued)

TABLE 1 (*Continued*)

First author, publication year (ref), country, year of field study	Study design, number of participants with vitamin B-12 results	Setting of study population, response rate	Vitamin B-12 supplement or multivitamin use, %	Vitamin B-12 cutoff, pmol/L	Vitamin B-12 insufficiency, %	Vitamin B-12, pmol/L
Cole, 1974 (57), Australia, N/R	CS, 130	Hospital, N/R	N/R	<148	12.3	272.3
Colman, 1975 (58), South Africa, N/R	CS, 106	Hospital, N/R	N/R	<295	0.9	524 ± 165
Areekul, 1976 (b) (50), Thailand, N/R	CS, 100	Hospital, N/R	N/R	<111	13	All trimesters: 211 ± 106
Fréry, 1992 (61), France, N/R	CS, 188	Hospital, N/R	N/R	<148	27.6	175 (74, 397)[5]
Giugliani, 1984 (63), Brazil, N/R	CS, 51	Hospital, 100%	51[2]	<165	21.6	251 ± 108
Ho, 1987 (69), Taiwan, N/R	CS, 221	Hospital, N/R	None	<110	3.6	228.6 ± 157.3
Knight, 1991 (b) (76), USA, 1985–1990	L, 218	Hospital, N/R	91[2]	<148	11.2	318 ± 216
Black, 1994 (55), Mexico, 1985–1987	CS, 85	Community, N/R	N/R	<74	15	228 ± 451
Açkurt, 1995 (b) (28), Turkey, 1991	L, 87	Hospital, 66% responded to invitation	35	<111	80.9	94.6 ± 107.8
Bruinse, 1995 (b) (56), Netherlands, N/R	L, 70	Hospital, N/R	None	<180	0	N/R
Pagán, 2002 (b) (87), USA, 1986–1988	L, 285	N/R, N/R	N/R	<148	2.1	285 ± 100
Ma, 2004 (82), China, 1999–2000	CS, 1019	Hospital, N/R	None	<148	10.5	N/R
García-Casal, 2005 (c) (31), Venezuela, 2001–2002	CS, 301	Hospital, N/R	N/R	<148	68.5	N/R
Hall, 2007 (66), Bangladesh, 2004–2005	CS, 95	Hospital, N/R	N/R	<185	58.9	180.0 ± 71.5
Gibson, 2008 (62), Ethiopia, N/R	CS, 83	Community, N/R	N/R	<150	23	268 (152, 372)[6]
Chemiluminescence						
Schulpis, 2004 (93), Greece, 1999–2002	CS, 1933	Hospital, N/R	None	<170	52.7	N/R
Koc, 2006 (77), Turkey, N/R	CS, 180	Hospital, N/R	19	<118	72	95.9 ± 45.5
Takimoto, 2007 (b) (96), Japan, 2001–2003	L, 82	Hospital, N/R	N/R	<148	16	265 ± 95
Barbosa, 2008 (53), Brazil, 2001–2003	CS, 275	Hospital, N/R	None	<179	75	N/R
Hussein, 2009 (71), Egypt, N/R	CS, 84	Hospital, N/R	N/R	<150	46.4	185.3 ± 113
Vanderjagt, 2009 (97), Nigeria, N/R	CS, 98	Hospital, N/R	None	<148	12.2	208 (25, 739)[8]
Halicioglu, 2012 (65), Turkey, 2008	CS, 208	Hospital, 88% consented	57[2]	<118	47.6	120 (N/R)[6]
Balcı, 2014 (52), Turkey, N/R	CS, 72	N/R, N/R	None	<148	70.8	120 ± 53.1
Enzyme immunoassay						
Guerra-Shinohara, 2004 (29), Brazil, N/R	CS, 119	Hospital, N/R	N/R	<132	52.9	130 (122, 138)[5]
Milman, 2006 (b) (84), Denmark, 1995–1996	L, 256	N/R, N/R	34[2]	<150	42.6	161 (71, 284)[7]
Wu, 2013 (b) (99), Canada, N/R	L, 220	Hospital, N/R	N/R	<148	23	224 ± 96.2

(Continued)

TABLE 1 (*Continued*)

First author, publication year (ref), country, year of field study	Study design, number of participants with vitamin B-12 results	Setting of study population, response rate	Vitamin B-12 supplement or multivitamin use, %	Vitamin B-12 cutoff, pmol/L	Vitamin B-12 insufficiency, %	Vitamin B-12, pmol/L
Others						
Abdelrahim, 2009 (49), Sudan, 2007–2009	CS, 55	Hospital, N/R	N/R	Immunofluorescence: <111	1.1	159.4 ± 66.5
Assay method not described						
Cook, 1971 (59), Latin America, N/R	CS, 899	N/R, N/R	N/R	<59	15.4	N/R
Shields, 2011 (b) (95), Scotland, 2008–2009	L, 77	Hospital, N/R	N/R	<156	60	153
Jacquemyn, 2014 (73), Belgium, 2011	CS, 78	Hospital, N/R	76[2]	<150	13	244 ± 93.9

[1]Key study characteristics and results from the 57 included studies classified according to the trimesters of pregnancy and vitamin B-12 measurement assay are presented. The lowercase letters in parentheses in the first column indicate the order of appearance in the table for studies that reported results from >1 trimester. CS, cross-sectional; L, longitudinal (i.e., the same participants had >1 vitamin B-12 measurement during pregnancy); N/R, not reported; ref, reference.

[2]The study states explicitly that the women consumed vitamin B-12 supplements or multivitamins containing vitamin B-12.

[3]Mean ± SD (all such values).

[4]Geometric mean; 10th, 90th centile in parentheses.

[5]Geometric mean; 5th, 95th centile in parentheses.

[6]Median; 25th, 75th centile or IQR in parentheses.

[7]Median; 5th, 95th centile in parentheses.

[8]Median; minimum, maximum in parentheses.

taking vitamin B-12 or multivitamin supplements. The 2 studies that reported median vitamin B-12 values showed a decrease of 13 and 64 pmol/L between the second and third trimesters (19, 84).

Standardized score

A total of 32 of the 57 studies reported mean vitamin B-12 values in addition to the insufficiency rates. Ten of these were longitudinal studies, providing results from >1 trimester; thus, a total of 43 results were available. In addition, 12 studies reported the median values that were used to estimate the mean. Two of these were longitudinal, yielding a total of 14 results. Combining the above 2 studies, we obtained 57 standardized scores (ratio of mean ÷ cutoff). Details of these studies with their standardized scores and corresponding vitamin B-12 insufficiency rates are shown in **Table 2**.

Linear regression was performed to test the degree to which the standardized score was a predictor of percentage of vitamin B-12 insufficiency reported in each study, in a model that included trimester, geographic region, and assay type. Two outlying results from a single study that gave very high standardized scores of >4.5 (due to a very low vitamin B-12 cutoff threshold of 59 pmol/L) were excluded (83). The model explained 72% of variance in the percentage insufficiency, and the standardized score was the only significant predictor of the former (adjusted B-coefficient: −136.5; 95% CI: −159.7, −113.3; $P < 0.001$) (**Supplemental Figure 1**). This confirms that, after internal correction for the assay type and cutoff, it was the same group of studies with the lower standardized scores (from specific geographic regions, namely the Indian subcontinent and Eastern Mediterranean) that also found higher prevalence rates of vitamin B-12 insufficiency, giving more weight to the results seen in the forest plots (Figures 2–4).

Study quality assessment

Studies were of differing quality, and the detailed quality assessments are shown in **Supplemental Table 2**. A total of 23 of the 57 of the studies were of good quality with minimal bias in reporting, a further 28 were of moderate quality, and 6 were of poor quality. When the breakdown of assessment criteria was looked at, most studies had a clearly defined question and outcomes but performed poorly in reporting the proportion of eligible participants consenting, the reliability and validity of biochemical assays used, and in accounting for confounders in analysis.

Vitamin B-12 insufficiency and BW

Study characteristics

A total of 1900 citations were obtained from the electronic database searches and by reviewing references of articles (Figure 1B). A total of 947 records were shortlisted for title and abstract screening, of which 148 full-text reviews were performed. A total of 23 studies met all of the inclusion criteria after full-text review and were included in the final analysis (23–25, 29, 60, 61, 87, 96, 102–116). **Tables 3–5** provide the characteristics of these studies and their results. The studies reported the association between maternal or cord vitamin B-12 concentrations and LBW in 3 different ways: *1*) logistic regression to analyze the OR of having an adverse-BW outcome (e.g., LBW, SGA, or IUGR) with low vitamin B-12 concentrations (n = 5; Table 3), *2*) comparison of mean/median vitamin B-12 values between adverse-BW cases and normal-BW

controls (n = 15; Table 4), and *3*) linear regression to study the association between vitamin B-12 and BW as continuous variables (n = 10; Table 5). Six studies reported their results by using a combination of the above 3 methods (23–25, 103, 108, 114) and were included as appropriate.

Odds of SGA or LBW with low vitamin B-12 concentrations

Five studies across the trimesters (23–25, 60) and cord blood (114) (8 results) reported the ORs of SGA or LBW with lower vitamin B-12 concentrations compared with higher concentrations (Table 3). Meta-analysis (n = 1482; 598 cases, 884 controls) of all of these studies showed that the OR of having an SGA/LBW infant was 1.70 (95% CI: 1.16, 2.50) with lower vitamin B-12 concentrations (**Figure 5**).

There were too few results from different countries and trimesters to provide subgroup analysis. Therefore, we conducted only the following 3 analyses: *1*) removal of the study reporting only cord blood vitamin B-12 concentrations (114) [because vitamin B-12 is actively transported across the placenta (117) and is expected to be higher than that of maternal vitamin B-12], *2*) combining the 6 results from India (from 3 studies) (25, 60, 114) because of the high prevalence of both vitamin B-12 insufficiency and SGA/LBW infants (26), and *3*) removal of the Muthayya et al. (25) study because it reported large effect sizes and contributed 21.5% to the pooled analysis. The ORs (95% CIs) were 1.59 (1.07, 2.36), 2.42 (1.50. 3.92), and 1.23 (0.90, 1.67), respectively, in these subgroup analyses. There were only 2 other results (from Australia and Holland), which did not show any association between vitamin B-12 and BW (23, 24).

Differences in mean/median vitamin B-12 concentrations and adverse BW

Fifteen studies reported either mean or median vitamin B-12 values between normal-BW and LBW/SGA groups. There were 8 cross-sectional and 7 longitudinal studies, yielding a total of 25 results (Table 4). Two subgroup meta-analyses by maternal concentrations (n = 1969; 487 cases and 1482 controls; 14 results) (**Figure 6**A) and cord blood concentrations (n = 896; 382

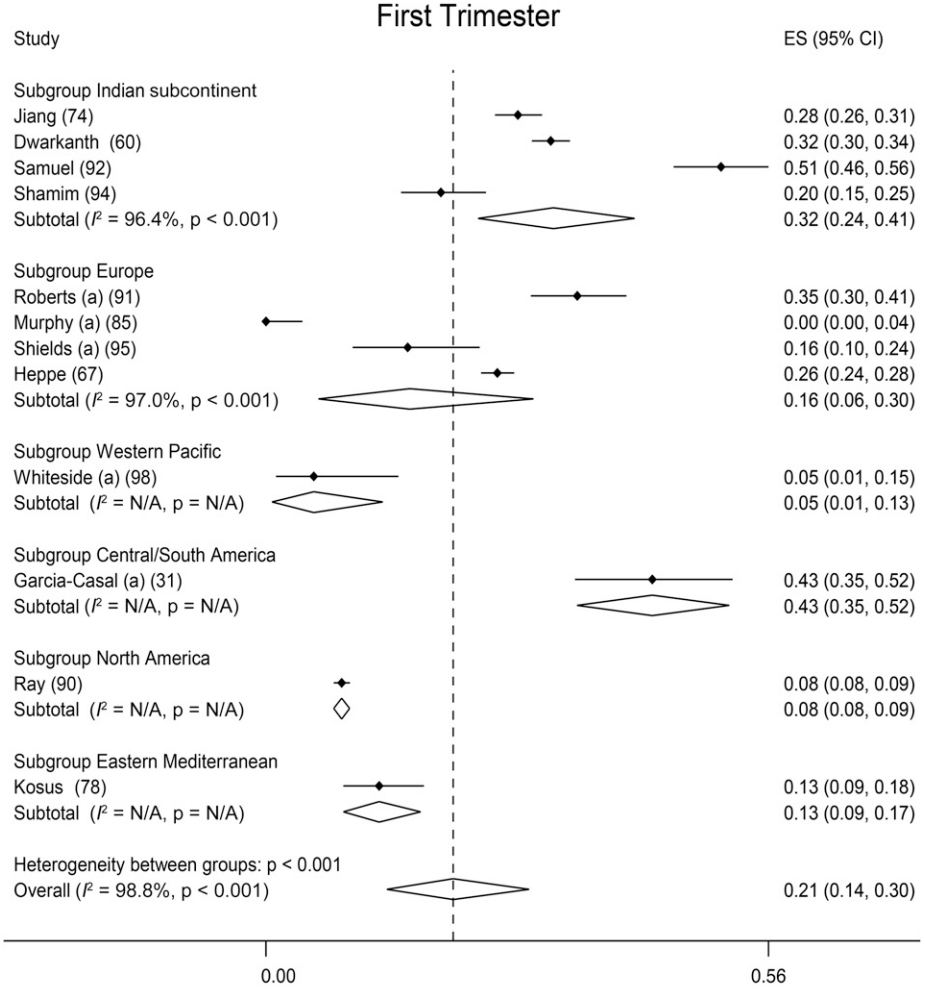

FIGURE 2 Meta-analysis of maternal vitamin B-12 insufficiency in the first trimester of pregnancy separated by subgroups of geographic regions (n = 12 results). Open diamonds represent the pooled proportions for each subgroup and the overall proportion for the trimester, and the solid diamonds in each study denote the proportion for that study (horizontal lines represent 95% CIs). The I^2 values refer to the statistical heterogeneity within each subgroup and the whole trimester combined. A random-effects model using generic inverse variance showed a pooled proportion insufficiency rate of 0.21 (0.14, 0.30). Studies which have reported results from >1 trimester have a lowercase letter in parentheses in the study reference to indicate their order of appearance in the Forest plots. ES, effect size; N/A, not applicable.

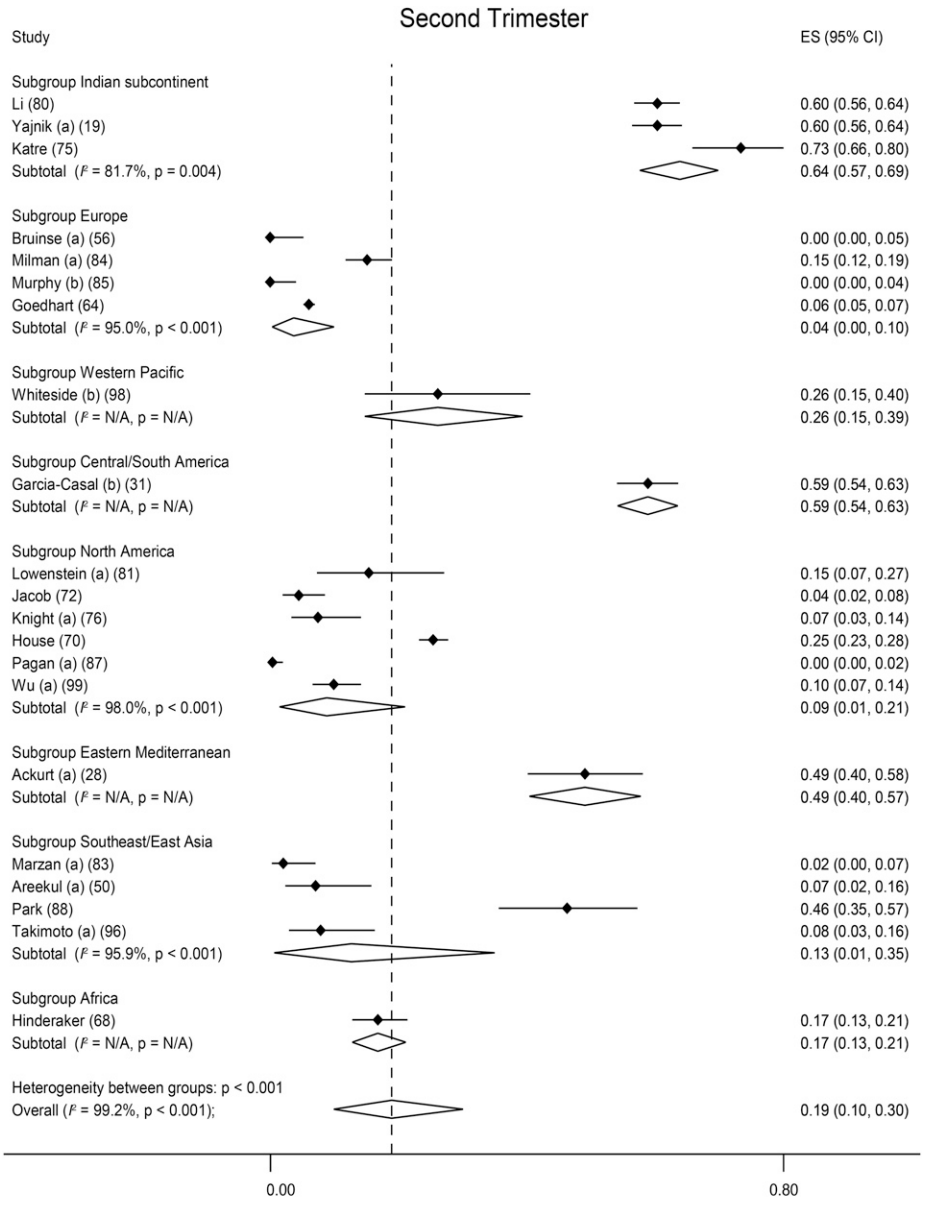

FIGURE 3 Meta-analysis of maternal vitamin B-12 insufficiency in the second trimester of pregnancy separated by subgroups of geographic regions (n = 21 results). Open diamonds represent the pooled proportions for each subgroup and the overall proportion for the trimester, and the solid diamonds in each study denote the proportion for that study (horizontal lines represent 95% CIs). The I^2 values refer to the statistical heterogeneity within each subgroup and the whole trimester combined. A random-effects model using generic inverse variance showed a pooled proportion insufficiency rate of 0.19 (0.10, 0.30). Studies which have reported results from >1 trimester have a lowercase letter in parentheses in the study reference to indicate their order of appearance in the Forest plots. ES, effect size; N/A, not applicable.

cases and 514 controls; 6 results) (Figure 6B) were conducted. Although the pooled estimates showed lower maternal vitamin B-12 concentrations in the adverse-BW group (particularly in the second trimester), these were not significant (Figure 6A). Similarly, the pooled meta-analyses of cord blood vitamin B-12 concentrations were not different between the BW groups (Figure 6B).

Three other studies (n = 880; 430 cases and 450 controls; 5 results) from the third trimester and cord blood showed differences in vitamin B-12 concentrations between the normal- and adverse-BW groups in terms geometric mean (107, 116), or mean difference in SD compared to the appropriate normal mean for gestation (102) (Table 4). One study showed a nonsignificant lower geometric mean vitamin B-12 concentration in the third trimester in SGA infants (116), but none of the other studies showed the positive association expected. These studies were not included in the pooled analysis of Figure 6A, B because the effect sizes could not be converted to a mean ± SD value.

Effect of vitamin B-12 concentrations across the spectrum on BW

Ten studies examined vitamin B-12 and BW as continuous variables (Table 5). In cases in which the studies performed both unadjusted linear correlations and adjusted regression coefficients we reported the latter (as B- or β-coefficients), but if only unadjusted correlations were carried out, we reported the former (as r coefficients). One study showed a significant positive correlation

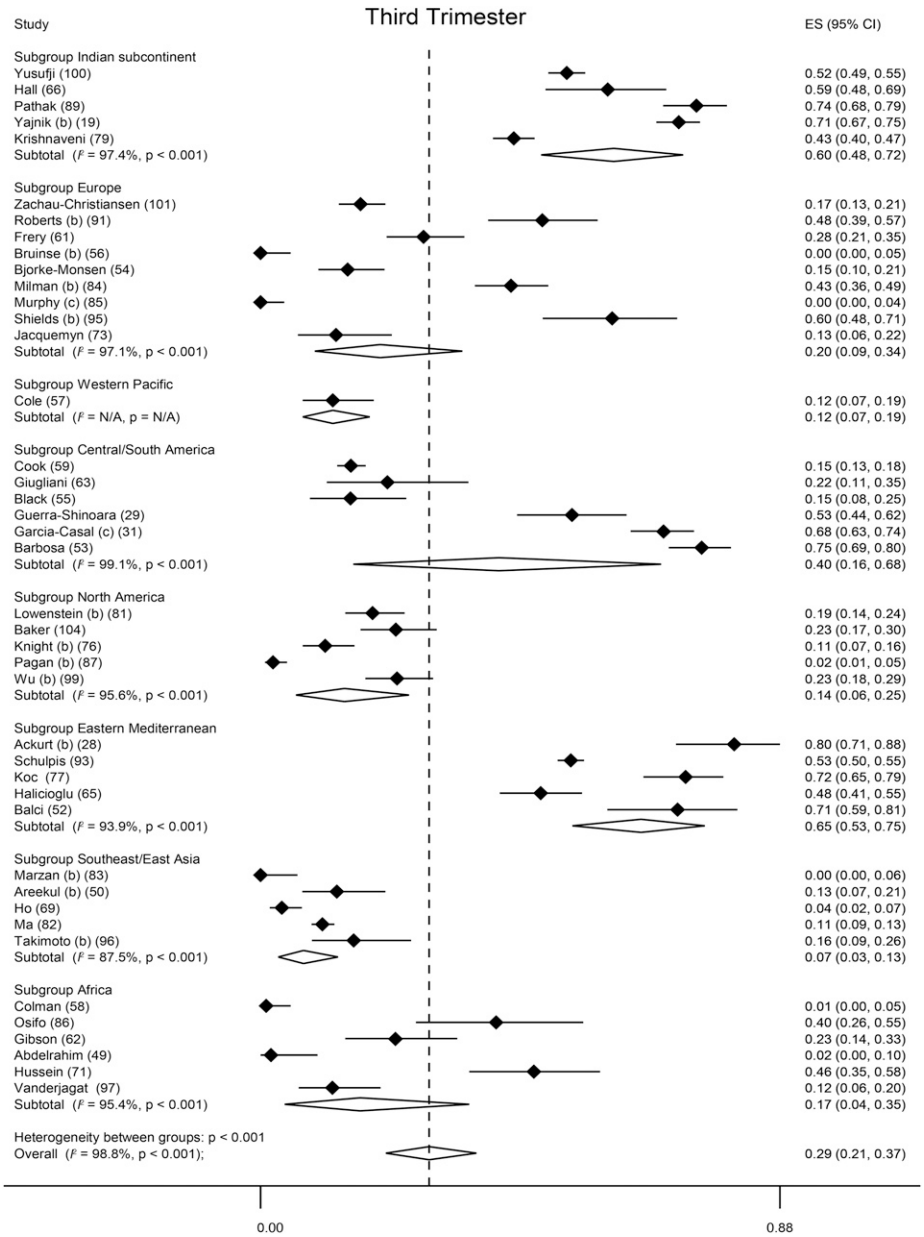

FIGURE 4 Meta-analysis of maternal vitamin B-12 insufficiency in the third trimester of pregnancy separated by subgroups of geographic regions ($n = 42$ results). Open diamonds represent the pooled proportions for each subgroup and the overall proportion for the trimester, and the solid diamonds in each study denote the proportion for that study (horizontal lines represent 95% CIs). The I^2 values refer to the statistical heterogeneity within each subgroup and the whole trimester combined. A random-effects model using generic inverse variance showed a pooled proportion insufficiency rate of 0.29 (0.21, 0.37). Studies which have reported results from >1 trimester have a lowercase letter in parentheses in the study reference to indicate their order of appearance in the Forest plots. ES, effect size; N/A, not applicable.

(17), and 9 showed no association between vitamin B-12 and BW [2 positive but nonsignificant (103, 108), 5 negative (24, 29, 61, 87, 105), and 2 showed varying associations at different time points (96, 113)].

Study quality assessment

Nine of the 23 studies were of good quality with minimal bias in reporting, a further 10 were of moderate quality, and 4 were of poor quality (**Supplemental Table 3**). Although the studies generally did well in reporting an appropriate study question and describing the source population, more than half did not report the number of eligible participants who consented or the rates of mothers/neonates lost to follow-up or performed a comparison between participants with available and missing data.

DISCUSSION

Prevalence of vitamin B-12 insufficiency

One of the striking findings of our systematic review was the high rate of vitamin B-12 insufficiency among certain populations

TABLE 2
Calculated standardized scores and corresponding vitamin B-12 insufficiency rates[1]

First author, year (reference)	Trimester (*n*)	Mean vitamin B-12, pmol/L	Vitamin B-12 cutoff, pmol/L	Vitamin B-12 insufficiency, %	Standardized score
Katre, 2010 (75)	2 (163)	119[2]	150	73	0.79
Koc, 2006 (77)	3 (180)	96	118	72	0.81
Balcı, 2014 (52)	3 (72)	120	148	70.8	0.81
Yajnik, 2008 (b) (19)	3 (594)	122[2]	150	71	0.81
Açkurt, 1995 (b) (28)	3 (87)	95	111	80.9	0.85
Yajnik, 2008 (a) (19)	2 (638)	135[2]	150	60	0.9
Hall, 2007 (66)	3 (95)	180	185	58.9	0.97
Guerra-Shinohara, 2004 (29)	3 (117)	130	132	52.9	0.98
Shields, 2011 (b) (95)	3 (77)	153	156	60	0.98
Samuel, 2013 (92)	1 (352)	149[2]	150	51.1	1
Halicioglu, 2012 (65)	3 (208)	120[2]	118	47.6	1.02
Milman, 2006 (b) (84)	3 (256)	161[2]	150	42.6	1.07
Krishnaveni, 2009 (79)	3 (774)	162[2]	150	43	1.08
Roberts, 1973 (b) (91)	3 (119)	134	118	48	1.14
Yusufji, 1973 (100)	3 (998)	117	103	52	1.14
Heppe, 2013 (67)	1 (2173)	175[2]	150	26	1.17
Fréry, 1992 (61)	3 (188)	175	148	27.6	1.18
Hussein, 2009 (71)	3 (84)	185	150	46.4	1.24
Açkurt, 1995 (a) (28)	2 (129)	141	111	48.8	1.27
Köşüş, 2012 (78)	1 (228)	200[2]	156	12.5	1.28
Shields, 2011 (a) (95)	1 (113)	215	156	16	1.38
Shamim, 2013 (94)	1 (285)	206	150	19.6	1.38
House, 2000 (70)	2 (1424)	180[2]	130	25.3	1.38
Roberts, 1973 (a) (91)	1 (320)	165	118	35	1.4
Osifo, 1976 (86)	3 (50)	208	148	40	1.41
Vanderjagt, 2009 (97)	3 (98)	208[2]	148	12.2	1.41
Baker, 1975 (51)	3 (174)	85	59	23	1.44
Abdelrahim, 2009 (49)	3 (55)	159	111	1.1	1.44
Lowenstein, 1960 (b) (81)	3 (252)	221	148	19	1.49
Murphy, 2007 (c) (85)	3 (84)	224	150	0	1.49
Milman, 2006 (a) (84)	2 (406)	225[2]	150	15	1.5
Wu, 2013 (b) (99)	3 (220)	224	148	23	1.51
Giugliani, 1984 (63)	3 (165)	251	165	21.6	1.52
Murphy, 2007 (b) (85)	2 (90)	230	150	0	1.53
Jiang, 2005 (74)	1 (1158)	237	150	28.3	1.58
Lowenstein, 1960 (a) (81)	2 (59)	235	148	15.2	1.59
Zachau-Christiansen, 1962 (101)	3 (365)	177	111	17	1.59
Jacquemyn, 2014 (73)	3 (78)	244	150	13	1.63
Bjørke Monsen, 2001 (54)	3 (169)	245[2]	150	15	1.63
Whiteside, 1968 (b) (98)	2 (50)	127	74	25	1.72
Murphy, 2007 (a) (85)	1 (88)	267	150	0	1.78
Colman, 1975 (58)	3 (106)	524	295	0.9	1.78
Takimoto, 2007 (b) (96)	3 (82)	265	148	16	1.79
Gibson, 2008 (62)	3 (83)	268[2]	150	23	1.79
Cole, 1974 (57)	3 (130)	272	148	12.3	1.84
Pagán, 2002 (b) (87)	3 (285)	285	148	2.1	1.93
Wu, 2013 (a) (99)	2 (264)	287	148	10	1.94
Ray, 2008 (90)	1 (2490)	249	125	8.5	1.99
Takimoto, 2007 (a) (96)	2 (77)	301	148	8	2.03
Ho, 1987 (69)	3 (221)	229	110	3.6	2.08
Knight, 1991 (b) (76)	3 (75)	318	148	11.2	2.15
Pagán, 2002 (a) (87)	2 (285)	357	148	0.35	2.41
Jacob, 1976 (72)	2 (182)	303	111	4.5	2.73
Whiteside, 1968 (a) (98)	1 (56)	217	74	5	2.93
Black, 1994 (55)	3 (85)	228	74	15	3.08
Marzan, 1971 (a) (83)	2 (100)	270	59	1.5	4.58
Marzan, 1971 (b) (83)	3 (57)	286	59	0	4.85

[1]The relations between the calculated standardized score (mean vitamin B-12 deficiency ÷ cutoff value) and corresponding vitamin B-12 insufficiency rate (n = 57 pairs of results) are shown. The studies are presented according to the standardized score for ease of comparison. The lowercase letters in parentheses in the first column indicate the order of appearance in the table for studies that reported results from >1 trimester.

[2]Mean estimated from the median (39).

TABLE 3
Odds of SGA or LBW with low vitamin B-12 concentrations[1]

First author, year (reference)	Country	Trimester[2]	Vitamin B-12 threshold	Birth outcome threshold	Cases, *n*	Controls, *n*	OR (95% CI)	Adjustments
Muthayya, 2006 (a) (25)	India	1	Tertile 1 vs tertile 3 (median: 116 vs 224 pmol/L)	IUGR: <10th centile for GA	45	45	5.98 (1.72, 20.74)	Maternal age, education, parity, weight
Dwarkanath, 2013 (a) (60)	India	1	Tertile 1 vs tertile 3 (median: 118 vs 284 pmol/L)	SGA: <10th centile for GA	107	103	1.43 (1.02, 2.17)	Age, education, parity, weight, energy intake
Muthayya, 2006 (b) (25)	India	2	Tertile 1 vs tertile 3 (median: 113 vs 210 pmol/L)	IUGR: <10th centile for GA	50	54	9.28 (2.90, 29.68)	Maternal age, education, parity, weight
Furness, 2013 (23)	Australia	2	N/R	EFW: <10th centile and serial tapering down of abdominal circumference	21	63	1.001 (0.996, 1.006)	Folate, homocysteine, age, smoking, BMI, DNA damage markers (e.g., micronuclei, nucleoplasmic bridges)
Dwarkanath, 2013 (b) (60)	India	2	Tertile 1 vs tertile 3 (median: 108 vs 245 pmol/L)	SGA: <10th centile for GA	96	96	1.45 (0.92, 2.27)	Age, education, parity, weight, energy intake
Muthayya, 2006 (c) (25)	India	3	Tertile 1 vs tertile 3 (median: 111 vs 182 pmol/L)	IUGR: <10th centile for GA	49	53	2.81 (1.01. 7.87)	Maternal age, education, parity, weight
Hogeveen, 2010 (24)	Netherlands	3	<134 pmol/L (Q1)	LBW: <3075 g (Q1)	92	274	0.70 (0.44, 1.11)	GA, smoking, sex
Sukla, 2013 (114)	India	Cord blood	N/R	LBW: <2500 g	138	196	2.41 (1.34, 4.5)	N/R

[1]Study characteristics and results from studies that describe an association between maternal or cord blood vitamin B-12 and birth outcomes by ORs are shown (n = 5 studies, 8 results). The studies are presented according to the trimester of pregnancy. The lowercase letters in parentheses in the first column indicate the order of appearance in the table for studies that reported results from >1 trimester. EFW, estimated fetal weight; GA, gestational age; IUGR, intrauterine growth restriction; LBW, low birth weight; N/R, not reported; Q1, quartile 1; SGA, small-for-gestational age.

[2]Refers to trimester of maternal vitamin B-12 unless specified as cord blood.

such as those from India, Nepal, Turkey, Greece, and parts of South America. The high rates in the studies from the Indian subcontinent can be explained by a predominantly vegetarian diet (79).

However, we also found high rates of vitamin B-12 insufficiency in 4 studies from Turkey and in 1 study from Greece (28, 52, 65, 77, 93). The "Mediterranean diet" contains plenty of fruit, vegetables, and pulses compared with meat (118), which may in part explain this observation because stricter adherence to the diet during pregnancy exacerbated vitamin B-12 deficiency (52). In addition, obesity rates are high in these regions (119), and obesity has been shown to be associated with low vitamin B-12 (79, 120–122). The link between obesity, gestational diabetes, and low vitamin B-12 concentrations has not been fully explored, but because the former 2 conditions are independently associated with fetal macrosomia, they may partly compensate for or mask the associations between vitamin B-12 and LBW (123, 124).

Plausible reasons for the observed decrease in vitamin B-12 during pregnancy are hemodilution, active transport to the fetus, and changes in binding proteins (56, 125). Holotranscobalamin, the functional form of vitamin B-12, is positively correlated with total cobalamin and the 2 biomarkers negatively correlate with methylmalonic acid (a marker of tissue-level vitamin B-12 insufficiency) during pregnancy (85). Although serum cobalamin decreases across the trimesters, holotranscobalamin has been shown to decrease in some studies and remain unchanged in others (85, 126). Therefore, the decrease in cobalamin may be due to a reduction in the fraction bound to haptocorrin (holo-haptocorin) (126). The tissue-level effects and clinical implications of a decrease in holo-haptocorin during pregnancy are unknown and warrant further studies.

Vitamin B-12 insufficiency and BW

Our review showed that the OR of LBW is 1.7 when vitamin B-12 insufficiency was present in maternal or cord blood (Figure 5). However, our results cannot confirm the association between low vitamin B-12 and LBW across the world because any positive observations may be isolated to Indian populations. Although all of the studies adjusted for most of the known confounding factors, the overall effect was driven by 1 study in India (25). It is important to note that only 27% of the women recruited into the study had vitamin B-12 concentrations measured, although the authors reported that they did not differ from the study population in their baseline characteristics. The 3 results from this study contributed a total of 21.5% to the pooled results (Figure 5), and when they were removed from the analysis the OR decreased from 1.70 to 1.23 (95% CI: 0.90, 1.67). Given the potential importance of the problem, further studies are needed to replicate or refute the magnitude of association found in this study.

TABLE 4
Differences in vitamin B-12 concentrations between NBW and adverse-BW outcomes[1]

First author, year (reference)	Country	Trimester[2]	Adverse-BW outcome threshold	Adverse-BW outcome		NBW outcome	
				n	Vitamin B-12, pmol/L	*n*	Vitamin B-12, pmol/L
Muthayya, 2006 (a) (25)	India	1	LBW: <2500 g; NBW: >3000 g	16	156 ± 65[3]	39	173 ± 58
Ubeda, 2011 (a) (115)	Spain	1	IUGR: BW <10th centile for GA	7	227.5 ± 132.4	48	260.4 ± 124.8
McGarry, 1972 (111)	UK	2	LBW: <2500 g; NBW: >2950 g	14	120 ± 33	331	136 ± 53
Muthayya, 2006 (b) (25)	India	2	LBW: <2500 g; NBW: >3000 g	19	139 ± 33	44	163 ± 46
Ubeda, 2011 (b) (115)	Spain	2	IUGR: BW <10th centile for GA	7	194.6 ± 75.9	48	209.5 ± 105.8
Furness, 2013 (23)	Australia	2	IUGR: EFW <10th centile and serial tapering down of abdominal circumference	21	205 ± 87.9	63	243 ± 135
Krishnaveni, 2014 (108)	India	2	LBW: <2500 g	126	191 ± 93	528	186 ± 102
Baker, 1977 (a) (104)	USA	3	LBW: <2500 g	50	95 ± 13	50	78 ± 13
Navarro, 1984 (a) (112)	France	3	LBW: <2500 g	31	295 ± 90	26	311 ± 58
Abbas, 1994 (a) (102)	UK	3	IUGR: abdominal circumference and EFW <5th centile	20	0.1 (0.21)[4]	20	N/A
Lindblad, 2005 (a) (109)	Pakistan	3	IUGR: EFW ≤11%	46	96 ± 41[5]	82	108 ± 48[5]
Yajnik, 2005 (116)	India	3	SGA: <10th centile for sex and GA	30	106 (87, 128)[6]	50	124 (100, 150)[6]
Mamabolo, 2006 (110)	South Africa	3	Tertile 1 vs tertile 3	66	176 ± 74	75	175 ± 78
Muthayya, 2006 (c) (25)	India	3	LBW: <2500 g; NBW: >3000 g	19	137 ± 38	42	156 ± 45
Ubeda, 2011 (c) (115)	Spain	3	IUGR: BW <10th centile for GA	7	139.9 ± 44.0	48	161.0 ± 95.6
Abraham, 2013 (103)	India	3	LBW: <2500 g	58	207 ± 94	58	203 ± 87
Baker, 1977 (b) (104)	USA	Cord blood	LBW: <2500 g	50	281 ± 43	50	439 ± 35
Navarro, 1984 (b) (112)	France	Cord blood	LBW: <2500 g	32	223 ± 61	26	255 ± 56
Abbas, 1994 (b) (102)	UK	Cord blood	IUGR: abdominal circumference and EBW <5th centile	20	0.9 (0.28)[4]	20	N/A
Lindblad, 2005 (b) (109)	Pakistan	Cord blood	IUGR: EFW ≤11%	46	190 ± 142[5]	82	171 ± 81[5]
Muthayya, 2006 (d) (25)	India	Cord blood	LBW: <2500 g; NBW: >3000 g	20	195 ± 63	47	236 ± 94
Gomes, 2010 (106)	Sri Lanka	Cord blood (preterm infants)	SGA: BW <10th centile for GA and sex	96	394 ± 169.3	113	409 ± 224.5
Hay, 2010 (a) (107)	Norway	Cord blood (nulliparous)	Quartile 1 vs quartile 4	180	363 (341, 420)[7]	180	242 (221, 311)[7]
Hay, 2010 (b) (107)	Norway	Cord blood (multiparous)	Quartile 1 vs quartile 4	180	365 (301, 423)[7]	180	258 (224, 297)[7]
Sukla, 2013 (114)	India	Cord blood	LBW: <2500 g	138	142.4 ± 60.5	196	157.9 ± 53.9

[1]Characteristics and results from studies that describe mean maternal or cord blood vitamin B-12 concentrations in adverse-BW and NBW outcome groups are shown (*n* = 15 studies, 25 results). The studies are presented according to trimester of pregnancy. The lowercase letters in parentheses in the first column indicate the order of appearance in the table for studies that reported results from >1 trimester. BW, birth weight; EFW, estimated fetal weight; GA, gestational age; IUGR, intrauterine growth restriction; LBW, low birth weight; N/A, not applicable; NBW, normal birthweight; SGA, small-for-gestational age.
[2]Refers to trimester of maternal vitamin B-12 unless specified as cord blood.
[3]Mean ± SD (all such values).
[4]Mean difference in SD between cases and controls; SEM in parentheses.
[5]Mean ± SD estimated from median (range) (39).
[6]Geometric mean; 25th, 75th centile in parentheses.
[7]Geometric mean; 95% CI in parentheses.

With regard to the 2 studies from Australia and Holland with negative results, it is notable that, when compared with the Indian studies, maternal vitamin B-12 concentrations in the Australian study were considerably higher (median: 239 pmol/L), whereas in the Dutch study a higher threshold was used to define LBW (<3075 g). In addition, the population characteristics differed [e.g., mean BMI (in kg/m^2) of women in the Australian study (23) was 28.5 compared with 22.0 in the Indian studies (25, 60)],

TABLE 5
Effect of vitamin B-12 concentrations across the spectrum on BW[1]

First author, year (reference)	Country	Trimester[2]	*n*	Unit vitamin B-12	Effect size	*P*	Adjustments
Relton, 2005 (a) (113)	UK	1	500	1 unit log vitamin B-12 (pg/mL)	$\beta = 0.03$[3] (−0.05, 0.12)	0.41	None
Takimoto, 2007 (a) (96)	Japan	1	51	1 pmol/L	B = −1.05	0.08	Age, parity, BMI
Pagán, 2002 (a) (87)	USA	2	285	1 pmol/L	B = −0.2	0.52	GA, race, BMI, smoking, sex
Takimoto, 2007 (b) (96)	Japan	2	77	1 pmol/L	B = −5.35	0.38	Age, parity, BMI
Faintuch, 2009 (105)	Brazil	2 (post–bariatric surgery)	13	1 pg/mL	$r = -0.846$	<0.001	None
Frery, 1992 (a) (61)	France	3 (all)	188	1 unit log vitamin B-12	$r = -0.05$	NS	None
		3 (smokers)	25		B = −507	0.03	Parity, ethnicity
Pagán, 2002 (b) (87)	USA	3	285	1 pmol/L	B = −0.2	0.53	GA, race, BMI, smoking, sex
Guerra-Shinohara, 2004 (a) (29)	Brazil	3	117	1 pmol/L	$r = -0.05$	0.52	None
Takimoto, 2007 (c) (96)	Japan	3	82	1 pmol/L	B = 0.776	0.44	Age, parity, BMI
Hogeveen, 2010 (24)	Netherlands	3	366	1 SD (69 pmol/L)	B = −37 (−100, 29)	NS	Age, GA, parity, smoking, sex, folate supplement
Abraham, 2013 (103)	India	3	116	1 pmol/L	$\beta = 0.22$	0.65	Diet, SES
Krishnaveni, 2014 (108)	India	3	654	1 SDS unit log vitamin B-12	$\beta = 1$: 0.02[4] (−0.05, 0.10) $\beta = 0.07$ (−0.003, 0.15)	NS	Sex, gestational age Above + BMI, GDM, SES, parity, religion
Fréry, 1992 (b) (61)	France	Cord blood (all)	154	1 unit log vitamin B-12	$r = -0.16$	<0.04	None
		Cord blood (smokers)	22		B = −414	0.06	Parity, ethnicity
Guerra-Shinohara, 2004 (b) (29)	Brazil	Cord blood	117	1 pmol/L	$r = -0.02$	0.80	None
Relton, 2005 (b) (113)	UK	Cord blood	522	1 unit log vitamin B-12 (pg/mL)	$\beta = -0.09$[3] (−0.17, −0.01)	0.02	None
Muthayya, 2006 (17)	India	Cord blood (37–39 wk gestation)	76	1 pg/mL	$r = 0.28$	0.01	None
	India	Cord blood (≥ to 40 wk gestation)	36	1 pg/mL	$r = -0.13$	0.45	None

[1]Characteristics and results from studies that describe an association between maternal or cord blood vitamin B-12 and birth outcomes by correlation/regression analysis are shown (n = 10 studies, 19 results). The studies are presented according to trimester of pregnancy. The effect sizes are reported as unadjusted correlation coefficients (r), standardised (β) or unstandardised (B) regression coefficients with 95% CI in parantheses (if reported). The lowercase letters in parentheses in the first column indicates the order of appearance in the table for studies that reported results from >1 trimester. BW, birth weight; GA, gestational age; GDM, gestational diabetes mellitus; SDS, SD score; SES, socioeconomic status.

[2]Refers to trimester of maternal vitamin B-12 unless specified as cord blood.

[3]BW z score.

[4]BW SDS.

which may have influenced their risk of having LBW/SGA infants.

In the studies that reported mean concentrations of vitamin B-12, a nonsignificant trend of lower concentrations was observed in women who delivered LBW or SGA infants, with a larger effect size found in the first and second compared with the third trimester. Because the heterogeneity between the studies was high and there were differences in the populations and vitamin B-12 assays, it was not possible to make meaningful conclusions with regard to a vitamin B-12 "threshold" that would be associated with lower fetal BW. In the 2 studies that reported results from >1 trimester (25, 115), the difference in maternal vitamin B-12 between normal-BW and LBW infants was greater in the first or second trimester than in the third trimester (−33 compared with −21 pmol/L and −24 compared with −19 pmol/L, respectively), suggesting that lower vitamin B-12 status earlier in pregnancy may be more detrimental for offspring weight.

The link between exposure to low vitamin B-12 conditions during pregnancy, BW, and noncommunicable diseases in the offspring can be explained by the DOHaD (Developmental Origins of Health and Disease) hypothesis, which suggests that the fetus is programmed to adapt to its in utero environment and disease can result if this is altered (1). The epigenetic modifications associated with low vitamin B-12 concentrations influence placental development from the early embryonic stage, so it is possible that vitamin B-12 has an effect on fetal growth and BW through this mechanism (127, 128). Vitamin B-12 also plays an important role in the myelination of fetal neurons, which maximally occurs from midgestation until 2 y of age (129). Hence, the critical window for vitamin B-12 adequacy continues throughout pregnancy and lactation.

Previous studies have shown an independent inverse relation between maternal homocysteine concentrations and LBW (OR: 1.25; B-coefficient: −31 g per 1 SD increase in homocysteine) (130) and hyperhomocysteinemia has been causally linked to LBW (131). In our prevalence subreview, 14 of the 57 studies reported homocysteine concentrations (19, 29, 53, 54, 62, 66, 75, 84, 87, 88, 92, 96, 97, 99). Although a detailed discussion of the associations between vitamin B-12

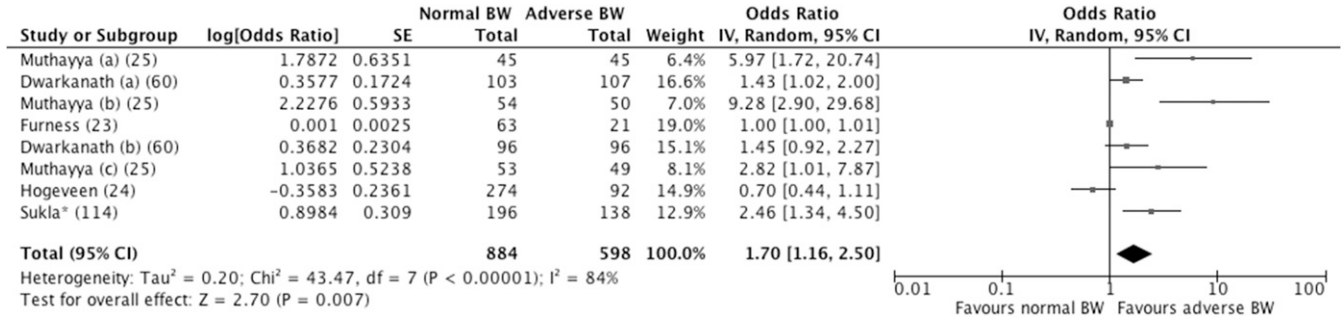

FIGURE 5 Meta-analysis of ORs of adverse BW in low maternal (or cord) vitamin B-12 cases and controls (n = 8 results). The letters in parentheses after the study ID refer to results from different trimesters within each study. The solid diamond represents the pooled OR, and the solid squares in each study denote the OR for that study (horizontal lines represent 95% CIs). Pooled and heterogeneity analyses were conducted on log-transformed ORs by using a random-effects model. The pooled OR (95% CI) was 1.70 (1.16, 2.50). *Vitamin B-12 measured in cord blood. BW, birth weight; IV, inverse variance.

and homocysteine is beyond the scope of this review, it is notable that among the studies with high vitamin B-12 insufficiency rates (>40%), average homocysteine was >6 μmol/L (19, 66, 88); and in 1 study, up to 40% of the women had homocysteine concentrations >10 μmol/L (92). It is therefore possible that the association of low vitamin B-12 status and LBW may be, at least in part, mediated through hyperhomocysteinemia during pregnancy.

In the absence of folate deficiency, vitamin B-12 deficiency is the strongest driver of high homocysteine concentrations (132).

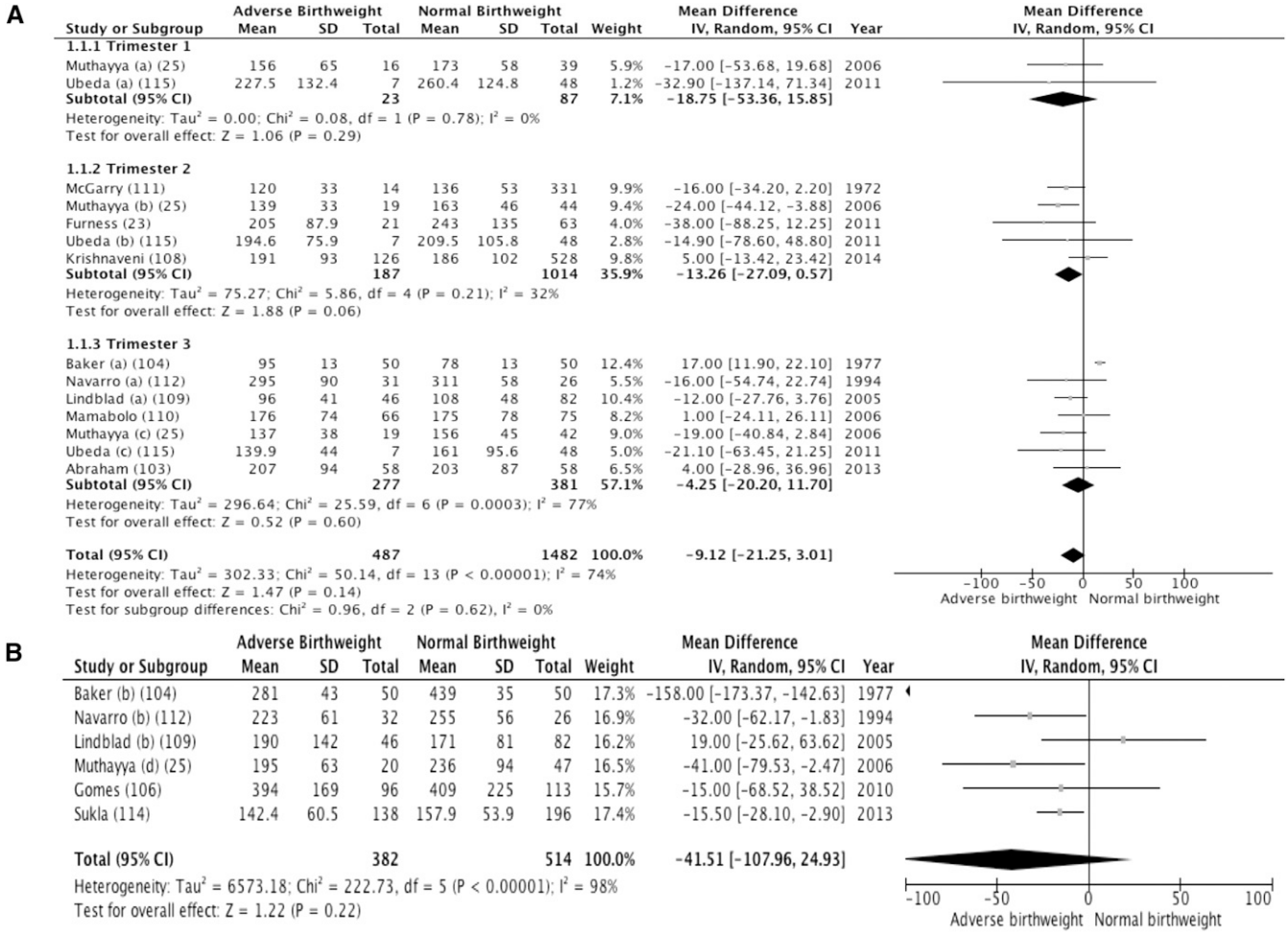

FIGURE 6 Meta-analysis of differences in mean vitamin B-12 concentrations between adverse- and normal-birth-weight infants. (A) Vitamin B-12 measured in maternal blood (divided in subgroups according to trimester of pregnancy; n = 14 results) and (B) vitamin B-12 measured in cord blood (n = 6 results). Solid diamonds represent the pooled difference for each subgroup and overall, and the squares in each study denote the mean difference for that study (horizontal lines represent 95% CIs). The I^2 values refer to the statistical heterogeneity within each subgroup and overall. Studies that have reported results from >1 trimester have a lowercase letter in parentheses in the study reference to indicate their order of appearance in the Forest plots. A random-effects model using generic inverse variance showed a pooled mean difference (95% CI) of −9.12 (−21.25, 3.01) in pregnancy and of −41.51 (−107.96, 24.93) in cord blood between adverse- and normal-birth-weight infants. IV, inverse variance.

In 12 studies with high rates of vitamin B-12 insufficiency, folate deficiency was <10% (19, 52, 55, 62, 63, 66, 70, 75, 77, 79, 88, 93). This observation was possibly due to adequate dietary intake (19, 88, 93) and antenatal consumption of folic acid (52, 66, 75). This imbalance between vitamin B-12 and folate is associated with lower neonatal BW and anthropometric measurements (133) as well as insulin resistance in offspring (19). Therefore, it is essential to address maternal vitamin B-12 status in addition to folate during pregnancy.

A meta-analysis of multiple micronutrient supplementation trials (typically containing 1 Recommended Daily Allowance of vitamin B-12) conducted in 12 low-income countries showed that supplementation was associated with a modest increase in BW (effect size: +22 g; $P = 0.002$) and reduced rates of LBW and SGA (134). Although this may not be due to optimizing concentrations of vitamin B-12 per se, it suggests that micronutrients in general are likely to contribute to increasing BW. Folic acid supplementation has been associated with higher BW, supporting the above explanation (135, 136).

The strengths of our study are that this is the first review, to our knowledge, to consider the vitamin B-12 status of pregnant women on a global level and link this to BW. We were able to show patterns in vitamin B-12 insufficiency rates and associations with BW in populations who broadly share dietary habits and inherent risk, although other differences may exist. One key limitation of our report is the vast heterogeneity between the studies in terms of vitamin B-12 measurement assays and cutoffs. This was partly addressed by devising the "standardized score," which allowed comparisons to be made between the studies after controlling for geographic region, trimester, and assay type. Another limitation is that, despite the large number of studies, the numbers were small in the subgroups (e.g., individual trimesters), highlighting the need for adequately powered longitudinal cohort studies with LBW or SGA as outcomes.

Conclusions

Our systematic review and meta-analysis in pregnant women showed that rates of vitamin B-12 insufficiency are high in certain populations (e.g., the Indian subcontinent and Eastern Mediterranean), including in nonvegetarian populations. The possible association between vitamin B-12 insufficiency and LBW/SGA warrants further investigation through larger cohort studies and randomized controlled trials. Even if the effect size of maternal vitamin B-12 on BW is modest, it has the potential to influence the health of future generations if a link is proven. The results of further studies will dictate practice with regard to vitamin B-12 supplementation in preconception and pregnancy, but until then, it would be sensible at least to measure vitamin B-12 concentrations when pregnant women first present to antenatal facilities across the world.

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