Intakes of Vitamin B-12 from Dairy Food, Meat, and Fish and Shellfish Are Independently and Positively Associated with Vitamin B-12 Biomarker Status in Pregnant Dutch Women

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Intakes of Vitamin B-12 from Dairy Food, Meat, and Fish and Shellfish Are Independently and Positively Associated with Vitamin B-12 Biomarker Status in Pregnant Dutch Women

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ABSTRACT

Background: The effect of vitamin B-12 from different animal foods on vitamin B-12 biomarker status has not previously been evaluated in pregnant women.

Objective: We examined the association of vitamin B-12 intake from dairy, meat, fish (including shellfish), and eggs with circulating concentrations of vitamin B-12 biomarkers and with the presence of vitamin B-12 deficiency in 1266 pregnant women participating in the KOALA Birth Cohort Study.

Methods: Blood samples were collected in weeks 34–36 of pregnancy, and vitamin B-12 intake from foods and supplements was estimated with a semiquantitative food-frequency questionnaire (FFQ). Total vitamin B-12, holotranscobalamin (holoTC), and methylmalonic acid (MMA) were determined in plasma. Vitamin B-12 deficiency was defined as holoTC <35 pmol/L and MMA >0.45 μmol/L. Associations were evaluated with linear and logistic regression analyses, adjusting for potential confounders.

Results: Significant dose-response relations were observed between vitamin B-12 intake from dairy, meat, and fish and plasma vitamin B-12, holoTC, and MMA [*P*-trend for (shell)fish with MMA = 0.002; *P*-trend for dairy, meat, and fish with all other markers < 0.001]. The OR (95% CI) of vitamin B-12 deficiency in the third compared with the first tertile of dairy-derived vitamin B-12 was 0.13 (0.04, 0.49), and the ORs for vitamin B-12 from meat and fish were 0.33 (0.11, 0.97) and 0.25 (0.08, 0.82), respectively. Egg-derived vitamin B-12 was only associated with holoTC. Additional analyses showed that self-defined vegetarians and FFQ-defined lacto-ovo-vegetarians had lower median total dietary vitamin B-12 intake and considerably worse vitamin B-12 biomarker status than omnivores and pescatarians.

Conclusions: In pregnant Dutch women, higher intakes of vitamin B-12 from dairy, meat, and fish were positively associated with vitamin B-12 status, suggesting that dairy, meat, and fish are good sources of bioactive vitamin B-12 in pregnancy. Nevertheless, for (lacto-)vegetarians, vitamin B-12 supplementation is recommended. *J Nutr* 2019;149:131–138.

Keywords: vitamin B-12 intake, pregnancy, plasma vitamin B-12, methylmalonic acid, holotranscobalamin, animal foods, vegetarian

Introduction

Vitamin B-12, or cobalamin, is an essential water-soluble micronutrient of microbial origin (1) predominantly found in animal foods including meat, (shell)fish, dairy products, and eggs (2). In humans, cobalamin is required as a cofactor for remethylation of homocysteine and isomerization of L-methylmalonyl-CoA to succinyl-CoA and is indispensable for proper RBC formation, normal neurological function, and DNA synthesis (3, 4).

Pregnant women and infants are especially vulnerable to vitamin B-12 deficiency. Maternal vitamin B-12 deficiency may cause infertility and recurrent spontaneous abortion (5). In addition, maternal vitamin B-12 adequacy is crucial for normal fetal development (6), and deficiency may increase the risk of birth defects such as neural tube defects (5). Moreover, infants from women with depleted vitamin B-12 stores are at high risk of developing vitamin B-12 deficiency (7, 8), with a negative impact on cognitive, motor, and growth outcomes (9). Low

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vitamin B-12 concentrations in breast milk from vitamin B-12–depleted mothers (10) may further increase deficiency risk in their breastfed infant. The estimated worldwide prevalence of vitamin B-12 deficiency during pregnancy was reported to be as high as 25% (11).

Vitamin B-12 deficiency can be caused by impaired gastrointestinal absorption or by insufficient dietary intake due to low consumption of animal foods (8, 12). Despite habitual dairy consumption, vegetarians often show lower concentrations of serum vitamin B-12 than omnivores (13–17), and vegetarianism was found to be associated with a relatively high prevalence of vitamin B-12 deficiency (18, 19). This raises the question to what extent dairy products, but also other animal foods, contribute to the prevention of a vitamin B-12 deficiency during pregnancy.

A few observational studies have evaluated the potential differential effect of vitamin B-12 from different types of animal foods on vitamin B-12 status in different adult populations (20–22), with conflicting results. For example, in the Norwegian Hordaland Homocysteine Study (20), vitamin B-12 from dairy and fish, but not from meat and eggs, was positively associated with plasma vitamin B-12 concentrations and negatively associated with the risk of plasma vitamin B-12 <200 pmol/L or impaired vitamin B-12 function, defined as plasma vitamin B-12 <200 pmol/L combined with methylmalonic acid (MMA) >0.27 μmol/L. In Dutch elderly individuals (21), vitamin B-12 from all aforementioned animal food groups, except from eggs, significantly contributed to serum vitamin B-12 concentrations and was negatively associated with low serum vitamin B-12 and impaired vitamin B-12 function (as defined in reference 20).

None of the previous studies on the relation between the intake of different animal foods and vitamin B-12 status included pregnant women. The interpretation of vitamin B-12 status during pregnancy is hampered by the pregnancy-related decline of vitamin B-12 concentrations during the course of pregnancy, which was consistently shown in biochemical studies (23–25). This decline may reflect a normal physiologic change, because it is not accompanied by major changes in other biomarkers of vitamin B-12 status (23–25). Therefore, when studying the effect of dietary vitamin B-12 on vitamin B-12 status in pregnant women, it is imperative to also include other vitamin B-12 biomarkers such as holotranscobalamin (holoTC), which reflects the vitamin B-12 fraction available for tissue uptake, and MMA as a substrate of a vitamin B-12–dependent reaction that accumulates in case of vitamin B-12 deficiency.

In the present study, we examined the association of vitamin B-12 intake from dairy, meat, fish and shellfish, and eggs with *1*) circulating concentrations of vitamin B-12 biomarkers and *2*) presence of vitamin B-12 deficiency in a Dutch population of women in the third trimester of pregnancy.

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Methods

Study population and design

The present cross-sectional study was embedded in the KOALA Birth Cohort Study, a prospective cohort study of mother-infant pairs in the Netherlands, of which the study design has been described in detail elsewhere (26). Briefly, between 2000 and 2002, healthy pregnant women in their 34th week of pregnancy were enrolled. Women were recruited from a prospective cohort study on pregnancy-related pelvic girdle pain (conventional recruitment group, n = 2343). To increase contrast in exposure variables including diet, the cohort was enriched with participants with alternative lifestyles who were recruited through several "alternative" channels such as organic food shops and Steiner schools (alternative recruitment group, n = 491). After inclusion, all women (n = 2834) were asked to complete a questionnaire on sociodemographic characteristics, health, and lifestyle habits; and those recruited from January 2002 onward (n = 1365) were asked to consent to biosampling. The present study was performed in a subsample of 1341 participants who provided a blood sample in weeks 34–36 of pregnancy. Participants whose blood sample was stored in improperly closed cryogenic vials (n = 55), had incomplete dietary intake data (n = 13), or were missing data on ≥1 vitamin B-12 biomarker (n = 7) were excluded, resulting in data for 1266 subjects being included in the present analysis. The study was approved by the Ethics Committee of the Maastricht University/University Hospital Maastricht. All participants gave written informed consent.

Dietary assessment

Dietary intake during late pregnancy was estimated with a semiquantitative FFQ completed at week 34 of pregnancy. This FFQ covered nearly 200 food items, including dairy products (28 items), meat (29 items), fish and shellfish (7 items, hereafter referred to as fish), and eggs (1 item), and was based on an existing validated FFQ (27). Participants estimated how often and in what quantity they had consumed these food items in the last month. Consumption of each food item was calculated as grams per day, and the Dutch Food Composition Database (NEVO) version 2011 (28) was used to calculate daily intake of energy (kilocalories per day) and vitamin B-12. First, vitamin B-12 intake (micrograms per day) from the separate animal food groups dairy, meat, fish, and eggs was calculated. Second, as a separate category, vitamin B-12 intake from mixed dishes and foods was calculated (e.g., meat or fish in a pizza, eggs and milk in a pancake, or vitamin B-12 added to certain types of meat substitutes; e.g., processed soy products). Because vitamin B-12 intake from this category cannot be allocated to one specific animal food, it was not analyzed separately but included as a covariate to adjust the analyses. Total dietary vitamin B-12 intake (micrograms per day) was defined as the sum of vitamin B-12 intake from the above separate animal food groups and mixed dishes (without supplements).

On the basis of data from the FFQ, we defined pescatarians as persons eating dairy, eggs, and fish and/or shellfish but no meat, and lacto-ovo-vegetarians as persons eating dairy and eggs but no meat and fish. Self-defined vegetarians were participants who, in a study questionnaire, indicated that they had adhered to a vegetarian diet over the past month.

Covariates

Sociodemographic data including age, highest attained level of education, height, and prepregnancy weight were reported by questionnaire. Prepregnancy BMI was calculated as the ratio of the self-reported prepregnancy body weight to height squared (kg/m^2). Current smoking habits, current alcohol consumption, and dietary supplement use between the seventh and ninth month of pregnancy were assessed by questionnaire in week 34 of pregnancy. Data on brand, type, and dose of all dietary supplements used during the third trimester of pregnancy were collected. To calculate the daily amount of supplemental vitamin B-12 intake (micrograms per day) of each participant, we used a database containing composition data of the supplements that were commercially available in the Netherlands at the time of the study (29). In rare cases in which compositional data could not be traced, the present vitamin B-12 concentration of the supplement of the same trademark was used.

Blood sampling and analysis

Maternal blood was collected in EDTA-coated tubes in week 34–36 of pregnancy during home visits by trained nurses. Blood samples were centrifuged immediately, transferred into cryogenic vials, transported at 4°C, and stored at −80°C in a biobank until further analysis.

Biochemical assays were performed in the Department of Clinical Chemistry, Erasmus MC Medical Center, Rotterdam, Netherlands. Plasma total vitamin B-12 was measured on a Roche Cobas 401 (Roche Diagnostics) according to the manufacturer's protocol. Plasma holoTC was measured on an Abbott Architect ci8200 (Abbott Diagnostics) according to the manufacturer's protocol. Plasma MMA was measured by LC–tandem MS (30). Between-run precision was <7% for total vitamin B-12, <15% for holoTC, and <8% for MMA.

Definition of vitamin B-12 deficiency

Vitamin B-12 deficiency was defined as plasma holoTC <35 pmol/L (31, 32) combined with plasma MMA above the upper limit of the 95% reference range as used in our analytical laboratory (i.e., 0.45 μmol/L). The reference range for MMA was determined in 100 healthy blood bank donors (50 men, 50 women) at the Erasmus MC Medical Center (33).

We did not use plasma total vitamin B-12 in our definition of vitamin B-12 deficiency, because previous research showed a gradual decline in serum total vitamin B-12 during pregnancy (23–25), which may reflect a normal physiologic change. In contrast, holoTC remained virtually unchanged during pregnancy (23, 24).

Statistical analyses

Statistical analyses were performed using the software package SPSS Statistics version 23.0 for Windows (SPSS, IBM Corp.). Characteristics of the study population are given as numbers (*n*) and proportions (%) for categorical variables and as means ± SDs or medians and IQRs for continuous variables with normal or skewed distributions, respectively. Spearman's rank correlation coefficient was used to assess the univariate association between holoTC and MMA. Linear regression models were used to determine the association of total dietary vitamin B-12 intake or vitamin B-12 intake from dairy, meat, fish, or eggs, with plasma concentrations of vitamin B-12, holoTC, and MMA. The positively skewed distributions of plasma vitamin B-12, holoTC, and MMA were log-transformed for analysis and back-transformed for presentation of geometric means and 95% CIs. Logistic regression models were used to determine the association between vitamin B-12 intake from the above-mentioned dietary sources and vitamin B-12 deficiency. Total dietary vitamin B-12 intake and vitamin B-12 intake from dairy, meat, and fish were categorized as quintiles, and vitamin B-12 from eggs was categorized as quartiles because of low egg consumption in the study population. Because of the low prevalence of vitamin B-12 deficiency (2%), tertiles were used in the models with that outcome. All bottom quantiles served as the reference category. The results of the linear regression analyses of plasma vitamin B-12, holoTC, and MMA were back-transformed to estimate the proportional difference in geometric mean for each quantile of vitamin B-12 intake compared with the reference group and are presented as proportional difference (%; 95% CI) relative to the reference group. Results of the logistic regression analyses are presented as ORs with corresponding 95% CIs. To evaluate dose-response effects, we performed a test for trend by repeating all analyses with the quantiles of vitamin B-12 intake entered as continuous variables. We also compared the contribution per 1-μg/d increment of dairy-, meat, or fish-derived vitamin B-12 to plasma concentrations of vitamin B-12 biomarkers by repeating the linear regression analyses with vitamin B-12 intake modeled continuously. This was not done for egg-derived vitamin B-12 because of low consumption (i.e., <1 μg/d for all participants). To evaluate whether results differed between the conventional and alternative recruitment groups, we tested for statistical interaction between the vitamin B-12 intake and recruitment groups. Because the interaction terms did not reach significance for any of the exposures and outcomes ($P > 0.05$), participants from the 2 recruitment sources were pooled for the analyses. Potential confounders that were significantly associated with vitamin B-12 biomarkers or vitamin B-12 deficiency or changed the effect estimate of vitamin B-12 intake by >10% were simultaneously included as covariates in all regression models to adjust the analyses (i.e., recruitment group; conventional, alternative): age (years); prepregnancy BMI (kg/m^2); highest level of education (low, intermediate, or high); current smoking (yes or no); current alcohol use (yes or no); vitamin B-12 intake from supplements (micrograms per day; log-transformed); energy intake (kilocalories per day); vitamin B-12 intake from dairy, meat, fish, and eggs separately (micrograms per day); and vitamin B-12 intake from mixed dishes and foods (micrograms per day).

As an additional analysis, we repeated all analyses for nonusers of vitamin B-12–containing dietary supplements. In addition, vitamin B-12 intake and status, as well as the contribution per 1-μg/d increment of dairy-derived vitamin B-12, were analyzed for vegetarians and omnivores separately.

TABLE 1 Characteristics of healthy pregnant women at weeks 34–36 of pregnancy[1]

Characteristics	Total population ($n = 1266$)
General	
Recruitment group, % conventional	70.8
Age, y	32.6 ± 3.8
Educational level, %	
Low	2.8
Intermediate	42.1
High	55.1
Prepregnancy BMI, kg/m^2	22.6 (20.8, 25.0)
Alcohol use, % yes	18.6
Smoking, % yes	5.1
Vitamin B-12 biomarkers	
Plasma total vitamin B-12, pmol/L	172 (138, 218)
Plasma holoTC, pmol/L	63.5 (47.0, 87.0)
Plasma MMA, μmol/L	0.22 (0.17, 0.29)
Vitamin B-12 status, %	
Plasma holoTC <35 pmol/L and MMA >0.45 μmol/L	2.0
Dietary intake	
Energy intake, kcal/d	2399 (2078, 2758)
Total dietary vitamin B-12, μg/d	5.0 (3.8, 6.5)
Dairy, g/d	474 (306, 596)
Vitamin B-12 from dairy, μg/d	1.9 (1.3, 2.7)
Meat, g/d	87.0 (53.5, 119)
Vitamin B-12 from meat, μg/d	1.3 (0.76, 1.8)
Fish,[2] g/d	34.5 (17.5, 56.0)
Vitamin B-12 from fish, μg/d	0.92 (0.34, 1.8)
Eggs, g/d	9.8 (6.3, 15.9)
Vitamin B-12 intake from eggs, μg/d	0.11 (0.05, 0.21)
Vitamin B-12 intake from mixed dishes and foods, μg/d	0.38 (0.28, 0.53)
Supplements	
Vitamin B-12–containing supplements in third trimester of pregnancy, % yes	46.1
Daily intake of vitamin B-12 from supplements (users only), μg	1.0 (1.0, 1.0)

[1]Values are proportions (%) for categorical variables, means ± SDs for continuous variables with a normal distribution, and medians (IQRs) for continuous variables with a skewed distribution. holoTC, holotranscobalamin; MMA, methylmalonic acid.
[2]Including shellfish.

Results

Population characteristics

Characteristics of the study population are shown in Table 1. The mean age of participants was 32.6 y, the majority had an intermediate or high level of education, and the mean

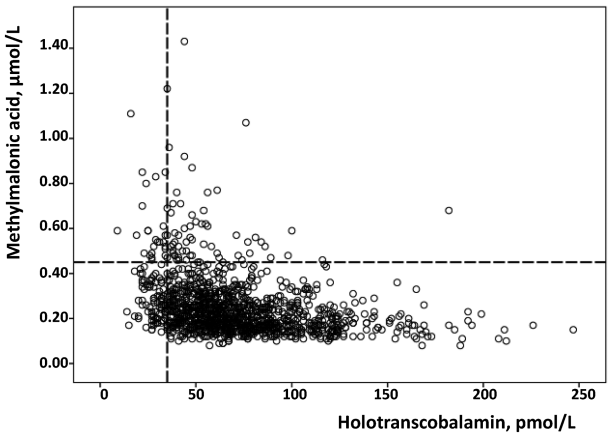

FIGURE 1 Scatterplot of the relation between holotranscobalamin and methylmalonic acid in healthy pregnant women at weeks 34–36 of pregnancy (n = 1266). The horizontal and vertical reference lines represent cutoff values for deficiency [i.e., 35 pmol/L for holotranscobalamin (31, 32) and 0.45 μmol/L for methylmalonic acid (33)], as explained in Methods.

(IQR) BMI is 22.6 (20.8–25.0). In late pregnancy, 5.1% of the participants smoked and 18.6% used alcohol. A vitamin B-12–containing dietary supplement was used by 46.1% of the participants and, of these, 74.1% used a daily dose of 1.0 μg supplemental vitamin B-12/d. None of the participants indicated having received vitamin B-12 by injections. Median plasma vitamin B-12 was 172 pmol/L, median plasma holoTC was 63.5 pmol/L, and median plasma MMA was 0.22 μmol/L. Two percent of the participants had a vitamin B-12 deficiency, defined as holoTC <35 pmol/L and MMA >0.45 μmol/L. A scatterplot of the relation between plasma concentrations of holoTC and MMA (Spearman's correlation coefficient = −0.39, P < 0.0001) is depicted in Figure 1.

Median total dietary vitamin B-12 intake was 5.0 μg/d; dairy was the largest absolute contributor to vitamin B-12 intake (i.e., 1.9 μg/d) and eggs had the smallest contribution (i.e., 0.11 μg/d) (Table 1). Median vitamin B-12 intake from meat was 1.3 μg/d and from fish was 0.92 μg/d. Median vitamin B-12 from mixed dishes and foods was 0.38 μg/d.

Association between vitamin B-12 intake and biomarkers of vitamin B-12 status

Linear regression analyses showed significant dose-response relations of total dietary vitamin B-12 as well as vitamin B-12 intakes from dairy, meat, and fish with plasma vitamin B-12, holoTC, and MMA (P-trend < 0.001 for dairy and meat, P-trend = 0.002 for fish) (Table 2). Between the highest quintile (quintile 5) and the bottom quintile (quintile 1) of dairy-derived vitamin B-12 intake, the multivariable adjusted proportional difference in plasma vitamin B-12 concentrations amounted to 29% (95% CI: 21%, 37%) and for holoTC this proportional difference was 53% (41%, 66%). For meat-derived vitamin B-12, the proportional difference between the fifth and the first quintile of intake was 15% (8%, 23%) for plasma vitamin B-12 and 20% (10%, 30%) for higher holoTC; for fish-derived vitamin B-12, the proportional difference was 7% (0.5%, 13%) for plasma vitamin B-12 and 15% (7%, 24%) for holoTC. With respect to the mean concentrations of MMA, the proportional differences observed for dairy-, meat-, and fish-derived vitamin B-12 were generally similar [e.g., with differences from the fifth compared with the first quintile of 21% (−27%, −14%) for dairy, 16% (−23%, −9%) for meat, and 15% (−21%, −8%) for fish. Egg-derived vitamin B-12 was not associated with plasma vitamin B-12 and MMA, but a significant 9% (1%, 17%) higher mean plasma holoTC was observed for the fourth compared with the first quartile of egg-derived vitamin B-12 in the fully adjusted model.

Linear regression analyses with vitamin B-12 intake as a continuous variable (Table 3) showed that, per 1-μg/d increment of vitamin B-12 intake, dairy-derived vitamin B-12 had the strongest contribution to plasma concentrations of vitamin B-12 and holoTC, followed by meat-derived vitamin B-12. For MMA, the contribution was similar for meat and dairy but less pronounced for fish.

Association between vitamin B-12 intake and vitamin B-12 deficiency

Logistic regression analyses showed significant inverse associations of total dietary vitamin B-12 (P-trend < 0.001), as well as vitamin B-12 from dairy (P-trend = 0.001), meat (P-trend = 0.036), and fish (P-trend = 0.011), with the presence of vitamin B-12 deficiency (defined as the combination of holoTC <35 pmol/L and MMA >0.45 μmol/L) (Table 4). Participants in the second compared with the first tertile of dairy-, meat-, or fish-derived vitamin B-12 showed a similar reduction in odds of vitamin B-12 deficiency [i.e., for dairy, a 68% reduction (OR: 0.32; 95% CI: 0.12, 0.86); for meat, a 72% reduction (OR: 0.28; 95% CI: 0.09, 0.88); and for fish, a 66% reduction (OR: 0.34; 95% CI: 0.12, 1.00)]. Those in the third compared with the first tertile of dairy-derived vitamin B-12 had 87% (OR: 0.13; 95% CI: 0.04, 0.49) lower odds of deficiency, whereas for vitamin B-12 from meat and fish this reduction was 65% (OR: 0.33; 95% CI: 0.11, 0.97) and 75% (OR: 0.25; 95% CI: 0.08, 0.82), respectively. Egg-derived vitamin B-12 was not associated with vitamin B-12 deficiency.

Additional analyses

Sensitivity analyses (**Supplemental Tables 1** and **2**) showed that, for dairy products and meat, the significant associations as reported for the total study population were also present in the subgroup of nonusers of vitamin B-12–containing supplements (i.e., 53.9% of the total study population). Moreover, the strength of the association was similar or even stronger than in the total study population. For fish-derived vitamin B-12, the association with biomarkers of vitamin B-12 status in the non–supplement users was attenuated and partly lost significance. Egg-derived vitamin B-12 was only associated with holoTC and MMA in nonusers. In addition, in the non–supplement users, absolute intake of total dietary vitamin B-12, as well as vitamin B-12 from dairy, meat, fish and eggs, was similar to that of the total study population.

Seventy-five participants (5.9%) indicated that they had adhered to a vegetarian diet during the past month (self-defined vegetarians), of whom 15 participants had consumed meat according to their FFQ and 50 had consumed fish. On the basis of the FFQ, 45 participants (3.6%) consumed fish but no meat (pescatarians), and 27 participants (2.1%) consumed neither meat nor fish (i.e., lacto-ovo-vegetarians). Both the self-defined vegetarians and the lacto-ovo-vegetarians had a lower median total dietary vitamin B-12 intake and considerably worse vitamin B-12 biomarker status than omnivores and pescatarians (**Supplemental Table 3**). Linear regression analyses with vitamin B-12 intake from dairy modeled continuously showed that for all groups, except for the lacto-ovo-vegetarians, dairy-derived vitamin B-12 significantly contributed to plasma concentrations

TABLE 2 Association between vitamin B-12 intake from different types of animal foods and biomarkers of vitamin B-12 status in healthy pregnant women at weeks 34–36 of pregnancy, by quintiles of vitamin B-12 intake[1]

	Plasma vitamin B-12		Plasma holoTC		Plasma MMA	
Vitamin B-12 intake from selected food groups,[2] μg/d	GM (IQR), pmol/L	% Difference (95% CI)[3]	GM (IQR), pmol/L	% Difference (95% CI)[3]	GM (IQR), μmol/L	% Difference (95% CI)[3]
Total diet						
Quintile 1 ($n = 255$): 2.7 ± 0.58 (0.83 to <3.5)	149 (143, 156)	0	53 (50, 56)	0	0.26 (0.25, 0.28)	0
Quintile 2 ($n = 252$): 4.1 ± 0.29 (≥3.5 to <4.6)	175 (167, 183)	18 (11, 26)***	59 (56, 62)	14 (6, 23)**	0.24 (0.22, 0.25)	−13 (−19, −6)***
Quintile 3 ($n = 252$): 5.0 ± 0.29 (≥4.6 to <5.6)	179 (171, 187)	23 (16, 31)***	68 (64, 72)	36 (25, 47)***	0.21 (0.20, 0.22)	−22 (−27, −15)***
Quintile 4 ($n = 253$): 6.2 ± 0.43 (≥5.6 to <7.1)	186 (179, 195)	28 (21, 37)***	71 (67, 75)	40 (30,52)***	0.22 (0.21, 0.23)	−20 (−26, −14)***
Quintile 5 ($n = 254$): 11.0 ± 4.4 (≥7.1 to 26.0)	178 (171, 185)	26 (18, 35)***	70 (66, 74)	44 (32, 56)***	0.21 (0.20, 0.22)	−24 (−30, −18)***
P-trend		0.000		0.000		<0.001
Dairy						
Quintile 1 ($n = 252$): 0.76 ± 0.30 (0.00 to <1.2)	155 (148, 163)	0	53 (50, 57)	0	0.25 (0.23, 0.26)	0
Quintile 2 ($n = 254$): 1.4 ± 0.15 (≥1.2 to <1.7)	166 (159, 174)	10 (4, 16)**	61 (57, 64)	18 (9, 27)***	0.24 (0.23, 0.25)	−5 (−11, 2)
Quintile 3 ($n = 254$): 1.9 ± 0.15 (≥1.7 to <2.2)	173 (167, 181)	14 (7, 21)***	61 (58, 64)	19 (10, 29)***	0.23 (0.22, 0.24)	−11 (−17, −4)**
Quintile 4 ($n = 254$): 2.5 ± 0.19 (≥2.2 to <2.9)	179 (172, 187)	19 (12, 26)***	70 (66, 74)	37 (27, 49)***	0.21 (0.20, 0.23)	−15 (−21, −9)***
Quintile 5 ($n = 252$): 3.6 ± 0.95 (≥2.9 to 12.5)	192 (184, 199)	29 (21, 37)***	75 (72, 79)	53 (41, 66)***	0.20 (0.19, 0.21)	−21 (−27, −14)***
P-trend		0.000		0.000		0.000
Meat						
Quintile 1 ($n = 250$): 0.25 ± 0.22 (0.00 to <0.64)	170 (162, 178)	0	62 (58, 65)	0	0.25 (0.24, 0.27)	0
Quintile 2 ($n = 253$): 0.87 ± 0.13 (≥0.64 to <1.1)	171 (164, 179)	6 (0, 12)	64 (60, 67)	8 (−0.2, 14)	0.22 (0.21, 0.24)	−10 (−17, −4)**
Quintile 3 ($n = 254$): 1.3 ± 0.11 (≥1.1 to <1.5)	178 (171, 186)	11 (5, 19)**	66 (63, 70)	14 (5, 23)**	0.23 (0.22, 0.24)	−10 (−17, −4)**
Quintile 4 ($n = 255$): 1.7 ± 0.15 (≥1. 5 to <2.0)	172 (164, 179)	10 (3, 17)**	61 (58, 65)	8 (−0.5, 17)	0.22 (0.21, 0.23)	−14 (−21, −8)***
Quintile 5 ($n = 254$): 2.9 ± 1.1 (≥2.0 to 9.0)	174 (166, 184)	15 (8, 23)***	65 (62, 69)	20 (10, 30)***	0.21 (0.20, 0.23)	−16 (−23, −9)***
P-trend		0.000		0.000		0.000
Fish[4]						
Quintile 1 ($n = 254$): 0.05 ± 0.08 (0.00 to <0.25)	158 (151, 165)	0	57 (54, 60)	0	0.25 (0.24, 0.27)	0
Quintile 2 ($n = 253$): 0.47 ± 0.13 (≥0.25 to <0.69)	166 (160, 173)	1 (−5, 7)	60 (57, 64)	3 (−5, 11)	0.22 (0.21, 0.23)	−13 (−19, −6)***
Quintile 3 ($n = 254$): 0.93 ± 0.15 (≥0.69 to <1.2)	182 (174, 191)	9 (3, 16)**	66 (62, 70)	10 (2, 19)*	0.23 (0.22, 0.24)	−10 (−16, −3)**
Quintile 4 ($n = 253$): 1.6 ± 0.29 (≥1.2 to <2.2)	179 (171, 188)	9 (3, 16)**	66 (62, 69)	11 (3, 20)**	0.22 (0.21, 0.24)	−12 (−18, −5)**
Quintile 5 ($n = 252$): 6.0 ± 4.3 (≥2.2 to 20.6)	180 (173, 188)	7 (0.5, 13)***	70 (67, 74)	15 (7, 24)***	0.21 (0.20, 0.22)	−15 (−21, −8)***
P-trend		0.002		0.000		0.000
Eggs[5]						
Quartile 1 ($n = 250$): 0.03 ± 0.03 (0.00 to <0.05)	170 (163, 178)	0	60 (57, 64)	0	0.24 (0.23, 0.25)	0
Quartile 2 ($n = 366$): 0.08 ± 0.03 (≥0.05 to <0.11)	172 (166, 178)	1 (−5, 6)	63 (61, 66)	4 (−3, 11)	0.23 (0.22, 0.24)	−4 (−10, 3)
Quartile 3 ($n = 328$): 0.13 ± 0.04 (≥0.11 to <0.21)	171 (164, 178)	1 (−5, 7)	62 (59, 65)	2 (−5, 10)	0.22 (0.21, 0.23)	−5 (−11, 2)
Quartile 4 ($n = 322$): 0.28 ± 0.12 (≥0.21 to 1.5)	179 (172, 186)	1 (−5, 7)	68 (65, 72)	9 (1, 17)*	0.22 (0.21, 0.23)	−5 (−12, 2)
P-trend		0.66		0.043		0.12

[1] $n = 1266$. Quartile 1 was the reference. IQRs were unadjusted. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. GM, geometric mean; holoTC, holotranscobalamin; MMA, methylmalonic acid.
[2] Values are means ± SDs (range).
[3] Multivariable-adjusted proportional difference in GMs relative to the reference group (lowest quintile of intake). Values were obtained by multiple linear regression analyses with adjustment for recruitment group, age, prepregnancy BMI, education, smoking, vitamin B-12 intake from supplements, alcohol use, energy intake, vitamin B-12 intake from mixed dishes, and vitamin B-12 intake from dairy, meat, fish, and eggs (except for the food group of interest).
[4] Including shellfish.
[5] Vitamin B-12 intake from eggs was categorized as quartiles because of low egg consumption in the study population.

of total dietary vitamin B-12, holoTC, and MMA. In lacto-ovo-vegetarians, only the association of dairy-derived vitamin B-12 with MMA reached significance.

Discussion

In the present study, we investigated the contribution of vitamin B-12 intake from different animal foods to plasma concentrations of selected vitamin B-12 biomarkers and vitamin B-12 deficiency in women in the late third trimester (34–36 wk) of pregnancy. Vitamin B-12 from dairy, meat, and fish, but not eggs, independently contributed to plasma concentrations of total vitamin B-12, holoTC, and MMA, as shown by significant dose-response relations. Vitamin B-12 intake from each of these product groups was also independently associated with a reduced odds of vitamin B-12 deficiency (holoTC <35 pmol/L and MMA >0.45 μmol/L). Egg-derived vitamin B-12 was only associated with holoTC.

The top compared with the bottom quintile of dairy-derived vitamin B-12 was associated with the largest proportional difference in mean concentrations of plasma vitamin B-12, followed by vitamin B-12 from meat and fish. These results with respect to dairy-derived vitamin B-12 are in line with the findings of the Framingham Offspring Cohort and the Hordaland Homocysteine Study (20, 22) but not fully with the study by Brouwer-Brolsma et al. (21), in which the difference in the mean concentration of serum vitamin B-12 for the top compared with the bottom tertile of dairy- and meat-derived vitamin B-12 was similar. Of note, in the latter study (21), the

TABLE 3 Proportional difference of plasma concentrations of vitamin B-12 biomarkers per 1-μg/d increment of vitamin B-12 intake from different animal food groups in healthy pregnant women at weeks 34–36 of pregnancy[1]

Vitamin B-12 intake from selected food groups per 1-μg/d increment	% Difference (95% CI)		
	Plasma vitamin B-12	Plasma holoTC	Plasma MMA
Total diet	1 (0.9, 2)***	3 (2, 3)***	−2 (−3, −1)***
Dairy	8 (6, 10)***	14 (11, 17)***	−6 (−9, −4)***
Meat	4 (2, 6)***	4 (2, 7)**	−4 (−7, −2)***
Fish[2]	0.5 (−0.02, 1)	0.9 (0.2, 2)*	−0.9 (−2, −0.2)*

[1]Values were obtained by linear regression analysis, multivariable adjusted for recruitment group, age, prepregnancy BMI, education, smoking, vitamin B-12 intake from supplements, alcohol use, energy intake, vitamin B-12 intake from mixed dishes, and vitamin B-12 intake from dairy, meat, fish, and eggs (except for the food group of interest); $n = 1266$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. holoTC, holotranscobalamin; MMA, methylmalonic acid.
[2]Including shellfish.

median vitamin B-12 intake both from dairy and from meat was ~1.2 μg/d, whereas in our study, this was 1.9 μg/d for dairy and 1.3 μg/d for meat. Comparable to the present study, the median intake of meat-derived vitamin B-12 in the group aged 41–47 y in the Hordaland Homocysteine Study was also lower than the intake of dairy-derived vitamin B-12 in that group (i.e., 1.5 compared with 1.3 μg/d), but was (in contrast to the present study) not associated with plasma vitamin B-12 concentrations (20). In the Framingham Offspring Cohort (22), a dose-response association for meat-derived vitamin B-12 was observed like in our study. In that study (22), but also in our study and 2 other studies (20, 21), fish-derived vitamin B-12 was positively associated with serum (21) or plasma (20) concentrations of total vitamin B-12, whereas egg-derived vitamin B-12 was not associated (20, 21).

TABLE 4 Association between vitamin B-12 intake from selected animal food groups and vitamin B-12 deficiency in healthy pregnant women at weeks 34–36 of pregnancy[1]

	Vitamin B-12 intake,[2] μg/d	OR (95% CI)
Total diet		
Tertile 1 ($n = 419$)	3.2 ± 0.71 (0.83 to <4.2)	1.00
Tertile 2 ($n = 424$)	5.0 ± 0.71 (≥4.2 to <5.9)	0.07 (0.02, 0.33)**
Tertile 3 ($n = 423$)	9.1 ± 4.1 (≥5.9 to 26.0)	0.10 (0.03, 0.37)**
P-trend		0.000
Dairy		
Tertile 1 ($n = 425$)	1.0 ± 0.38 (0.00 to <1.5)	1.00
Tertile 2 ($n = 419$)	2.0 ± 0.25 (≥1.5 to <2.4)	0.32 (0.12, 0.86)*
Tertile 3 ($n = 422$)	3.2 ± 0.89 (≥2.4 to 12.5)	0.13 (0.04, 0.49)**
P-trend		0.001
Meat		
Tertile 1 ($n = 422$)	0.5 ± 0.32 (0.00 to <0.95)	1.00
Tertile 2 ($n = 420$)	1.3 ± 0.19 (≥0.95 to <1.6)	0.28 (0.09, 0.88)*
Tertile 3 ($n = 424$)	2.4 ± 0.99 (≥1.6 to 9.0)	0.33 (0.11, 0.97)*
P-trend		0.036
Fish[3]		
Tertile 1 ($n = 417$)	0.18 ± 0.18 (0.00 to <0.54)	1.00
Tertile 2 ($n = 426$)	0.93 ± 0.25 (≥0.54 to <1.4)	0.34 (0.12, 1.00)*
Tertile 3 ($n = 423$)	4.3 ± 3.9 (≥1.4 to 20.6)	0.25 (0.08, 0.82)*
P-trend		0.011
Eggs		
Tertile 1 ($n = 419$)	0.04 ± 0.02 (0.00 to <0.05)	1.00
Tertile 2 ($n = 440$)	0.10 ± 0.01 (≥0.05 to <0.11)	0.45 (0.16, 1.25)
Tertile 3 ($n = 407$)	0.026 ± 0.12 (≥0.11 to 1.5)	0.56 (0.20, 1.56)
P-trend		0.20

[1]ORs were obtained by logistic regression analysis, with adjustment for recruitment group, age, prepregnancy BMI, education, smoking, vitamin B-12 intake from supplements, alcohol use, energy intake, vitamin B-12 intake from mixed dishes, and vitamin B-12 intake from dairy, meat, fish, and eggs (except for the food group of interest). Tertile 1 was the reference. Deficiency was defined as plasma holoTC <35 pmol/L (31, 32) and plasma MMA >0.45 μmol/L (33), as explained in Methods. *$P < 0.05$, **$P < 0.01$. holoTC, holotranscobalamin; MMA, methylmalonic acid.
[2]Values are means ± SDs (range).
[3]Including shellfish.

In the Hordaland Homocysteine Study, the results for plasma MMA (34) were in sharp contrast to those for plasma total vitamin B-12 (20): dairy-derived vitamin B-12 was not associated with plasma MMA, whereas vitamin B-12 from meat and fish was inversely associated with MMA (34). In our study, the dietary associations with plasma MMA were consistent with plasma total vitamin B-12, but the dietary associations with holoTC were generally stronger than with plasma total vitamin B-12. This is of interest because, in contrast to total vitamin B-12, holoTC represents the fraction of vitamin B-12 available for tissue uptake. At least 2 other studies (35, 36) also observed a higher correlation of total dietary vitamin B-12 intake with holoTC than with plasma total vitamin B-12 concentrations.

Per 1-μg/d increment of intake in our study, dairy-derived vitamin B-12 was the most potent contributor to plasma vitamin B-12 and holoTC concentrations, and dairy and meat for concentrations of MMA. For plasma concentrations of vitamin B-12, this corroborates the findings of the Hordaland Homocysteine Study (20), in which dairy-derived vitamin B-12 showed the steepest dose-response curve. Although previously mentioned (20), and suggested by our results, that the bioavailability of dairy-derived vitamin B-12 may be higher than vitamin B-12 from other food sources, a note of caution is needed, because the vitamin B-12 concentrations of the various food products were determined with different biochemical assays.

No reference values exist for vitamin B-12 biomarkers during pregnancy. Previous studies showed a gradual decrease in plasma total vitamin B-12 concentrations during the course of pregnancy combined with steady concentrations of the biologically active holoTC (23–25). Therefore, it has been suggested that holoTC and not vitamin B-12 should be used as a marker of vitamin B-12 status during pregnancy (23). In the present study, median plasma total vitamin B-12 (172 pmol/L) was near the lower limit of the 95% reference range (145–637 pmol/L), whereas median plasma holoTC (63.6 pmol/L) fell in the middle of the reference range (21–117 pmol/L) for nonpregnant healthy individuals as used in our analytical laboratory. Although we do not have data on vitamin B-12 biomarkers in early pregnancy from our study population, the observed low concentration of plasma total vitamin B-12 relative to holoTC in late pregnancy is in line with previous studies (23–25) and justifies our choice of using holoTC

instead of total plasma vitamin B-12 to define vitamin B-12 deficiency.

We observed a strong and consistent negative association of vitamin B-12 intake from dairy, meat, and fish with vitamin B-12 deficiency (holoTC <35 pmol/L and MMA >0.45 μmol/L). For example, the 3-fold higher dairy-derived vitamin B-12 intake in the top tertile compared with the bottom tertile was associated with a 87% reduced odds of vitamin B-12 deficiency, the 5-fold higher intake of meat-derived vitamin B-12 in the top compared with the bottom tertile was associated with a 67% reduced odds, and the 24-fold higher intake of fish-derived vitamin B-12 with a 75% reduced odds of vitamin B-12 deficiency.

The large majority of participants had a dietary vitamin B-12 intake above the current RDA of 3.2 μg/d for pregnant women in the Netherlands (37). Dairy intake according to recommended levels (e.g., 40 g Dutch cheese and 300 mL milk and 150 mL yogurt) contains ∼2.7 μg vitamin B-12/d. Our results would suggest that dairy products may be a good source of vitamin B-12, with bioavailability and biological activity not less than vitamin B-12 from meat and fish. Nevertheless, it must be noted that, in our study, a total dietary vitamin B-12 intake of ≥4.2 μg/d was associated with an ∼90% reduced odds of deficiency compared with a dietary vitamin B-12 intake of <4.2 μg/d. This would suggest that the current RDA might be too low, as was also endorsed recently by European Food and Safety Authority, which set an Adequate Intake of 4.5 μg/d (38) for pregnant women. If we combine this higher Adequate Intake of 4.5 μg/d with our finding of a 10-fold higher odds of deficiency in pregnant women with dietary vitamin B-12 intake <4.2 μg/d, vitamin B-12 intake from dairy products alone would thus generally be too low to prevent vitamin B-12 deficiency in pregnant women. This would imply that lacto-vegetarians need additional sources of vitamin B-12 (e.g., from dietary supplements) to achieve this vitamin B-12 intake.

A major strength of the present study is the large size of the cohort, combined with the availability of detailed information on intake of specific animal food sources and information on 3 vitamin B-12 biomarkers in blood. This enabled us to study the independent associations of vitamin B-12 from different animal food groups and to define vitamin B-12 deficiency by combining ≥1 biomarker of circulating vitamin B-12 concentrations with ≥1 functional biomarker, as was suggested by, for example, Carmel (39). Other strengths are the thorough adjustment for potential confounders.

A limitation is that 46% of the study population used vitamin B-12–containing food supplements. For this reason, vitamin B-12 intake from supplements was quantified in all subjects and adjusted for in all analyses. In addition, the level of supplementation was generally low (median: 1.0 μg/d) and results for dairy and meat remained similar or were even stronger in the non–supplement users, further substantiating our results. Another limitation is that we did not correct for creatinine, whereas impaired renal function is a strong determinant of elevated MMA (40, 41). However, in this study population of healthy pregnant women, the association of vitamin B-12 intake with plasma MMA closely resembled that of the other biomarkers and confounding by impaired renal function would bias our results toward the null. In addition, because only 6.8% of this population had an MMA concentration >0.45 μmol/L, and we only defined participants as vitamin B-12–deficient who had a combination of low holoTC with high MMA concentrations, the chance of a false-positive deficiency can be considered as low. Finally, because vitamin B-12 metabolism in pregnant women is clearly different from that in the general population (23–25), which may include, for example, differences in intestinal and cellular uptake, utilization, and entero-hepatic recycling and excretion, transfer of our findings to the general population should be done with caution.

In conclusion, in this study population of Dutch pregnant women, higher intakes of vitamin B-12 from dairy, meat, and fish were independently associated with better vitamin B-12 biomarker status, as shown by plasma concentrations of vitamin B-12, holoTC, and MMA, as well as reduced odds of vitamin B-12 deficiency. Results were similar in the population subgroup (46%) who did not use a vitamin B-12–containing supplement. Therefore, dairy, meat, and (shell)fish can be considered good sources of bioavailable and bioactive vitamin B-12 in pregnant women. Because, to our knowledge, this is the first study of this kind that included pregnant women, results need confirmation in other pregnant populations as well as in the general population. For lacto-vegetarians, as in vegans, the use of a vitamin B-12 supplement is recommended, because our findings provide strong indications that the amount of vitamin B-12 generally provided by dairy products is too low to prevent deficiency.

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