Vitamin B-12 and homocysteine status in a folate-replete population: results from the Canadian Health Measures Survey

Amanda J MacFarlane, Linda S Greene-Finestone, and Yipu Shi

ABSTRACT

Background: Vitamin B-12 is an important cofactor required for nucleotide and amino acid metabolism. Vitamin B-12 deficiency causes anemia and neurologic abnormalities—a cause for concern for the elderly, who are at increased risk of vitamin B-12 malabsorption. Vitamin B-12 deficiency is also associated with an increased risk of neural tube defects and hyperhomocysteinemia. The metabolism of vitamin B-12 and folate is interdependent, which makes it of public health interest to monitor biomarkers of vitamin B-12, folate, and homocysteine in a folic acid–fortified population.

Objective: The objective was to determine the vitamin B-12, folate, and homocysteine status of the Canadian population in the period after folic acid fortification was initiated.

Design: Blood was collected from a nationally representative sample of ~5600 participants aged 6–79 y in the Canadian Health Measures Survey during 2007–2009 and was analyzed for serum vitamin B-12, red blood cell folate, and plasma total homocysteine (tHcy).

Results: A total of 4.6% of Canadians were vitamin B-12 deficient (<148 pmol/L). Folate deficiency (<320 nmol/L) was essentially nonexistent. Obese individuals were less likely to be vitamin B-12 adequate than were individuals with a normal BMI. A total of 94.9% of Canadians had a normal tHcy status (<13 μmol/L), and individuals with normal tHcy were more likely to be vitamin B-12 adequate and to have high folate status (>1090 nmol/L).

Conclusions: Approximately 5% of Canadians are vitamin B-12 deficient. One percent of adult Canadians have metabolic vitamin B-12 deficiency, as evidenced by combined vitamin B-12 deficiency and high tHcy status. In a folate-replete population, vitamin B-12 is a major determinant of tHcy.

INTRODUCTION

Vitamin B-12 is an essential water-soluble B vitamin that acts as a coenzyme in the conversion of Hcy to methionine, a folate-dependent reaction, and the conversion of L-methylmalonyl-coenzyme A to succinyl-CoA. Vitamin B-12 is also required for nucleotide synthesis through its interaction with folate metabolism. Vitamin B-12 deficiency results in hyperhomocysteinemia, megaloblastic anemia, and neurodegeneration, and, when left undiagnosed, cognitive decline (1). The elderly are vulnerable to vitamin B-12 deficiency because of malabsorption, the risk of which increases with age (1, 2). Vitamin B-12 deficiency is also associated with an increased risk of NTDs (3–5). Because of these associations, calls have been made to fortify the food supply with vitamin B-12 in addition to folic acid.

Folate, also an essential water-soluble B vitamin, is required for nucleotide and methionine biosynthesis (6). Folate deficiency is associated with NTDs, other birth defects, cardiovascular disease, some cancers, megaloblastic anemia, and cognitive impairment in the elderly (6). The use of folic acid–containing supplements in the periconceptional period greatly reduces the risk of NTDs (7), but many women do not take them during this critical period (8). Therefore, Canada and the United States mandated the addition of folic acid to white flour in 1998 to increase folic acid intake in women of childbearing age and to decrease the risk of NTDs. Indeed, mandatory fortification in Canada has proved to be a successful public health intervention; folate deficiency has been essentially eliminated (9), and the prevalence of NTDs has decreased by ~45% (10). However, the folate status of a significant proportion of the general population could be considered high (9). High folic acid intake has been associated with an increased risk of some chronic diseases, including some cancers (11–14), although the evidence is equivocal at this point in time (15–18). The metabolism of folate and vitamin B-12 interact significantly, and a high folic acid intake has been shown to exacerbate the symptoms of vitamin B-12 deficiency. High folic acid intake can mask the hematologic symptoms of vitamin B-12 deficiency and worsen vitamin B-12–dependent neurodegeneration (19). High folate status in vitamin B-12–deficient individuals was also associated with further impairment of vitamin B-12 metabolism, including evidence of decreased activity of the 2 vitamin B-12–dependent enzymes
and increased circulating methylmalonic acid and tHcy, a biomarker of numerous chronic diseases including cardiovascular disease and cancer (20, 21). The recent CHMS, cycle 1 provides a unique opportunity to determine national estimates for vitamin B-12 status and its interaction with folate and tHcy in the Canadian population for the first time in the post-folic acid fortification period.

SUBJECTS AND METHODS

Survey design and study population

The CHMS cycle 1 is a comprehensive direct health measures survey that collects information on sociodemographic characteristics, risk factors, and health outcomes and includes blood, urine, and anthropometric measures (22). The CHMS cycle 1 represents ~96.3% of the Canadian population aged 6–79 y living at home and residing in the 10 provinces and 3 territories but excludes persons living in remote areas and areas with a low population density, persons living on reserves or other Aboriginal settlements, persons living in institutions, or full-time members of the Canadian Forces. Data from ~5600 persons over a 2-y period (2007–2009) at 15 sites were collected with a minimum of 500 respondents for each sex from 5 age groups (6–11, 12–19, 20–39, 40–59, and 60–79 y). Collection sites were stratified into 5 regions to ensure national representation. A household interview included general demographic information and an in-depth health questionnaire. A visit to a mobile examination center included physical measure tests and the collection of blood and urine samples. All processes of cycle 1 of the CHMS were reviewed and approved by the Health Canada Research Ethics Board. Participation in the survey was voluntary, and written informed consent was obtained from individual participants.

Blood analyses

Hematocrit was measured and whole blood, plasma, and serum were collected and processed at the mobile examination center. Samples were shipped frozen to the Health Canada Nutrition Laboratory for analysis. For RBC folate analysis, samples were thawed and diluted 1:26 with 0.5% ascorbic acid solution. After being mixed, hemolysates were allowed to stand for 180 min at room temperature. Folate was analyzed by using the Immulite 2000 immunoassay (Siemens Canada), as described by the manufacturer. Serum vitamin B-12 was analyzed by using the Immulite 2000 immunoassay (Siemens Canada), as described by the manufacturer. Plasma tHcy was analyzed by using the OrthoClinical Diagnostics VITROS 5.1 FS, as described by the manufacturer. Cutoffs for B-12 status were deficient (<148 pmol/L), marginal (148–220 pmol/L), and adequate (>220 pmol/L) (23). Cutoffs for folate status were deficient (<320 nmol/L), adequate (320–1090 nmol/L), and high (>1090 nmol/L). The high folate cutoff was set to approximate RBC folate in individuals consuming 2.1 mg DFEs/d based on data from Quinlivan and Gregory, where they determined the relation between dietary folate intake and serum and RBC folate (24). The 2.1 mg DFE/1090 nmol/L cutoff represents the combined intake of 0.4 mg DFEs, the Recommended Dietary Allowance for adults (not including pregnant or lactating women), and a 1-mg folic acid supplement, equivalent to 1.7 mg DFEs because of its increased bioavailability and which can be purchased in Canada without a prescription. Standard cutoffs for tHcy status were normal (≤13 μmol/L) and high (>13 μmol/L).

Associated factors

Age groups for the analysis were 6–11, 12–19, 20–45, 46–59, and 60–79 y. Ethnicity, income, and BMI were derived variables (25). Ethnicity was defined as whites and nonwhites, including Aboriginals. Household income was categorized into 3 groups: 2 groups designated as low and middle/high were calculated on the basis of both total family income from all sources and total household members, and a third income category was created for individuals with missing data. The BMI categories (in kg/m²) for adults were defined as <18.5 (underweight), 18.5–24.99 (normal weight), 25–29.99 (overweight), and ≥30 (obese). The BMI cutoffs for children were derived variables based on CDC growth charts (26) and were defined as ≤5th percentile (underweight), 5–85th percentile (normal weight), 86–95th percentile (overweight), and ≥95th percentile (obese). We chose the CDC-defined BMI cutoffs for children and adolescents to allow for comparisons with studies based on NHANES data. For the purpose of our analysis, underweight and normal BMI categories were grouped for both children and adults.

Statistics

The CHMS used a complex sampling strategy. The strategy allows for nationally representative estimates with 15 collection sites stratified in 5 regions to ensure a cross-country representative distribution of the sample (27). The number of collection sites was relatively low because of logistical and cost constraints and restricted the statistical analysis to 11 df. Prevalence estimates were calculated by using data weighted to represent the Canadian population aged 6–79 y. Descriptive statistics (means, percentiles, and prevalences) were determined by using defined cutoffs, age, sex, ethnicity, income, and/or BMI. Bootstrap weights were applied, and the 11 df defined for all variance estimations for geometric means, percentiles and proportions to account for the complex sample design (27). The serum vitamin B-12 distribution was skewed; therefore, the data were log transformed for comparisons. Student’s t test was used to test differences between proportions and geometric means. Significance was defined as a P value <0.05. The analyses were performed by using SAS Enterprise Guide 4 software (SAS Institute Inc) and BOOTVAR 3.2-SAS version (Statistics Canada; www.statcan.gc.ca/rdc-cdr/bootvar_sas-eng.htm).

RESULTS

Vitamin B-12 status in the Canadian population

We estimated the geometric mean serum vitamin B-12 concentration to be 309 pmol/L for all age and sex groups combined (Table 1). The reference values, the 5th and 95th percentiles for serum vitamin B-12 in the Canadian population, were 150 and 629 pmol/L, respectively (Table 1). We observed a significant age effect in that children aged 6–11 y had higher mean serum
vitamin B-12 concentrations than did all other age groups, regardless of sex (Table 1). Sex differences were observed in older age categories, such that women aged 46–79 y had a significantly higher mean serum vitamin B-12 than did age-matched males. Also, women aged 60–79 y had higher serum vitamin B-12 than did younger women aged 20–45 y.

Whereas we observed significant sex effects on the mean serum vitamin B-12 estimates, we did not observe a sex effect on the prevalence of vitamin B-12 norms. Therefore, sex groups were collapsed to determine the association of age with the prevalence of vitamin B-12 norms. The prevalence of vitamin B-12 deficiency (<148 pmol/L; 23) was estimated to be 4.6% in

### Table 1

<table>
<thead>
<tr>
<th>Geometric mean and distribution of serum vitamin B-12 estimates by sex and age in the Canadian population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex and age</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>All 6–79 y</td>
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<tr>
<td>All 6–11 y</td>
</tr>
<tr>
<td>12–19 y</td>
</tr>
<tr>
<td>20–45 y</td>
</tr>
<tr>
<td>46–59 y</td>
</tr>
</tbody>
</table>

1 Respondents included subjects with a valid serum vitamin B-12 value.
2 Because of the complex study design, differences between the percentiles were not tested.
3 Serum vitamin B-12 data were log transformed, and differences between age and sex groups were determined by using Student’s t test. Significance was defined as P < 0.05. Values in a column and within a sex group with different superscript letters are significantly different.
4 Indicates a significant effect of sex for the age group.

### Table 2

<table>
<thead>
<tr>
<th>Prevalence of serum vitamin B-12 and RBC folate norms by age in the Canadian population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>No. of subjects</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>6–11 y</td>
</tr>
<tr>
<td>12–19 y</td>
</tr>
<tr>
<td>20–45 y</td>
</tr>
<tr>
<td>46–59 y</td>
</tr>
<tr>
<td>60–79 y</td>
</tr>
</tbody>
</table>

1 Respondents included subjects with a valid serum vitamin B-12 or RBC folate concentration. Because of the lack of sufficient respondents with an RBC folate concentration, these values were excluded. Sex had no significant effect on either measure; therefore, the data for males and females were combined. Differences between age groups were determined by using Student’s t test. Significance was defined as P < 0.05. Values within a column with different superscript letters are significantly different. RBC, red blood cell.
2 CV > 33.3, data suppressed.
3 CV of 16.6–33.3, interpret with caution.
We sought to determine the association of sociodemographic factors with vitamin B-12 status in the Canadian population. In children, adolescents, and adults, sex and income were not associated with vitamin B-12 status (Table 3). Nonwhite individuals (aged 6–19 y) were less likely than were white individuals to have marginal vitamin B-12 status (7.7 compared with 12.5%, respectively). Similarly, fewer nonwhite adults (14.6%) had marginal vitamin B-12 status than did white adults (20.9%). Of note, obesity was negatively associated with vitamin B-12 status. Significantly fewer obese children and adolescents were vitamin B-12 adequate (79.6%) in comparison with normal-weight children and adolescents (88.3%). Also, a significantly smaller proportion of adults with a BMI ≥30 (66.7%) were observed to have adequate vitamin B-12 status than were adults with a BMI of 25–29.99 (79.2%) or BMI <25 (76.9%) (Table 3).

The general Canadian population (Table 2). Significantly more children (96.2%) than adolescents (88.3%) had vitamin B-12 adequacy (>220 pmol/L). Vitamin B-12 deficiency was too uncommon in children to estimate; marginal status (148–220 pmol/L) was observed in 3.6% of children (96.2%) than adolescents (88.3%) or adults (20–59 y). Adequate than were adults aged 20–59 y but not than were older adults (60–79 y) (Table 2). Adolescents aged 12–19 y had a 3.6% prevalence of deficiency and a 15.8% prevalence of marginal status. Adults aged ≥20 y consistently had a 5.0% prevalence of vitamin B-12 deficiency, a 19.7% prevalence of marginal status, and a 75.3% prevalence of adequacy (Table 3). We did not observe an effect of age on the prevalence of vitamin B-12 deficiency in older adults (60–79 y) in comparison with younger adults (20–59 y) (Table 2).

### Table 3

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<tr>
<th>Category</th>
<th>No. of subjects</th>
<th>Deficient (&lt;148 pmol/L)</th>
<th>Marginal (148–220 pmol/L)</th>
<th>Adequate (&gt;220 pmol/L)</th>
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<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>Estimate</td>
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</tr>
<tr>
<td></td>
<td>Female</td>
<td>858</td>
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</tr>
<tr>
<td>BMI</td>
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<td>Suppressedⁱ</td>
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<tr>
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<td>86th–95th percentile</td>
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<td></td>
<td>&gt;95th percentile</td>
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<td>Mid/high</td>
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<td>Suppressedⁱ</td>
<td>Suppressedⁱ</td>
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<td></td>
<td>Missing</td>
<td>142</td>
<td>Suppressedⁱ</td>
<td>Suppressedⁱ</td>
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<tr>
<td>Adults (20–79 y)</td>
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<td>3.6, 6.5</td>
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<td>4.1²</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1708</td>
<td>6.1²</td>
<td>3.5, 8.6</td>
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<td>Ethnicity</td>
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<td>5.1</td>
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<td></td>
<td>Nonwhite</td>
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<tr>
<td>BMI</td>
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<td>&lt;25 kg/m²²</td>
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<tr>
<td></td>
<td>25–29.99 kg/m²²</td>
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<tr>
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<td>≥30 kg/m²³</td>
<td>857</td>
<td>6.2²</td>
<td>3.5, 8.9</td>
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<tr>
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<td>Mid/high</td>
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<td>3.6, 6.5</td>
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<tr>
<td></td>
<td>Missing</td>
<td>173</td>
<td>Suppressedⁱ</td>
<td>Suppressedⁱ</td>
</tr>
</tbody>
</table>

¹ Respondents included subjects with a valid serum vitamin B-12 concentration and excluded those who were 20–79 y of age with impaired renal function (serum creatinine >133 μmol/L for men or >115 μmol/L for women) and pregnant women. Differences between groups were determined by using Student’s t test. Significance was defined as P ≤0.05. Values in the same column within a category with different superscript letters are significantly different.

² CV of 16.6–33.3, interpret with caution.

³ CV >33.3, data suppressed.

⁴ BMI cutoffs for children were based on values derived from NHANES data by the CDC (26): <85th percentile, normal; 85th–95th percentile, overweight; >95th percentile, obese.
Folate status in the Canadian population

We did not observe a significant sex effect on RBC folate and therefore presented the data by age groups only. Less than 1% of the population had an RBC folate status indicative of folate deficiency (Table 2; 9). We found that 63.5% of the Canadian population had a high folate status indicative of total folate intake >2.1 mg DFE/d (>1090 nmol/L; Table 2). Adolescents were the least likely to demonstrate high folate status among the age groups, although their status was only significantly different from adults aged 60–79 y. Among adults, the prevalence of high folate status increased with age (60.2%, 66.7%, and 73.0% for 20–45, 46–59, and 60–79-y-olds, respectively); the difference between 20–45-y-olds and 60–79-y-olds was significant.

Association of vitamin B-12 and folate status in the adult population

Because of the very low prevalence of folate deficiency, we were not able to accurately estimate the proportion of the Canadian population with combined vitamin B-12 deficiency/marginal status and folate deficiency (Table 4). Among individuals with a high folate status (>1090 nmol/L), 3.1% had vitamin B-12 deficiency compared with 8.8% of individuals with adequate folate status (320–1090 nmol/L). Similarly, vitamin B-12 marginal status was observed in fewer individuals with high folate status than in those with adequate folate status: 18.5% and 23.4%, respectively. Consequently, individuals with high folate status were more likely to have vitamin B-12 adequacy than were individuals with adequate folate status (78.4% compared with 67.9%, respectively).

Homocysteine status in the Canadian population

A significant majority, representing 94.9% of Canadians, had a normal tHcy status (Table 5). We observed a significant effect of both sex and age on the proportion of individuals with high tHcy status (>13 μmol/L; Table 5). More than twice as many males than females had high tHcy status (7.0% compared with 3.3%, respectively). Less than 1% of children and adolescents had high tHcy compared with 3.2–13.8% of adults. Also, we observed a more than 2- to 4-fold increase in the prevalence of older adults (60–79 y; 13.8%) with high tHcy compared with younger adults aged 46–59 (5.8%) and 20–45 (3.2%) y, respectively.

We sought to determine the association of vitamin B-12 and folate status with tHcy in adults aged 20–79 y (Table 6). We
observed an association between high circulating tHcy and vitamin B-12 deficiency. Among folate-replete adults (≥320 nmol/L), 20% and 29.8% of individuals with high tHcy had a vitamin B-12-deficient or marginal status, respectively, in comparison with 4.1% and 19.3% of individuals with normal tHcy status (Table 6). Conversely, individuals with high tHcy were significantly less likely to be vitamin B-12 adequate. The 20% of individuals with high tHcy who were vitamin B-12 deficient, defined as functional vitamin B-12 deficiency, represented ~1% of the total adult population (data not shown). Of note, high folate status (>1090 nmol/L) was associated with a higher prevalence of normal tHcy status (79.7%) in comparison with adequate folate status (320–1090 nmol/L; 70.6%) among vitamin B-12–adequate individuals (Table 6).

**DISCUSSION**

Vitamin B-12 is an essential nutrient required to prevent anemia and neurodegeneration and, and along with folate, is required for nucleotide and methionine metabolism. We present the first national level data on vitamin B-12 status and its association with folate and tHcy in the Canadian population in the post–folic acid fortification era. We observed that most Canadians have an adequate vitamin B-12 status, but 5% have serum vitamin B-12 concentrations indicative of deficiency. Functional vitamin B-12 deficiency, defined as both a serum vitamin B-12 concentration <148 pmol/L and a plasma tHcy concentration >13 μmol/L, was evident in ~1% of adult Canadians. Our data indicate that vitamin B-12 is a significant determinant of tHcy status in a folate-replete population.

Poor vitamin B-12 status is often associated with older age because of malabsorption of vitamin B-12 from food, which is common in the elderly. Malabsorption results from atrophy of the gastric mucosa and reduced gastric acid, which is required for the release of vitamin B-12 from food (reviewed in reference 1). An increasing prevalence of serum vitamin B-12 deficiency with age was reported on the basis of data from NHANES 1999–2002 (23, 28); <1% of children and adolescents, ≤3% of adults aged 20–39 y, and ~6% of adults aged ≥70 y had vitamin B-12 deficiency (23, 28). The Hordaland Homocysteine Study also showed that older adults (aged 71–74 y) were more likely to be vitamin B-12 deficient than were younger adults (aged 47–49 y) (29). Whereas Canadian children and adolescents were less likely to be vitamin B-12 deficient than were adults, we observed a consistent prevalence of ~5% vitamin B-12 deficiency and ~20% marginal status among adults. Our data are limited to Canadians ≤79 y of age, a notable caveat because the prevalence of vitamin B-12 deficiency was observed to increase to 10% in individuals aged ≥75 y in the United Kingdom (30). Our data suggest that older Canadian adults (≥60–79 y) have better vitamin B-12 status than do their American and European counterparts.

One limitation of our study was that we did not consider vitamin B-12 intake from diet or supplements—major determinants of vitamin B-12 status (29, 31). Canadian women are less likely than men to meet the estimated adequate requirement for vitamin B-12 intake from food sources alone (32). However, ~40% of Canadians take vitamin and mineral supplements, the use of which is higher among older adults, especially older women (29, 33–35), which could explain the lack of an age effect on the prevalence of vitamin B-12 deficiency. This interpretation is supported in part by the observed higher mean serum vitamin B-12 concentration in older women than in younger women and men (Table 1). The difference in vitamin B-12 status between adults and children may be attributed to increased consumption of vitamin B-12–rich foods, such as milk.
and other dairy products (36), or to an increased prevalence of multivitamin use among children (37, 38).

We explored the association of a limited suite of socioeconomic factors with vitamin B-12 status. Sex and income were not associated with vitamin B-12 status; however, ethnicity had a significant effect such that nonwhite individuals were less likely to have marginal vitamin B-12 status, perhaps an indication of different dietary patterns. We also observed a significant effect of BMI on vitamin B-12 status. Obese children, adolescents, and adults were less likely to have adequate vitamin B-12 status and more likely to demonstrate marginal status in comparison with individuals with a normal BMI (Table 3). A similar association was not observed in NHANES III (39). The decrease in the proportion of obese Canadians with adequate status could reflect poor diet quality (40), a decreased prevalence of vitamin B-12 supplement use (38, 41), or a physiologic effect of obesity on nutrient absorption and/or metabolism. The CHMS collected information pertaining to vitamin B-12 intake from foods and supplements, allowing for a future study of the relations between obesity, diet quality, supplement use, and vitamin B-12 status.

Vitamin B-12 deficiency has been identified as an independent risk factor for NTDs (3–5). Very low serum vitamin B-12 was associated with a 4-fold increase in NTD risk in a high-risk region of China (5), and Canadian women with low vitamin B-12 status had a 2–3-fold increased NTD risk independent of folate status (3). Molloy et al (4) found that women with serum vitamin B-12 <148 pmol/L had a 3-fold increased risk of an NTD compared with women with a serum vitamin B-12 concentration >295 pmol/L. They estimated that a periconceptional serum vitamin B-12 concentration of 221 pmol/L would reduce most of the NTD risk. If applied to our population, ~75–80% of Canadian women of childbearing age (15–45 y) would be protected from vitamin B-12–responsive NTDs. However, the 5% of women with vitamin B-12 deficiency, a prevalence consistent with a previous study from Ontario (42), may be at increased risk of having a child with an NTD. The relatively high prevalence of vitamin B-12 deficiency and marginal status have raised the possibility for fortification of the Canadian food supply with vitamin B-12 (43). However, a randomized controlled clinical trial for the prevention of vitamin B-12–dependent NTDs should be performed to support the implementation of a fortification program.

Mandatory folic acid fortification has successfully eliminated folate deficiency in Canada. However, 22% of Canadian women of childbearing age had an RBC folate concentration <906 mmol/L, the concentration above which is associated with protection from NTDs (9). Concurrently, the folate status of a significant proportion of the population could be considered high. High folate intake was recently shown to be above which is associated with negative health outcomes, including CRC. Temporal associations were made between the introduction of folic acid fortification and a decrease in the rate of decline in CRC incidence in Canada, the United States, and Chile (11, 12). Clinical trial data also suggested that prolonged supplementation with 1 mg folic acid/d increased recurrence of multiple and more progressive CRC (13) as well as the risk of prostate cancer (14). However, the association with CRC was not supported by a second clinical trial (15).

Adding to the complexity, there is no consensus on an acceptable high RBC folate cutoff. Depending on the cutoff used, 40–65% of Canadians have a high folate status (Table 2; 9). However, the intake of dietary folate in the adult Canadian population was estimated to range from ~300 to 800 DFEs/d (32, 37), which suggests that vitamin supplements likely contribute significantly to high folate status. Indeed, folic acid supplement intake was shown to drive high serum folate status (44), and the intake of supplements containing >400 μg folic acid resulted in intakes above the upper level in most individuals (45). These findings highlight the need to define high folate status with preference for a cutoff associated with excessive folic acid intake or a folic acid–dependent clinical health risk. In the meantime, it remains prudent to continue monitoring the folate status of Canadians and its relation with chronic disease.

Folate and vitamin B-12 metabolism interact significantly and are inversely associated with tHcy—a biomarker of many chronic diseases, including cardiovascular disease and cancer. Green and Miller (46) proposed that vitamin B-12 deficiency is the major cause of elevated plasma tHcy in folate-replete populations; vitamin B-12 deficiency was associated with an increased risk of hyperhomocysteinemia in older adults aged ≥60 y in the postfolic acid fortification period (46). Among folate-replete adult Canadians, high tHcy was associated with vitamin B-12 deficiency or vitamin B-12 marginal status in 5-fold and 50% more individuals, respectively, than those with normal tHcy. Whereas we cannot rule out an effect of riboflavin and vitamin B-6, additional determinants of tHcy, our data indicate that vitamin B-12 is a significant determinant of tHcy in this folate-replete population.

Low vitamin B-12 status combined with high folic acid intake/folate status has been associated with exacerbation of vitamin B-12–dependent clinical symptoms, including elevated tHcy (19). However, we found that high folate status was associated with an increased prevalence of vitamin B-12 adequacy and normal tHcy in comparison with adequate folate status. Whereas our data do not exclude the possibility that combined high folate/low vitamin B-12 status has a negative effect on tHcy metabolism, it indicates that the likelihood of presenting with high folate/low vitamin B-12 status decreases with increasing folate status. Yang et al (45) made a similar observation, and the positive association between vitamin B-12 and folate status, especially among individuals with high folate status, was attributed to the consumption of combined vitamin B-12/folic acid–containing supplements.

The strengths of the current study can be attributed to the features of the CHMS cycle 1, including a large representative sample size, a population-based design, and a high response rate. Limitations of the study included the lack of methylmalonic acid data to assess functional/metabolic vitamin B-12 deficiency, an age limit of 79 y (because older individuals are more likely to be vitamin B-12 deficient), the lack of a gastrin measure to assess gastric atrophy and malabsorption, the lack of consideration for vitamin B-12 deficient), the lack of a gastrin measure to assess functional/metabolic vitamin B-12 deficiency, an age limit of 79 y (because older individuals are more likely to be vitamin B-12 deficient), the lack of a gastrin measure to assess gastric atrophy and malabsorption, the lack of consideration for vitamin B-12 deficiency was associated with an increased risk of hyperhomocysteinemia in older adults aged ≥60 y in the postfolic acid fortification period (46). Among folate-replete adult Canadians, high tHcy was associated with vitamin B-12 deficiency or vitamin B-12 marginal status in 5-fold and 50% more individuals, respectively, than those with normal tHcy. Whereas we cannot rule out an effect of riboflavin and vitamin B-6, additional determinants of tHcy, our data indicate that vitamin B-12 is a significant determinant of tHcy in this folate-replete population.

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REFERENCES


