Nutrient Requirements

Long-Term Ovo-Lacto Vegetarian Diet Impairs Vitamin B-12 Status in Pregnant Women[1]

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ABSTRACT A well-planned vegetarian diet has been stated to be adequate during pregnancy. The aim of the present study was to compare serum vitamin B-12 and homocysteine concentrations in pregnant women (n = 109) consuming vegetarian and Western diets and to evaluate the adequacy of current dietary reference intakes of vitamin B-12 for these women. Pregnant women adhering to vegetarian diets for at least 3 y, with subgroups of ovo-lacto vegetarians (OLVs; n = 27), low-meat eaters (LME, n = 43), and women eating an average Western diet (control group, n = 39), were recruited. Dietary vitamin B-12 intake, serum vitamin B-12, and plasma total homocysteine (tHcy) concentrations were measured in wk 9–12, 20–22, and 36–38 of pregnancy. During pregnancy serum vitamin B-12 concentrations of ovo-lacto vegetarians ($P < 0.001$) and low-meat eaters ($P = 0.050$) were lower than those of the control group. We observed the combination of low serum vitamin B-12 concentrations and elevated plasma tHcy in 22% of ovo-lacto vegetarians, in 10% of low-meat eaters, and in 3% of controls ($P = 0.003$). In OLVs, serum vitamin B-12 predicted 60% of the plasma tHcy variation ($P < 0.001$), but in LMEs and controls only <10% (NS). Serum vitamin B-12 concentrations increased and plasma tHcy decreased sharply with increasing dietary intake of vitamin B-12 toward a cutoff point of 3 μg/d. Pregnant women consuming a long-term predominantly vegetarian diet have an increased risk of vitamin B-12 deficiency. Current recommended dietary intakes urgently need reevaluation. J. Nutr. 134: 3319–3326, 2004.

KEY WORDS: • *pregnancy* • *vitamin B-12* • *homocysteine* • *vegetarian diets*

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Natural sources of vitamin B-12 in human diets are restricted to foods of animal origin (1,2). Vegetarian diets, characterized by a reduced consumption of foods of animal origin and therefore lower intakes of vitamin B-12, are popular (3). One of the reasons for the increased popularity of vegetarian diets is the recent reports of lower morbidity and mortality rates from several chronic degenerative diseases in vegetarians relative to nonvegetarians (4–10). A well-planned lacto-ovo-vegetarian diet is often stated to be adequate during pregnancy, i.e., to meet the vitamin B-12 needs of both the mother and the fetus (11,12). Nevertheless, some studies have demonstrated a vitamin B-12 deficiency in infants born to mothers adhering to a strict vegetarian (vegan) diet (13–15). During pregnancy, low serum vitamin B-12 concentration is an independent risk factor for neural tube defects, pre-eclampsia, and other pregnancy-related complications (16–19). Low serum vitamin B-12 concentrations also lead to consequences for the mother such as macrocytic anemia, neurological complications, and cognitive disabilities (20). The breast-fed infant of a vitamin B-12–deficient mother is at risk for severe developmental abnormalities, growth failure, and anemia (21). Despite the increased risk of vitamin B-12 deficiency in utero, the intake of vitamin B-12 during pregnancy is not the focus of nutritional research because in mixed Western diets with high intake of foods of animal origin the intake of vitamin B-12 is well above the current recommended dietary intakes (22–24). There is evidence of increased intestinal absorption of vitamin B-12 during pregnancy, and fetal needs are thought to be very low (25,26). On the other hand, it has been speculated that vitamin B-12 from maternal tissue stores does not cross the placenta (13,14).

The dietary reference intakes for pregnant women are calculated mainly on the basis of data on fetal deposition and increased maternal absorption (24). The estimated average requirement (EAR)[3] for vitamin B-12 intake is 2 μg/d for nonpregnant women. For pregnant women, the EAR is only increased by 0.2 μg/d (24). Despite this definition of EAR,

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[3] Abbreviations used: EAR, estimated average requirement; GEE, generalized estimating equation; holo-HC, holohaptocorrin; holo-TC, holotranscobalamin; LME, low-meat eater; OLV, ovo-lacto vegetarian; tHcy, total homocysteine.

Manuscript received 23 July 2004. Initial review completed 26 August 2004. Revision accepted 16 September 2004.

convincing studies on vitamin B-12 requirements during pregnancy are lacking. Information about the consequences of long-term vegetarian and predominantly vegetarian diets, characterized by relatively low intake of vitamin B-12 (14), is therefore urgently needed.

The aim of the present study was to compare vitamin B-12 status in healthy pregnant women adhering to a predominantly vegetarian and plant-based diet for a long period with that of women consuming an average Western diet. In addition, the occurrence of vitamin B-12 deficiency, defined as low serum vitamin B-12 accompanied by elevated plasma total homocysteine (tHcy) concentrations, and their correlation to the dietary intake of vitamin B-12 was investigated.

SUBJECTS AND METHODS

Study design and subjects. The present study was designed as a prospective cohort study in which pregnant women were monitored. Subject recruitment and selection has been described in detail elsewhere (27). Briefly, pregnant women of each trimester of pregnancy responding to announcements in health magazines and to study information handed out by their gynecologists were recruited for this study. Included were pregnant women who were apparently healthy, with <4 prior pregnancies. Participants could enter the study at any stage of pregnancy and were followed until delivery. Information on dietary intake and blood samples was collected in wk 9–12, 20–22, and 36–38 of gestation. A questionnaire regarding their nutritional behavior, food consumption (semi-quantitative food frequency list, considering the usual dietary intake before pregnancy), anthropometric and sociodemographic data, use of oral contraceptives, parity, smoking, and physical activities, as well as prevalent diseases, was sent to all participants.

From 1995 to 1997 pregnant women in each trimester of pregnancy were recruited and divided into 3 diet groups: women adhering to a plant-based diet, subdivided into ovo-lacto vegetarians and low-meat eaters, and women consuming an average Western diet (control group). Using a priori defined selection criteria subjects were assigned to diet groups. A total of 109 women participated in the study: 70 women consuming a plant-based diet (27 ovo-lacto vegetarians, 43 low-meat eaters) and 39 controls. Women consuming a plant-based diet were characterized by a high consumption of raw vegetables (>100 g/d), preference of whole-grain products (ratio of refined grain products/whole-grain products < 0.95) and limited meat consumption (meat < 300 g/wk; meat products < 105 g/wk). Of these, the subgroup of ovo-lacto vegetarians were defined as subjects who completely omitted meat and meat products from their diets. Participants in both of these groups were only included if they had not changed their diet substantially for at least 3 y. The diet of the control group was similar to that of the average German population as defined in the German National Consumption Study (28). Briefly, this diet consisted mainly of refined grain products (ratio of refined grain products/whole-grain products > 1.05) and of >300 g meat and 105 g meat products per week and <100 g unheated vegetables per day; participants in the control group were only included if they did not follow any special diet.

Due to pregnancy-related and organizational reasons, of 109 participants only 60 were assessed 3 times throughout pregnancy. After the first trimester 1 ovo-lacto vegetarian and 1 low-meat eater and after the second trimester 1 ovo-lacto vegetarian dropped out from the study after a miscarriage. After the second trimester 7 ovo-lacto vegetarians, 9 low-meat eaters, and 5 women from the control group were not assessed because of relocation or birth of the child before the last blood sampling date.

The study was approved by the Ethics Committee of the Division of Human Medicine, University of Giessen, Germany. Written informed consent was obtained from all participants.

Dietary assessment. In wk 9–12, 20–22, and 36–38 of gestation, participants recorded their dietary intake for 4 d (Sunday through Wednesday) using a validated semi-quantitative food record. The validation procedure was described previously (27,29,30). Most participants recorded food intake preceding blood sampling, but for organizational reasons, 7 women recorded their food intake immediately after blood sampling. The food record included categories of 152 food items with descriptions and photographs of portion sizes estimated by typical household measures (27,29,30). Food supplement and medical drug intakes were assessed using a study diary during the whole study period.

At the time of this study, no systematic food fortification was carried out in Germany except for multivitamin-fortified juices. Calculation of dietary folate and vitamin B-12 intake was performed using the German Food Code and Nutrition Data Base BLS II.3 (31); vitamin B-12 concentrations in multivitamin-fortified juices were taken from producers' data.

Blood analyses. In wk 9–12, 20–22, and 36–38 of gestation venous blood samples were drawn after an overnight fast into vacutainers with and without EDTA. Within 2 h of venepuncture, serum/plasma was separated from blood cells by centrifugation at 2000 × *g* for 10 min and then stored frozen (−18°C) until assayed. To quantify erythrocyte folate concentrations, a chemiluminescent competitive protein binding assay (ACS Folate Assay, Ciba Corning Diagnostics GmbH) was used (CV 3.9%). tHcy in plasma was determined according to Ubbink et al. (32) and Araki and Sako (33) (CV 4.0%). Serum vitamin B-12 concentrations were determined using the IMx cobalamin assay (Abbot Diagnostics Division), which incorporates microparticles coated with porcine intrinsic factor to bind cobalamin. Holotranscobalamin (holo-TC) was removed from whole serum by a modification of the method recommended by Das et al. (34), as described by Wickramasinghe and Fida (35). Briefly, a slurry containing synthetic amorphous precipitated silica (Sipernet 283 LS, PQ Corporation) in distilled water was prepared and stored at 4°C. Holo-TC was absorbed by adding silicea to serum, vortexing the mixture, and leaving it for 10 min. The mixture was centrifuged, and the supernatant fluid was assayed for holohaptocorrin (holo-HC) by the IMx cobalamin assay (CV serum vitamin B-12 4.2%; holo-HC 4.3%).

Statistical analyses. All statistical analyses were performed using SAS 8.2 (SAS Institute). All values are arithmetic means ± SEM or medians with 25th and 75th percentiles in parentheses. BMI was calculated as reported pregravid weight/height (kg/m^2).

The vitamin B-12 status of the diet groups was compared using the SAS generalized estimating equation (GEE) procedure. GEE models allow an appropriate analysis of longitudinal data with repeated measurement and missing values. For all models a dependent working correlation matrix was chosen. Data were log-transformed to normalize distribution of serum vitamin B-12. The risk of vitamin B-12 deficiency and the risk of elevated plasma tHCY levels were computed by a logistic regression analysis with repeated measurement design (GEE), and odds ratios and 95% confidence intervals are presented. Linear regression analysis was performed to calculate variance of tHCY explained by serum vitamin B-12 and erythrocyte folate. For this analysis, the mean blood concentrations during pregnancy were used. Cutoff values for low plasma vitamin B-12 concentrations were defined according to Koebnick et al. (36) as lower 95% confidence limits in omnivorous subjects for each trimester: <130 pmol/L in the first trimester, <120 pmol/L in the second trimester, and <100 pmol/L in the third trimester. Elevated plasma tHcy levels were defined as upper 95% confidence intervals in omnivorous healthy pregnant subjects based on data from Murphy et al. (37): >9.0 μmol/L in the first trimester and >7.8 μmol/L in the second and third trimesters.

RESULTS

Compared to the omnivorous control group, ovo-lacto vegetarians and low-meat eaters had similar mean age and parity, but showed lower prepregnancy BMI and marginal differences in smoking habits (**Table 1**).

Ovo-lacto vegetarians had followed a plant-based diet for a mean of 8.7 ± 0.8 y (range 3–18 y) and low-meat eaters for a mean of 7.5 ± 0.7 y (range 3–24). The plant-based diet of ovo-lacto vegetarians and low-meat eaters was characterized by a higher intake of whole-grain products, vegetables, and

TABLE 1

General characteristics, food consumption, and dietary intake of vitamin B-12 in pregnant ovo-lacto vegetarians (OLVs), low-meat eaters (LMEs), and subjects consuming an average Western diet (control group)

	Plant-based diet			*P*-value	
	OLV (n = 27)	LME (n = 43)	Control group (n = 39)	OLV vs. control	LME vs. control
General characteristics[1]					
Age, *y*	30.8 ± 0.9	30.6 ± 0.6	29.1 ± 0.6	0.143	0.275
BMI, *kg/m²*	20.5 ± 0.4	21.5 ± 0.3	23.1 ± 0.7	0.002	0.031
Parity, *n*	1.7 ± 0.2	1.7 ± 0.1	2.0 ± 0.2	0.217	0.111
Smokers, %	3.7	9.1	15.4	0.226	0.507
Food consumption during pregnancy,[2,3] *g/d*					
Bread, cereal, rice, pasta	282 (198–374)	273 (218–365)	277 (224–345)	0.888	0.793
Whole-grain products	193 (124–275)	185 (125–247)	56 (19–100)	0.437	<0.0001
Vegetables	277 (188–422)	280 (209–359)	175 (121–256)	0.647	<0.0001
Fruits	408 (188–516)	337 (193–430)	222 (163–319)	0.114	<0.0001
Milk, yogurt, cheese	291 (116–506)	328 (227–418)	324 (245–451)	0.303	0.647
Meat and fish	0 (0–0)	39 (30–76)	133 (104–168)	<0.0001	<0.0001
Eggs	14 (0–20)	14 (0–27)	14 (0–27)	0.998	0.999
Vitamin B-12 intake during pregnancy[2,3]					
Total dietary intake,[2] *μg/d*	2.5 (1.3–3.8)	3.8 (3.0–4.9)	5.3 (4.3–6.3)	<0.001	<0.001
From milk, yogurt, cheese	2.1 (0.1–3.2)	2.3 (1.8–3.0)	2.0 (1.4–2.6)	0.569	0.102
From meat, fish, eggs	0.3 (0.0–0.5)	1.3 (0.7–2.0)	3.2 (2.2–4.1)	<0.001	<0.001
Vitamin B-12 supplement user, %	32.1	27.9	20.5	0.889	0.559

[1] Values are means ± SEM.
[2] Values are medians and interquartile ranges.
[3] Calculated as the mean of all dietary records during pregnancy.

fruits than the control group diet (Table 1). As expected, the diet groups differed in the consumption of meat, fish, and eggs. By definition, ovo-lacto vegetarians completely omitted meat from their diet. Although the diet groups did not differ significantly with respect to the consumption of milk and dairy products, ovo-lacto vegetarians exhibited the widest consumption range. Further details of dietary intake of the study groups have been described by Koebnick et al. (27).

Dietary intakes of vitamin B-12 and the vitamin B-12 intake derived from foods of animal origin by the diet groups were determined (Table 1). The diet groups differed significantly with regard to dietary vitamin B-12 intake in all trimesters of pregnancy. Vitamin B-12 intake was lowest in ovo-lacto vegetarians, followed by low-meat eaters, and highest in the control group. The estimated average vitamin B-12 requirement (EAR = 2.2 μg/d) as proposed by the U.S. Institute of Medicine (24) from dietary vitamin B-12 alone was met by 60% of ovo-lacto vegetarians, 94% of low-meat eaters, and all women from the control group throughout pregnancy ($P < 0.001$). Including vitamin B-12 from supplements, EAR was met by 61% of ovo-lacto vegetarians and 95% of low-meat eaters. For ovo-lacto vegetarians and low-meat eaters, the main sources of dietary vitamin B-12 were milk and dairy products (74.2 and 59.5% of total vitamin B-12 intake). In the control group, only 35.0% of dietary vitamin B-12 intake was derived from milk and dairy products (Table 1).

Serum vitamin B-12 concentrations. Serum vitamin B-12, holo-TC, and holo-HC concentrations decreased during pregnancy ($P < 0.001$) (**Table 2**). After controlling for effects of supplemental vitamin B-12, maternal age, and pregnancy stage (first, second, or third trimester), serum vitamin B-12 concentrations of ovo-lacto vegetarians ($P < 0.001$) and low-meat eaters ($P = 0.050$) were lower than those of the control group. Holo-HC concentrations were also lower in ovo-lacto vegetarians than in the control group ($P = 0.009$), whereas low-meat eaters and the control group did not differ ($P = 0.192$).

During pregnancy, low serum concentrations of vitamin B-12 (<130 pmol/L in the first trimester, <120 pmol/L in the second trimester, and <100 pmol/L in the third trimester) in at least 1 trimester were found in 39% of ovo-lacto vegetarians, in 9% of low-meat eaters, and in 3% of the control group ($P < 0.001$) (**Fig. 1**). In all trimesters, the frequency of low serum vitamin B-12 levels was highest in ovo-lacto vegetarians, followed by low-meat eaters; the frequency of low serum vitamin B-12 levels was lowest in the first trimester and highest in the third trimester. The odds ratio of having a low serum vitamin B-12 levels in at least 1 trimester was 3.9 (95% CI, 1.9–6.1) for ovo-lacto vegetarians and 1.8 (1.0–3.9) for low-meat eaters, using the control group as a reference.

Plasma total homocysteine. Over the total pregnancy, plasma tHcy concentrations were higher in ovo-lacto vegetarians than in the control group ($P = 0.032$) and tended to be higher in the low-meat eaters than in the control group ($P = 0.061$) (Table 2).

The frequency of elevated plasma tHcy concentrations did not differ among the diet groups (Fig. 1). We observed the combination of elevated concentrations of plasma tHcy and low serum concentrations of vitamin B-12 in at least 1 trimester in 25.0% of ovo-lacto vegetarians, in 10.0% of low-meat eaters, and in 2.6% of controls ($P = 0.003$).

Relation among vitamin B-12 intake, serum B-12, and tHcy. With regard to food intake, the strongest predictors of serum vitamin B-12 concentrations were intake of vitamin B-12 (μg/d) from milk and dairy products (GEE estimate ± SE for log serum vitamin B-12 (log mmol/L): 0.0190 ± 0.0085; $P = 0.026$), from supplements (0.0212 ± 0.0101, $P = 0.036$), and from meat and fish (0.0016 ± 0.0015, $P = 0.073$). In contrast, the strongest predictors of plasma tHcy concentrations were intake of vitamin B-12 from meat and fish (GEE

TABLE 2

Concentrations of serum vitamin B-12 and plasma tHcy in pregnant ovo-lacto vegetarians (OLVs), low-meat eaters (LMEs), and pregnant women consuming an average Western diet (control group), according to trimester of pregnancy[1,2]

	Plant-based diet					
Trimester	OLV	*n*	LME	*n*	Control	*n*
			Serum vitamin B-12, *pmol/L*[a,b]			
1	176 (100–317)	16	209 (160–293)	29	249 (201–319)	31
2	176 (102–271)	25	215 (151–269)	42	238 (190–305)	39
3	127 (90–184)	19	164 (125–208)	34	169 (141–213)	38
			Serum holo-transcobalamin, *pmol/L*			
1	10 (0–51)	16	17 (0–42)	29	19 (9–46)	31
2	9 (0–38)	25	14 (2–29)	42	20 (6–44)	39
3	5 (0–23)	19	6 (0–18)	34	4 (0–16)	38
			Serum holo-haptocorrin, *pmol/L*[a]			
1	172 (93–313)	16	191 (153–259)	29	239 (177–291)	31
2	165 (101–260)	25	208 (141–261)	42	193 (176–269)	39
3	119 (81–169)	19	161 (121–197)	34	162 (139–206)	38
			Plasma t-Hcy, *μmol/L*[a]			
1	7.5 (6.4–9.5)	16	7.3 (6.7–7.8)	29	6.7 (5.7–8.2)	31
2	6.9 (5.2–9.3)	25	6.5 (5.1–7.5)	42	5.7 (4.8–6.4)	39
3	5.7 (5.1–7.9)	19	7.0 (6.3–7.8)	34	6.4 (5.2–7.6)	38

[1] Values are shown as medians with 25th and 75th percentiles in parentheses.
[2] Different from control group ($P < 0.05$; a indicates a difference of OLV vs. control; b indicates a difference of LME vs. control).

estimate ± SEE for plasma tHcy (μmol/L): −0.1778 ± 0.0587, $P = 0.003$) and from supplements (−0.2610 ± 0.0873, $P = 0.003$), whereas vitamin B-12 from milk and dairy products only marginally predicted plasma tHcy (−0.1962 ± 0.1191, $P = 0.099$). The effects on serum vitamin B-12 or plasma tHcy observed among milk, yogurt, and cheese did not differ. The intake of vitamin B-12 from eggs did not affect serum vitamin B-12 of plasma tHcy concentrations. No significant interactions were observed between intake of vitamin B-12 from different food groups and diet group.

In analyses of all participants combined, plasma tHcy concentrations were inversely related to both serum vitamin B-12 (GEE estimate of log-B-12 ± SEE for plasma tHcy (μmol/L): −2.6842 ± 0.8636; $P = 0.002$) and erythrocyte folate concentrations (−0.0016 ± 0.0007; $P = 0.023$). An interaction was also observed between diet group and serum vitamin B-12, suggesting a greater effect of serum vitamin B-12 concentrations on tHCY in ovo-lacto vegetarians ($P = 0.032$) when the control group was used as reference. The relation between serum vitamin B-12 and plasma tHcy did not differ significantly between low-meat eaters and the control group. In ovo-lacto vegetarians, serum vitamin B-12 predicted 61% of plasma tHcy variation, in low-meat eaters 11%, and in controls 6% (**Fig. 2**).

Serum vitamin B-12 concentrations were positively (GEE estimate of log-B-12 ± SEE for total dietary vitamin B-12 intake (μg/d):1.1180 ± 0.03098; $P = 0.003$) and plasma tHcy concentrations negatively (GEE estimate of tHcy ± SEE for total dietary vitamin B-12 intake (μg/d): −1.6096 ± 0.7113; $P = 0.024$) related to total dietary vitamin B-12 intake. Statistically significant effects of vitamin B-12 intake on both serum vitamin B-12 and plasma tHcy concentrations were seen at a dietary intakes of up to 4.0 and 4.5 μg/d (**Fig. 3**), as shown by lower serum vitamin B-12 and higher plasma tHcy concentrations at lower intake levels relative to a vitamin B-12 intake > 6 μg/d. Low serum vitamin B-12 levels were only found in women with an intake of vitamin B-12 < 4.0 μg/d. These results were similar in all trimesters (data not shown). All participants with a vitamin B-12 intake < 1.0 μg/d, 57% of women with an intake of 1.0–2.0 μg/d, 22% of women with an intake of 2.0–3.0 μg/d, and 21% of women with an intake of 3.0–4.0 μg/d showed a low serum vitamin B-12 level. In addition, in all participants with an intake < 1.0 μg/d, elevated plasma tHcy levels were observed.

DISCUSSION

Vitamin B-12 status is an independent risk factor for neural tube defects (16–18,38). But the importance of an adequate vitamin B-12 status is often underestimated because the dietary intake of vitamin B-12 is usually far above dietary reference intakes in mixed Western diets. At present, little is known about the consequences of long-term predominantly vegetarian diets in pregnant women.

The present study shows that a long-term ovo-lacto vegetarian diet resulted in lower serum vitamin B-12 and higher plasma tHcy concentrations during pregnancy and therefore in an increased risk of vitamin B-12 deficiency. The present data also suggest that the current dietary reference intakes are too low and that an optimal dietary intake of vitamin B-12 during pregnancy should be at least above 3 μg vitamin B-12/d.

Most national and international dietary guidelines recommend a well-balanced diet with ample fruits and vegetables and moderate amounts of meat (39,40). A plant-based diet decreases the risk for many nutrition-related diseases such as coronary heart diseases and diseases of the so-called "metabolic syndrome" (4,6–8). Although it is usually stated that a vegetarian diet during pregnancy is adequate or even "health-conscious" (11,12,41), surprisingly little is known about the consequences of a predominantly vegetarian diet in pregnant women on vitamin and mineral status.

The pregnant women consuming a plant-based diet in the

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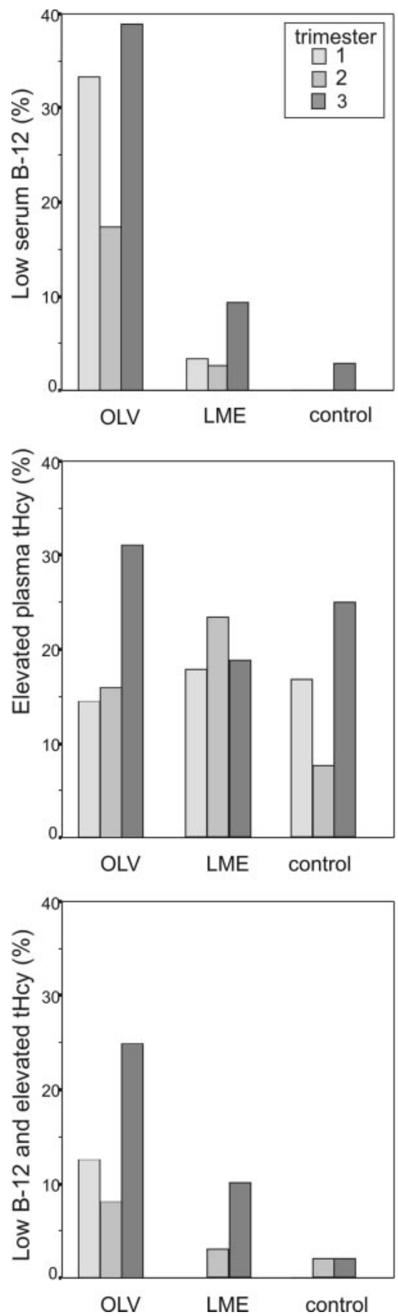

FIGURE 1 Occurrence of low serum vitamin B-12 concentrations, elevated plasma tHcy concentrations, and the simultaneous occurrence of both low serum vitamin B-12 and elevated plasma tHcy concentrations in pregnant ovo-lacto vegetarians (OLVs, $n = 27$), low-meat eaters (LMEs, $n = 43$) and women consuming an average Western diet (controls, $n = 39$). Vitamin B-12 deficiency is defined as <130 pmol/L in the first trimester, <120 pmol/L in the second trimester, and <100 pmol/L in the third trimester according to Koebnick et al (36), and elevated plasma tHcy concentrations is defined as >9.0 μmol/L in the first trimester and >7.8 μmol/L in the second and in the third trimesters according to Murphy et al. (37).

present study followed a low-meat or an ovo-lacto vegetarian diet, with a relatively high consumption of fruits and vegetables and whole-grain products and a low consumption of meat and meat products compared to the control diet (27). In the ovo-lacto vegetarian group, meat and meat products were omitted completely; low-meat eaters included a maximum of 2 portions/wk (i.e., on average 39 g/d), whereas controls consumed about 1 portion (133 g)/d. Although the mean intake of dairy products did not differ among diet groups, the ovo-lacto vegetarians exhibited the widest range in the consumption of dairy products. These differences in dietary habits among the diet groups were also reflected by the nutrient intake. Dietary intake of vitamin B-12 was lowest in the ovo-lacto vegetarians and a considerable proportion of women were far below the EAR for vitamin B-12 intake during pregnancy.

Recently, we showed that the folate status in pregnant women adhering to a plant-based diet was better than that in women following an average Western diet (27). But we also observed low erythrocyte folate concentrations in some women who reported a low dietary intake of vitamin B-12 (<3 μg/d) despite a high intake of dietary folate, suggesting a

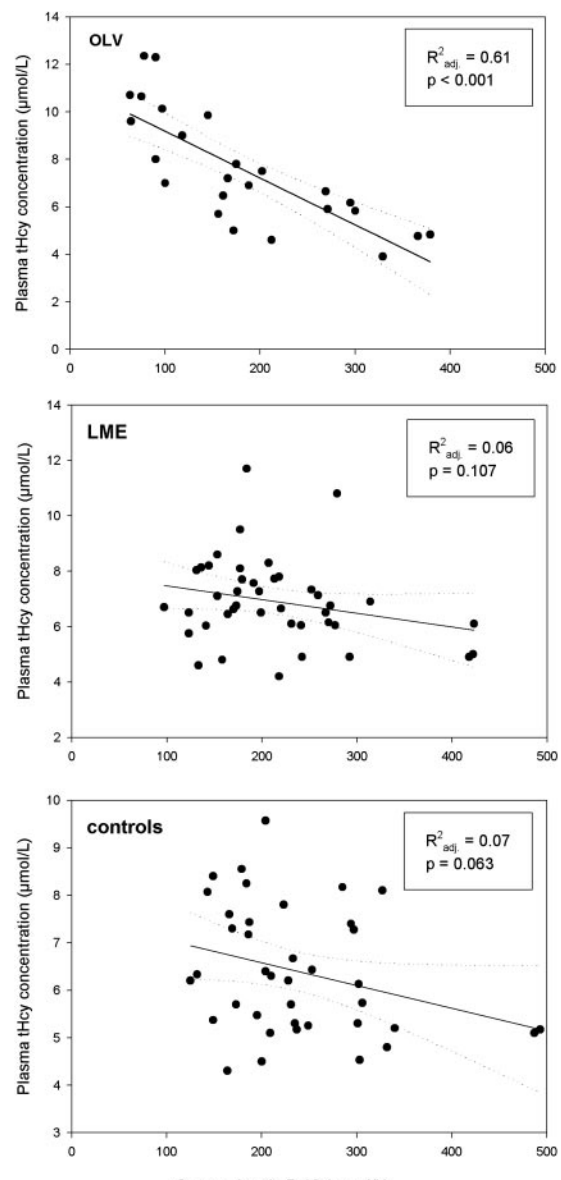

FIGURE 2 Relation between plasma *t*-homocysteine (tHcy) and serum vitamin B-12 concentrations in pregnant ovo-lacto vegetarians (OLVs, $n = 27$), low-meat eaters (LMEs, $n = 43$), and pregnant women consuming an average Western diet (controls, $n = 39$). Data are means of values throughout pregnancy.

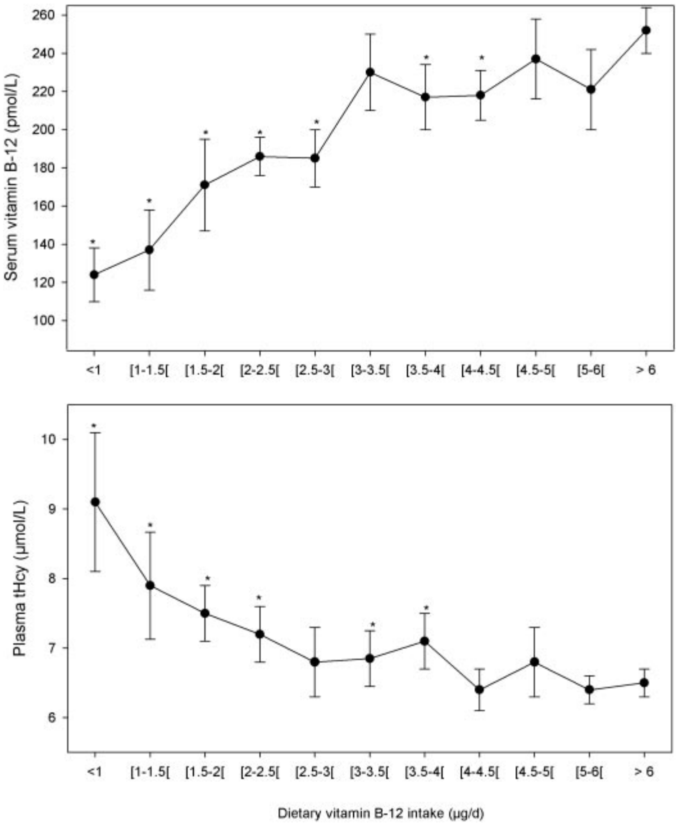

FIGURE 3 Serum vitamin B-12 and plasma tHcy concentrations according to dietary intake of vitamin B-12 in pregnant women (n = 109). Data are means of values in all trimesters. *$P < 0.05$ compared with values at a vitamin B-12 intake >6 μg/d estimated from the GEE model. Plasma tHcy concentrations are adjusted for erythrocyte folate concentrations.

functional folate deficiency as a consequence of vitamin B-12 deficiency (27).

The present data clearly show that serum vitamin B-12 concentrations were lowest and risk of low serum vitamin B-12 concentrations was highest in the group of pregnant ovo-lacto vegetarians. However, the prevalence of elevated plasma tHcy concentrations was equally distributed among diet groups; also, mean fasting plasma tHcy concentrations in all diet groups were higher than that reported by Murphy et al. (37). We previously reported (27) a higher folate intake in ovo-lacto vegetarians and low-meat eaters relative to the control group. Unfortunately, we did not have the opportunity to determine methylmalonic acid in plasma, which is a more specific marker for vitamin B-12 deficiency than plasma tHcy, which is determined by both vitamin B-12 and folate status. Nevertheless, when we define a functional vitamin B-12 deficiency as the combination of low serum vitamin B-12 and elevated plasma tHcy, the prevalence of vitamin B-12 deficiency was highest in the pregnant ovo-lacto vegetarians. Moreover, dietary vitamin B-12 intake was strongly positively related to serum vitamin B-12 concentrations and inversely related to plasma tHcy concentrations only in pregnant ovo-lacto vegetarians. This would suggest that both the pregnant low-meat eaters and the control group were adequately supplied with vitamin B-12, whereas pregnant ovo-lacto vegetarians were, at least in part, not adequately supplied with this vitamin.

The results of the present study indicate that dietary recommended intakes for vitamin B-12 for pregnant women may be too low. The recommendations are based upon the fetal deposition of 0.1 to 0.2 μg/d vitamin B-12 throughout pregnancy (24,42–44). In the present study, serum vitamin B-12 concentrations sharply increase from dietary vitamin B-12 intakes <1 μg to intakes of 3 μg and further slightly increase to an intake of <4.5 μg/d, compared to an intake of >6 μg/d. For plasma tHcy, a similar although less distinct tendency could be observed up to a dietary vitamin B-12 intake of 3.0 μg/d. These results suggest that the cutoff level for an optimal intake of vitamin B-12 during pregnancy should be at least >3.0 μg/d.

The results of the present study must be interpreted with some caution due to several limitations. First, the small sample size limited the precision of our estimation of optimal vitamin B-12 intake. Additionally, some comparisons shown in the present study are more cross-sectional than longitudinal due to the study design with an inconsistent sample size in different trimesters. Although it is not possible to avoid drop-outs from early birth, a higher sample size would capture those effects to some extent. Further studies are also needed to exactly specify differences among dietary groups in the progress of pregnancy. Second, high folate intake and folate supplementation during pregnancy may cover the real effects of low vitamin B-12 intake on plasma tHcy levels due to the trapping of 5-methyltetrahydrofolate, the so-called folate trap (45). Third, no validated cutoff values for pregnant women indicating a deficiency for serum vitamin B12 and plasma tHcy exist in the literature. Therefore, we used 95% CIs as a cutoff, which may result in an underestimation of the prevalence of vitamin B-12 deficiency in the present study.

Although further studies will be needed to confirm our results, the present data do strongly suggest that the requirement for vitamin B-12 during pregnancy has been underestimated and that the current EAR for vitamin B-12 (2.2 μg/d) as well as the recommended dietary allowances (2.6 μg/d) (24) need reevaluation. Since fetal and maternal serum vitamin B-12 concentrations are strongly related (46), low intake of vitamin B-12 may result in a vitamin B-12 deficiency in newborns. An impaired vitamin B-12 status in newborns and a subsequent low intake of vitamin B-12 from breast-feeding may have unfavorable effects on cognitive and psychomotor development (47). If a low vitamin B-12 status at birth is followed by a continuing low intake of vitamin B-12 due to breastfeeding by a vitamin B-12–deficient mother, these unfavorable effects may be aggravated (15,48,49). Moreover, neurological complications for the expectant mother may occur. However, further studies focusing on these consequences for mother and infant are needed.

Several studies have shown biochemical evidence of vitamin B-12 deficiency based on elevated plasma tHcy and methylmalonic acid in vegans and ovo-lacto vegetarians, indicating that the risk of vitamin B-12 deficiency in these groups has been underestimated (15,50–59). In one of these studies, the degree of vitamin B-12 deficiency was related to the degree of animal product restriction, resulting in the highest risk for vegans (50).

As already mentioned, we previously reported that the low-meat variant of the plant-based diet of our study population did not result in an improved folate status compared to an average Western diet (27). Only the ovo-lacto vegetarian diet was associated with a significantly reduced risk for developing a folate deficiency during pregnancy. On the other hand, the present findings show that the same ovo-lacto vegetarian diet resulted in an increased risk for an impaired vitamin B-12 status. How could this dilemma be solved? One possible solution would be to keep the advantages of the ovo-lacto vegetarian diet while avoiding its disadvantages. Because the main sources of vitamin B-12 in an ovo-lacto vegetarian diet are dairy products and eggs, the dietary guidelines for women at childbearing age could focus on increased consumption of milk and dairy products to ensure an adequate vitamin B-12 supply. Maybe a vitamin B-12 supplement must also be recommended for ovo-lacto vegetarian mothers in general. The present results suggest that, at least in a part of the ovo-lacto vegetarian population, milk and dairy products did contribute substantially to serum vitamin B-12 concentrations, an observation that is in accordance with other studies (22). However, it is noteworthy that the effects of milk and dairy products on plasma tHcy concentrations were much less pronounced than those on vitamin B-12 intake. This effect cannot be explained by the present study. In human breast milk (48) and algae (2) there was a high amount of nonbioactive cobalamin analogues, and other studies also suggest a high occurrence of vitamin B-12 deficiency in ovo-lacto vegetarian Seventh-Day Adventists (58). Therefore, further longitudinal studies in larger cohorts of pregnant women are needed before definitive recommendations can be given.

In conclusion, our study demonstrates that vitamin B-12 deficiency is frequent in pregnant women consuming ovo-lacto vegetarian diets and, as expected, closely related to vitamin B-12 intake from foods and supplements. Our results suggest that current recommendations for an adequate intake of vitamin B-12 during pregnancy urgently need reevaluation in order to avoid vitamin B-12 deficiency and its consequences for the newborns of lacto-vegetarian mothers.

LITERATURE CITED

1. Herbert, V. (1988) Vitamin B-12: plant sources, requirements, and assay. Am. J. Clin. Nutr. 48: 852–858.

2. Dagnelie, P., van Staveren, W. A. & van den Berg, H. (1991) Vitamin B-12 from algae appears not to be bioavailable. Am. J. Clin. Nutr. 53: 695–697.

3. Haddad, E. H. & Tanzman, J. S. (2003) What do vegetarians in the United States eat? Am. J. Clin. Nutr. 78: 626S–632S.

4. Key, T. J., Davey, G. K. & Appleby, P. N. (1999) Health benefits of a vegetarian diet. Proc. Nutr. Soc. 58: 271–275.

5. Knutsen, S. F. (1994) Lifestyle and the use of health services. Am. J. Clin. Nutr. 59: 1171S–1175S.

6. Rajaram, S. & Sabate, J. (2000) Health benefits of a vegetarian diet. Nutrition 16: 531–533.

7. Hu, F. B. (2003) Plant-based foods and prevention of cardiovascular disease: an overview. Am. J. Clin. Nutr. 78: 544S–551S.

8. Jenkins, D. J., Kendall, C. W., Marchie, A., Jenkins, A. L., Augustin, L. S., Ludwig, D. S., Barnard, N. D. & Anderson, J. W. (2003) Type 2 diabetes and the vegetarian diet. Am. J. Clin. Nutr. 78: 610S–616S.

9. Liu, R. H. (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr. 78: 517S–520S.

10. Lampe, J. W. (2003) Spicing up a vegetarian diet: chemopreventive effects of phytochemicals. Am. J. Clin. Nutr. 78: 579S–583S.

11. American Dietetic Association & Dietitians of Canada (2003) Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. J. Am. Diet Assoc. 103: 748–765.

12. Mangels, R. (2002) Position Paper: Vegetarian Diets in Pregnancy, p. 2. Vegetarian Nutrition Group of the American Dietetic Association, Chicago, IL.

13. Specker, B. L., Black, A., Allen, L. & Morrow, F. (1990) Vitamin B-12: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. Am. J. Clin. Nutr. 52: 1073–1076.

14. Allen, L. H. (1994) Vitamin B-12 metabolism and status during pregnancy, lactation and infancy. Adv. Exp. Med. Biol. 352: 173–186.

15. Dagnelie, P. C., van Staveren, W. A., Vergote, F. J., Dingjan, P. G., van den Berg, H. & Hautvast, J. G. (1989) Increased risk of vitamin B-12 and iron deficiency in infants on macrobiotic diets. Am. J. Clin. Nutr. 50: 818–824.

16. Kirke, P. N., Molloy, A. M., Daly, L. E., Burke, H., Weir, D. G. & Scott, J. M. (1993) Maternal plasma folate and vitamin B-12 are independent risk factors for neural tube defects. Q. J. Med. 86: 703–708.

17. Steen, M. T., Boddie, A. M., Fisher, A. J., Macmahon, W., Saxe, D., Sullivan, K. M., Dembure, P. P. & Elsas, L. J. (1998) Neural-tube defects are associated with low concentrations of cobalamin (vitamin B12) in amniotic fluid. Prenat. Diagn. 18: 545–555.

18. Steegers-Theunissen, R. P., Wathen, N. C., Eskes, T. K., van Raaij-Selten, B. & Chard, T. (1997) Maternal and fetal levels of methionine and homocysteine in early human pregnancy. Br. J. Obstet. Gynaecol. 104: 20–24.

19. Sanchez, S. E., Zhang, C., Rene Malinow, M., Ware-Jauregui, S., Larrabure, G. & Williams, M. A. (2001) Plasma folate, vitamin B-12, and homocyst(e)ine concentrations in preeclamptic and normotensive Peruvian women. Am. J. Epidemiol. 153: 474–480.

20. Savage, D. G. & Lindenbaum, J. (1995) Neurological complications of acquired cobalamin deficiency: clinical aspects. Baillieres Clin. Haematol. 8: 657–678.

21. Stabler, S. P. & Allen, R. H. (2004) Vitamin B12 deficiency as a worldwide problem. Annu. Rev. Nutr. 24: 299–326.

22. Tucker, K. L., Rich, S., Rosenberg, I., Jacques, P., Dallal, G., Wilson, P. W. & Selhub, J. (2000) Plasma vitamin B-12 concentrations relate to intake source in the Framingham Offspring study. Am. J. Clin. Nutr. 71: 514–522.

23. Yates, A. A. (1998) Process and development of dietary reference intakes: basis, need, and application of recommended dietary allowances. Nutr. Rev. 56: S5–S9.

24. Institute of Medicine (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. National Academy Press, Washington, DC.

25. Hellegers, A., Okuda, K., Nesbitt, R.E.I., Smith, D. W. & Chow, B. F. (1957) Vitamin B-12 absorption in pregnancy and in the newborns. Am. J. Clin. Nutr. 5: 327–333.

26. Robertson, J. A. & Gallagher, N. D. (1983) Increased intestinal uptake of cobalamin in pregnancy does not require synthesis of new receptors. Biochim. Biophys. Acta 757: 145–150.

27. Koebnick, C., Heins, U. A., Hoffmann, I., Dagnelie, P. C. & Leitzmann, C. (2001) Folate status during pregnancy in women is improved by long-term high vegetable intake compared with the average western diet. J. Nutr. 131: 733–739.

28. Projektträgergemeinschaft Forschung im Dienste der Gesundheit (1991) Die Nationale Verzehrsstudie. Ergebnisse der Basisauswertung [German; The National Food Survey. The results of the basic evaluation]. Schriftenreihe zum Programm der Bundesregierung, Bonn, Germany.

29. Hoffmann, I., Kohl, M., Groeneveld, M. & Leitzmann, C. (1994) Development and validation of a new instrument to measure food intake. Am. J. Clin. Nutr. 59: 284S.

30. Hoffmann, I., Groeneveld, M. J., Boeing, H., Koebnick, C., Golf, S., Katz, N. & Leitzmann, C. (2001) Giessen Wholesome Nutrition Study: relation between a health-conscious diet and blood lipids. Eur. J. Clin. Nutr. 55: 887–895.

31. Federal Institute for Health Protection of Consumers and Veterinary Medicine (1999) The German Food Code and Nutrient Data Base (BLS II. 3): conception, structure and documentation of the data base blsdat. BgVV, Berlin, Germany.

32. Ubbink, J. B., Vermaak, W. J. & Bissbort, S. H. (1991) Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J. Chromatogr. 565: 441–446.

33. Araki, A. & Sako, Y. (1987) Determination of free and total homocysteine in human plasma by high- performance liquid chromatography with fluorescence detection. J. Chromatogr. 422: 43–52.

34. Das, K. C., Manusselis, C. & Herbert, V. (1991) Determination of vitamin B-12 (Cobalamin) in serum and erythrocytes by radioassay, and of holo-transcobalamin II (holo-TC II) and holo-haptocorrin (holo-TC I and III) in serum by adsorbing holo-TC II on microfine silicea. J. Nutr. Biochem. 2: 455–464.

35. Wickramasinghe, S. N. & Fida, S. (1993) Correlations between holo-transcobalamin II, holo-haptocorrin, and total B-12 in serum samples from healthy subjects and patients. J. Clin. Pathol. 46: 537–539.

36. Koebnick, C., Heins, U. A., Dagnelie, P. C., Wickramasinghe, S. N., Ratnayaka, I. D., Hothorn, T., Pfahlberg, A., Hoffmann, I., Lindemans, J. & Leitzmann, C. (2002) Longitudinal Levels of vitamin B-12 and Vitamin B-12 binding proteins during normal pregnancy. Clin. Chem. 48: 928–933.

37. Murphy, M. M., Scott, J. M., McPartlin, J. M. & Fernandez-Ballart, J. D. (2002) The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. Am. J. Clin. Nutr. 76: 614–619.

38. Steegers-Theunissen, R. P., Boers, G. H., Blom, H. J., Nijhuis, J. G., Thomas, C. M., Borm, G. F. & Eskes, T. K. (1995) Neural tube defects and elevated homocysteine levels in amniotic fluid. Am. J. Obstet. Gynecol. 172: 1436–1441.

39. Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Kris-Etherton, P., Goldberg, I. J. et al. (2001) AHA Scientific Statement: AHA Dietary Guidelines: Revision 2000: A Statement for Healthcare Professionals From the Nutrition Committee of the American Heart Association. J. Nutr. 131: 132–146.

40. Johnson, R. K. (2000) The 2000 Dietary Guidelines for Americans: foundation of US nutrition policy. Nutr. Bull. 25: 741–748.

41. Messina, V. K. & Burke, K. I. (1997) Position of The American Dietetic Association: Vegetarian diets. J. Am. Diet Assoc. 11: 1317–1321.

42. Baker, S. J., Jacob, E., Rajan, K. T. & Swaminathan, S. P. (1962) Vitamin-B12 deficiency in pregnancy and the puerperium. Br. Med. Jnl. 5293: 1658–1661.

43. Loria, A., Vaz-Pinto, A., Arroyo, P., Ramirez-Mateos, C. & Sanchez-Medal, L. (1977) Nutritional anemia. VI. Fetal hepatic storage of metabolites in the second half of pregnancy. J. Pediatr. 91: 569–573.

44. Vaz Pinto, A., Torras, V., Sandoval, J. F., Dillman, E., Mateos, C. R. & Cordova, M. S. (1975) Folic acid and vitamin B12 determination in fetal liver. Am. J. Clin. Nutr. 28: 1085–1086.

45. Scott, J. & Weir, D. (1981) The methyl-folate trap and the supply of S-adenosylomethionine. Lancet 2: 755–756.

46. Frery, N., Huel, G., Leroy, M., Moreau, T., Savard, R., Blot, P. & Lellouch, J. (1992) Vitamin B-12 among parturients and their newborns and its relationship with birthweight. Eur. J. Obstet. Gynecol. Reprod. Biol. 45: 155–163.

47. Higginbottom, M. C., Sweetman, L. & Nyhan, W. L. (1978) A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B12-deficient breast- fed infant of a strict vegetarian. N. Engl. J. Med. 299: 317–323.

48. Dagnelie, P. C. & van Staveren, W. A. (1994) Macrobiotic nutrition and child health: results of a population-based, mixed-longitudinal cohort study in The Netherlands. Am. J. Clin. Nutr. 59: 1187S–1196S.

49. Dagnelie, P. C., van Dusseldorp, M., van Staveren, W. A. & Hautvast, J. G. (1994) Effects of macrobiotic diets on linear growth in infants and children until 10 years of age. Eur. J. Clin. Nutr. 48 (suppl. 1): S103–S111.

50. Herrmann, W., Schorr, H., Obeid, R. & Geisel, J. (2003) Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. Am. J. Clin. Nutr. 78: 131–136.

51. Rogers, L. M., Boy, E., Miller, J. W., Green, R., Sabel, J. C. & Allen, L. H. (2003) High prevalence of cobalamin deficiency in Guatemalan schoolchildren: associations with low plasma holotranscobalamin II and elevated serum methylmalonic acid and plasma homocysteine concentrations. Am. J. Clin. Nutr. 77: 433–440.

52. Misra, A., Vikram, N. K., Pandey, R. M., Dwivedi, M., Ahmad, F. U., Luthra, K., Jain, K., Khanna, N. & Devi, J. R. et al. (2002) Hyperhomocysteinemia, and low intakes of folic acid and vitamin B-12 in urban North India. Eur. J. Nutr. 41: 68–77.

53. Hung, C. J., Huang, P. C., Lu, S. C., Li, Y. H., Huang, H. B., Lin, B. F., Chang, S. J. & Chou, H. F. (2002) Plasma homocysteine levels in Taiwanese vegetarians are higher than those of omnivores. J. Nutr. 132: 152–158.

54. Herrmann, W., Schorr, H., Purschwitz, K., Rassoul, F. & Richter, V. (2001) Total homocysteine, vitamin B-12, and total antioxidant status in vegetarians. Clin. Chem. 47: 1094–1101.

55. Herbert, V. (1994) Staging vitamin B-12 (cobalamin) status in vegetarians. Am. J. Clin. Nutr. 59: 1213S–1222S.

56. Barr, S. I. & Broughton, T. M. (2000) Relative weight, weight loss efforts and nutrient intakes among health-conscious vegetarian, past vegetarian and nonvegetarian women ages 18 to 50. J. Am. Coll. Nutr. 19: 781–788.

57. Donaldson, M. S. (2000) Metabolic vitamin B-12 status on a mostly raw vegan diet with follow-up using tablets, nutritional yeast, or probiotic supplements. Ann. Nutr. Metab. 44: 229–234.

58. Hokin, B. D. & Butler, T. (1999) Cyanocobalamin (vitamin B-12) status in Seventh-day Adventist ministers in Australia. Am. J. Clin. Nutr. 70: 576S–578S.

59. Janelle, K. C. & Barr, S. I. (1995) Nutrient intakes and eating behavior scores of vegetarian and nonvegetarian women. J. Am. Diet Assoc. 95: 180–186.