British Journal of Nutrition (2016), **116**, 1236–1245
doi:10.1017/S000711451600307X

Folate intakes from diet and supplements may place certain Canadians at risk for folic acid toxicity

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(Submitted 14 April 2016 – Final revision received 27 July 2016 – Accepted 1 August 2016 – First published online 9 September 2016)

Abstract

To examine the prevalence of folate inadequacy and toxicity based on usual intakes from food and supplements, as well as biomarkers of folate, secondary data analyses were performed using cross-sectional, nationally representative data from the Canadian Community Health Survey, Cycle 2.2 (*n* 32 776), as well as biomarker data from the Canadian Health Measures Survey, Cycles 1, 2 and 3 (*n* 15 754). On the basis of unfortified food sources, Canadians would struggle to consume adequate amounts of folate. When folate intakes from all food sources were considered, the overall prevalence of folate inadequacy was low across all age/sex groups, with the exception of females >70 years. However, >10 % of supplement users were above the tolerable upper intake level, increasing to almost 18 % when overage factors were accounted for. In addition, between 20 and 52 % of supplement users had elevated erythrocyte folate concentrations, depending on the cut-off used. Results from this study suggest that insufficient dietary intakes of folate in Canadians have been ameliorated because of the fortification policy, although folate inadequacy still exists across all age groups. However, supplement users appear to be at an increased risk of folic acid (FA) overconsumption as well as elevated erythrocyte folate. As such, the general population should be informed of the potential risks of FA overconsumption resulting from supplement use. This study suggests a need for more careful assessment of the risks and benefits of food fortification, particularly fortification above mandated levels, and FA supplement use in the general population.

Key words: Folate: Food fortification: Health surveys: Supplements

Folate (vitamin B_9) is a water-soluble vitamin, necessary for cell division and growth, which occurs naturally in foods such as leafy green vegetables, fruits and legumes. Its synthetic form, folic acid (FA), is found in supplements and fortified foods[1]. Folate deficiency during the periconceptional period (3–4 weeks after conception) has been associated with an increased risk for neural tube defects (NTD) such as spina bifida and anencephaly[2–4], and several studies have corroborated the effect of FA supplementation on lowering the prevalence of NTD and other birth defects[5–7]. As such, adequate intakes of folate are essential for women of childbearing age (WCBA), and the Institute of Medicine (IOM) recommends that WCBA consume 400 µg (0·4 mg) a day of FA in addition to consuming foods naturally rich in folate[8,9]. In 1996, the United States Food and Drug Administration announced that it would allow for the fortification of flour and other cereal grain products with FA, deeming fortification mandatory in 1998. The very same year, Canada also mandated the addition of FA to white flour and enriched pasta and maize meal at 0·15 mg FA/100 g of flour and 0·20 mg FA/100 g of pasta[9], predicting an overall intake increase of 100 µg/d[10]. After fortification, results showed a dramatic decrease (approximately 40 %) in national rates of NTD[11,12] and an improvement in folate status[13,14].

In spite of this success, there exists some controversy regarding possible health concerns associated with FA overconsumption. The tolerable upper intake level (UL) is defined as the 'maximum level of chronic nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population'[15,16]. On the basis of the metabolic interactions between folate and vitamin B_{12}, the Food and Nutrition Board (FNB) and the IOM established an UL for *synthetic* forms of folate (µg of FA) available in dietary supplements and fortified foods for each life-stage group[1].

Recently, FA fortification has come under scrutiny because of accumulating evidence suggesting that the general population of the USA has been exposed to unprecedented levels of FA above the UL[17]. This may be attributed to the fact that high

Abbreviations: CCHS 2.2, Canadian Community Health Survey, cycle 2.2; CHMS, Canadian Health Measures Survey; DFE, dietary folate equivalent; DRI, dietary reference intakes; EAR, estimated average requirement; FA, folic acid; IOM, Institute of Medicine; NHANES, National Health and Nutrition Examination Survey; NTD, neural tube defects; POFI, prevalence of folate inadequacy; UL, tolerable upper intake level; WCBA, women of childbearing age.

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plasma folate has been associated with an exacerbation of both clinical and biochemical signs of B_{12} deficiency, potentially permitting cognitive impairment to occur[18,19]. A recent review article outlined the increased risk of anaemia and cognitive issues seen in older adults with high serum folate but poor B_{12} status, implying that excessive FA intake is not safe and is associated with adverse clinical outcomes in the elderly[18,19]. This is of particular concern in older adults who are more susceptible to vitamin B_{12} deficiency[20]. However, older adults are not the only ones at risk of adverse health outcomes: an increase in insulin resistance has been linked to high maternal erythrocyte folate among Indian mothers[21]. In addition, animal studies have shown that excessive FA may be harmful, reducing natural killer cell cytotoxicity in mice[22] and also disturbing their immune response and resistance to malaria[23]. Trends also suggest that after fortification, North America has experienced a 'reversal' of the downward trend in the incidence of colorectal cancer at a statistically significant rate of 4–6 cases/100 000 individuals, although causality cannot be inferred[24]. However, even though there is evidence that excess FA may be associated with advanced colorectal adenomas[25] and recurrent colorectal adenomas[24,26], a recent meta-analysis study does not support this[27]. Nevertheless, results from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial observed that women who reported consuming ≥400 μg/d of FA had a 20 % greater risk of developing breast cancer[28]. The hypothesised mechanism behind these phenomena suggests that, although FA aids in normal DNA replication, in malignant cells excess amounts may cause cell proliferation[29]. As it passes through the intestinal wall, FA is converted to a natural biological form of the vitamin, resulting in the circulating form – 5-methyltetrahydrofolate[30,31]. It has been shown that doses of FA in physiological quantities can 'saturate' this conversion mechanism, resulting in measurable levels of circulating FA, which may be detrimental[31].

Mandatory fortification has resulted in increased exposure to circulating FA[17], and it has been observed that increased concentrations of plasma FA in elderly women who took FA-containing supplements (>400 μg) were inversely associated with decreases in the cytotoxicity of circulating natural killer cells[32]. Although the biochemical and physiological consequences of overconsumption remain unclear, there remains concern over the adverse effects of high levels of FA[24,33]. Previous studies have indicated that it is permissible for manufacturers to fortify at higher levels than the mandated amount, and Shakur *et al.* have estimated that the amount of FA in Canada's fortified foods may be as high as 50 % more than the quantity expected based on the government-authorised levels[34–36]. Thus, it is critical not only to observe the dietary intake of folate but also to measure erythrocyte folate concentrations to obtain a clearer picture of the folate status of the Canadian population in a post-fortification era. The aim of this study was to determine whether there are groups at risk for toxicity or deficiency of this vitamin. In addition, we assessed the intake of supplement users separately from non-supplement users, as this particular group could be at an increased risk of FA overconsumption as well as elevated levels of erythrocyte folate[37,38].

Methods

Data source

This study used data from the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2) 2004, as well as data from the Canadian Health Measures Survey, Cycles 1, 2 and 3 (CHMS). In total, the CCHS 2.2 surveyed 35 107 respondents, each of whom completed a general health questionnaire as well as a 24-h dietary recall, administered by trained interviewers. A subsample of 10 786 respondents completed a second 24-h dietary recall, 3–10 d later[39]. This study utilised the CCHS 2.2 Share File, restricting the analysis to the 95·3 % respondents who agreed to share their information for research purposes[39,40]. Further details on the CCHS 2.2 survey methodology and sampling design can be found elsewhere[40]. In addition to demographic details, the general health component of the CCHS 2.2 captured information on vitamin and mineral supplement use during the 30 d before the interview[41]. Exclusion criteria for this study included women who were pregnant or breast-feeding at the time of the survey, those who had an invalid or missing dietary recall, those who reported consuming only breast milk and those who did not report any food items consumed during the 24-h recall period, resulting in a final sample size of *n* 32 776. CCHS 2.2 data were used for folate intake analysis.

The CHMS is a comprehensive direct health measures survey, which includes blood, urine and anthropometric measures and banks specimens for future measurements and genetic research. The CHMS has collected data in three cycles (2007–2009, 2009–2011 and 2012–2013) with a minimum of 500 respondents for each sex from 10 age groups of Canadians – 6–79 years (Cycle 1) and 3–79 years (Cycles 2 and 3). Cycles 1, 2 and 3 surveyed 5604, 6395 and 5785 respondents, respectively. The CHMS also captured the intake of vitamin and mineral supplements 30 d before the clinic visit[42,43]. CHMS data were used for the frequency of supplement use and erythrocyte folate level estimates.

Folate intake estimates

In order to determine folate consumption habits of Canadians, folate intake was categorised as follows: naturally occurring folate from foods (food folate) and the synthetic form from supplements or from fortification (FA). The dietary folate equivalents (DFE) for synthetic FA sources were calculated, a method introduced in 1998 by the FNB and IOM to take into account the higher bioavailability of synthetic FA compared with natural folate[44]. The DFE for specific foods was calculated as the amount of food folate (μg) plus 1·7 times the amount of synthetic FA (μg) from the 24-h dietary recall, thus taking into account both natural folate and synthetic FA. As supplements taken in the CCHS 2.2 were assumed to have been consumed on an empty stomach, the aforementioned calculation was modified for supplemental FA, resulting in a conversion factor of 2. The DFE values for natural folate and FA from food fortification were also kept as separate values to estimate intake from these different sources.

Folate intakes were calculated for separate dietary reference intakes (DRI) life-stage groups by FA supplement use (total folate intake from all sources was also calculated). In addition,

the amount of folate from fortified sources was re-calculated with an 'overage' factor, defined as the 'potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage'[36]. For key food groups (breads, baked goods, etc.), the amount of folate in the product, plus overage, was calculated by multiplying initial amounts by an adjustment factor based on the food group category[36]. Folate intakes for the three sources and for all the sources, combined, were then compared with the estimated average requirement (EAR) for DRI life-stage groups, which is the amount of a nutrient expected to meet the needs of 50 % of the population, and the UL[15,16]. Nutrient and food intake data were analysed using SAS software, version 9.1 (SAS Institute Inc.). Version 1.11 of the Software for Intake Distribution Estimation (SIDE)-IML program was used in conjunction with SAS to generate an estimation of the usual dietary intake by using both the first and the second dietary recall[45,46] along with bootstrapping weights to estimate variance.

Blood folate estimates

A total of three cycles of CHMS data were pooled to provide a larger data set for statistical evaluation of erythrocyte folate analysis. Biomarker analysis is described briefly in this study and in detail elsewhere[47]. Blood samples for erythrocyte folate analysis were collected by a certified phlebotomist. Erythrocyte folate content was measured using the Immulite 2000 assay (Siemens Healthineers)[42,43]. Participants who did not give blood or had unusable samples were excluded from the analysis, as were respondents under 6 years of age, to ensure compatibility between cycles (only Cycles 2 and 3 had participants aged 3 years and above), resulting in a final sample size of 15 754. SPSS, version 22, and STATA 13 software were used to generate an estimation of Canadians with elevated erythrocyte folate concentrations along with bootstrapping weights to estimate variance, by life-stage group as well as by supplement use. Erythrocyte folate status was assessed using cut-off values for elevated blood levels that have been proposed in the literature.

Recently, it has been suggested that erythrocyte folate concentrations differ depending on the assay used, depending on certain variations or calibrations[48]. Using a sample of 152 individuals and two different assays (Immulite 2000 and the microbiological assay), Colapinto *et al.* used the Deming regression method to create the following equation:

$$\text{Predicted microbiological assay concentration} = -22{\cdot}95 \times (0{\cdot}81) \times \text{Immulite 2000 assay concentrations.}$$

Given that results from differing assay methods can be large, and that the microbiological assay is deemed the gold standard for determining erythrocyte folate concentrations, it seemed prudent to utilise the adjustment method proposed by Colapinto to convert Immulite 2000 assay results to those on par with the microbiological assay used in the National Health and Nutrition Examination Survey (NHANES) or 'NHANES level'. However, the results must be interpreted with caution, as converting CHMS Immulite 2000 assay to microbiological assay values lowers erythrocyte folate concentrations, and thus may under-represent the number of Canadians who have elevated levels of erythrocyte folate.

Results

Overall dietary folate equivalent intake

The average DFE intake for Canadians, excluding supplements, was 442 µg. When overage factors were added to the total DFE, intake increased to 487 µg. The highest intakes were seen among males aged 14–50 years, ranging from 521 to 576 µg (Table 1). Among all life-stage groups, average DFE intakes surpassed the EAR values, irrespective of the calculation method. When DFE intake was calculated with the addition of FA from dietary supplements, the overall intake was significantly higher for the supplement user group ($P < 0{\cdot}001$) compared with non-supplement users by a difference of approximately 900 µg. Intake was also significantly higher among supplement users in all age/sex groups when compared with non-supplement users in the same group (Table 1). Overall, non-supplement using WCBA had mean DFE intakes of 417 (SE 113) µg, whereas women who consumed supplements had an intake of 1474 (SE 501) µg.

Supplemental folate use

Both CCHS 2.2 and CHMS data showed comparable proportions of Canadians who take FA-containing supplements (Table 2). Overall, the CCHS reported that 25 % of Canadians consumed a supplement containing FA, ranging by age group from 13 to 38 %. Slightly lower results were observed by age group using the combined CHMS cycles (22 %). The highest user rate defined by the CCHS was found in children aged 4–8 years (38 %), and the lowest rate of supplement use occurred among males aged 14–18 years (13 %). The CHMS also found high rates among children <8 years (29 %) as well as in females >70 years (38 %). In all, 23 % of males and 28 % of females reported use of a supplement containing FA in the CCHS and 19 % and 25 % reported supplement use in the CHMS, respectively. With the exception of males aged 9–13 years, females in every other age group had higher rates of FA supplement use according to CCHS 2.2 data. Similar patterns were found using CHMS pooled cycles, with females reporting higher supplement use in every age group with the exception of those under 8 years. Females >31 years reported higher supplement use proportion than the national average. CHMS data observed the same trend (data not shown). Overall, comparable proportions were reported by WCBA (24 % in the CCHS and almost 23 % in the CHMS) and in the general population (26 % in the CCHS and 22 % in the CHMS).

Assessment of the prevalence of folate inadequacy: dietary folate

Folate inadequacy among Canadians was calculated by measuring the proportion of individuals in each DRI life-stage group who did not meet EAR criteria for folate (Fig. 1(a)). Overall, the prevalence of folate inadequacy (POFI) was low (<6 %) among female respondents <13 years and males <50 years, regardless of the calculation method or supplement use status.

Table 1. Folate intake of Canadians by source and supplement use based on results from the Canadian Community Health Survey, cycle 2.2 (Mean values with their standard errors)

	Natural folate		DFE		DFE + overage‡		Folic acid		Folic acid + overage‡	
Life-stage groups	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Overall (*n* 32 776)										
1–3 years (*n* 2117)	144	59	280	103	320	117	114	76	137	83
4–8 years (*n* 3235)	173	59	381	105	444	114	162	72	196	79
Male: 9–13 years (*n* 2080)	213	69	466	123	541	136	182	81	222	90
Female: 9–13 years (*n* 1980)	181	58	403	108	470	119	161	81	196	88
Male: 14–18 years (*n* 2288)	255	98	563	182	651	208	231	131	275	144
Female: 14–18 years (*n* 2256)	198	77	432	150	495	177	198	136	231	148
Male: 19–30 years (*n* 1804)	272	101	576	171	653	189	259	170	298	181
Female: 19–30 years (*n* 1854)	210	82	410	124	456	133	232	216	254	219
Male: 31–50 years (*n* 2596)	262	90	521	155	588	173	241	197	279	209
Female: 31–50 years (*n* 2686)	228	99	406	137	446	147	279	286	300	285
Male: 51–70 years (*n* 2550)	263	109	463	155	515	169	279	276	305	281
Female: 51–70 years (*n* 3200)	223	82	381	113	418	125	281	329	300	329
Male: 71 years and over (*n* 1520)	224	87	394	123	447	139	267	305	292	309
Female: 71 years and over (*n* 2610)	195	75	327	102	363	110	268	351	286	352
Overall (*n* 32 776)	230	96	442	160	487	181	217	215	274	238
Supplement users (*n* 8390)§										
1–3 years (*n* 603, 28 %)	163	61	523***	168	566***	169	191***	73	215***	74
4–8 years (*n* 1243, 38 %)	171	54	580***	162	643***	170	221***	73	254***	79
Male: 9–13 years (*n* 462, 22 %)	218	64	728***	256	810***	264	275***	120	317***	128
Female: 9–13 years (*n* 425, 22 %)	196	54	694***	302	767***	308	271***	146	308***	155
Male: 14–18 years (*n* 293, 15 %)	266	91	1227***	586	1318***	603	502***	272	548***	282
Female: 14–18 years (*n* 340, 13 %)	223	69	1178***	638	1249***	640	501***	301	537***	301
Male: 19–30 years (*n* 313, 15 %)	290	112	1497***	484	1585***	519	632***	231	678***	250
Female: 19–30 years (*n* 452, 24 %)	213	70	1328***	652	1370***	645	578***	326	599***	327
Male: 31–50 years (*n* 541, 21 %)	263	81	1451***	652	1517***	667	616***	337	652***	344
Female: 31–50 years (*n* 812, 30 %)	220	82	1506***	814	1533***	805	659***	408	675***	404
Male: 51–70 years (*n* 631, 25 %)	287	113	1654***	767	1702***	762	702***	372	727***	370
Female: 51–70 years (*n* 1065, 33 %)	238	86	1589***	1014	1623***	1002	299·5	329·4	688***	506
Male: 71 years and over (*n* 372, 25 %)	268	98	1644***	885	1703***	886	291·5	308·7	704***	432
Female: 71 years and over (*n* 838, 32 %)	214	85	1560***	1042	1595***	1035	286·3	351·5	685***	517
Overall (*n* 8390, 26 %)	235	95	1344***	824	1395***	819	274·3	238·1	546***	395
Non-supplement users (*n* 24 386)										
1–3 years (*n* 1514)	136	58	269	111	307	127	78	42	100	53
4–8 years (*n* 1992)	173	58	387	102	451	114	126	37	160	48
Male: 9–13 years (*n* 1618)	212	70	470	127	543	138	152	46	191	56
Female: 9–13 years (*n* 1555)	176	59	396	111	461	122	129	35	164	40
Male: 14–18 years (*n* 1995)	252	98	565	180	653	205	186	59	231	75
Female: 14–18 years (*n* 1916)	193	77	426	146	487	171	138	51	171	67
Male: 19–30 years (*n* 1491)	269	98	568	159	644	165	177	55	215	65
Female: 19–30 years (*n* 1402)	209	83	417	136	465	147	122	50	148	64
Male: 31–50 years (*n* 2055)	262	88	527	163	594	180	155	63	189	76
Female: 31–50 years (*n* 1874)	231	100	419	138	464	142	111	37	135	44
Male: 51–70 years (*n* 1919)	253	101	445	139	498	151	114	46	140	58
Female: 51–70 years (*n* 2135)	215	77	378	103	418	116	94	26	116	40
Male: 71 years and over (*n* 1148)	208	79	371	116	418	131	97	42	122	53
Female: 71 years and over (*n* 1772)	185	68	317	97	352	107	78	37	97	46
Overall (*n* 24 386)	228	95	442	168	503	182	112	55	600	399

DFE, dietary folate equivalent.
*** $P < 0{\cdot}001$ compared with non-supplement users.
‡ Overage is defined as the potential extra amount of folic acid added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage.
§ Percentages in brackets reflect the proportion of supplement users in each age group.

Table 2. Proportion of folic acid supplement users* from the Canadian Community Health Survey, cycle 2.2 (CCHS 2.2), and the Canadian Health Measures Survey (CHMS)

	CCHS 2.2 (%)		CHMS combined cycles (%)	
Life-stage groups	Males (*n* 2612)	Females (*n* 3932)	Males (*n* 1556)	Females (*n* 1978)
1–3 years	28·4		–	
4–8 years	38·4		28·5	
9–13 years	22·2	21·5	17·5	20·1
14–18 years	12·8	15·1	11·5	14·5
19–30 years	17·4	24·4	13·1	18·6
31–50 years	20·8	30·2	18·4	27·0
51–70 years	24·7	33·3	22·8	28·7
71 years and over	24·5	32·1	26·0	38·1
Overall	24·7		22·1	

* Reported vitamin or supplements use in the 30 d before interview.

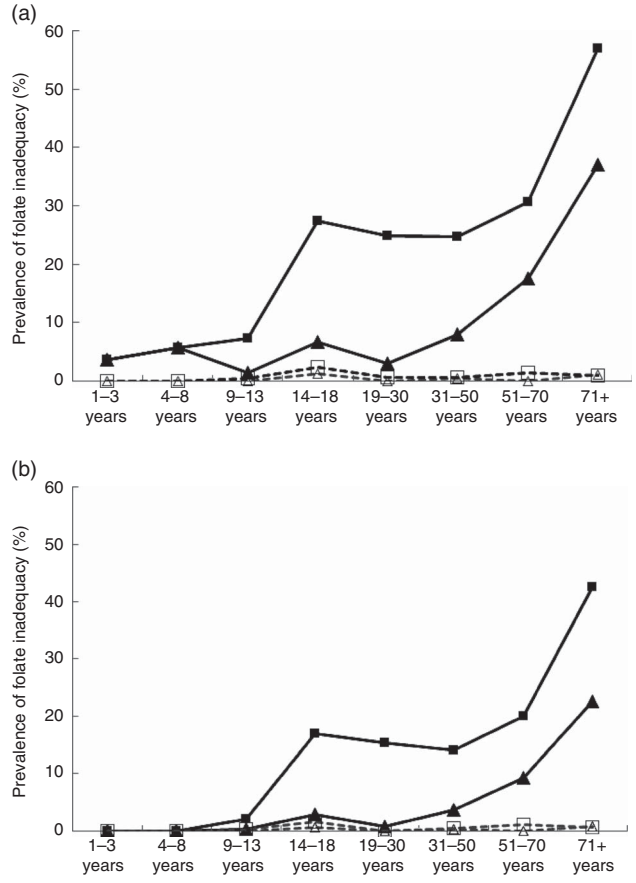

Fig. 1. Prevalence of folate inadequacy by dietary reference intakes life-stage group based on intake levels below the estimated average requirement (EAR) by sex and folic acid (FA) supplement use based on general fortification (a) and based on general fortification plus overage (b). Overage is defined as the potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage. a: —▲—, Male non-supplement user (supp); —■—, female non-supp; --△--, male supp; --□--, female supp; b: —▲—, male non-supp + overage; —■—, female non-supp + overage; --△--, male supp + overage; --□--, female supp + overage. EAR values (μg): 1–3 years (120), 4–8 years (160), 9–13 years (250), 14–18 years (330), 19+ years (320) (source: Institute of Medicine).

Among supplement users, the POFI was also very low (<5 %) for all adolescent and adult DRI life-stage groups, regardless of calculation method. Among non-supplement users, however, the POFI increased with age and was highest among older adults >50 years, with the highest proportion occurring in females >70 years. When POFI was estimated using only folate from unfortified food sources, inadequacy levels were very high, ranging from 38 % to a staggering 94 % among females aged 14–18 years (results not shown). When estimated with overage factors, POFI was low among supplement users (<2 %), but rose to 43 % in older females who did not report supplement use. In addition, almost one-quarter (23 %) of older males aged 70 years and above had intakes below the EAR (Fig. 1(b)).

Assessment of the prevalence of folate inadequacy: erythrocyte folate

Results from the observed erythrocyte folate concentrations showed that folate deficiency among Canadians (erythrocytes folate <305 nmol/l) was virtually non-existent (<1 %), regardless of whether or not the conversion method was used. This level (305 nmol/l) has been deemed by the IOM to denote a deficiency state, based on its association with megaloblastic anaemia[49,50]. Among WCBA, approximately 23 % showed erythrocyte folate concentrations below those deemed 'optimal' for maximal NTD risk reduction (<906 nmol/l).

Assessment of the prevalence of folate overconsumption: dietary folate

The UL for folate established by the IOM are based on the DFE intake of synthetic forms of folate (i.e. FA). Intake estimates to be compared against the UL do not include natural sources of folate, because high intakes from food have not been linked to adverse health effects[15]. Among non-supplement users, intake above the UL was virtually non-existent, even with overage additions taken into account (Figs. 2(a) and (b)). Among supplement users, however, the percentage with FA intakes above the UL was highest in respondents >13 years of age, regardless of sex, and the prevalence rate of folate above UL reached 18 % among male supplement users aged 14–18 years when overage factors were considered.

Assessment of the prevalence of folate over-concentration: erythrocyte folate

High erythrocyte folate concentrations do exist among certain subgroups of the Canadian population (Fig. 3). Using the previously mentioned lower cut-off values determined by

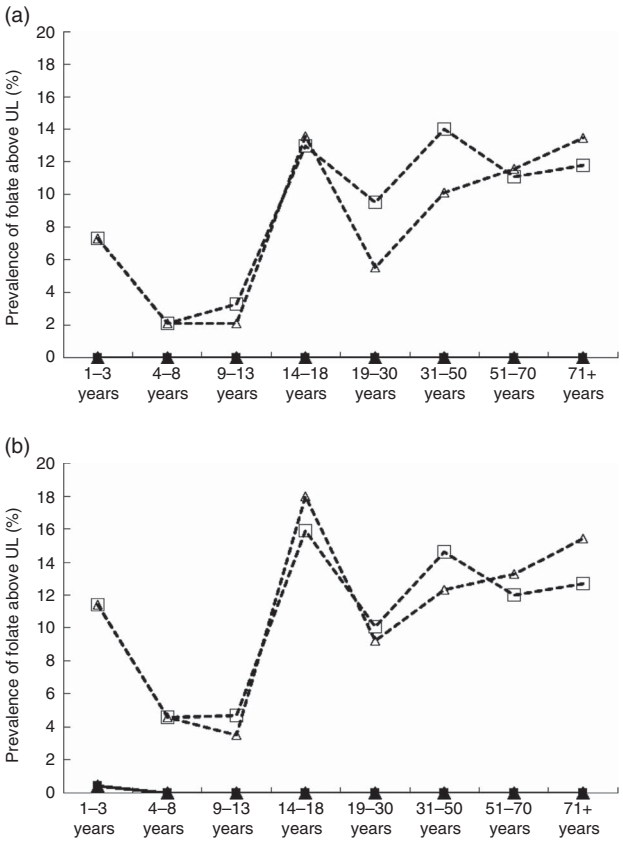

Fig. 2. Prevalence of folic acid overconsumption by dietary reference intakes life-stage group based on intake levels above the tolerable upper intake level (UL) by sex and folic acid (FA) supplement use based on general fortification (a) and based on general fortification plus overage (b). Overage is defined as the potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage. a: —▲—, Male non-supplement user (supp); --△--, male supp; --□--, female supp; —■—, female non-supp; b: —▲—, male non-supp + overage; --△--, male supp + overage; --□--, female supp + overage; —■—, female non-supp + overage. UL values (μg): 1–3 years (300), 4–8 years (400), 9–13 years (600), 14–18 years (800), 19+ years (1000) (source: Institute of Medicine).

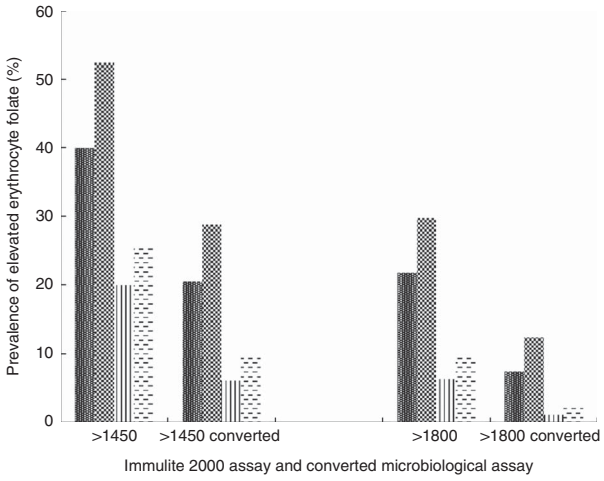

Fig. 3. Prevalence of elevated erythrocyte folate concentrations by sex and supplement use status based on proposed cut-offs, as well as by conversion factor. Converted microbiological assay concentration = −22·95 × (0·81) × Immulite 2000 assay concentrations[49]. ▩, Male supplement user (supp); ▩, female supp; ▥, male non-supp; ▥, non-female supp.

Colapinto *et al.*[49], in conjunction with their proposed conversion method, almost 30 % of female supplement users and 20 % of male supplement users had blood folate concentrations >1450 nmol/l compared with 10 and 6 % of female and male non-supplement users, respectively. Using the middle level cut-off, 12 and 7 % of female and male supplement users had blood folate concentrations >1800 nmol/l, compared with 2·5 and 1 % of non-supplement users of the corresponding sex. Using the highest cut-offs, 4 % of supplement using Canadians had blood folate levels >2150 nmol/l, compared with <1 % of their non-supplement using counterparts (results not shown).

Discussion

Folate inadequacy

The results of this study demonstrate that mandatory folate fortification has led to significant improvements in the overall intake of folate in the Canadian population, as the overall POFI (dietary and clinical) was found to be low. However, non-supplement users, particularly adults >70 years, may still be at risk for folate deficiency. Rates of POFI were comparable with previous studies by Bailey *et al.*[51] and Shakur *et al.*[34], with the highest POFI occurring among females >70 years, and may reflect the fact that older adults tend to consume less food overall. These results are similar to recent findings that showed up to one-third of elderly Irish men and women (>64 years) did not meet the requirements for optimal intake[52]. Overall, estimates of total DFE intake were comparable with those previously published by Shakur *et al.* using results from the CCHS 2.2[34]. Any small differences may be attributed to disparities in inclusion criteria as well as the fact that their study used the CCHS 2.2 Master File (*n* 35 107), whereas this study utilised the Share File (*n* 33 469), which is a slightly smaller subset of the Master file containing data on respondents willing to share responses with the survey Share partners[53]. Similarly, overall prevalence of erythrocyte folate concentrations <305 nmol/l (used to estimate deficiency) as well as that of WCBA were similar to the results by Colapinto *et al.*[47] using cycle 1 of the CHMS. The unique contribution of this study is that it utilised both the 24-h dietary recall data and the blood sample estimates to assess folate inadequacy. POFI based on 24-h dietary recall data were shown to be comparable with the estimates based on erythrocyte folate data. Results from both surveys showed 24·5 and 23 % of WCBA below the optimal level of folate for maximal NTD risk reduction, indirectly validating the 24-h recall method on estimating nutrient intake and status.

Supplement use and folate intake

Results from both the CCHS and the CHMS showed similar proportions of FA supplement users. Although the highest user rate appeared among respondents aged 4–8 years in the CCHS, the CHMS results showed that females >70 years had the highest reported rates of FA supplement use, followed by females aged 51–70 years and children under 8 years of age.

In our study, the prevalence of supplement use among children aged 4–8 years (38 %) was comparable with the 36 % supplement use prevalence observed in US children ≤13 years of age based on NHANES data[51], although slightly lower rates (29 %) were observed among children in the CHMS. This difference could be due to the smaller sample size of children in the CHMS. Our FA supplement use percentages also corresponded with previous CCHS 2.2 analyses[34].

CCHS supplement users were unlikely to be folate deficient. However, as demonstrated by our analysis, it is critical that supplement users should be observed separately from non-supplement users, as this particular group is at an increased risk for possible overconsumption[54]. Intakes of folate in non-supplement using adults in this study were higher than those generated from the NHANES 1999–2000 data (≥20 years, *n* 2121), perhaps due to demographic differences (such as including a younger age range of 2–18 years) or variations in food sources consumed by both populations[55]. Conversely, mean folate intakes from this study were somewhat lower than those previously reported in a study that used data from NHANES 2003–2006 to examine folate intake in Americans ≤13 years of age[7,51]. Paediatric intakes from this study are similar to intakes in Ontario pre-school children aged 3–5 years (*n* 254), whose average intake of DFE was calculated to be 336 μg[56]. Results from both surveys showed that <25 % of WCBA consumed FA-containing supplements, similar to the findings of Shuaibi *et al.*[14] who observed that only 26 % of females aged 18–25 years used supplements. However, although supplement use was found to ameliorate folate inadequacy, it should be noted that CCHS 2.2 data also provide evidence that certain subgroups are consuming levels of FA above the UL.

Folate consumption above the tolerable upper limit and elevated erythrocyte folate concentration

The pooled CHMS data show that certain Canadians have elevated erythrocyte folate concentrations. Rates of intake above the UL for FA reached 18 % in male supplement users aged 14–18 years (when potential overages were accounted for), and up to 25 % of supplement users have elevated blood folate levels. In light of a recent speculation that high levels of FA may be potentially linked to increased cancer risk and cognitive decline, this may be cause for concern. Although there are little data on the implications of FA toxicity among growing children and adolescents, some researchers have suggested consideration be given to removing FA from supplements designed for children and men[36].

Previous studies have suggested that FA intakes greater than the UL are considered safe and that care must be taken when interpreting risks of intakes above the UL[54], because the body of literature used to derive UL for most nutrients is limited[54,57]. However, recent observations from the Framingham study demonstrate that regular users of vitamin supplements have a mean concentration of unmetabolised FA in fasting plasma that is approximately 40 % higher than non-users[17]. Moreover, >80 % of regular vitamin users have detectable levels of unmetabolised FA in their plasma[17,32], which may decrease the cytotoxicity of lymphocytes thought to play a role in the destruction of neoplastic cells in older women[32] – a group that this study shows is consuming FA above the UL. This same population is also more susceptible to vitamin B_{12} deficiency[9] and potentially masking this problem with adequate FA intake[9,33,58]. A unique contribution of this study is the estimation and comparison of the prevalence of folate over-consumption and elevated folate status. The proportion of folate consumption above the UL based on dietary intake data was higher than the estimates based on elevated erythrocyte folate concentrations using the middle or higher cut-offs, but lower than the estimates using the lower cut-off. Nevertheless, both estimates (24-h dietary recall and erythrocyte folate concentration) revealed that higher-than-optimal folate intakes and elevated blood concentrations exist in the Canadian population.

The data presented in this report are subject to a few limitations. In the CCHS 2.2, only a subset of respondents provided second-day dietary intake data on which usual intake estimates were based, although the use of SIDE software aids in remedying this by using the subset of 2nd-d recalls to adjust variance estimates and generate stable usual intake estimates for groups. Although the use of overage factors have been based on a rather limited amount of fortified foods (*n* 92), the resulting overage percentages reported are comparable with those determined previously by other authors[34–36]. In addition, the CCHS 2.2 is a self-reported survey, and non-sampling errors such as non-response, recall bias and social desirability may affect the validity of results. Although the five-step multiple-pass method utilised during the 24-h recall has been shown to enhance accuracy and assist the respondent in remembering what and how much food they consumed[59], it has been reported that the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10 %, with a greater under-reporting rate among respondents who were overweight or obese, adults compared with teenagers and women compared with men[60]. Finally, a limitation of this study is that there does not exist a universal cut-off for elevated erythrocyte folate, and it must be noted that using higher cut-offs may discount individuals with potentially high folate statuses. Consequently, using slightly higher cut-offs will also provide information on those subgroups in which high folate status is most prevalent.

The reference range of erythrocyte folate varies by age, but generally falls between 317 and 1422 nmol/l[61]. Researchers have used quantiles of study populations to postulate cut-offs for erythrocyte folate[47,62,63]. MacFarlane *et al.*[62] proposed an erythrocyte folate cut-off of 1090 nmol/l based on data from Quinlivan & Gregory[63], who determined the relationship between dietary folate intake and erythrocyte folate to be 2·1 mg DFE/1090 nmol/l. This reflects a combined intake of 0·4 mg DFE (based on RDA) and a 1 mg FA supplement (bioavailability 1·7 mg). Using results from the CHMS 2007–2009, they observed that 63·5 % of Canadians had erythrocyte folate levels above the 1090 nmol/l cut-off[62]. However, a more stringent cut-off of 1360 nmol/l, which was based on the concentration at the 97th percentile of the NHANES 1988–2010 suggested by Colapinto, reduced this proportion to 40 %[47]. Pfeiffer *et al.*[64] suggested three cut-offs using NHANES data (erythrocyte folate

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concentrations obtained via a microbiological assay) using upper erythrocyte folate concentrations at the 90th, 95th and 97·5th percentiles of 1820, 2140 and 2490 nmol/l, respectively. Colapinto mimicked these percentiles using converted CHMS erythrocyte folate data, obtaining slightly lower cut-offs than Pfeiffer. For the purpose of practicality, this study uses the cut-offs established by Colapinto *et al.*[49], which were based on post-fortification NHANES data of 1450, 1800 and 2150 nmol/l that occurred at regular intervals (350 nmol/l apart) within this range of postulated cut-offs. In comparison with MacFarlane's results, using the conversion factor and more stringent high erythrocyte folate cut-offs decreased the proportion of Canadians with high erythrocyte folate status to 16·4 %[49]. It is evident that both fortified foods and FA supplements are increasing the erythrocyte folate status of Canadians. With this in mind, it is possible that a future erythrocyte folate cut-off based on these higher population intakes may be established. However, any cut-offs established in the future must be treated carefully and be paired with extensive research and long-term studies, particularly in light of recent data showing cognitive impairment associated with high plasma folate[18]. It will be necessary to strike a balance between establishing a cut-off that reflects the dietary changes of the population at hand, but also protects this same population from adverse health effects.

A strength of this project is that the POFI as well as toxicity was based on a combination of dietary, supplemental and clinical measures of status. The results of both survey data showed comparable results on folate intakes below EAR and above UL as well as those above the recommended cut-offs. Therefore, the results of this study are generalisable to other similar populations with a similar food supply environment.

Conclusions

Results of several studies leave little doubt that mandated folate fortification has led to significant improvements in the overall intake of folate in North America and to impressive decreases in the incidence of NTD. Ideally, one's diet should provide sufficient intake of folate, and data provide evidence that, although folate intake of Canadians has increased after fortification, consumption of folate from naturally occurring and fortified food sources may not be enough to achieve the desired levels of folate as recommended by the IOM, particularly for WCBA. In light of our results that 23 % of WCBA had erythrocyte folate concentrations below those deemed 'optimal' for maximal NTD risk reduction, it should be suggested that females aged 15–45 years who do not consume a FA-containing supplement on a daily basis should start to do so if a pregnancy is expected or planned.

Although previous studies have suggested that FA intakes above the UL are considered safe, a careful evaluation of the risks and benefits of food fortification, particularly above mandated levels, is necessary. Recent research shows cognitive impairment to be associated with high erythrocyte folate, and emerging animal studies highlight other adverse outcomes linked with excessive FA. In light of increasing evidence suggesting that certain subgroups of the population may be at risk by being subjected to high levels of FA, it seems prudent that persons of all ages who may be considering a FA-containing supplement should be cautioned about the potential risk involved with FA consumption above the UL. In particular, it may be suggested that vitamins targeted towards children, men, women past childbearing age and older adults not include FA. Future research and studies monitoring excessive folate intake are imperative in order to find a healthy balance to achieve optimal intake, and well-designed longitudinal studies are needed to draw definitive conclusions regarding the adverse effects of consuming excess FA. Consequently, it is important to universally define high blood folate status in order to better inform health professionals as well as the public about the benefits and risks of increased FA intake and elevated status.

Acknowledgements

The authors thank Dr Ian Clara and Kelly Cranswick at the Manitoba Research Data Centre for their statistical help and support for this project. The authors thank Dr Amanda MacFarlane at Nutrition Research Division, Health Canada, for her thorough review and constructive suggestions for this paper and Linda Greene-Finestone from the Public Health Agency of Canada for her assistance and suggestions.

Funding for this study was provided by the Public Health Agency of Canada.

All the authors were responsible for the study design and assisted in manuscript revision. M. d. G. and N. Y. were responsible for project conception. A. N. M. and N. Y. were responsible for data analyses and interpretation of the results. A. N. M., N. Y., H. M. A. and M. d. G. were responsible for drafting the manuscript and critical revision. The interpretation of results or points of view expressed in this paper are those of the authors and do not necessarily represent the opinions or position of the Public Health Agency of Canada.

The authors declare that there are no conflicts of interest.

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