Dietary sources of vitamin B-12 and their association with plasma vitamin B-12 concentrations in the general population: the Hordaland Homocysteine Study

Anna Vogiatzoglou, A David Smith, Eha Nurk, Paula Berstad, Christian A Drevon, Per M Ueland, Stein E Vollset, Grethe S Tell, and Helga Refsum

ABSTRACT
Background: Limited information is available on the association between vitamin B-12 status and intake from different dietary sources. Objective: We investigated the relation of dietary intake of different food items with plasma vitamin B-12 concentrations in the general population. Design: A cross-sectional, population-based study of 5937 subjects in 2 age groups (47–49 and 71–74 y) from the Hordaland Homocysteine Study in Norway was conducted by using a food-frequency questionnaire and measurements of plasma vitamin B-12 concentrations. Results: A significant difference in plasma vitamin B-12 concentrations was observed with increasing total vitamin B-12 intake. A plateau was reached at an intake of ~10 μg/d. Plasma vitamin B-12 was associated with intakes of increasing amounts of vitamin B-12 from dairy products or fish (P for trend <0.001 for both) but not with intakes of vitamin B-12 from meat or eggs. For the same content of vitamin B-12, intake from dairy products led to the greatest increase in plasma vitamin B-12. Total intake of vitamin B-12, particularly from milk and fish, decreased the risk of vitamin B-12 concentrations <200 pmol/L and impaired vitamin B-12 function (vitamin B-12 <200 pmol/L and methylmalonic acid >0.27 μmol/L) in the total group and in 71–74- y-old subjects. Conclusions: Dietary intake of dairy products and fish are significant contributors to plasma vitamin B-12 and may improve plasma vitamin B-12 status. Vitamin B-12 appears to be more bioavailable from dairy products; guidelines for improving vitamin B-12 status should take this into consideration. Am J Clin Nutr 2009;89:1078–87.

INTRODUCTION
Low vitamin B-12 status may be associated with megaloblastic anemia and neurologic disorders, such as neuropathy, myelopathy, memory impairment, dementia, depression, brain atrophy, and cerebrovascular disease (1–6). Recent studies indicate that subnormal or borderline plasma vitamin B-12 concentrations can occur in infants (7) and are highly prevalent among the elderly (8–12). Prevention of low vitamin B-12 status is important because some of the neurologic signs and psychiatric symptoms can occur in patients with vitamin B-12 concentrations within the range formerly considered low-normal and without associated anemia or macrocytosis (1, 2, 6, 13, 14). Furthermore, increasing evidence suggests that low vitamin B-12 status may be of particular concern in subjects with a high intake of folate, as is often the case in the era of folic acid fortification (15).

Typical causes of cobalamin deficiency in the elderly include pernicious anemia and food-bound malabsorption, but these causes explain less than half of the low vitamin B-12 concentrations (16). Dietary insufficiency is considered rare (17) and is often ignored, because the dietary intake of vitamin B-12 is usually above dietary reference intakes in subjects consuming a mixed Western diet. However, these dietary reference intakes were defined as the amount needed to prevent the overt vitamin B-12 deficiency that causes megaloblastic anemia and to maintain blood concentrations >150 pmol/L (18). Because several morbidities may be associated with low-normal vitamin B-12 status >150 pmol/L, it is important to establish whether dietary interventions can increase vitamin B-12 status throughout the normal range.

The relation between dietary intake and vitamin B-12 status has been investigated in different populations, with conflicting results. One study concluded that low vitamin B-12 status in the
elderly was not related to inadequate intake (17), whereas other reports showed significant associations between intake of vitamin B-12 and plasma concentrations (19–22). These discrepancies may arise in part from the different bioavailabilities of vitamin B-12 because the proportion absorbed differs according to food source, even in healthy adults (20, 23).

Foods, including breakfast cereals, were not fortified with vitamin B-12 in Norway for the duration of the study. Consequently, the vitamin B-12 intake in this population derives from natural food products or from vitamin supplements, the latter containing only low doses of vitamin B-12 (3–4 μg). We previously briefly reported correlations between components of the diet and plasma vitamin B-12 concentrations in subjects in the Hordaland Homocysteine Study (HHS) (24). In the present study, we investigated these associations in greater depth with a view to identifying the best dietary sources of bioavailable vitamin B-12 and to examining whether high vitamin B-12 intakes from certain foods can reduce the risk of low vitamin B-12 concentrations.

SUBJECTS AND METHODS

Study population

The HHS is a large population-based study of subjects aged 40–67 y living in the county of Hordaland in western Norway (25). Participant recruitment and examination were conducted in 2 periods ∽6 y apart. The first investigation (HHS-I) took place in 1992–1993. In 1997–1999, a follow-up study (HHS-II) was carried out in subjects living in Bergen and its surroundings and was part of the Hordaland Health Study (HUSK). The present study population includes participants from the HHS-II. All subjects underwent a brief health examination and provided a nonfasting blood sample. Information on diet, lifestyle, and medical history was collected via self-administered questionnaires. In total, 6140 of 7074 (87%) subject completed a food-frequency questionnaire (FFQ). Participants who provided questionnaires that were considered invalid were excluded from further analyses, which limited the total number of subjects to 5991. Of these, 5961 had plasma vitamin B-12 measured. We excluded 24 subjects who reported having injections of vitamin B-12, which left a total of 5937 subjects for analysis. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All subjects gave their written consent to participate in the study.

Health examination and analytic procedures

The health examination included measurements of height, weight, and blood pressure. Blood was drawn from nonfasting subjects, and tubes with plasma containing EDTA were stored at −80°C for biochemical analyses. Plasma total homocysteine was measured by automated HPLC with fluorescence detection (26). The concentrations of plasma folate and vitamin B-12 were measured by Lactobacillus casei (27) and Lactobacillus leichmannii microbiological assays (28). Plasma methylmalonic acid (MMA) was measured by using a modified gas chromatography–mass spectrometry method based on ethylchloroformate derivatization (29). The concentration of serum creatinine was measured with a standard alkaline picrate colorimetric assay.

Assessment of dietary intake

Dietary data were collected with the use of a validated self-administered FFQ developed by the Department of Nutrition, University of Oslo (30). The FFQ was validated for the Norwegian adult population (30). Although not validated for vitamin B-12 intake, the protein intake obtained by the FFQ corresponded very well with the protein intake obtained by 14-d weighed records, which suggested that this FFQ was well suited for the assessment of vitamin B-12 intake. It included 169 food items, grouped according to Norwegian meal patterns, and was designed to obtain information on usual food intake during the past year. The FFQ includes frequency alternatives (from once a month to several times per day) and the number of units eaten or portion sizes (eg, slices, cups, pieces, spoons, and deciliters). The information from the FFQ is presented as individual food items, food groups (individual food items combined), and nutrients. The FFQ also includes questions about common brands of single-vitamin and multivitamin supplements. Subjects using at least one dose of a B vitamin–containing supplement per day regularly during the past year were classified as B vitamin supplement users (n = 928). Daily nutrient intakes were computed from a database and software system developed at the Department of Nutrition, University of Oslo (KOSTBEREGNINGSSYSTEM, version 3.2; University of Oslo, Oslo, Norway) (30). The nutrient database is mainly based on the official Norwegian food-composition table (31). For most of the foods, vitamin B-12 was determined at the National Institute of Nutrition and Seafood Research (Norway) using microbiological assays (meat, Lactobacillus mesenteroides; milk and fish, Lactobacillus delbrueckii). Vitamin B-12 in some food products was determined in other places, as listed in the food tables. Estimated total intake of vitamin B-12 includes cobalamin from natural dietary sources and supplements (fortification with vitamin B-12 was not permitted in Norway at that time). Intake was calculated without losses from preparation methods. Vitamin B-12 intake in supplements was calculated from information on the contents of vitamin supplements for sale during 1997–1999 (usual supplement dose of vitamin B-12 was 3–4 μg).

Statistical analyses

All calculations were performed by using SPSS 15.0 (SPSS Inc, Chicago, IL) if not otherwise stated. Results are expressed as means or geometric means and 95% CIs or medians with 25th and 75th percentiles. Student’s t test, Mann-Whitney U test, analysis of variance (ANOVA), Kruskal-Wallis test, and chi-square test were used to compare independent groups. Adjustment for multiple comparisons was performed by using the Bonferroni correction. Spearman’s rank correlation coefficients were used to assess simple correlations between estimated dietary intake of food groups and plasma vitamin B-12. In the Spearman correlations, we compared the significance between the correlations using the Fisher r-to-z transformation.

For the association between plasma vitamin B-12 and diet, we used ANOVA according to groups of dietary factors divided into quartiles. Values for plasma vitamin B-12 were log-transformed for analysis and back transformed for presentation of geometric
means and 95% Cls. We adjusted for sex (men or women), age (young or old), energy intake (continuous), intake of other vitamin B-12–containing foods, and intake of supplements with B vitamins (yes or no). We also examined the potential confounding effect by smoking status (current or noncurrent smoker) and alcohol intake (continuous). Using multivariate-adjusted logistic regression, we calculated the odds ratio for plasma vitamin B-12 <200 pmol/L or impaired vitamin B-12 function (vitamin B-12 <200 pmol/L combined with MMA >0.27 μmol/L) according to 3 categories of vitamin B-12 intake from the selected food items. The categories of intake (ranked according to sex) were chosen to examine whether a high intake (>87.5th percentile of intake) could reduce the risk of vitamin B-12 <200 pmol/L or impaired vitamin B-12 function, using <12.5th percentile of intake as reference. All regression models were adjusted for age, sex, energy intake, and intake of other vitamin B-12–containing food items. We also adjusted for creatinine (continuous) because it is a strong determinant of elevated MMA (11, 32). All values were 2-sided, and values <0.05 were considered significant.

Gaussian generalized additive regression models implemented in S-PLUS for WINDOWS software (version 8.0; Insightful Corporation, Seattle, WA) were used to generate graphic representations of the dose-response relations between the vitamin B-12 intake from different food items and plasma vitamin B-12, after adjustment for age, sex, energy intake, intake of supplements with B vitamins and other vitamin B-12–containing food items. On the y axis, this nonparametric model generates a reference value of zero that approximately corresponds to the vitamin B-12 concentration associated with the mean intakes of food items for all subjects. Corresponding P values were obtained from multiple linear regression analyses.

**RESULTS**

**Subject characteristics and dietary intake**

Characteristics of the study population in the 4 age-sex groups are listed in Table 1. Plasma vitamin B-12 was significantly higher in younger than in older men and higher in women than in men within each age group. In all subjects, 4.9% had plasma vitamin B-12 concentrations <200 pmol/L and only 1.0% <150 pmol/L; in the elderly, the respective proportions were 6.5% and 1.7%, which indicated a good vitamin B-12 status in this population. Moreover, only 1.9% of all subjects and 3.1% of the elderly had plasma vitamin B-12 <200 pmol/L combined with plasma MMA values >0.27 μmol/L (impaired vitamin B-12 function). The distribution of plasma vitamin B-12 in the 4 age-sex groups is shown in Figure 1.

Food intake (kJ/d) and total vitamin B-12 intake (μg/d) from food were higher in men and in the younger groups (P < 0.001 for all). The mean total intake of vitamin B-12 ranged from 5.0 to 7.3 μg/d in the 4 age-sex groups. A lower intake of vitamin B-12 than recommended in the Nordic Nutritional Recommendations (2 μg/d) was observed among 2.3% (n = 135) of the subjects, but most of these subjects (n = 125) also had a very low energy intake (<6,500 kJ/d), which suggested either that they were dieting or did not complete their questionnaire correctly.

**Dietary sources of vitamin B-12**

Main dietary sources of vitamin B-12 are listed in Table 2. Intakes of meat and eggs were significantly higher among the younger groups and men. However, old men consumed more fish than did young men, P < 0.001. Milk intake was higher in men, whereas consumption of dairy products was significantly lower in men than in women.

**TABLE 1**

Characteristics of the study population: the Hordaland Homocysteine Study

<table>
<thead>
<tr>
<th>Characteristics of the Study Population: the Hordaland Homocysteine Study</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
<td>47–49 y (n = 1329)</td>
<td>71–74 y (n = 1310)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 (25.9, 26.3)</td>
<td>26.0 (25.8, 26.1)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>32.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Vitamin supplement users (%)</td>
<td>15.0</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Plasma variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>354 (348, 360)</td>
<td>340 (332, 348)</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>6.5 (6.4, 6.7)</td>
<td>6.4 (6.3, 6.6)</td>
</tr>
<tr>
<td>Total homocysteine (μmol/L)</td>
<td>10.4 (10.3, 10.6)</td>
<td>12.4 (12.2, 12.6)</td>
</tr>
<tr>
<td>Methylmalonic acid (μmol/L)</td>
<td>0.16 (0.16, 0.17)</td>
<td>0.20 (0.19, 0.20)</td>
</tr>
<tr>
<td><strong>Nutrient intake</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>10,183 (10,032, 10,337)</td>
<td>8,216 (8086, 8349)</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>94 (92, 95)</td>
<td>77 (75, 78)</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>88 (86, 89)</td>
<td>68 (66, 69)</td>
</tr>
<tr>
<td>Carbohydrates (g/d)</td>
<td>294 (290, 299)</td>
<td>244 (240, 248)</td>
</tr>
<tr>
<td>Vitamin B-12 (μg/d)</td>
<td>7.3 (7.1, 7.5)</td>
<td>6.9 (6.6, 7.0)</td>
</tr>
<tr>
<td>Folate (μg/d)</td>
<td>255 (250, 259)</td>
<td>220 (215, 224)</td>
</tr>
</tbody>
</table>

1 ANOVA or chi-square test followed by pairwise comparison with Bonferroni corrections.
2 Values are means; 95% CIs in parentheses.
3 Significant difference between sexes within the age group, P < 0.05.
4 Significant difference between age groups within sex, P < 0.05.
5 Use of multivitamins or other supplements that contained B vitamins.
6 Values are geometric means; 95% CIs in parentheses.
higher in old than in young women. In all 4 age-sex groups, fish, meat, and milk represented the most important sources of vitamin B-12, followed by cheese and eggs. The use of supplements that contained B vitamins was reported by 15.6% of the population and was significantly higher in women and in the younger age groups. Vitamin B-12 intake from supplements represented approximately 1% of the mean total intake for the whole sample (data not shown).

### Simple correlations

Spearman’s rank correlation coefficients between plasma vitamin B-12 and vitamin B-12–containing food items are shown for the 4 sex-age groups as supplemental data (see Table 1S under “Supplemental Data” in the online issue). For all subjects, the strongest positive correlations were observed between plasma vitamin B-12 and total vitamin B-12 intake ($r = 0.11$), intake of dairy products ($r = 0.13$), and in particular milk ($r = 0.13$), followed by fish ($r = 0.07$). Intakes of milk and fish were also associated with plasma vitamin B-12 in each age-sex group, whereas intakes of meat or cheese were not significantly associated with plasma vitamin B-12 in any subgroup. There was a significant difference between the correlations for total vitamin B-12 intake and some food items between old and young men, consistent with weaker associations in older men, but not between men and women in either age group. A separate analysis in older subjects, in whom associations of plasma vitamin B-12 with food intake tended to be weaker, confirmed the significance of the associations after the exclusion of subjects with a high

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Relative contribution of vitamin B-12 intake from different food groups to total vitamin B-12 intake: the Hordaland Homocysteine Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men 47–49 y old (n = 1329)</td>
</tr>
<tr>
<td>Meat and meat products</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>138 (96, 182)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>1.6 (1.2, 2.5)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>84 (54, 118)$^4$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>2.8 (1.6, 4.6)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>16 (9, 27)$^{3, 4}$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>0.2 (0.1, 0.4)$^4$</td>
</tr>
<tr>
<td>Dairy products $^5$</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>389 (218, 571)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>1.8 (1.1, 2.6)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>320 (144, 488)$^{2, 4}$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>1.3 (0.6, 1.9)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Yogurt</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>7 (0, 27)$^{3, 4}$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>0.0 (0.0, 1.0)$^{2, 4, 4}$</td>
</tr>
<tr>
<td>Cheese</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>26 (13, 48)$^4$</td>
</tr>
<tr>
<td>Vitamin B-12 intake (μg/d)</td>
<td>0.3 (0.2, 0.6)$^4$</td>
</tr>
</tbody>
</table>

$^1$ Kruskal-Wallis test followed by pairwise comparison with Bonferroni corrections.
$^2$ Median; 25th and 75th percentile in parentheses (all such values).
$^3$ Significant difference between sexes within the age group, $P < 0.05$.
$^4$ Significant difference between age groups within sex, $P < 0.05$.
$^5$ Includes milk, yogurt, cheese, cream, and ice cream.

**FIGURE 1.** Histograms of the frequency distribution of plasma vitamin B-12 concentrations in the 4 age-sex groups. Medians with 25th and 75th percentiles are presented for each age-sex group. Significance between the 4 age-sex groups was first tested by the Kruskal-Wallis test ($P < 0.001$) followed by pairwise comparison between the relevant groups with Bonferroni correction. The median plasma vitamin B-12 concentration differed significantly between young men and old women ($P < 0.001$ for both).
MMA (>0.27 μmol/L) concentration (n = 437). Likewise, after the exclusion of subjects with possible malabsorption, defined as a vitamin B-12 intake greater than the median combined with plasma vitamin B-12 <200 pmol/L (n = 72) or a high MMA concentration (n = 181), the associations changed only modestly but with weaker significance (data not shown).

## Plasma vitamin B-12 and dietary intake of vitamin B-12

The adjusted associations between plasma vitamin B-12 and vitamin B-12 intake from different foods are presented in Table 3 for the total group and the 2 age groups. The dose-response curves for total vitamin B-12 intake and vitamin B-12 intake from fish, dairy products, and meat for all subjects are shown in

### Table 3

Adjustment mean and 95% CI plasma vitamin B-12 concentrations by quartile of vitamin B-12 intake of selected food items in subjects who participated in the Hordaland Homocysteine Study.

<table>
<thead>
<tr>
<th>Dietary vitamin B-12 intake from food items</th>
<th>All subjects (n = 5937)</th>
<th>Subjects aged 47–49 y (n = 3067)</th>
<th>Subjects aged 71–74 y (n = 2861)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin B-12</strong></td>
<td>pmol/L</td>
<td>pmol/L</td>
<td>pmol/L</td>
</tr>
<tr>
<td>≤4.2 μg/d</td>
<td>329 (322, 336)</td>
<td>323 (314, 332)</td>
<td>334 (323, 345)</td>
</tr>
<tr>
<td>4.3–5.9 μg/d</td>
<td>346 (340, 353)</td>
<td>348 (340, 356)</td>
<td>345 (334, 356)</td>
</tr>
<tr>
<td>6.0–8.3 μg/d</td>
<td>362 (355, 369)</td>
<td>365 (357, 373)</td>
<td>358 (346, 370)</td>
</tr>
<tr>
<td>≥8.4 μg/d</td>
<td>375 (367, 383)</td>
<td>385 (376, 395)</td>
<td>364 (351, 378)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from dairy products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.9 μg/d</td>
<td>324 (318, 330)</td>
<td>331 (324, 338)</td>
<td>318 (309, 328)</td>
</tr>
<tr>
<td>1.0–1.4 μg/d</td>
<td>340 (334, 346)</td>
<td>343 (335, 351)</td>
<td>335 (327, 344)</td>
</tr>
<tr>
<td>1.5–2.1 μg/d</td>
<td>356 (350, 362)</td>
<td>372 (363, 381)</td>
<td>341 (332, 349)</td>
</tr>
<tr>
<td>≥2.2 μg/d</td>
<td>373 (366, 380)</td>
<td>388 (378, 397)</td>
<td>358 (348, 368)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from milk (μg/d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.3</td>
<td>323 (317, 329)</td>
<td>331 (324, 338)</td>
<td>317 (307, 327)</td>
</tr>
<tr>
<td>0.4–0.8</td>
<td>340 (334, 346)</td>
<td>345 (337, 353)</td>
<td>335 (326, 344)</td>
</tr>
<tr>
<td>0.9–1.4</td>
<td>354 (347, 360)</td>
<td>367 (358, 376)</td>
<td>340 (331, 349)</td>
</tr>
<tr>
<td>≥1.5</td>
<td>373 (366, 380)</td>
<td>388 (378, 397)</td>
<td>357 (347, 367)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from cheese</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.1 μg/d</td>
<td>342 (336, 349)</td>
<td>346 (338, 355)</td>
<td>337 (328, 347)</td>
</tr>
<tr>
<td>0.2–0.3 μg/d</td>
<td>346 (340, 351)</td>
<td>355 (347, 362)</td>
<td>336 (328, 344)</td>
</tr>
<tr>
<td>0.4–0.5 μg/d</td>
<td>345 (339, 352)</td>
<td>349 (341, 358)</td>
<td>341 (331, 350)</td>
</tr>
<tr>
<td>≥0.6 μg/d</td>
<td>358 (351, 364)</td>
<td>370 (362, 378)</td>
<td>343 (332, 355)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.008</td>
<td>0.001</td>
<td>0.707</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from meat and meat products</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.5 μg/d</td>
<td>349 (342, 356)</td>
<td>355 (344, 366)</td>
<td>340 (332, 349)</td>
</tr>
<tr>
<td>0.6–1.0 μg/d</td>
<td>344 (338, 350)</td>
<td>349 (342, 357)</td>
<td>338 (329, 347)</td>
</tr>
<tr>
<td>1.1–1.8 μg/d</td>
<td>345 (338, 351)</td>
<td>356 (348, 363)</td>
<td>332 (322, 343)</td>
</tr>
<tr>
<td>≥1.9 μg/d</td>
<td>353 (347, 359)</td>
<td>362 (354, 369)</td>
<td>342 (332, 354)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.140</td>
<td>0.189</td>
<td>0.522</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from fish and shellfish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1.4 μg/d</td>
<td>330 (324, 336)</td>
<td>339 (331, 346)</td>
<td>321 (311, 330)</td>
</tr>
<tr>
<td>1.5–2.5 μg/d</td>
<td>344 (338, 350)</td>
<td>354 (346, 361)</td>
<td>333 (324, 343)</td>
</tr>
<tr>
<td>2.6–4.1 μg/d</td>
<td>349 (343, 355)</td>
<td>361 (353, 369)</td>
<td>336 (327, 345)</td>
</tr>
<tr>
<td>≥4.2 μg/d</td>
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<td>375 (366, 385)</td>
<td>359 (350, 369)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vitamin B-12 intake from eggs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤0.1 μg/d</td>
<td>348 (343, 354)</td>
<td>356 (350, 363)</td>
<td>339 (331, 347)</td>
</tr>
<tr>
<td>0.2–0.3 μg/d</td>
<td>349 (343, 355)</td>
<td>356 (348, 364)</td>
<td>342 (333, 352)</td>
</tr>
<tr>
<td>0.3–0.4 μg/d</td>
<td>345 (337, 352)</td>
<td>352 (342, 362)</td>
<td>338 (326, 340)</td>
</tr>
<tr>
<td>&gt;0.4 μg/d</td>
<td>346 (340, 353)</td>
<td>358 (349, 366)</td>
<td>334 (324, 344)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.818</td>
<td>0.837</td>
<td>0.677</td>
</tr>
</tbody>
</table>

1 ANOVA adjusted for sex, age groups (except within the age groups), energy, and the use of supplements containing B vitamins.
2 Includes milk, yogurt, cheese, cream, and ice cream.
3 Also adjusted for total intakes of fish, meat, and eggs.
4 Also adjusted for total intakes of fish, meat, cheese, and eggs.
5 Also adjusted for total intakes of fish, meat, milk, and eggs.
6 Also adjusted for total intakes of fish, dairy products, and eggs.
7 Also adjusted for total intakes of dairy products, meat, and eggs.
8 Also adjusted for total intakes of fish, meat, and dairy products.
Figure 2. The associations with plasma vitamin B-12 were first evaluated for the intake of the selected food items (see Table 2S under “Supplemental data” in the online issue) and without adjustment for the vitamin B-12 intake from the other vitamin B-12–containing food groups. The associations, in both cases, were similar to those presented. There were positive associations between vitamin B-12 intakes from fish and dairy products (specifically from milk) and plasma vitamin B-12. However, the differences in the mean of plasma vitamin B-12 across quartiles of vitamin B-12 intakes were higher for the young group. The association between vitamin B-12 intake from cheese and plasma vitamin B-12 was statistically significant but only after adjustment for the vitamin B-12 intake from other food items. Vitamin B-12 intakes from meat and eggs were not significantly associated with plasma vitamin B-12 in any of the groups. For all subjects, the difference in plasma vitamin B-12 between the top and bottom quartiles of vitamin B-12 intakes was 14.0% (47 pmol/L) for total vitamin B-12 intake, 15.2% (50 pmol/L) for vitamin B-12 from dairy products, and 11.2% (37 pmol/L) for vitamin B-12 intake from fish. The magnitude of the mean differences in plasma vitamin B-12 between the highest and lowest quartiles of vitamin B-12 intake from foods listed in Table 2 ranged from 4.5% to 15.3%. Further adjustment for smoking and alcohol intake did not change the associations (data not shown).

For total vitamin B-12 intake, we observed a dose-response curve that reached a plateau after an intake of 10 μg/d (Figure 2). In the graphs in Figure 2 we used similar scales on both axes to allow comparison of bioavailability. There is a steep dose-response curve for vitamin B-12 from dairy products, which indicates that its bioavailability from this source, specifically milk (see Supplemental Figure 1 under “Supplemental Data” in the online issue), appears to be higher than that from the other sources. Too few subjects consumed yogurt in this population for meaningful analysis. For the intake of vitamin B-12 from fish, the dose-response curve is not as steep, and, for higher intakes, the 95% CI increasingly widens (Figure 2). We observed an apparent decrease in plasma vitamin B-12 for intakes of vitamin B-12 >4 μg from meat. However, the 95% CIs were very wide. The difference in plasma vitamin B-12 between those not consuming dairy products and those eating the maximum amount was ~75 pmol/L, whereas for fish intake the respective difference was ~35 pmol/L (see Supplemental Figure 2 under “Supplemental data” in the online issue).

Risk of low vitamin B-12 status according to dietary intake of vitamin B-12

Subjects with plasma vitamin B-12 <200 pmol/L (n = 291) had lower vitamin B-12 intakes and lower intakes of fish and dairy products than did those with a plasma vitamin B-12 intake >200 pmol/L (P < 0.001; data not shown). Logistic regression analysis showed that subjects with the highest intake of vitamin B-12 had only one-third the risk of having plasma vitamin B-12 <200 pmol/L (Table 4). Similarly, those with the highest intake of vitamin B-12 from milk had only one-fourth the risk of having plasma vitamin B-12 <200 pmol/L. A similar, but weaker, effect was found for high intakes of vitamin B-12 from fish but not for high intakes of vitamin B-12 from meat, eggs, and cheese. Further analysis on subjects who also had high plasma MMA (>0.27 μmol/L), consistent with impaired vitamin B-12 function, showed similar but weaker reductions in risk with a high intake. Use of vitamin B-12–containing supplements reduced the risk of plasma vitamin B-12 <200 pmol/L but not of impaired vitamin B-12 function, perhaps because of infrequent use among those with plasma vitamin B-12 <200 pmol/L (n = 18).

Separate analysis in the old group, i.e., in whom a low plasma vitamin B-12 concentration is more likely to be due to malabsorption, showed that a high vitamin B-12 intake, particularly from milk, but also from fish, was associated with a reduced risk of vitamin B-12 <200 pmol/L and of impaired vitamin B-12 function. As for the total group, use of vitamin B-12–containing supplements tended to protect against plasma vitamin B-12 <200 pmol/L but not against impaired vitamin B-12 function.

DISCUSSION

In this large population-based study, we found a significant association between total dietary intake of vitamin B-12 and plasma vitamin B-12 concentrations. Notably, plasma vitamin B-12 was related to the vitamin B-12 intake from dairy products (especially from milk) and from fish, but not from meat and eggs. These findings have possible implications for nutritional guidelines.

The mean total intake (diet plus supplements) of vitamin B-12 was ~6 μg/d, comparable with that of free-living American and northern European subjects (20, 22, 33). Only 2.3% of the subjects did not meet the Norwegian recommended intake (34) of 2.0 μg/d. The use of supplements and intake of vitamin B-12 from supplements were lower than in American studies (20, 21, 35), but were similar to those in northern European studies (22, 33). Our
### TABLE 4
Odds ratios and 95% CIs of subjects with plasma vitamin B-12 concentrations <200 pmol/L or impaired vitamin function (vitamin B-12 <200 pmol/L and methylmalonic acid >0.27 µmol/L) according to contrasting intakes of vitamin B-12 from selected food items in all and old subjects1

<table>
<thead>
<tr>
<th>Dietary vitamin B-12 intake from food items</th>
<th>Vitamin B-12 &lt;200 pmol/L (n = 291)</th>
<th>Impaired vitamin B-12 function (n = 109)</th>
<th>Vitamin B-12 &lt;200 pmol/L (n = 186)</th>
<th>Impaired vitamin B-12 function (n = 87)</th>
</tr>
</thead>
</table>

#### Vitamin B-12

- **≤4.1 µg/d (men), ≤2.9 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **4.2–12.6 µg/d (men), 3.0–9.2 µg/d (women)**
  - 0.54 (0.39, 0.75)
  - 0.47 (0.29, 0.78)
  - 0.58 (0.39, 0.81)
  - 0.45 (0.26, 0.78)

- **>12.6 µg/d (men), ≥9.3 µg/d (women)**
  - 0.36 (0.21, 0.63)
  - 0.41 (0.17, 0.95)
  - 0.40 (0.20, 0.87)
  - 0.43 (0.17, 1.08)

  **P for trend**
  - 0.001
  - 0.012
  - 0.004
  - 0.025

#### Vitamin B-12 intake from dairy products2–4

- **≤0.6 µg/d (men), ≤0.5 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **0.7–2.9 µg/d (men), 0.6–2.4 µg/d (women)**
  - 0.54 (0.39, 0.74)
  - 0.79 (0.45, 1.38)
  - 0.63 (0.42, 0.96)
  - 0.72 (0.33, 1.56)

- **≥3.0 µg/d (men), ≥2.5 µg/d (women)**
  - 0.25 (0.14, 0.45)
  - 0.43 (0.17, 1.06)
  - 0.41 (0.20, 0.84)
  - 0.41 (0.16, 1.09)

  **P for trend**
  - <0.001
  - 0.074
  - 0.008
  - 0.089

#### Vitamin B-12 intake from milk4

- **≤0.1 µg/d (men), 0.0 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **0.2–2.3 µg/d (men), 1.8–2.4 µg/d (women)**
  - 0.48 (0.35, 0.66)
  - 0.53 (0.31, 0.89)
  - 0.46 (0.31, 0.74)
  - 0.55 (0.33, 1.01)

- **≥2.4 µg/d (men), 1.9 µg/d (women)**
  - 0.22 (0.12, 0.42)
  - 0.25 (0.10, 0.62)
  - 0.29 (0.15, 0.57)
  - 0.34 (0.13, 0.88)

  **P for trend**
  - <0.001
  - <0.001
  - <0.001
  - 0.017

#### Vitamin B-12 intake from cheese5

- **0.0 µg/d (men), 0.0 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **0.1–0.8 µg/d (men), 0.1–0.7 µg/d (women)**
  - 0.91 (0.62, 1.34)
  - 0.68 (0.39, 1.18)
  - 0.98 (0.61, 1.57)
  - 0.69 (0.38, 1.28)

- **≥0.9 µg/d (men), ≥0.8 µg/d (women)**
  - 0.80 (0.47, 1.36)
  - 0.66 (0.29, 1.53)
  - 0.99 (0.49, 1.98)
  - 0.75 (0.29, 1.89)

  **P for trend**
  - 0.419
  - 0.251
  - 0.938
  - 0.405

#### Vitamin B-12 intake from meat and meat products6

- **≤0.4 µg/d (men), ≤0.2 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **0.5–3.4 µg/d (men), 0.3–2.2 µg/d (women)**
  - 1.04 (0.72, 1.52)
  - 0.91 (0.53, 1.56)
  - 1.02 (0.68, 1.53)
  - 0.87 (0.49, 1.53)

- **≥3.5 µg/d (men), ≥2.3 µg/d (women)**
  - 0.80 (0.47, 1.38)
  - 0.92 (0.41, 2.08)
  - 0.84 (0.44, 1.60)
  - 0.82 (0.33, 2.03)

  **P for trend**
  - 0.450
  - 0.775
  - 0.705
  - 0.588

#### Vitamin B-12 intake from fish and shellfish7

- **≤1.0 µg/d (men), ≤0.7 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **1.1–6.6 µg/d (men), 0.8–4.6 µg/d (women)**
  - 0.57 (0.42, 0.79)
  - 0.49 (0.29, 0.79)
  - 0.79 (0.51, 1.24)
  - 0.54 (0.31, 0.97)

- **≥7.5 µg/d (men), ≥4.7 µg/d (women)**
  - 0.40 (0.24, 0.67)
  - 0.28 (0.12, 0.65)
  - 0.55 (0.29, 1.07)
  - 0.29 (0.12, 0.76)

  **P for trend**
  - <0.001
  - <0.001
  - 0.072
  - 0.009

#### Vitamin B-12 intake from eggs8

- **0.0 µg/d (men), 0.0 µg/d (women)**
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)
  - 1.00 (ref)

- **0.1–0.4 µg/d (men), 0.1–0.3 µg/d (women)**
  - 0.73 (0.49, 1.08)
  - 0.59 (0.34, 0.93)
  - 0.71 (0.45, 1.11)
  - 0.67 (0.36, 1.26)

- **≥0.5 µg/d (men), ≥0.4 µg/d (women)**
  - 0.59 (0.37, 0.98)
  - 0.61 (0.29, 1.27)
  - 0.69 (0.39, 1.25)
  - 0.69 (0.31, 1.55)

  **P for trend**
  - 0.051
  - 0.238
  - 0.256
  - 0.406

(Continued)
All subjects ($n = 5937$) Subjects aged 71–74 y ($n = 2866$)

<table>
<thead>
<tr>
<th>Vitamin B-12</th>
<th>Impaired vitamin B-12 function ($\bar{\alpha} = 87$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;$200 pmol/L</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>$\geq$200 pmol/L</td>
<td>0.74 (0.38, 1.48)</td>
</tr>
<tr>
<td>$&gt;$2.0 mg/d</td>
<td>0.75 (0.38, 1.48)</td>
</tr>
<tr>
<td>$&gt;$5.0 mg/d</td>
<td>0.75 (0.38, 1.48)</td>
</tr>
</tbody>
</table>

Values adjusted for age (except for the 71–74-y-old subjects), sex, energy intake, and creatinine. Except for supplement use, the categories were chosen so that the reference (ref) group included the lowest 12.5th percentile of intake, whereas the high intake group included the top 12.5th percentile of intake based on the total population. The 3 categories of each food group were ranked according to sex.

Dietary vitamin B-12 intake from food items

1. Includes milk, yogurt, cheese, cream, and ice cream.
2. Also adjusted for total intakes of fish, meat, and dairy products.
3. Also adjusted for total intakes of fish, meat, cheese, and eggs.
4. Also adjusted for total intakes of fish, meat, dairy products, and eggs.
5. Also adjusted for total intakes of fish, meat, and eggs.
6. Also adjusted for total intakes of fish, meat, dairy products, and eggs.

We found an association between plasma vitamin B-12 and intake of fish. There is a high consumption of fish in this population (46). Although fish consumed in Norway is usually cooked, the loss of vitamin B-12 in fish from cooking is not high.
In the Norwegian food database, vitamin B-12 contents of certain fish are high (2.1–12.7 μg/100 g) (31).

In the Framingham Offspring Study (20) and in a study of Hispanic elderly (21), intake of supplements was associated with increased vitamin B-12 status. In our study, vitamin B-12 intake from supplements (>2 μg/d) in subjects with plasma vitamin B-12 <200 pmol/L improved their vitamin B-12 status, but not in the elderly. This may have been due to the low dose in the supplements and to the fact that few subjects used them, which made it difficult to detect associations.

Our findings may have implications for guidelines for the maintenance of a good vitamin B-12 status. Whereas supplementation is recommended for those at immediate risk of deficiency, the options for increasing the overall vitamin B-12 status of the population are either fortification or targeted dietary recommendations. Our confirmation that dairy foods provide a highly bioavailable source of vitamin B-12 and that increased milk intake can raise plasma concentrations within the normal range suggests that the promotion of a high intake of dairy products would be a good strategy. It would avoid potential pitfalls of fortification and could be applied in less-developed regions. By increasing the overall vitamin B-12 status of a large portion of the population vulnerable to vitamin B-12 deficiency without malabsorption (elderly, pregnant and lactating women, infants, vegetarians, and persons from parts of the world with a low intake of animal-derived products), not only could morbidities associated with overt deficiency be prevented, but so too could the proposed subtle harmful effects attributable to low-normal concentrations of vitamin B-12 in persons with adequate vitamin B-12 absorption. Although the proportional increase in mean plasma vitamin B-12 concentrations was modest at ∼14.4% (48 pmol/L) from the bottom to the top quartile of dairy product intake (Table 3), such an increase would be beneficial in subjects with low-normal concentrations of vitamin B-12. Furthermore, correction of an imbalance between folate and vitamin B-12 (47), as may occur in countries that have fortified flour with folic acid (15), or in vegetarians (48) may possibly be achieved by this nutritional strategy.

Advantages of our study include its large study population and the use of a validated FFQ, which provides information about nutrient intake over a period of a year (49), although we recognize its limitations (50). Another weakness in our study relates to the inability to include the preparation losses of vitamin B-12 from food items. Inclusion of an estimate of losses relates to the inability to include the preparation losses of vitamin B-12. Furthermore, correction of an imbalance between folate and vitamin B-12 (47), as may occur in countries that have fortified flour with folic acid (15), or in vegetarians (48) may possibly be achieved by this nutritional strategy.

In conclusion, we suggest that, even in a well-nourished population, dietary intake is an important contributor to plasma vitamin B-12. It appears that vitamin B-12 in meat is less bioavailable than is that in milk and fish, which may have implications for recommendations about how to maintain a good vitamin B-12 status. On the basis of our observations and of previously published findings (20, 22), it seems that daily dietary vitamin B-12 intakes between 6 and 10 μg ensures the maximal plasma vitamin B-12 concentration in persons with adequate vitamin B-12 absorption. This amount is considerably larger than the current recommended daily intakes. We suggest that the guidelines for improving vitamin B-12 status should focus on the intake of dairy products, particularly milk, because vitamin B-12 appears to be more bioavailable from these sources.

We thank Kari Juul and Elfrid Błomdal for help with setting up the data files and with literature support. Kari Solvoll, Elin Bjørgø Løken, and Gunnar H. Åmlid are gratefully acknowledged for their work with the food frequency questionnaires.

The authors’ responsibilities were as follows—HR, SEV, and GST: participated in the study design and organization of data collection; CAD: assessed food intakes and helped develop the food-frequency questionnaire; AV and HR: developed the concept, conducted the statistical analysis, and wrote the first draft of the manuscript; PB: contributed to the calculation of food intakes; and AV, EN, PB, CAD, HR, ADS, GST, PMU, and SEV: interpreted the results and contributed to the study design and critical revision of the manuscript. None of the authors reported a conflict of interest.


