Prevalence and correlates of high red blood cell folate concentrations in the Canadian population using three proposed cut-points

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<th>Journal:</th>
<th>Applied Physiology, Nutrition, and Metabolism</th>
</tr>
</thead>
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<td>Manuscript ID:</td>
<td>apnm-2015-0191.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>29-May-2015</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Colapinto, Cynthia; Children’s Hospital of Eastern Ontario Research Institute, Healthy Active Living and Obesity Research Institute; O’Connor, Deborah; Sick Kids Hospital, Dubois, Lise; Ottawa Institute of Population Health; Tremblay, Mark; Children’s Hospital of Eastern Ontario Research Institute,</td>
</tr>
<tr>
<td>Keyword:</td>
<td>folic acid, RBC folate, Canadian Health Measures Survey, high folate status</td>
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</table>
Correlates of high folate status

Title: Prevalence and correlates of high red blood cell folate concentrations in the Canadian population using three proposed cut-points

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Abstract

**Introduction:** A distinct shift towards higher folate concentrations has emerged in Canada. These higher concentrations have known benefits, including neural tube defect prevention, but concerns have been raised regarding potential associations with adverse health outcomes. The aim of this research was to propose cut-offs for high RBC folate concentrations and identify their correlates. **Methods:** RBC folate was measured in a nationally representative, cross-sectional sample of Canadians (N=5248), aged 6 to 79 years. RBC folate concentrations were adjusted from Immulite 2000 immunoassay to microbiologic assay. The population was characterized at three RBC folate cut-offs, 1450 nmol/L, 1800 nmol/L and 2150 nmol/L. We used t-tests to examine differences by age, sex, income and body mass index (BMI) at each cut-off and logistic regression to explore associations with folic acid supplement intake.

**Results:** Participants had 16%, 6% and 2% prevalence of having high RBC folate, at thresholds of 1450 nmol/L, 1800 nmol/L and 2150 nmol/L, respectively. Females, those aged 60 to 79 years and overweight or obese participants had the greatest prevalence of having high RBC folate at each cut-off. Folic acid supplement users were more likely than non-users to have high RBC folate concentrations.

**Conclusions:** Older age, higher BMI and folic acid supplement use were identified as correlates of high folate status. A high RBC folate concentration cut-off will advance the field towards consistent measurement and reporting of high folate status. This may facilitate future investigation of associations between RBC folate concentrations at the upper end of the distribution and health outcomes.

**Keywords:** folic acid; folate; red blood cell folate; RBC folate; high folate; Canadian Health Measures Survey; CHMS
Introduction

Folate is a nutrient with a significant population health impact. While best known for its role in reducing neural tube defects, folate has also been implicated in the etiology of other beneficial health outcomes, such as decreased risk of congenital heart defects and oral clefts and, at adequate levels, lower risk of breast, lung and prostate cancer. (De-Regil et al. 2010; Goh and Koren 2008; Mason 2011) Folate deficiency (red blood cell [RBC] folate <305 nmol/L) is virtually non-existent in Canada, likely due to the 1998 introduction of mandatory fortification of certain grain products with folic acid and increased use of folic acid-containing nutritional supplements. (Berry et al. 2010; Colapinto et al. 2011; Institute of Medicine 1998; Shakur et al. 2010) Mandatory folic acid fortification has been associated with a 46% reduction in neural tube defect births. (De Wals et al. 2008)

Women of childbearing age were the target of folic acid interventions; however, fortification strategies increase folic acid intake for the entire population. (Berry et al. 2010) Amidst this public health success story some speculate that widespread usage of folic acid supplements, in addition to intake of fortified foods, may lead to adverse health outcomes. Dissipating interest in deficiency has been replaced with the need to consider the possible health effects of elevated RBC folate concentrations. (Colapinto et al. 2011; Crider et al. 2011; Kim 2007) Proposed adverse effects include increased risk of cognitive impairment in older adults with unmetabolized folic acid in their serum, colorectal cancer in those with pre-existing neoplasms and prostate cancer in men taking folic acid supplements. (Baggott et al. 2012; Fife et al. 2011; Morris et al. 2010; Vollset et al. 2013; Wien et al. 2012)

Studies proposing adverse health outcomes in association with folic acid supplement use have been refuted, for example, recent systematic reviews and meta-analyses have demonstrated a beneficial or neutral effect in the etiology of cancer. (Science and Risk Directorate of the Ministry for Primary Industries 2012; Vollset et al. 2013) The lack of consensus in this field confirms that the study of high folate concentrations is an important, albeit controversial, area of investigation that will ultimately inform future clinical practice, research on health outcomes and folic acid policies. Though high RBC
Correlates of high folate status

Folate concentration cut-offs have been postulated (Colapinto et al. 2011; MacFarlane et al. 2011), a definition of high folate status does not exist. The aim of this research was to propose cut-offs for high RBC folate concentrations and define their correlates.

Methods

Data from the 2007-09 Canadian Health Measures Survey (CHMS) were used for these analyses. The survey methodology is described briefly here and in greater detail elsewhere. (Bryan et al. 2007; Tremblay et al. 2007)

CHMS sampling

The CHMS used a complex, multi-stage, cluster sampling protocol to achieve a nationally representative cross-sectional sample. The final sample included 5604 Canadians aged 6 to 79 years balanced by sex in each of the following age groups: 6 to 11, 12 to 19, 20 to 39, 40 to 59, and 60 to 79 years and is representative of approximately 96% of the Canadian population. People living in institutions, on Indian reserves or Crown lands, full-time members of the Canadian Armed Forces, and residents of certain remote regions were excluded.

Survey methods

A Statistics Canada interviewer administered a detailed in-home health questionnaire that included sociodemographic characteristics. One day to six weeks later, blood samples were taken by a certified phlebotomist in a mobile examination centre for later determination of a variety of analytes, including RBC folate. (Bryan et al. 2007) Of the 8,772 dwellings selected, 70% agreed to participate and 88% of those participated in the household survey. Of the household survey participants, 85% attended the mobile clinic. The overall response rate was 52%. Implied consent occurred in the household and a comprehensive consent process was employed in the clinic. (Day et al. 2007)

Ethics approval for the CHMS was obtained from the Health Canada Research Ethics Board and we obtained institutional ethics approval for this secondary data analysis.

Determination of RBC folate content and high RBC folate cut-offs
Blood was taken from 5373 of the 5604 CHMS participants with 5248 providing a usable sample for RBC folate measurement, weighted to represent 28 million Canadians who met the CHMS inclusion criteria. Participants who refused to participate in the blood draw or did not have a usable sample were excluded (n=356). RBC folate allows for an estimate of tissue folate stores. (Institute of Medicine 1998) Venipuncture samples were collected in EDTA-treated vacutainers, then immediately processed on-site. After hematocrit measurement, aliquots of whole blood were frozen, stored at -20°C and shipped weekly to the Health Canada Nutrition Laboratory on dry ice. (Bryan et al. 2007) Samples were thawed, diluted (1-in-26) with 0.5% ascorbic acid solution, allowed to incubate for 180 minutes at room temperature and then analyzed for RBC folate using the Immulite 2000 immunoassay (Siemens Canada Ltd., Mississauga) (Siemens Canada 2009) RBC folate was calculated from the measured whole-blood folate concentration adjusting for RBC volume, without correction for plasma folate. Accuracy and reproducibility of these procedures was assessed using the manufacturers’ serum controls (Con6: Tri-level multi-constituent control and tri-level BioRad ImmunoassayPlus) and whole blood controls (BioRad Lyphochek Whole Blood, 2 levels; BioRad Laboratories, Hercules, CA). Serum controls had an inter-assay coefficient of variation of <8% and all analyzed controls (serum, whole blood) were within 10% of target values.

Microbiological assay is considered the gold standard for determining RBC folate concentration, and this method measures RBC folate lower than Immulite 2000 immunoassay. (Icke et al. 2004) Thus, CHMS data were adjusted using an equation (Colapinto et al. 2014) that converts RBC folate concentrations from Immulite 2000 immunoassay to microbiologic assay medium containing Lactobacillus rhamnosus (NCIB 10463) and a calibration curve generated using 5-methyltetrahydrofolate, as per the method of Molloy et al., with modification. (Molloy and Scott 1997; Pfeiffer et al. 2011)

A scan of the literature revealed that few studies have postulated a cut-off for high RBC folate concentrations. (Colapinto et al. 2011; MacFarlane et al. 2011) Researchers have also used quantiles of the study population to examine high serum folate (Selhub et al. 2009), thus we also considered the
Correlates of high folate status upper RBC folate percentiles (90\textsuperscript{th}, 95\textsuperscript{th} and 97.5\textsuperscript{th}) from the post-fortification NHANES data, adjusted to microbiologic assay.(Pfeiffer et al. 2012) We estimated three cut-offs – 1450 nmol/L, 1800 nmol/L and 2150 nmol/L – that occurred at regular intervals, approximately 350 nmol/L apart, within this range of postulated cut-offs (Table 1) to analyse correlates for high RBC folate concentrations.

Potential correlates of high folate concentrations:

Selected sociodemographic factors: The CHMS sample age groups were used for these analyses.(Bryan et al. 2007) Socioeconomic status was examined by per person household income equivalents that grouped respondent income into quartiles after adjusting for family size and composition.

Behavioural factors: The CHMS collected drug identification and natural health product numbers from supplement containers during household interviews and these were verified at the mobile clinic visit.(Statistics Canada 2011)

Folic acid supplement use in the 30 days prior to the clinic visit and the supplement dose were determined by matching the drug and national health product numbers with the supplement composition information found in the Health Canada Drug Product Database and Licensed Natural Health Product Database.(Health Canada (a) 2013; Health Canada (b) 2015; Statistics Canada 2011) This process required a special request to access drug identification and natural health product number data that are not available on the CHMS master file.

Self-reported smoking status and history were used to categorize participants as a current, former or never smoker.

Clinical factors: Measured height and weight were used to calculate body mass index (BMI, kg/m\textsuperscript{2}), by which participants were then classified as neither overweight nor obese or overweight/obese. The BMI status of adults, excluding pregnant women, was classified using Health Canada’s guidelines (25 to 29.9 indicates overweight, ≥30 indicates obesity).(Health Canada 2003) Participants aged 6 to 17 years
Correlates of high folate status were classified into two categories, neither overweight nor obese or overweight or obese, based on the World Health Organization cut-offs. (Dietitians of Canada et al. 2010)

Serum vitamin B\textsubscript{12} was assessed using the Immulite 2000 immunoassay (Siemens Canada Ltd., Mississauga), a solid phase, competitive chemiluminescent enzyme immunoassay involving an automated alkaline denaturation procedure. The cut-off for marginal Vitamin B\textsubscript{12} status was determined to be \( \leq 221 \text{ pmol/L} \). (Allen 2009; Siemans Canada 2006) Serum controls had an interassay coefficient of variation of 9.6% (manufacturer’s %CV) and all analyzed controls were within 10% of target values.

**Statistical Analysis:**

Descriptive statistics (frequencies, percentiles) were used to characterize the population at each of the proposed high RBC folate concentration cut-offs. Where sample sizes were adequate, we used t-tests to examine differences between sociodemographic, behavioural and clinical factors and logistic regression analyses were used to examine folic acid supplement use as a correlate of high folate concentrations. All estimates were based on weighted data to represent the Canadian population.

Variance estimation (95% confidence intervals) and significance testing were based on the bootstrap technique to account for the complex sampling design. Analyses were conducted in SAS 9.1.3 (SAS Institute Inc., Cary, NC) and SUDAAN v.10.0 (RTI International, Research Triangle Park, NC), using DDF=11 in the SUDAAN procedure statements. Given the 11 degrees of freedom available for variance estimation, Satterthwaite-adjusted statistics were used to test the significance of each regression model’s coefficients. (Statistics Canada 2011) Significance was defined as a p-value of <0.05 and a Bonferroni adjustment was applied in cases of multiple comparisons.

**Results**

*Prevalence at each proposed high cut-off*

Prevalence data for the population are presented in Table 2. In the general population, 16%, 6% and 2% of participants had RBC folate concentrations of 1450 nmol/L, 1800 nmol/L and 2150 nmol/L, respectively. Higher prevalence of being above each RBC folate cut-points occurred in females as
Correlates of high folate status compared to males, participants 60 to 79 years versus younger age group, those who were overweight or obese in comparison with those who were neither overweight or obese, and folic acid supplement users versus non-users. Daily and former smokers had a higher prevalence of high RBC folate at the lowest cut-off, but this did not persist at the two upper cut-offs. No consistent difference was found for income in the general population. Those with replete vitamin B\textsubscript{12} concentrations had a higher prevalence of RBC folate concentrations above the 1450 nmol/L and 1800 nmol/L cut-offs than those who with low to marginal vitamin B\textsubscript{12} concentrations.

Supplement use as a correlate of high folate status

As we previously reported, 17\% of folic acid supplement users consumed doses of ≥1000 µg/d. (Colapinto et al. 2012) Less than 1\% of the population reported use of a folic acid supplement ≥5000 µg/d. Further, approximately 51\% of the general population reported consuming doses of folic acid in the range of 400 to 999 µg/d. Folic acid supplement users were more likely than non-users to have high RBC folate, regardless of the cut-off. Supplement users had 5.67 times the odds of having folate concentrations >1450 nmol/L than non-supplement users (95\%CI 3.96, 7.82). Folic acid supplement users were also more likely than non-users to be above the proposed high cut-off at 1800 nmol/L (OR 7.54, 95\%CI 4.22, 13.46) and 2150 nmol/L (OR 7.03, 95\%CI 3.37, 14.65). These odds persisted after controlling for age, sex and income at 1450 nmol/L, though the sample size was too small to conduct multivariate analysis at 1800 nmol/L and 2150 nmol/L. The small sample size also did not support an analysis of folic acid supplement dose at each cut-off.

Discussion

This study provides important information to characterize high RBC folate concentrations in the Canadian population. Using the proposed cut-offs of 1450, 1800 and 2150 nmol/L it was determined that less than 20\% of Canadians may have high folate status. Folic acid supplement use, older age and overweight and obesity were correlates of high RBC folate concentrations, indicating a need to
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examine these associations further. Use of these proposed high RBC folate concentration cut-offs in future studies may serve as a starting point for the examination of relationships between high RBC folate concentrations and adverse health outcomes.

It is paramount to consider that high folate concentrations in the general population should not overshadow the reduced risk of neural tube defect birth when women consume folic acid supplements three months prior to conception and continue for 21 to 28 days into their pregnancy.(Health Canada 2009) Considering that an estimated 50% of pregnancies are unplanned, Canadian guidelines recommend that women capable of becoming pregnant consume a folic acid (400 µ/d) containing multi-vitamin supplement, in addition to folate-rich foods. Further, RBC folate concentrations greater than the deficiency cut-off of 305 nmol/L have been associated with lower risk of NTD.(Crider et al. 2014; Daly et al. 1995) For example, a 2014 study used Bayesian modeling to demonstrate a population level dose-response relationship between RBC folate concentrations >1000 nmol/L in women of childbearing age and a substantially attenuated risk for NTDs, though the associations modeled demonstrated that RBC folate concentrations in excess of 1300 to 1500 nmol/L do not appear to provide additional benefit for NTD prevention.(Crider et al. 2014)

We observed that folic acid supplement use was a strong modifiable risk factor for high RBC folate concentrations, regardless of the proposed cut-off selected. By definition the Institute of Medicine’s Tolerable Upper Intake Level (UL) is the highest intake of a nutrient thought to pose no adverse health effects in healthy individuals.(Bradbury et al. 2012; Institute of Medicine 1998; Nguyen et al. 2009) For folate, this is set at 1000 µg/d of synthetic folic acid based on the lowest observed adverse effect level (5000 µg/d), a dose that could potentially mask neurological symptoms of vitamin B₁₂ deficiency and an uncertainty factor of 5, which accounts for the limited evidence and uncertainty in the process of defining a UL.(Zlotkin 2006) The proposed high folate cut-offs that we estimated are similar to the RBC folate concentrations — recently reported by ourselves and others — of participants consuming folic acid as a supplement at or above the UL. For example, we previously reported the RBC folate concentrations in a sample of healthy women, (18–45 years) from Toronto, randomly assigned to
Correlates of high folate status consume daily supplements of 1100 µg/d or 5000 µg/d (a dose approximately equal to the tolerable UL and the lowest observed adverse effect level, respectively) for 30 weeks. At the end of the study period the mean RBC folate concentrations (measured by microbiologic assay) rose from 1035 ± 273 nmol/L and 1121 ± 410 nmol/L to 1625 ± 339 nmol/L and 2339 ± 782 nmol/L, among women randomized to the 1100 µg/d and 5000 µg/d folic acid supplement respectively. (Nguyen et al. 2009) In another study – originally designed to examine folate supplementation and cognitive function – adults >65 years of age in New Zealand (n=276) were given a daily placebo or 1 mg/d folic acid supplement over a two year period as part of a double-blind randomized controlled trial to assess time to achieve steady-state concentrations. The supplemented group demonstrated considerable increases in RBC folate concentrations (measured by microbiologic assay) from 980 nmol/L at baseline to 2750 nmol/L at 6 months and 3010 nmol/L at 12 months. (Bradbury et al. 2012) It is notable at the time of this latter study there was no mandatory folic acid fortification program in New Zealand. Nonetheless, the Toronto and New Zealand studies together suggest that supplemental folic acid doses in the range of the UL elicit RBC folate concentrations similar to or above our proposed high cut-offs of 1800 and 2150 nmol/L.

High doses of folic acid (4000 to 5000 µg/d) are recommended for women at increased risk of NTD, such as those who have had a previous NTD birth and obese women, though the etiology is unclear. (Wilson et al. 2007) Further, obese women are more likely to have lower serum folate concentrations than non-obese women. (Bird et al. 2015) Our study demonstrates that the prevalence of high RBC folate concentrations was greater in obese participants than non-obese participants, suggesting that serum folate concentrations may not be reflective of folate stores in this sub-group. Further, older adults, who are not a target population for folic acid supplementation, had a greater prevalence of having high RBC folate concentrations at each cut-off. These findings require further investigation to understand appropriate folic acid supplement recommendations for these sub-groups.

The primary strengths of this study include a large, nationally representative sample, directly measured biomarkers and in-person collection of folic acid supplement information. Further, the
CHMS data were harmonized with the proposed cut-offs by adjusting the RBC folate concentrations from Immulite 2000 immunoassay to microbiologic assay. Interassay differences in RBC folate measures can be profound and harmonizing results to a common assay method should be considered when examining high folate concentrations. The overall response rate for the CHMS was slightly above 50%. While the survey weights ensured that the sample was representative of the target population, bias might exist if the RBC folate concentrations of non-respondents differed systematically from those of respondents.

In the present paper, correlates of three high RBC folate concentration cut-offs are examined. The lowest cut-off selected is more than four times the cut-off for folate deficiency (305 nmol/L). The highest two cut-offs are similar to recent controlled trials where a folic acid supplement was provided at the UL proposed by the U.S. Institute of Medicine.(Bradbury et al. 2012; Nguyen et al. 2009)

Identifying a high RBC folate concentration cut-off will advance the field towards consistent measurement and reporting of high folate status. This may facilitate future investigation of associations between RBC folate concentrations at the upper end of the distribution and health outcomes, particularly in the sub-population identified to have a greater prevalence of high folate status. This cut-off will also inform the debate on appropriate public health interventions and clinical guidelines for folic acid.
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References


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Table 1: Proposed high red blood cell (RBC) folate cut-offs

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<thead>
<tr>
<th>RBC folate cut-off (nmol/L)</th>
<th>Rationale</th>
<th>Citation</th>
</tr>
</thead>
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<tr>
<td>1090</td>
<td>This cut-off was estimated in correspondence to a relationship between DFEs and RBC folate determined by Quinlivan and Gregory, here representing 2.1 DFEs, or a combined intake of the recommended daily allowance for the general population (0.4 mg DFEs) and a 1 mg folic acid supplement</td>
<td>MacFarlane et al. 2011</td>
</tr>
<tr>
<td>1360</td>
<td>Examination of Canadian Health Measures Survey (CHMS) folate data using a cut-off based on the 97th percentile of BioRad 1999-2004 NHANES, as recent national Canadian data were unavailable.</td>
<td>Colapinto et al., 2011</td>
</tr>
<tr>
<td>1820</td>
<td>Represents the 90th percentile 2005-2010 NHANES adjusted to microbiologic assay.</td>
<td>Pfeiffer et al. 2012</td>
</tr>
<tr>
<td>2140</td>
<td>Represents the 95th percentile 2005-2010 NHANES adjusted to microbiologic assay.</td>
<td>Pfeiffer et al. 2012</td>
</tr>
<tr>
<td>2490</td>
<td>Represents the 97.5th percentile 2005-2010 NHANES adjusted to microbiologic assay.</td>
<td>Pfeiffer et al. 2012</td>
</tr>
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Table 2: Prevalence of the Canadian population above proposed high RBC folate cut-offs by sociodemographic, behavioural and clinical factors

(n=5248, unless otherwise indicated)

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<thead>
<tr>
<th>Population distribution</th>
<th>N</th>
<th>Weighted N</th>
<th>&gt;1450 nmol/L</th>
<th>&gt;1800 nmol/L</th>
<th>&gt;2150 nmol/L</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>&gt;16.4(12.6,21.0)</td>
<td>&gt;5.9(4.1,8.4)</td>
</tr>
<tr>
<td>Total population</td>
<td>5248</td>
<td>100</td>
<td>6.0 (3.9, 9.1)†</td>
<td>suppressed†</td>
<td>suppressed†</td>
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<tr>
<td>Sociodemographic factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-11</td>
<td>911</td>
<td>1830800</td>
<td>17.4</td>
<td>6.0 (3.9, 9.1)†</td>
<td>suppressed†</td>
</tr>
<tr>
<td>12-19</td>
<td>945</td>
<td>3110000</td>
<td>18.0</td>
<td>5.6 (3.2, 9.5)†</td>
<td>suppressed†</td>
</tr>
<tr>
<td>20-39</td>
<td>1150</td>
<td>8749000</td>
<td>21.9</td>
<td>12.0 (8.7, 16.4)*</td>
<td>3.6 (1.8, 6.9)*†</td>
</tr>
<tr>
<td>40-59</td>
<td>1202</td>
<td>9546000</td>
<td>22.8</td>
<td>20.0 (14.7, 26.6)*</td>
<td>7.3 (5.2, 10.3)*†</td>
</tr>
<tr>
<td>60-79</td>
<td>1040</td>
<td>4700500</td>
<td>19.8</td>
<td>28.2 (21.4, 36.2)§</td>
<td>12.5 (7.7, 19.5)§</td>
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<tr>
<td>Income quartile</td>
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</tr>
<tr>
<td>Q1</td>
<td>1368</td>
<td>6434800</td>
<td>26.1</td>
<td>13.0 (9.1, 18.2)*</td>
<td>5.0 (3.1, 8.0)*†</td>
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<tr>
<td>Q2</td>
<td>1242</td>
<td>6523600</td>
<td>23.7</td>
<td>16.3 (11.0, 23.4)†</td>
<td>5.9 (2.8, 11.9)†</td>
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<tr>
<td>Q3</td>
<td>1196</td>
<td>6543400</td>
<td>22.9</td>
<td>15.5 (10.6, 22.1)†</td>
<td>5.3 (2.7, 10.1)†</td>
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<tr>
<td>Q4</td>
<td>1110</td>
<td>6546400</td>
<td>21.6</td>
<td>20.8 (15.2, 27.8)*</td>
<td>7.1 (4.2, 11.6)*†</td>
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<td>15.4 (8.9, 25.4)†</td>
<td>6.5 (3.2, 12.9)*</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Female</td>
<td>2705</td>
<td>13910400</td>
<td>51.5</td>
<td>18.4 (13.7, 24.3)*</td>
<td>7.6 (5.2, 11.2)*†</td>
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<tr>
<td>Male</td>
<td>2543</td>
<td>14025800</td>
<td>48.5</td>
<td>14.3 (11.0, 18.5)§</td>
<td>4.2 (2.5, 7.0)*†</td>
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<td>Behavioural factors</td>
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<td>Folic acid supplement</td>
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<td>(last 30 days)</td>
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<td>Yes (at least one)</td>
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<td>6869500</td>
<td>24.0</td>
<td>37.1 (30.4, 44.4)*</td>
<td>16.2 (11.4, 22.4)*§</td>
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<td>No</td>
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<td>21066700</td>
<td>76.0</td>
<td>9.6 (6.6, 13.7)*§</td>
<td>2.5 (1.4, 4.4)*§</td>
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<td>Smoking status</td>
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<td>Daily</td>
<td>780</td>
<td>5349400</td>
<td>20.5</td>
<td>5.5 (2.9, 10.7)*</td>
<td>suppressed†</td>
</tr>
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<td>Former</td>
<td>1111</td>
<td>7068800</td>
<td>27.1</td>
<td>14.4 (9.1, 21.9)†</td>
<td>7.5 (4.7, 7.5)†</td>
</tr>
<tr>
<td>Never</td>
<td>2446</td>
<td>13687100</td>
<td>52.4</td>
<td>10.6 (7.8, 14.1)*§</td>
<td>4.7 (3.1, 6.7)*§</td>
</tr>
<tr>
<td>Clinical factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=5217)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neither overweight nor</td>
<td>2524</td>
<td>12172550</td>
<td>43.9</td>
<td>13.1 (9.8, 17.2)*</td>
<td>4.4 (2.7, 7.1)*†</td>
</tr>
<tr>
<td>obese</td>
<td>2693</td>
<td>15570370</td>
<td>56.1</td>
<td>18.5 (14.0, 24.1)§</td>
<td>7.0 (5.0, 9.8)§</td>
</tr>
<tr>
<td>Overweight/Obese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12 concentrations (N=4988)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low to marginal (≤ 221 pmol/L)</td>
<td>1013</td>
<td>6150600</td>
<td>20.3</td>
<td>9.0 (5.6, 14.3)*§</td>
<td>1.5 (0.8, 2.6)*§</td>
</tr>
<tr>
<td>Replete (&gt;221 pmol/L)</td>
<td>3975</td>
<td>20611200</td>
<td>79.7</td>
<td>17.8 (14.1, 22.3)*§</td>
<td>6.6 (4.6, 9.4)§</td>
</tr>
</tbody>
</table>

* Significantly different from the reference group
† Interpret with caution (high sampling variability; coefficient of variation ≥ 16.6 and < 33.3)
‡ Estimate suppressed because of extreme sampling variability or small sample size.
§ Reference group
¶ Estimates rounded to the nearest hundred

Note: Canadian Health Measures Survey RBC folate data was adjusted to be comparable to a microbiologic assay (Colapinto et al. 2014)