Unmetabolized folic acid prevalence is widespread in the older Irish population despite the lack of a mandatory fortification program[1–3]

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ABSTRACT

Background: In 2006 the Food Safety Authority of Ireland recommended mandatory folic acid fortification of flour for the prevention of neural tube defects in addition to the existing extensive voluntary folic acid fortification culture in place there. This recommendation is now suspended until further scientific evidence surrounding safety becomes available. The safety issues include concerns about the masking of vitamin B-12 deficiency and potential cancer acceleration, both of which may be of concern for the elderly population.

Objective: The aim of this study was to measure the basal (fasted) concentrations of unmetabolized folic acid in the plasma of an elderly population group exposed to this liberal voluntary fortification of foodstuffs in Ireland.

Design: We invited participants aged 60–86 y from the Lifeways Cross-Generation Cohort Study to participate in this project. After providing informed consent, the participants were invited to provide fasting blood samples and to complete a standard food-frequency questionnaire and a questionnaire on recent and habitual intakes of folic acid. Samples were assayed for total plasma folate, red blood cell folate, homocysteine, and unmetabolized folic acid.

Results: A total of 137 subjects with a mean age of 67.4 y were studied. Unmetabolized folic acid was detected in 94.1% of the cohort with a mean concentration of 0.39 nmol/L (range: 0.07–1.59 nmol/L), accounting for 1.3% of total plasma folate.

Conclusion: These results indicate unmetabolized folic acid in plasma in most of this elderly Irish cohort, even after an overnight fast. These results should be considered carefully by those legislating in this area. *Am J Clin Nutr* 2012;96:613–21.

INTRODUCTION

The discovery by the Medical Research Council (1) Group in 1991 that a large proportion (72% in that study) of neural tube defects (NTDs)[4] could be prevented by maternal periconceptional consumption of folic acid led to a major health promotion campaign in many Western countries aimed specifically at women, with the objective of getting them to consume preconceptionally a 400-μg folic acid supplement daily. This campaign largely failed in its objective, in part because more than half of all pregnancies are unplanned (2). This led in turn to some policymakers, including those in the United States and Canada, moving to a more generalized approach in the form of mandatory folic acid fortification. Other countries, such as Ireland and the United Kingdom, did not adopt this mandatory approach and a culture of widespread voluntary fortification emerged instead, with many food staples being fortified to varying degrees with folic acid. According to a recent report (3), ∼200 foodstuffs on Irish supermarket shelves are fortified. After 20 y, the risks and/or benefits associated with either of these strategies remains unclear, and the debate continues.

Traditionally, the safety issue of most concern related to the masking effect of folic acid on vitamin B-12 deficiency, such as in pernicious anemia—a particular problem for the elderly population (4). Untreated pernicious anemia can result in fatal neuropathy (5–12). One milligram is now established as the tolerable upper intake level for daily folic acid intakes (13, 14). Newer safety concerns, related to cancer acceleration of the colon (15) and prostate (16), have arisen as a result of a randomized controlled trial conducted in 2007. In light of these concerns, mandatory folic acid fortification previously recommended for Ireland and the United Kingdom is now suspended until more definitive scientific evidence becomes available (17–19). Other studies, conversely, have found a protective effect of folic acid on colorectal cancer risk (20). Indeed, data from animal studies and clinical observation suggest that folate possesses dual modulatory effects, preventative and accelerant, on colorectal cancer, depending on the timing and doses of the intervention (21). Oral folic acid above certain threshold doses (∼200 μg) results in unmetabolized folic acid (UFA) in plasma

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[4] Abbreviations used: DHFR, dihydrofolate reductase; FFQ, food-frequency questionnaire; MTHFR, methylenetetrahydrofolate reductase; NTD, neural tube defect; SVUH, St Vincent's University Hospital; UFA, unmetabolized folic acid.

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(22), and several studies have shown its presence (23–26) The risks and benefits of this are unknown, but a recent study showed a relation between UFA and lower cognitive test scores in American seniors (27). No data exist for older Irish people. It has been speculated (28) that the magnitude of response to folic acid consumption in terms of how much folic acid appears in the circulation after consumption of dietary folic acid may be related to genes involved in folate metabolism, specifically dihydrofolate reductase (DHFR). The primary aim of this study was to measure the persistent (fasting) concentrations of UFA in a healthy, noninstitutionalized elderly population group exposed to liberal voluntary fortification in Ireland. In addition, we examined whether genes involved in folate metabolism are associated with UFA concentrations in plasma.

SUBJECTS AND METHODS

Recruitment

After ethical approval was obtained, we invited participants aged between 60 and 86 y from the Lifeways Cross-Generation Cohort Study to take part. The Lifeways Study was funded by the Health Research Board, and details on recruitment were reported previously (29, 30). The oldest cohort of grandparents was selected because they were most likely to be in the relevant age range. We contacted 254 potential participants from the greater Dublin area who had previously had a dietary assessment at recruitment and invited participation by letter initially, which was followed up by a phone call. Of these, 137 agreed to partake (54% response rate). An appointment was set up for the subjects to undergo early-morning blood sampling in the phlebotomy unit of the St Vincent's University Hospital (SVUH). It was explained to all subjects by telephone that they should abstain from all food, drinks, and vitamin supplements from at least 2400h before the blood sampling. Regular supplement users were not asked to refrain from supplements other than the night before the blood sampling. Ethical approval was obtained from the research ethics committee of our host university (University College Dublin) and SVUH, Dublin, and was in accordance with the Helsinki Declaration of 1975 as revised in 1983.

The research interview

Participants provided an early-morning fasting blood sample, collected by the trained phlebotomy staff employed at SVUH. A researcher was present at the interview and initially ascertained that the subjects had not consumed any food, drink, or supplements since 2400h. After the samples had been taken and the 2 dietary questionnaires (*see* below) completed, participants proceeded to the restaurant in the hospital where they received a complimentary breakfast and a taxi journey home that had been arranged for them.

Dietary questionnaires

Two dietary intake questionnaires were administered face to face with the participants. The first was a semiquantitative 149 item food-frequency questionnaire (FFQ), derived from the internationally validated instrument developed in the Harvard School of Public Health, which was adapted and validated for an Irish population. It has been used in all the National Surveys of Lifestyle, Attitudes and Nutrition in Ireland since 1998 (31, 32) and also has been used in the Lifeways Cross-Generation Cohort Study since 2001. This survey records average intake over the previous 7 d and hence is a measure of habitual (long-term) dietary intake data from all food sources.

The second FFQ specifically captured synthetic folic acid intakes from all sources of fortified foods and from supplements. It was developed and used by the authors in a previous study and showed good correlation with plasma folic acid measurements (24). For the purposes of developing the latter questionnaire, the authors undertook a comprehensive identification of all sources of supplemental and fortified products containing folic acid in the Irish Market. This amounted to 349 foodstuffs and 309 supplements. A questionnaire that captured typical and recent intakes of these products was developed based on the main food groups containing folic acid, which are breads, milks, so-called energy bars/drinks, spreads, breakfast cereals, and multi- or single vitamin supplements. The frequency of consumption and amount and dose of folic acid and the product name were captured by the questionnaire, by framing the questions in the same way as the FFQ. This allowed us to estimate typical intakes of fortified and supplemental folic acid in a typical day as well as estimate their intakes in the previous 24-h period. This instrument was administered to all respondents assisted by the researcher.

Sampling and laboratory analysis

Whole blood was collected into 3 mL EDTA, light proof containers. Samples were immediately sent to the hematology laboratory, where they were centrifuged within 1 h of collection and then stored on ice while they were transported to the Vitamin Research Laboratory at the Trinity Medical Centre. They were then portioned into aliquots and stored frozen at -80°C. Plasma was separated from the whole blood by centrifugation at $1251 \times g$ for 12 min. Samples were separated into 1.5-mL tubes and stored at -80°C until batch analysis. Plasma folate and red blood cell folate analyses were conducted by using the *Lactobacillus casei* microbiological assay described by Molloy and Scott (33). UFA was measured by a column-switching HPLC method by using fluorescence detection after postcolumn coulometric oxidation (24, 34). The lower limit of detection of the assay is $\sim$0.07 nmol/L. Vitamin B-12 analysis was by microbiological assay (35), and homocysteine analysis was performed by using a commercially available kit supplied by Abbott Diagnostics based on fluorescence polarization immunoassay (36). Genotyping of the *MTHFR* (methylenetetrahydrofolate reductase) 677C>T polymorphism (rs1801133) and the MTHFD1L (mitochondrial 10-formyltetrahydrofolate synthetase) deletion insertion polymorphism [c.781–6823 ATT (7–9); rs3832406] was carried out by using Hybprobes and melting curve analysis on the Roche Lightcycler 480 instrument (primers and conditions are available on request). The DHFR intron A 19-bp deletion insertion polymorphism was genotyped as previously described (37).

Statistical methods

Throughout the analysis, the terms *folate* and *folic acid* are used. *Folate* refers only to the natural form of the vitamin. *Folic*

acid refers only to the synthetically produced folic acid added to foodstuffs (ie, fortified foods) or folic acid in the form of supplements. The terms are not used interchangeably.

Folic acid intake from fortified foods and from vitamin supplements was combined to give a total habitual folic acid intake and total recent folic acid intake. The lowest level of detection of UFA was 0.07 nmol/L, and any values below this were taken as zero.

Because a proportion of subjects had no folic acid intake (from vitamins or fortified food), there were many zeros in these data, and dealing with them as continuous variables would have violated the assumptions of parametric analyses. To overcome this, we categorized (total) habitual and recent folic acid intake to create two 5-category variables. The first category was no folic acid intake, and the remaining 4 categories were defined by the quartile cutoffs in the nonzero data.

In addition, all of the continuous variables showed a marked positive skew and when analyzed as continuous variables were each log transformed (base 10). To avoid taking the log of zero, any zeros were replaced by a value equal to half the smallest observation for the relevant variable. Although all analyses with continuous data used logged values, for simplicity we present means and medians rather than geometric means. Groups were compared by using the independent *t* test or a 1-factor ANOVA as appropriate.

Interrelations between the log-transformed variables were examined with scatter plots and Pearson product-moment correlations (excluding pairwise subjects with zero values of folic acid intake). Multiple regressions were performed with logUFA as the dependent variable and continuous variables or dummy indicator variables as predictors. A 2-sided level of statistical significance at $P < 0.05$ was taken throughout, and 95% CIs are presented. PASW Statistics 18 was used for data analysis (release 18.0.3; IBM Inc).

RESULTS

A total of 137 subjects were studied with a mean age of 67.4 y (median: 66; SD: 5.9; range: 60–86 y), only 135 of whom completed the standard FFQ. The general dietary intakes of our cohort members were compared with reported intakes from comparably aged people in the 2007 national Survey of Lifestyle, Attitudes and Nutrition population study, which used the same standardized methods to assess dietary intake, and are shown in **Table 1** (31, 38). The table illustrates good comparability in

TABLE 1
Sociodemographic and nutritional profile of our study cohort compared with a national population profile (SLAN 2007 survey)[1]

	Study cohort		SLAN 2007[2]			
	Male (n = 54)	Female (n = 81)	Male	Male	Female	Female
Age (y)	60–81	60–81	45–64	≥65	45–64	≥65
Sociodemographic profile						
Age (y)	67.7 ± 5.4[3]	66.9 ± 5.4				
Current cigarette smokers (%)	16	26	23	17	27	13
Compliance with Food Pyramid shelves (no. of standard-portion servings/d)						
Top shelf, <3 servings/d (% complied)	9.3	9.9	14 (18- to >65-y age groups)			
Top shelf (% overconsumed)	90.7	90.1	86 (18- to >65-y age groups)			
Meats shelf, 2 servings/d (% complied)	44.4	53.1	39.7	46.3	45.0	47.0
Meats shelf (% overconsumed)	40.7	28.4	40.7	37.0	31.7	79.0
Dairy shelf, 3 servings/d (% complied)	9.3	7.4	18.7	20.0	19.7	16.7
Dairy shelf (% underconsumed)	88.9	87.7	65.0	65.0	70.0	72.7
Fruit and vegetable shelf, ≥5 servings/d (% complied)	53.7	70.4	58.7	54.0	74.0	65.3
Cereals shelf, ≥6 servings/d (% complied)	72.2	67.9	28.3	31.0	26.7	27.0
Nutrient intakes						
Energy (kcal)	2396 ± 908	2211 ± 743	2117	2057	2108	1930
Protein (g)	101 ± 40	92 ± 25	96[4]	93[4]	93[4]	87[4]
Protein (% of energy)	17	17	18	17	17	17
Fat (g)	96 ± 50	87 ± 38	84	82	79	73
Fat (% of energy)	36	35	35	34	35	33
Carbohydrate (g)	301 ± 113	282 ± 106	257	253	259	243
Carbohydrate (% of energy)	50	51	48	49	49	50
Fiber (g)	31 ± 13	30 ± 11	25	27	25	25
Folate (μg)	384 ± 117	351 ± 128	342	342	361	335
Vitamin B-12 (μg)	5.7 ± 4.4	4.3 ± 3.3	6.3	6.9	6.2	5.9
Vitamin C (mg)	135 ± 80	154 ± 92	147	132	182	146
Vitamin D (μg)	4.2 ± 2.5	3.8 ± 2.2	3.5	3.5	3.4	3.2
Iron (mg)	15 ± 5	14 ± 5	13	13	13	12
Calcium (mg)	669 ± 292	617 ± 228	904	892	870	823

[1] The top shelf refers to the top shelf of the Food Pyramid, a commonly used lay resource that provides guidelines on the amounts of each food group to be consumed daily. Source: Irish Nutrition and Dietetic Institute (http://www.indi.ie/index.php?page=32). SLAN, Surveys of Lifestyle, Attitudes and Nutrition.
[2] Morgan et al (2008) (38) and Harrington et al (2008) (31) SLAN 2007 Reports.
[3] Mean ± SD (all such values).
[4] Median values by SLAN 2007.

TABLE 2
Distrubution of folate, homocysteine, and vitamin B-12 status measurements in the study cohort[1]

	UFA	RCF	Plasma folate	Plasma Hcy	Vitamin B-12
	nmol/L	*nmol/L*	*nmol/L*	*μmol/L*	*nmol/L*
No. of valid observations	136	136	136	137	133
Mean	0.39	1423.1	29.8	12.6	267.4
Median	0.34	1254.6	22.9	11.5	243.2
SD	0.27	696.5	21.0	4.4	122.7
Percentiles					
25th	0.23	898.8	12.2	9.5	190.2
50th	0.34	1254.6	22.9	11.5	243.2
75th	0.53	1842.6	43.1	14.6	318.9

[1] Hcy, homocysteine; RCF, red blood cell folate; UFA, unmetabolized folic acid.

terms of macro- and micronutrient intakes, which suggests that our sample was reasonably typical in their dietary habits of the older Irish population. UFA had one missing value but was detected (limit of detection: 0.07 nmol/L) in all but 8 of the samples, giving an overall UFA prevalence of 94.1%. The mean concentration was 0.39 nmol/L (range: 0.07–1.59 nmol/L), accounting for 1.3% of total plasma folate. The distribution and the number of valid observations for the biochemical factors and the folic acid intakes examined are shown in **Tables 2** and **3**. One subject with a valid UFA value had a plasma folate concentration well outside normal values, and the value was considered missing. Only 19.0% of the sample used vitamin supplements with folic acid habitually, whereas only 8.0% had taken a supplement recently. Overall, 90.5% had some intake of folic acid habitually and 78.8% had a recent intake. Thirteen (9.6%) of the subjects reported no recent or habitual folic acid consumption.

When the correlations between the study factors were examined (logged values: **Table 4**), all correlations were positive except those with plasma homocysteine. UFA was highly significantly correlated with all factors ($P < 0.0005$) except vitamin B-12. Vitamin B-12 showed a very weak and nonsignificant relation with each of the other factors, except plasma folate and plasma homocysteine, for which a significant relation was obtained. Recent folic acid intake was strongly associated with habitual intake ($r = 0.81$, $P < 0.0005$), and the relation of each of these to the other factors was very similar. All of the relations with folic acid intake, except those with plasma homocysteine and vitamin B-12, were also significant ($P < 0.0005$). Of note, the correlation of each of the folic acid intake variables with red blood cell folate was higher than that with plasma folate.

In the 13 subjects with no recent or habitual folic acid intake, UFA values were significantly lower than in subjects who had some recent or habitual intake (mean ± SE: 0.11 ± 0.03 compared with 0.43 ± 0.02 nmol/L; $P < 0.0005$). How UFA was related to each of the sources of folic acid (fortified food or supplements) and whether it was consumed habitually or in the previous 24 h are shown in **Table 5**. Interpretation of this table is difficult because the habitual and recent folic acid intakes are so highly correlated and because of the small numbers taking supplements. Each of the folic acid–fortified food intake factors (habitual and recent) was significantly related to UFA, but neither of the supplement factors were.

Mean and median UFA values in the (total) recent and habitual folic acid intake categories, defined by quartile cutoffs, are shown in **Table 6**. A multiple regression analyses for these data with recent and habitual intake as predictors showed that habitual folic acid intake was significantly related to UFA ($P < 0.0005$) but that the effect of recent folic acid was not significant ($P = 0.108$).

The results of the genotyping analysis are shown in **Table 7**. No significant correlations with *MTHFR* 677C>T, DHFR 19-bp intron deletion, or MTHFD1L were found.

The relative contributions of the main voluntarily fortified foodstuffs and supplements to folic acid intake in this cohort are shown in **Figure 1**. Folic acid in single or multivitamin

TABLE 3
Distribution of folic acid intake in 137 subjects[1]

	Recent[2] folic acid supplement	Recent[2] folic acid food	Recent[2] folic acid total	Habitual folic acid supplement	Habitual folic acid food	Habitual folic acid total
	μg	*μg*	*μg*	*μg/d*	*μg/d*	*μg/d*
No. of zeros[3]	126	32	29	111	17	13
Mean	1081.8	176.5	281.8	653.0	177.2	308.4
Median	200.0	139.0	152.0	200.0	126.1	162.2
SD	1939.5	142.8	669.2	1346.6	153.8	654.4
Percentiles						
25th	100.0	72.0	76.0	39.1	67.6	75.3
50th	200.0	139.0	152.0	200.0	126.1	162.2
75th	400.0	242.0	263.5	425.0	250.6	332.0

[1] Statistics based on nonzero values only (no missing data).
[2] Recent intake refers to intake within the past 24 h but not in the previous 8 h.
[3] The zeros denote no folic acid intake from this source.

TABLE 4
Correlations between folic acid intake data (recent and habitual) and measurements of folate, homocysteine, and vitamin B-12 status (Pearson product moment)[1]

	UFA	RCF	Plasma folate	Plasma Hcy	Vitamin B-12	Recent[2] folic acid intake	Habitual folic acid intake
UFA	1	0.579[3]	0.559	−0.267	0.091[4]	0.443	0.517
RCF	0.579	1	0.645	−0.380	0.092[4]	0.466	0.473
Plasma folate	0.559	0.645	1	−0.388	0.254	0.360	0.378
Plasma Hcy	−0.267	−0.380	−0.388	1	−0.179[5]	−0.175[4]	−0.066[4]
Vitamin B-12	0.091	0.092	0.254	−0.179[5]	1	−0.009[4]	0.014[4]
Recent[2] folic acid intake	0.443	0.466	0.360	−0.175[4]	−0.009[4]	1	0.811
Habitual folic acid intake	0.517	0.473	0.378	−0.066[3]	0.014[4]	0.811	1

[1] Correlations are based on pairwise deletion. The folic acid intake variables exclude the zero values. All variables were log transformed to base 10. Hcy, homocysteine; RCF, red blood cell folate; UFA, unmetabolized folic acid.
[2] Recent intake refers to intake within the past 24 h but not in the previous 8 h.
[3] $P < 0.005$ for all correlations except those indicated.
[4] $P > 0.05$ (NS).
[5] $P < 0.05$.

forms contributes the most, followed by fortified milk, spreads (margarine), breads, cereals, and then multivitamin drinks.

A scatter plot of UFA compared with habitual folic acid intake on a logged scale is provided in **Figure 2**. The multiple zeros show the difficulty with a regression analysis on the full data. No threshold effect was seen. To determine a dose response between habitual folic acid intake and UFA, we performed a regression in those subjects who had nonzero habitual folic acid intake. The relation is as follows: log (UFA) = −1.167 + 0.327 (log daily habitual folic acid intake). From this it can be shown that a doubling of folic acid intake results in a 14.27% (95% CI: 9.7%, 18.8%) increase in UFA. This relation was expected but is quantified by this analysis.

Having established that habitual folic acid intake, rather than recent intake, is the important factor related to UFA, we performed a multiple regression with logUFA as a dependent variable and 2 variables indicating whether the source of habitual folic acid was fortified food and/or vitamin supplements. In this model, the supplement effect was not significant ($P = 0.501$), whereas that for fortified food was highly significant ($P < 0.0005$).

DISCUSSION

Both Ireland and the United Kingdom are examples of countries that have extensive voluntary fortification of foodstuffs with folic acid, including breakfast cereals, breads, milks, yogurts, energy bars, energy drinks, and dairy spreads. Our results in this analysis indicate the persistent presence of low concentrations of UFA in plasma in most (94.1%) of this elderly Irish cohort in the current context of voluntary fortification, even after an overnight fast, which is likely to reflect steady state or chronic concentrations. This corresponds with our previous work in fasted pregnant women, in whom 90% of those sampled had UFA in plasma and 98% of unfasted blood donors had UFA in plasma (24).

Our results show clearly that UFA is an issue that needs to be considered in this elderly Irish group. In terms of biochemical correlates of UFA in plasma, red blood cell folate concentrations were more strongly related than concentrations of plasma folate. Our regression analysis of dietary factors shows convincingly that, in these fasting subjects, concentrations of UFA are related to habitual folic acid intake rather than to intake within the previous 24 h. The strong correlation of UFA with red blood cell folate supports this view. There seems to be no threshold effect, and the amount of UFA increases with folic acid intake. We showed that a doubling of folic acid intake results in a 14% rise in UFA. UFA seems to be more driven by folic acid–fortified food intake rather than intake of supplements, but this could be attributed to the small number ($n = 13$) of people consuming supplements only in our cohort.

We do not know whether the presence of UFA in the circulation is actively harmful, but it is difficult to see how mandatory fortification on top of widespread voluntary fortification would confer any additional health benefits to this elderly population. A recent meta-analysis found no evidence that routine use of folic

TABLE 5
Mean and median UFA concentrations by source of folic acid in 136 subjects[1]

	UFA		
	Median	Mean ± SE	P^2
	nmol/L	*nmol/L*	
Recent[3] folic acid supplement			0.228
Yes ($n = 0.40$)	0.40	0.48 ± 0.09	
No ($n = 0.33$)	0.33	0.39 ± 0.02	
Recent[3] fortified food			<0.0005
Yes ($n = 0.39$)	0.39	0.45 ± 0.03	
No ($n = 0.18$)	0.18	0.22 ± 0.04	
Habitual folic acid supplement			0.549
Yes ($n = 0.39$)	0.39	0.42 ± 0.06	
No ($n = 0.33$)	0.33	0.39 ± 0.03	
Habitual fortified food			<0.0005
Yes ($n = 0.37$)	0.37	0.42 ± 0.02	
No ($n = 0.11$)	0.11	0.21 ± 0.07	

[1] UFA, unmetabolized folic acid.
[2] Reflects the comparison of means with an independent *t* test (134 df).
[3] Recent intake refers to intake within the past 24 h but not in the previous 8 h.

TABLE 6
Mean and median UFA concentrations by recent and habitual intakes of folic acid in 136 subjects[1]

	UFA		
	Median	Mean	P^2
	nmol/L	*nmol/L*	
Recent[3] folic acid intake[4] (μg)			<0.0005
None (n = 29)	0.18	0.19	
≤76.0 (n = 28)	0.29	0.33	
76.1–152.0 (n = 26)	0.32	0.35	
152.1–263.5 (n = 26)	0.49	0.51	
≥263.6 (n = 27)	0.55	0.62	
Habitual folic acid intake[4] (μg/d)			<0.005
None (n = 13)	0.08	0.11	
≤75.3 (n = 31)	0.26	0.30	
75.4–162.2 (n = 31)	0.29	0.32	
162.3–332.0 (n = 31)	0.42	0.47	
≥332.1 (n = 30)	0.55	0.61	

[1] UFA, unmetabolized folic acid.
[2] Reflects the comparison of means with 1-factor ANOVA (4, 131 df).
[3] Recent intake refers to intake within the past 24 h but not in the previous 8 h.
[4] Cutoffs based on quartiles (25th, 50th, and 75th percentiles) of non-zero values.

acid for 5 y has any material effect on cardiovascular or non-cardiovascular events in the North American and European populations studied (39).

Oral doses of folic acid produce a transient elevation of UFA in plasma, the extent of which is dependent on the dose. Even with doses as low as 0.4 mg, the baseline concentration of persistent plasma UFA may not be reestablished until after ≥8 h, especially with higher doses (SW Bailey and JE Ayling, unpublished observations, 2005). Therefore, measurement of the persistent concentration of UFA, as opposed to transient concentrations from recent intake of supplement or fortified foods, must be carried out by using subjects that are not only sufficiently food fasted, but remote from any intake of supplement. A few previous studies appear to meet this requirement and can thus be compared with the current results. Kalmbach et al (40) examined subjects from the Framingham Heart Study, both before and after the establishment of the US folic acid–fortification program. This study also further divided subjects into those who used supplements and those who did not. The median concentration of UFA in those not consuming supplements before US fortification (0.25 nmol/L) was similar to that in those in the current study who were not habitual consumers of fortified food or supplements (median: 0.08 nmol/L; mean: 0.12 nmol/L; n = 13 subjects). Conversely, habitual consumers of supplements by the current Irish subjects had mean and median UFA concentrations of 0.42 and 0.39 nmol/L, respectively. This is similar to the median UFA concentrations found in supplement users before fortification of 0.54 nmol/L by Kalmbach et al (40). Note that the highest concentration of UFA observed in any subject was 1.59 nmol/L, whereas Kalmbach et al reported values as high as 34 nmol/L. Such high UFA may have been a result of noncompliance with the fasting instructions. The median UFA concentration in the placebo group studied by Obeid et al (41) varied between 0.07 nmol/L (before treatment) and 0.17 nmol/L (after placebo treatment). These values are also consistent with those who were not habitual consumers of fortified food or supplements in the current study, because Germany does not have a fortification policy, although the intake of supplements or voluntarily fortified food was not reported.

The above values are in contrast with those found in serum samples from similarly aged participants in NHANES, in which a median of 1.2 nmol UFA/L was reported for all subjects (26). This value is higher than that reported by Kalmbach et al (40) for postfortification supplement consumers (0.68 nmol/L), despite the use of nominally the same assay method, and whose

TABLE 7
Relation between plasma unmetabolized folic acid and genes involved in folate metabolism inclusive of *MTHFR* 677C>T (rs1801133), DHFR 19-bp deletion, MTHFD1L DIP [C.781–6823 ATT (7–9); rs3832406][1]

Genotype	Median	Range	No. of individuals
MTHFR 677C>T (rs1801133)			
CC	0.32	0.06–1.59	61
TT	0.36	0.02–1.04	13
CT	0.34	0.05–1.40	62
DHFR 19-bp deletion[2]			
WT/WT	0.42	0.08–1.40	40
DEL/DEL	0.28	0.05–0.67	29
WT/DEL	0.32	0.02–1.59	67
MTHFD1L DIP [C.781–6823 ATT (7–9); rs3832406][3]			
1/1	0.40	0.02–1.59	55
1/2	0.32	0.05–0.86	39
1/3	0.24	0.05–0.90	26
2/2	0.54	0.25–0.95	6
2/3	0.37	0.08–0.67	7
3/3	0.26	0.25–0.33	3

[1] bp, base pair; DEL, deletion; DHFR, dihydrofolate reductase; DIL, deletion insertion polymorphism; MTHFD1L, mitochondrial 10-formyltetrahydrofolate synthetase; MTHFR, methylenetetrahydrofolate reductase; WT, wild type.
[2] DEL denotes an allele with a 19-bp deletion, and WT denotes an allele with no deletions.
[3] 1 denotes an allele with 7 ATT repeats, 2 denotes an allele with 8 ATT repeats, and 3 denotes an allele with 9 ATT repeats.

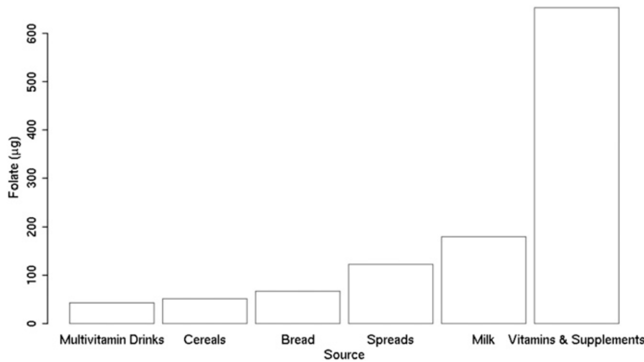

FIGURE 1. Contributions of the various commercially available folic acid–fortified foodstuffs (inclusive of multivitamin drinks, breakfast cereals, breads, spreads, and milks) and folic acid supplements to total daily folic acid intake in our study cohort.

subjects had folic acid intakes at least as high. The total daily habitual folic acid intakes (from food and supplements) by the subjects in the current study were similar to those in the NHANES group, although a smaller percentage of these were consumers of supplements (19% compared with 47%). Despite the higher median concentration, UFA was detected in only 38% of the NHANES subjects compared with 94% in the current study. If the limit of detection of the current study had been 0.18 nmol/L (as in NHANES) instead of 0.07 nmol/L, 81% of our subjects would have been considered to have detectable UFA. The reason for the higher median UFA values found in the NHANES subjects than by Kalmbach et al (40) is not clear. A greater genetic diversity inherent in the NHAHES group was suggested as a possibility (26), although the concentrations found in supplement users in the current study of an elderly Irish population are more consistent with those found by Kalmbach et al (40).

In the current study, and in the studies by Kalmbach et al (40) and Obeid et al (41), low concentrations of persistent UFA are found in many subjects supposedly not exposed to either fortified foods or supplements. Conceivably, this may be attributable to an inability to account for all intakes of folic acid. The current study and the other studies show that persistent UFA increased as a result of higher folic acid intake, but the question remains as to what would be observed in a population that has no access to folic acid–containing products.

Voluntary fortification in Ireland has escalated recently, with many food products containing folic acid (3). The evidence implies that folic acid intake in the general population, most likely from voluntarily fortified sources, has increased and it appears to have caused a reduction in NTD incidence (3). Whereas NTD incidence has decreased from 1.0 to 1.3 per 1000 pregnancies during 1997–2001 to an estimated 0.92 cases per 1000 pregnancies during 2005–2007, infants are still being born with NTDs in Ireland every year. There is a question as to whether these rates can be improved further without increasing the risk of harm to other nontargeted individuals exposed passively to excess folic acid. In the original Medical Research Council study of 1991 (1), which reported a 72% reduction in rates from periconceptional consumption of folic acid, it was speculated that the other 28% of NTDs may not have been responsive to folic acid. More recent work suggests that the percentage reduction depends on the rate before the intervention. Heseker et al (42) suggest that the rate of folic acid non-responsive cases may be 4 per 10,000 births or 7 per 10,000 births plus abortions. As a society it may be necessary to accept that NTD incidence can never be eliminated totally without causing some unintentional hazard to others. The rate of NTDs may always be relatively higher in Ireland than elsewhere because of the higher prevalence of the thermolabile (677TT) genotype (43), which is associated with reduced plasma and red blood cell folate status and also to the fact that termination of affected pregnancies remains illegal in that jurisdiction.

The question of how much folic acid can be added to the food chain to maximize the benefit for one group while minimizing the risks for others has yet to be answered. In the meantime, the culture of voluntary fortification that has flourished in Ireland should now be addressed more systematically, including consideration by food manufacturers to use stricter controls to fortification and more consideration also on the risks and benefits of adding folic acid to produce. In addition, it may be time to revisit ways in which to persuade women at risk of occurrence or recurrence of NTDs to take more folic acid periconceptually. A limitation to our study was the relatively small sample of participants. However, we selected respondents from an established and well-documented cohort that recruited family members through a nationally representative sample of pregnant mothers initially (29, 30). Although there was some evidence at recruitment of higher morbidity in the older cohort members, the general dietary habits of the sample in our study were comparable with those of the general Irish population; thus, it is therefore reasonable to extrapolate our findings on folic acid as also being more widely representative.

In conclusion, on the basis of our findings, most of the elderly population in Ireland appears to have persistent UFA in serum despite the lack of mandatory fortification. The implications of exposure to higher intakes of folic acid that would result from mandatory fortification are unclear, but these results merit careful consideration by policymakers in this area.

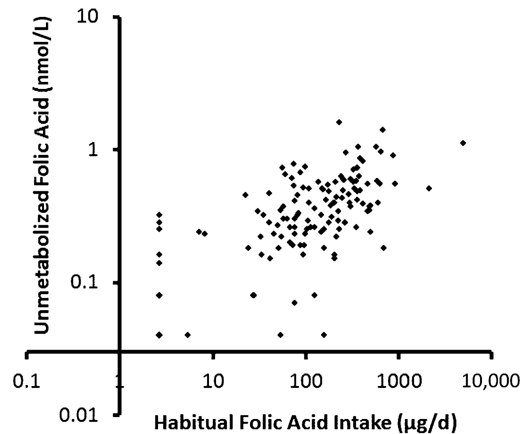

FIGURE 2. Scattergram of unmetabolized folic acid concentrations and habitual folic acid intake in the 136 subjects. Data are presented on a log scale, and the zero on the axis represents a small value to avoid taking the log of zero while showing the data. Only 7 data points are shown for a zero intake of habitual folic acid rather than 13 because some of the subjects had identical concentrations of unmetabolized folic acid.

The authors' responsibilities were as follows—AB: undertook the field work component of the study and performed the data entry; A Staines: provided support at the study design phase of the study; CCK (Director of the National Nutrition Surveillance Centre and Principal Investigator of the Lifeways Study): facilitated access to the elderly cohort via the Lifeways Study, provided the medical indemnity needed to undertake the sample collection at SVUH, and contributed to the data collection and the interpretation and writing of the manuscript; LD: provided statistical support throughout all stages of the study and performed the statistical analysis of the data; IS: facilitated the use of the phlebotomy services at SVUH and organized the centrifugation of the homocysteine samples within 1 h of collection and transport of the samples to the vitamin research laboratory; SWB and JEA: provided support at the study design phase and oversaw the analysis of the metabolized folic acid; PBA: performed the laboratory analysis of UFA; APM and AM: undertook the genotyping analysis; A Shrivastava (Health Research Board PhD fellow on Lifeways Study): undertook the food-frequency questionnaire analysis under the direction of CK; JMS: contributed significantly to the design of the study, and his vitamin research laboratory conducted the red blood cell folate, plasma folate, plasma vitamin B-12, and homocysteine analyses; and MRS (Principal Investigator): had the original idea for the study, designed the study, obtained the ethical approval at both centers, managed the fieldwork component, assisted with the data analysis and interpretation, and drafted the manuscript. All authors contributed to the writing of the manuscript and revised it for intellectual content. None of the authors declared a conflict of interest.

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