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Folate intake and folate serum levels in men and women from two European populations: The IMMIDIET project

George Pounis M.Sc. [a], Augusto F. Di Castelnuovo Ph.D. [a], Michel de Lorgeril M.D. [b], Vittorio Krogh M.D. [c], Alfonso Siani M.D. [d], Jozef Arnout M.D., Ph.D. [e], Francesco P. Cappuccio M.D. [f], Martien van Dongen Ph.D. [g], Bruno Zappacosta M.D. [h], Maria Benedetta Donati M.D., Ph.D. [a], Giovanni de Gaetano M.D., Ph.D. [a], Licia Iacoviello M.D., Ph.D. [a,*] on behalf of the European Collaborative Group of the IMMIDIET Project

[a] *Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Pozzilli (IS), Italy*
[b] *Université Joseph Fourier-CNRS, Faculté de Médecine, La Tronche, France*
[c] *Istituto Nazionale dei Tumori, Milan, Italy*
[d] *Institute of Food Sciences, CNR, Avellino, Italy*
[e] *Katholieke Universiteit Leuven, Flanders, Belgium*
[f] *Warwick Medical School, Coventry, United Kingdom*
[g] *Maastricht University, Maastricht, The Netherlands*
[h] *U.O.C. Laboratorio Analisi, Fondazione di Ricerca e Cura "Giovanni Paolo II," Università Cattolica del Sacro Cuore, Campobasso, Italy*

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ABSTRACT

Objective: Folate status has been associated with neural tube defects and cerebrovascular disease. The aim of this study was to evaluate possible differences in folate status in two European Union countries and to assess their possible association with dietary patterns and/or other lifestyles.
Methods: In the framework of the European Union-funded IMMIDIET Project, 1068 individuals (534 male–female pairs), ages 26 to 64 y, were enrolled in Italy and the United Kingdom. One-year-recall food frequency questionnaire was used to evaluate dietary intake. Reduced rank regression analysis was used to derive a dietary pattern better describing high dietary folate intake.
Results: Of the total participants, 11.3% of the Italians and 45.1% of the British exceeded the optimal dietary folate intake of 400 μg/d (Recommended Dietary Allowance). Of the women, 66.7% and 22.1% of Italian and British women, respectively, all at childbearing age, had folate serum levels <6.62 ng/mL ($P = 0.01$). The percentage of total variance of dietary folate intake explained by food group consumption was 14.2% and 16.3% in Italy and the United Kingdom, respectively. Reduced rank regression analysis indicated a healthy pattern that was positively associated with folate serum levels in both countries (for all β-coefficients >0; $P < 0.001$): 100 μg/d increase in dietary folate intake was associated with 13.8% and 10.5% increase in folate serum levels in the Italian and British population, respectively (for 100 μg/d increase $e^{\beta\text{-coef}} = 1.138$ and 1.105; $P < 0.001$). Smoking habit was negatively but physical activity positively associated with folate serum levels ($P < 0.05$).
Conclusions: An inadequate dietary folate intake and subsequent serum levels were observed in the Italian participants. High consumption of food sources of folate was positively associated with folate serum levels, explaining a good proportion of its variability.

*IMMIDIET Project Investigators are listed in the Appendix.

MdL, VK, AS, JA, FPC, and LI were responsible for the conception and design of the study. GP, AFDC, MdL, VK, AS, JA, FPC, MvD, and LI generated, collected, assembled, analysed, and/or interpreted the data: GP, AFDC, MdL, VK, AS, JA, FPC, MvD, MBD, GdG, and LI were involved in the drafting or revision of the manuscript. GP, AFDC, MdL, VK, AS, JA, FPC, MvD, MBD, GdG, and LI approved the final version of the manuscript.

* Corresponding author: Tel.: +39 086 592 9664; fax:. +39 0865 92575.
E-mail address: Licia.iacoviello@neuromed.it (L. Iacoviello).

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Introduction

Folate is a water-soluble B vitamin and one of the micronutrients included in the Mediterranean diet [1]. Reduced folate levels have been associated with cardiovascular disease [2–4], possibly through increases in homocysteine; however, trials aiming at reducing homocysteine with folate supplementation have shown overall negative results [5], except for a reduction in stroke prevention [6,7].

More consistent are the data on the association between low folate levels in pregnant women and neural tube defects (NTD) or other adverse birth outcomes [8]. The increase in serum folate with the diet or by supplementation drastically reduces the birth prevalence of NTDs. In the United States, Canada, Chile, and Costa Rica, which all made mandatory the fortification of flour between 1998 and 2000, the drop in NTD rates among live newborn babies was between 23% and 78% [9]. However, the fortification is still not mandatory in several European countries mainly due to a concern that folate fortification may harm people with undiagnosed vitamin B_{12} deficiency [10].

In Italy, food folate fortification is not mandatory and folate supplementation of women of childbearing age or health promotion strategies targeted at increasing intake of dietary sources are not major public health issues.

On the contrary, in England, public health promotion strategies force consumers to prefer fortified foods as a health protection choice. In 2007, the U.K. Food Standards Agency, considering the report of Scientific Advisory Committee on Nutrition [11], recommended the mandatory fortification of bread or flour with folate to reduce the risk for NTDs in fetuses. However, to date the health ministers have not yet made such a decision.

There are many foods containing folate; however, the relation between folate food intake and folate serum levels is weak. There is limited evidence as to which food sources would introduce the appropriate daily amount of folate to achieve the desired serum levels [12]. Additionally, it is important to better understand factors, other than diet, that might affect folate levels.

This study aims first at describing the present status of dietary folate intake and serum levels in a population of men and women from Italy and the United Kingdom, a southern and a northern European country, respectively, with no mandatory food fortification strategy for folate. Second, it aims at identifying food patterns that better describe a high folate intake in these two countries and at evaluating if and how such patterns are associated with folate serum levels.

Materials and methods

Study population

The IMMIDIET Project [13,14] and participant recruitment procedures were previously described. The IMMIDIET study is a population-based, cross-sectional study; apparently healthy pairs were male–female spouses or partners living together, recruited through local general practices. To protect against selection bias, the selection of eligible pairs was randomized in each center. Between October 2001 and October 2003, 271 pairs in the Abruzzo region in Italy and 263 in southwest London ages 26 to 64 y (mean ± SD, men 48 ± 7, women 45 ± 7) were randomly enrolled [13,14]. The participation rates were 85% in Italy and 90% in London. The ethical committees of all participating institutions using the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments approved the study. All study participants agreed to participate by written informed consent before their inclusion into the study.

Measurements

Interviews were taken using a standardized questionnaire previously adopted [15].

Participants were classified as non-smokers (if they had never smoked cigarettes), ex-smokers (if they had smoked cigarettes in the past), and current smokers if they were currently smoking one or more cigarettes per day on a regular daily basis. Physical activity rate was assessed by a standardized questionnaire [15]. Participants were grouped in two categories of physical activity ("low" or "high") according to the median rate of each population. Socioeconomic status (SES) was defined as a score (0–5) based on three variables: Education, job, and housing. The higher the score, the higher was the level of SES. Participants were grouped in two categories of SES ("low" or "high") according to the median of each population. Women were divided into groups of premenopausal and menopausal, according to self-report.

Body weight and height were measured on a standard beam balance scale with an attached ruler, in participants wearing no shoes and only light indoor clothing. Body mass index was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Blood samples were obtained between 0700 and 1000 h from participants who had been fasting overnight and had refrained from smoking for at least 6 h.

Folate serum levels were determined by a chemoluminescent assay at microparticles capture, Axsym (Abbott) (adequate levels, folate > 6.62 ng/mL) [16]. The assay sensitivity was lower than 0.8 ng/mL, interassay coefficient of variability <10%.

Dietary assessment

Either the validated Italian or the English European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaire (FFQ) [17] were used to evaluate dietary intake. A computer program, Nutrition Analysis of FFQ [18] was developed by the Epidemiology and Prevention Unit, Fondazione IRCCS, Istituto Nazionale dei Tumori, Milan to convert questionnaire dietary data into frequencies of consumption and average daily quantities of foods, energy, folate, and vitamin B_6 intake. Nutrition Analysis of FFQ was linked either to the McCance Food Composition Tables (FCT) for U.K. data [19], or to the Italian Food Composition Tables, for Italian data [20]. According to Recommended Dietary Allowance (RDA) for adults [21], dietary folate intake of 400 and 200 μg/d were considered as optimal and lowest recommended intake, correspondingly. From the 164 food items included in the EPIC-FFQ, food sources of folate were categorized in 15 major food groups as: "leafy vegetables," "broccoli & root vegetables," "tomato & other vegetables," "legumes," "citrus fruits," "other fruits," "fruit & vegetable juices," "dried fruits," "pasta rice & cereals," "potatoes & bread," "breakfast cereals," "nuts & seeds," "red meat & products," "white meat & egg," "fish," and "dairy." The use of vitamin supplements was evaluated in the U.K. population as a binary factor (yes or no). Data on their exact prescription was missing.

Statistical analysis

Normally distributed continuous variables are presented as mean ± SD, skewed as median (first, third quartiles) and categorical variables as frequencies. Comparisons of continuous variables between two groups of study were performed using the independent Student's t test, for the normally distributed variables and the Mann-Whitney test, for the skewed ones. Associations between categorical variables were tested using the Pearson's χ^2 test.

Reduced rank regression (RRR) was used to derive dietary patterns (for Italy and the United Kingdom) including major food sources of folate, better describing a high dietary folate intake. RRR extracts linear functions of predictors (named factors) that explain as much response variation as possible [22]. RRR produces as many factors as there are dependent variables, which in our case is only one. The correlations between each extracted factor and foods are called factor loadings; we characterized the factor using the foods with an absolute factor loading >0.20. Each participant received, for each pattern, a factor score (RRR score), calculated by summing the observed intake of the 15 food groups, each weighed by factor loadings [22]. An increase in this score represents increasing adherence to the factor-dietary pattern that better describes the high dietary folate intake.

Linear regression analysis was used separately for Italian and U.K. participants, to evaluate the association between dietary folate intake and food group consumption. Unadjusted models with main outcome dietary folate intake and independent factors each food group consumption were derived. Furthermore, to evaluate the percentage of the total variance of dietary folate intake that was explained by food group consumption, multiple regression models including all food items were performed. Partial R^2% conducted from these analyses indicated the aforementioned percentage. Using the same setting standardized β-coefficients was also produced to comparatively evaluate the effects of independent factors to the main outcome. They were reported as a percent of absolute values. The same regression analyses were performed to evaluate the association between dietary folate intake and other participant characteristics. Unadjusted and multiadjusted linear regression analyses separated by either country also were performed to evaluate possible associations of folate serum

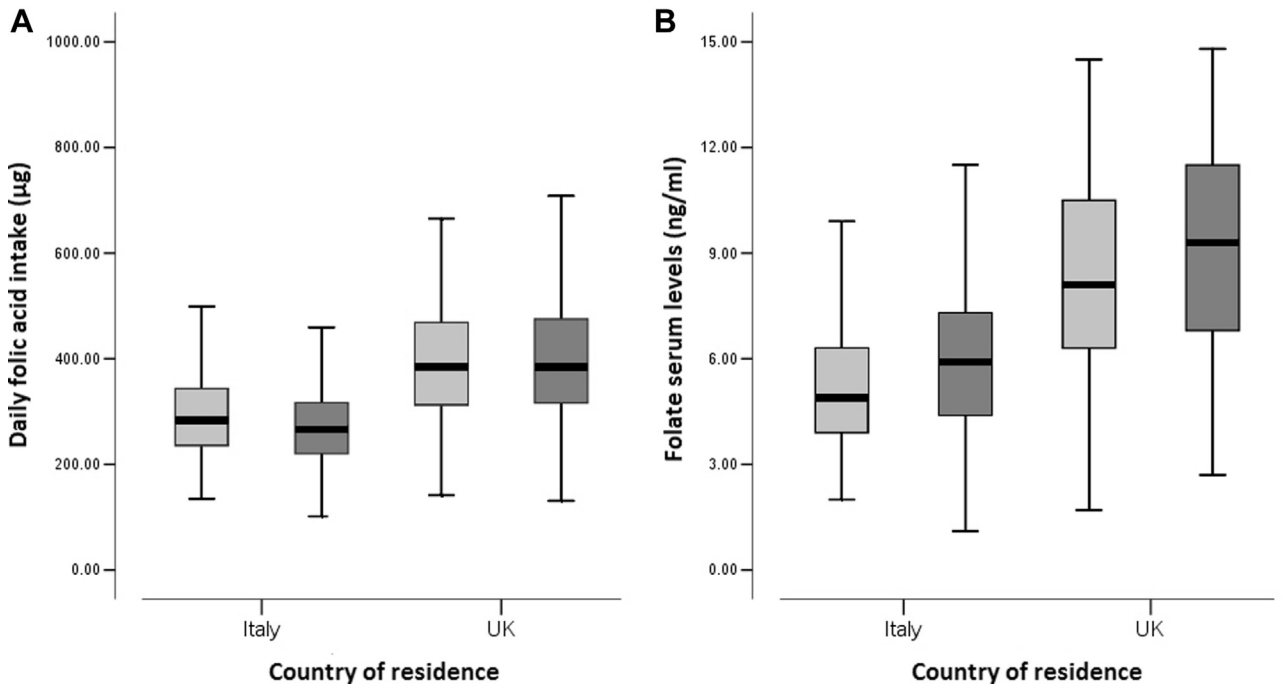

Fig. 1. Distribution of dietary folate intake and folate serum levels in men and women participants according to country of residence: (a) Italy, (b) United Kingdom.
Daily folate intake (μg) by country and sex: Median (first, third quartile)
Italy, men: 284 (235, 344); Italy, women: 266 (220, 317); U.K., men: 385 (312, 469); U.K. women: 384 (316, 476)
Folate serum levels (ng/mL) by country and sex: Median (first, third quartile)
Italy, men: 4.9 (3.9, 6.3); Italy, women: 5.9 (4.4, 7.3) U.K., men: 8.1 (6.3, 10.5); U.K. women: 9.3 (6.8, 11.5).

levels with food group consumption, dietary folate intake, and other characteristics. Because of the log-transformation of the dependent factor in each of the aforementioned cases, the one-unit increase in the independent factor caused the β-coefficient (β-coef) to be equal with the log ratio of the dependent situations after and before the one-unit increase. So the $e^{\beta\text{-coef}}$ should be equal to the ratio of the dependent situations after and before the one-unit increase in the independent factor; results were presented as $e^{\beta\text{-coef}}$, describing how many times 1 unit increase in the independent factor increased or decreased the dependent.

In all regression models normality of residuals, homoscedasticity, and multiple colinearity were evaluated by plotting standardized residuals against the predicted values. All tested hypotheses were two-sided. *P*-value < 0.05 was considered as statistically significant. STATA version 9 software was used for all calculations (STATA Corp., College Station, Texas, USA) except from RRR where SAS software (version 9.1.3 for Windows, Cary, NC, USA: SAS Institute Inc. 2000-2004) was used.

Results

Dietary intake of folate in Italy and United Kingdom

Figure 1A presents the distribution of dietary folate intake in men and women according to either country. Mean folate intake was higher in Italian men than women (β-coef = 29.4; $P < 0.001$), whereas no sex difference was observed in the United Kingdom group (β-coef = −7.6; $P = 0.49$). In both sexes, English people had greater dietary folate intake than Italians ($P < 0.001$ for country differences). The percentage of participants that reached the lowest recommended intake of 200 μg/d was 83% of Italian and 96% of English participants ($P < 0.001$). However, only 11.3% (15.1% of men and 7.4% of women; $P < 0.001$) of Italians exceeded the optimal recommended intake of 400 μg/d (RDA for adults) compared with 45.1% of U.K. participants (44.9% of men and 45.3% of women; $P = 0.93$; $P < 0.001$). Further analysis showed that in Italy, menopausal women had greater dietary folate intake (362 [273, 455] μg/d) than premenopausal women (298 [244, 378] μg/d), ($P < 0.001$), whereas no significant difference was observed in the United Kingdom ($P = 0.18$). The percentage of premenopausal women who reached the recommended levels of dietary folate intake (RDA, 400 μg/d) was only 8.1% in Italy but 41.4% in the United Kingdom ($P < 0.001$).

Figure 2 presents the partial R^2% calculated from multivariate analysis in Italian and U.K. participants. Total variance of dietary folate intake that was explained by food groups was 88.3% in the Italian group and 89.5% in the U.K. group. In Italians, potatoes and bread explained 61.5% of folate intake variance, followed by leafy vegetables, fruits and vegetables, juices, pasta and rice, and citrus fruit. In the British, at variance, broccoli and root vegetables explained more of the dietary intake folate variance (partial R^2% = 59.1) followed by breakfast cereals, potatoes and bread, dairy, and leafy vegetables.

In Italy, a factor-dietary pattern was derived that could explain 85.9% of the total variation of dietary folate intake and 15.7% of the total variation between food groups (Table 1). The "Italian dietary folate intake pattern" was described by high consumption of every different type of vegetable, legume, potato and bread, non-citrus fruits, and white meat. The results of RRR in the United Kingdom also revealed a factor-dietary pattern explaining 82% of the total variation of dietary folate intake and 14% of the total variation between food groups. The "U.K. dietary folate intake pattern" was described by high consumption of vegetables, non-citrus fruits, potatoes and bread, breakfast cereals, and dairy.

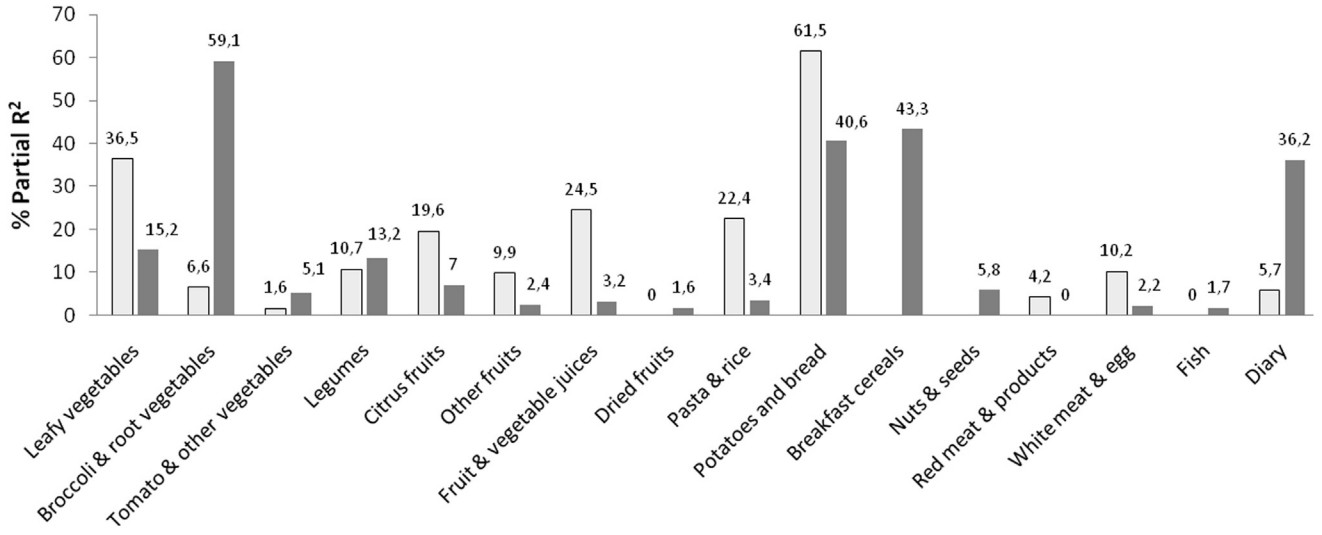

Fig. 2. Contribution of food group consumption to dietary folate intake in Italy and UK.

Folate serum levels in Italy and the United Kingdom

Figure 1B presents the distribution of folate levels in men and women according to either country. In both genders, British participants showed higher folate levels than Italians ($P < 0.001$ for country differences). This was also indicated by the percentage of participants who had a favorable folate status (serum levels > 6.62 ng/mL or 15 nmol/L) Italy 29% and United Kingdom 75.5% ($P < 0.001$). Menopausal women had higher folate levels (8.8 [6.3, 11.1] ng/mL) than premenopausal (6.7 (4.9, 9.2) ng/mL). Additionally, only 33.3% of Italian women of childbearing age had favorable folate status, whereas in 77.9% of the U.K. group did ($P < 0.001$).

Multivariate analyses showed that Italian participants who were classified in the higher quartile of folate levels had greater daily consumption of citrus fruits, leafy vegetables, and fish compared with those in the lowest quartile (Table 2A). On the contrary, red meat, legumes, and dairy products were consumed less. The total percentage of variance of folate levels explained by food group consumption was 14.2% (R^2% from multivariable model). The "Italian high folate dietary pattern" was also positively associated with serum folate levels in the multivariable model ($P < 0.001$).

Italians who were classified in the highest quartiles of folate levels were older, more frequently women, had a higher rate of high physical activity and a lower rate of current smoking habits, as well as lower energy intake ($P < 0.05$ for all) (Table 2B). According to both R^2% and |standardized β-coef%, the "Italian high folate dietary pattern" was the most important factor explaining folate levels variability. Moreover, energy intake accounted for a relatively high percentage of explained variability ($R^2\% = 3.7$). On the contrary, physical activity was the least important factor.

U.K. participants with the highest folate levels had greater daily consumption of citrus fruit, breakfast cereals, and fish and a lower intake of red meat ($P < 0.05$ for all) (Table 3A). The total percentage of variance of folate levels explained by food group consumption was 16.3% (R^2% from multivariable model).

U.K. participants with the highest folate levels showed higher adherence to the "English high folate dietary pattern" and more frequent use of vitamin supplements ($P < 0.05$ for all) (Table 3B). Moreover, they were less frequently smokers and had both lower energy intake and body mass index. According to both R^2% and standardized β-coef% the "English high folate dietary pattern" was the most important factor in explaining folate level variability. Additionally, the use of vitamin supplements ($R^2\% = 3.6$) and tobacco ($R^2\% = 2.9$) accounted for a relatively high percentage of explained variability. In contrast, energy intake was the least important ($R^2\% = 1.1$).

In both populations, dietary folate intake was positively associated with folate levels after adjustments for confounders ($P < 0.05$ for all). An increase of 100 μg/d in dietary folate intake was associated with 13.8% and 10.5% increase in folate levels in the Italian and U.K. populations, respectively (for 100 μg/d increase $e^{\beta\text{-coef}} = 1.138$ and 1.105, $P < 0.001$).

Table 1
Results from reduced rank regression analyses that evaluated the dietary pattern, which is associated with folate dietary intake of Italian and English IMMIDIET population

	Factor loadings*	
	Italy (n = 542)	U.K. (n = 526)
Food groups (g/d)		
Leafy vegetables	39.2	30.4
Broccoli & root vegetables	28.6	43.9
Tomato & other vegetables	30.2	37.1
Legumes	29.3	
Citrus fruits		
Other fruits	32.7	23.4
Fruit & vegetable juices		
Dried fruits		
Pasta rice & cereals		
Potatoes & bread	37.6	28.9
Breakfast cereals		34.6
Nuts & seeds		
Red meat & products		
White meat & egg	28.0	
Fish		
Dairy		26.7

* Factor loadings lower than 20 were not presented for simplicity.

Table 2A
Distribution of food group consumption of Italian participants according to folate serum levels*

	Q1 (< 4.2 ng/mL)	Q2 (4.2–5.3 ng/mL)	Q3 (5.3–6.9 ng/mL)	Q4 (≥ 6.9 ng/mL)	*P* for differences†	*P* from multivariable model‡	$e^{\beta\text{-coef}}$§
Food groups (g/d)							
Leafy vegetables	25 (13.3, 41.3)	22.2 (12.3, 42.3)	28 (19.1, 44.6)	33 (50.8, 21.3)	<0.001	0.04	1.01 (10 g increase)
Broccoli & root vegetables	12.7 (6.7, 22.5)	14.0 (7, 28.7)	15.6 (7.4, 28.5)	15.8 (8.3, 29.9)	0.14	0.26	
Tomato & other vegetables	58.4 (40, 82)	71.1 (38.1, 108)	71.4 (49.6, 99.3)	66.4 (44.4, 103)	0.03	0.10	
Legumes	20.7 (12.9, 31.3)	20.0 (11.2, 28.9)	20.1 (15.8, 31.8)	20.3 (13.5, 28.7)	0.53	0.01	0.99 (10 g increase)
Citrus fruits	58.0 (34.1, 93.4)	67.9 (41.1, 116)	81.3 (54.5, 110)	83.9 (54.7, 139)	<0.001	<0.001	1.02 (25 g increase)
Other fruits	194 (110, 272)	215 (140, 323)	216 (156, 316)	252 (175, 353)	<0.001	0.35	
Fruit & vegetable juices	34.5 (0, 104)	44.0 (0, 116)	35.7 (0, 83.3)	35.7 (4.2, 92.3)	0.63	0.70	
Dried fruits	0.1 (0, 0.1)	0.1 (0, 0.1)	0.1 (0, 0.1)	0.1 (0, 0.1)	0.14	0.51	
Pasta & rice	69.7 (42.4, 115)	68.6 (42.2, 98.1)	64.1 (40.8, 87.6)	61.6 (35.1, 94.2)	0.14	0.16	
Potatoes & bread	146 (94.3, 247)	144 (92, 235.1)	134 (69.3, 189)	128 (70.2, 191)	0.03	0.06	
Breakfast cereals	–	–	–	–	–		
Nuts & seeds	0.2 (0.2, 0.3)	0.2 (0.2, 1)	0.2 (0.2, 0.7)	0.2 (0.2, 1)	0.13	0.07	
Red meat & products	104 (67.5, 133)	75.7 (54.8, 124)	79.2 (50, 132)	64.3 (43.9, 88)	<0.001	<0.001	0.96 (25 g increase)
White meat & egg	56.5 (40.1, 75.2)	61.2 (38.9, 91.7)	62.2 (42.1, 87.5)	54.7 (38.9, 76)	0.24	0.63	
Fish	26.7 (16.4, 36.6)	22.9 (13.7, 36.19)	27.6 (18.8, 42.1)	27.5 (14.4, 38.1)	0.26	0.04	1.18 (10 g increase)
Dairy	185 (74.7, 279)	183 (73.9, 298)	172 (86.9, 295)	171 (59.1, 262)	0.44	0.02	0.98 (100 g increase)

* Skewed food group intake data are presented as median (first, third quartile).
† *P*-value for differences between quartiles of folate serum levels derived through univariate analysis.
‡ *P*-value derived through multiple linear regression analysis with main outcome the log-transformed folate serum levels and independent variables all food groups intake.
§ Delivered from the multiple regression model. Coefficients of non-significant results were not included for simplicity.

Discussion

Dietary folate intake in Italy and the United Kingdom

In this study, an inadequate dietary folate intake was observed in southern Italian participants, whereas in the individuals from southwest London, the folate status appeared to be better. Only 11.3% of Italians but 45.1% of English participants exceed the recommended intake of 400 μg/d, as an indication of a better but non-optimal dietary profile.

The average European intake (United Kingdom excluded), according to recent evidence from EPIC study, was 307 μg/d in men and 252 μg/d in women [23]; whereas a recent review demonstrated the dietary folate intake did not exceed 320 μg/d [24]. EPIC data also have indicated that an English health-conscious group (mainly vegetarians or vegans) had markedly greater intake than other European Union populations [23].

The percentage of women of childbearing age who did not reach the appropriate intake of folate was quite high in both countries. Indeed, 91.9% and 58.6% of Italian and English women of childbearing age did not succeed in reaching an intake of 400 μg/d, which is quite safe at a reproductive age. In the United States, after the fortification strategy of grain and cereal products, spina bifida and anencephaly rates were reduced by about 20% [25].

To understand possible factors responsible for the low intake of folate in the two populations, we examined food sources of folate.

Potatoes and bread explained 61.5% of the total variance of dietary folate intake in Italians followed by leafy vegetables; whereas the corresponding food groups in the U.K. group were broccoli and root vegetables (59.1% of total variance), followed by breakfast cereals. Parallel pattern analysis resulted in similar conclusions. The Italian dietary folate intake pattern, which was characterized by high intake of vegetables, potatoes, bread, fruits, and white meat, described more precisely the high dietary folate intake. The corresponding U.K. pattern differed only in legumes and white meat consumption, which was replaced by breakfast cereals and dairy.

These findings are in agreement with previous European data that indicated vegetables, cereals, and cereal products as the most important folate sources, in both locations [23].

Additionally, both dietary patterns extracted by the present a posteriori dietary analysis included high consumption of food groups common with those included in the traditional Mediterranean diet, a pattern recently associated to a better folate nutritional adequacy [26].

Folate serum status in Italy and the United Kingdom

As far as folate intake is concerned, folate serum levels were generally inadequate in the Italian population, whereas U.K. participants showed a better profile. Again, women of childbearing age had lower folate serum levels than those at menopause, probably because of a lower dietary intake.

A recent study [16] indicated that when using the same cutoff (6.62 ng/mL or 15 nmol/L) in Germany, Sweden, the United Kingdom, and Spain, folate serum levels seemed to be adequate. The three Italian studies included in that European survey reported an alarming situation. Similarly, a more recent analysis [27] indicated that only 22.5% of a southern Italy population had adequate serum folate levels. On the contrary, results from the United States after the mandatory fortification strategy revealed a more adequate folate serum profile [28].

Possible reasons for such difference include dietary intake of folate and the fortification strategies that had been followed by national health policies. Dietary intake profile of United Kingdom participants was more adequate than that of the Italians. In the United Kingdom, public health promotion efforts have been made the in the past 2 decades recommending consumers choose fortified foods for health protection. In 2007, the U.K. Food Standards Agency approved the suggestion for food industries to fortify bread or flour with folate; however, there is still no decision for a mandatory fortification [11].

Table 2B
Distribution of other dietary factors and environmental characteristics of Italian participants according to folate serum levels*

	Q1 (< 4.2 ng/mL)	Q2 (4.2–5.3 ng/mL)	Q3 (5.3–6.9 ng/mL)	Q4 (≥ 6.9 ng/mL)	*P* for differences†	*P* from multivariable model‡	$e^{\beta\text{-coef}}$§	R^2%§‖	St. β-coef§
Dietary factors									
RRR score from pattern analysis	−0.33 (−1.00, 0.71)	−0.19 (−0.83, 0.90)	−0.19 (−0.88, 0.72)	−0.16 (−0.85, 0.51)	0.81	<0.001	1.06 (0.5 units increase)	4.9	15.3
Total energy intake (kcal/d)	2465 (2021, 2998)	2411 (2064, 3141)	2327 (1830, 2711)	2091 (1815, 2513)	<0.001	<0.001	0.99 (100 Kcal increase)	3.7	12.5
Alcohol intake (g/d)	11.2 (0.56, 26.2)	8.5 (0.34, 24.7)	2.3 (0.31, 16.3)	5.4 (0.34, 16.6)	0.07	0.05			
Vitamin B_6 intake (mg/d)	1.92 (1.61, 2.44)	2.02 (1.60, 2.40)	1.95 (1.65, 2.33)	1.79 (1.51, 2.18)	0.03	0.05			
Other environmental characteristics									
Age (y)	44 (38, 49)	43 (39, 48)	45 (39, 51)	45 (41, 51)	0.02	<0.001	1.03 (5 y increase)	1.6	4.7
Male Sex (%)	59.9	54.5	46.5	36.8	<0.001	0.01	0.90	1.5	5.2
Body mass index (kg/m²)	26.9 (24.5, 29.5)	26.4 (23.8, 29.8)	26.9 (23.8, 30.8)	27.0 (24.2, 29.5)	0.68	0.98			
High social status (%)‖	14.8	17.9	14.7	10.5	0.40	0.10			
High physical activity (%)¶	21.1	29.9	28.7	36.8	0.04	0.01	1.09	1.3	4.1
Current smokers (%)	47.9	34.3	25.6	16.5	<0.001	<0.001	0.84	4.8	7.9

* Skewed continuous data are presented as median (first, third quartile) and categorical as frequencies.
† *P*-value for differences between quartiles of folate serum levels derived through univariate analysis.
‡ *P*-value derived through multiple linear regression analysis with main outcome the log-transformed folate serum levels and independent variables other dietary factors and environmental characteristics.
§ Delivered from the multiple regression model. Coefficients, R^2% and |standardized β-coefficients|% of non-significant results were not included for simplicity.
‖ Socioeconomic status was defined as a score (0–5) based on education, job, and housing. The higher the score, the higher was the level of socioeconomic status. Participants were grouped in two categories of socioeconomic status ("low" or "high") according to the median of each population.
¶ Physical activity rate was assessed by a standardized questionnaire [15]. Participants were grouped in two categories of physical activity ("low" or "high") according to the median rate of each population.

Food group consumption in Italy and the United Kingdom explained respectively 14.2% and 16.3% of serum folate variability. Similarly, the adherence to a high folate dietary pattern was positively associated with folate serum levels in both countries. Conversely, red meat, legumes, and dairy, although containing folate, were inversely associated with folate serum levels. Taking into account that the use of an FFQ may not always allow to identify strong associations of dietary and serum folate, the intake of food rich in folate seems to result somehow in higher folate serum levels. Available data both from cross-sectional [29,30] and diet-intervention studies [31,32] suggest a positive association between folate status and dietary patterns characterized by high consumption of fruits, vegetables, and low consumption of red meat and dairy. Probably the increase in consumption of the latter might be an indicator of an unhealthy dietary pattern with limited servings of fruits, vegetables, and cereals. Additionally, bioavailability of folate in different food groups, which has not yet been fully understood [33], might explain the reported proportion of explained variability of serum folate levels by food intake.

U.K. participants with the highest folate levels showed more frequent use of vitamin supplements. This could in part explain the better profile. However, data about exact prescription of vitamins was missing.

In both European Union populations, energy intake was positively associated with serum folate levels. Indeed, in the Italian population, energy consumption explained 3.7% of the total folate levels variability. Indeed, lower energy intake has been associated with better quality of diet through lower energy-dense and healthier foods choices [34]. This might lead to a better and well-absorbed folate intake. Additionally, low energy reporting is usually associated to a healthier lifestyle [35] (e.g., lower smoking habits, greater physical activity), which, according to the present results, was also associated with higher folate levels.

Among non-dietary environmental factors, not smoking was associated with better folate profile. In a study with a healthy Greek population, non-smokers had 13% greater folate serum levels than smokers [36]. Similar findings were observed in a population of pregnant women where non-smokers had 22% higher levels of folate serum levels than smokers [37]. The EPIC study demonstrated the same conclusion [23]. The underlined biological mechanism of this association could be explained by the chemical components found in tobacco smoke, which interact with folate in blood by transforming them into inactive compounds, reducing their active concentration in biological fluids and possibly altering the ability of the cell to store and metabolize them [38].

Physical activity was positively associated with folate serum levels in Italians, as already observed in southern Italy, where more active individuals had significant greater folate serum levels compared with inactive people [27]. According to our knowledge there is no biological mechanism explaining the positive association of physical activity rate with serum folate levels. However, physically active populations seemed to use tobacco less frequent [39], whereas they had greater adherence to healthy dietary patterns, including high quantities of fruits, vegetables, and cereals [40]; factors that, in our study, were associated with higher folate levels.

Limitations of this study

Although the data reported here have important public health implications, this study has some limitations. First, the cross-

Table 3A
Distribution of food group consumption of English participants according to folate serum levels*

	Q1 (< 6.7 ng/mL)	Q2 (6.7–8.6 ng/mL)	Q3 (8.6–11.2 ng/mL)	Q4 (≥ 11.2 ng/mL)	*P* for differences†	*P* from multivariable models‡	$e^{\beta\text{-coef}}$§
Food groups (g/d)							
Leafy vegetables	26.8 (5.8, 31.5)	28.3 (10, 39.7)	28.3 (10.6, 40.8)	27.2 (10.7, 27.2)	0.02	0.84	
Broccoli & root vegetables	79.2 (53.7, 132)	104 (72.3, 144)	101 (71.4, 148)	104 (66.7, 141)	0.03	0.48	
Tomato & other vegetables	64.2 (35.5, 101)	73.7 (55.3, 116)	80 (50.1, 122)	82.2 (53.2, 115)	0.03	0.75	
Legumes	37.3 (18.9, 67.4)	37.2 (17.5, 55)	31.5 (14, 51.5)	37.3 (14.6, 70)	0.07	0.57	
Citrus fruits	5 (1.2, 31.2)	11.3 (2, 33.8)	29.8 (3.5, 59)	21.5 (3.5, 41.3)	<0.001	0.03	1.02 (10 g increase)
Other fruits	94 (32.2, 172)	122 (72.5, 254)	135 (71.2, 212)	145 (89.4, 213)	<0.001	0.80	
Fruit & vegetable juices	24.1 (6.3, 107)	59.5 (8.3, 127)	55.3 (17.9, 126)	98.3 (18.9, 143)	<0.001	0.25	
Dried fruits	0.8 (0, 0.8)	0.8 (0, 2.5)	0.8 (0, 3.1)	0.8 (0.4, 3.8)	<0.001	0.47	
Pasta & rice	24.3 (8.5, 45.2)	33.9 (15.5, 52.6)	25 (15, 52.6)	26.3 (15.5, 52.6)	0.03	0.78	
Potatoes & bread	166 (112, 207)	177 (118, 209)	150 (110, 209)	144 (92.7, 197)	0.18	0.26	
Breakfast cereals	5.7 (1, 20)	17.9 (2.9, 33.9)	31.4 (7.3, 40)	31.4 (17, 40.3)	<0.001	< 0.001	1.06 (10 g increase)
Nuts & seeds	1.2 (0.6, 3)	1.2 (0.7, 5.7)	1.2 (0.7, 4)	0.8 (0.7, 2.5)	0.52	0.18	
Red meat & products	49.9 (25.8, 79.8)	33.9 (23.4, 64.7)	34.1 (17.8, 54.9)	28.7 (14, 47.8)	<0.001	<0.001	0.96 (20 g increase)
White meat & egg	52.4 (32, 65.1)	54.4 (29.8, 70.9)	51 (26, 70.2)	53.8 (31.5, 70.2)	0.51	0.35	
Fish	7.7 (3.7, 16.8)	14.3 (5, 31.5)	16.3 (7, 31.5)	16.3 (4.5, 31.5)	<0.001	<0.001	1.01 (5 g increase)
Dairy	353 (286, 569)	385 (290, 536)	381 (298, 545)	382 (293, 501)	0.86	0.05	

* Skewed food group intake data are presented as median (first, third quartile).
† *P*-value for differences between quartiles of folate serum levels derived through univariate analysis.
‡ *P*-value derived through multiple linear regression analysis with main outcome the log-transformed folate serum levels and independent variables all food group intake.
§ Delivered from the multiple regression model. Coefficients of non-significant results were not included for simplicity.

sectional design of the IMMIDIET study does not enable determination of causality. Second, possible errors due to misreporting by participants should be acknowledged. The use of FFQ as a dietary assessment method may not always allow identification of strong associations of intake and folate levels. Additionally, the use of different food composition tables might insert possible error in the evaluation of folate intake in the two European Union populations. Generalization of the present conclusions to the total Italian or United Kingdom populations should be avoided because the recruitment of study participants, although randomly made from the general population, was limited to the Abruzzo region of Italy and to southwest London.

Table 3B
Distribution of other dietary factors and environmental characteristics of English participants according to folate serum levels*

	Q1 (<6.7 ng/mL)	Q2 (6.7–8.6 ng/mL)	Q3 (8.6–11.2 ng/mL)	Q4 (≥ 11.2 ng/mL)	*P* for differences†	*P* from multivariable models‡	$e^{\beta\text{-coef}}$§	R^2%§	|St. β-coef|%§
Dietary factors									
RRR score from pattern analysis	−0.61 (−1.42, 0.52)	0.04 (−0.75, 0.90)	0.22 (−0.74, 1.07)	0.10 (−0.44, 0.92)	<0.001	<0.001	1.05 (0.5 units increase)	3.7	11.7
Total energy intake (kcal/d)	2093 (1707, 2581)	2201 (1858, 2655)	2102 (1737, 2530)	2057 (1724, 2532)	0.31	0.02	0.99 (100 Kcal increase)	1.1	7.0
Alcohol intake (g ethanol/d)	12.2 (9.9, 14.2)	13.1 (10.9, 15.4)	12.6 (9.9, 14.7)	12.7 (10, 14.9)	0.24	0.78			
Dietary Vitamin B_6 (mg/d)	2.3 (1.9, 2.9)	2.7 (2.2, 3.4)	2.6 (2.2, 3.1)	2.6 (2.1, 3.1)	<0.001	0.85			
Supplementary vitamin intake (%)	21.2	24.4	29.6	50.4	<0.001	<0.001	0.86	3.6	6.9
Other environmental characteristics									
Age (y)	48 (41, 55)	47 (42, 54)	50 (43, 55)	50 (42, 54)	0.71	0.48			
Male Sex (%)	54	55	49.6	41.2	0.10	0.97			
Body mass index (kg/m^2)	26.8 (24, 30)	25.3 (23.5, 27.6)	25.9 (23.8, 28.7)	25.3 (22.7, 28.4)	0.02	0.01	0.95	1.6	4.4
High social status (%)‖	32.9	44.3	41.6	44.3	0.18	0.25			
High physical active (%)¶	38.7	58	50.4	52.7	0.01	0.30			
Current smokers (%)	32.9	7.6	12	11.5	<0.001	<0.001	0.45	2.9	6.0

* Skewed continuous data are presented as median (first, third quartile) and categorical as frequencies.
† *P*-value for differences between quartiles of folate serum levels derived through univariate analysis.
‡ *P*-value derived through multiple linear regression analysis with main outcome the log-transformed folate serum levels and independent variables other dietary factors and environmental characteristics.
§ Delivered from the multiple regression model. Coefficients, R^2% and |standardized β-coefficients|% of non-significant results were not included for simplicity.
‖ Socioeconomic status was defined as a score (0–5) based on education, job, and housing. The higher the score, the higher was the level of socio-economic status. Participants were grouped in two categories of socioeconomic status ("low" or "high") according to the median of each population.
¶ Physical activity rate was assessed by a standardized questionnaire [15]. Participants were grouped in two categories of physical activity ("low" or "high") according to the median rate of each population.

Conclusions

In this study, both inadequate dietary folate intake and serum levels were observed in Italian participants, whereas in individuals from southwest London, folate status appeared somewhat better. Between-country differences in food group consumption as good sources of folate could in part explain this phenomenon. Non-smoking habits and physical activity were the two non-dietary, lifestyle characteristics positively associated with folate serum levels.

Folate is a potentially relevant factor in the prevention of a number of diseases. The evidence linking folate to NTD prevention must lead to the introduction of public health strategies to increase folate intake, in particular in countries with evidence of low intake and low folate serum levels. Different approaches should be used, such as pharmacologic supplementation and/or mandatory or voluntary fortification of staple foods with folate, although they should never replace dietary improvement strategies.

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Appendices. European Collaborative Group of the IMMIDIET Project

Project Coordinator: Licia Iacoviello[a]

Scientific Committee: Jef Arnout,[c] Frank Buntinx,[d] Francesco P. Cappuccio,[e] Pieter C. Dagnelie,[f] Maria Benedetta Donati,[a] Michel de Lorgeril,[g] Vittorio Krogh,[h] Alfonso Siani[i]

Coordinating secretariat: Carla Dirckx[c,d]

Data management and statistics: Augusto Di Castelnuovo[a]

Dietary assessment and analysis: Martien van Dongen[f]

Communication and dissemination: Americo Bonanni[a]

Recruitment: Carla Dirckx,[c,d] Pit Rink,[e] Branislav Vohnout,[b] Francesco Zito[b]

External advisory committee: Mario Mancini, Napoli, Italy; Antonia Trichopoulou, Athens, Greece

The IMMIDIET group, collaborative centers and associated investigators (2012)

a IRCCS Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Isernia, Italy (Licia Iacoviello, Mari Benedetta Donati, Giovanni de Gaetano Amalia De Curtis, Augusto Di Castelnuovo, Americo Bonanni)

b Fondazione di Ricerca e Cura "Giovanni Paolo II," Catholic University, Campobasso, Italy (Francesco Zito, Branislav Vohnout, Marco Olivieri, Agnieszka Pampuch)

c Centre for Molecular and Vascular Biology, Katholieke Universiteit Leuven, Leuven, Belgium (Jef Arnout, Carla Dirckx, Ward Achten)

d Department of General Practice, Katholieke Universiteit Leuven, Leuven, Belgium (Frank Buntinx, Carla Dirckx, Jan Heyrman)

e Clinical Sciences Research Institute, Warwick Medical School, Coventry, United Kingdom (Francesco P. Cappuccio, Michelle A Miller); Division of Community Health Sciences, St George's, University of London, United Kingdom (Pit Rink, Sally C Dean, Clare Harper)

f Department of Epidemiology, NUTRIM Subdivision of Nutritional Epidemiology, Maastricht University, Maastricht, The Netherlands (Peter Dagnelie, Martien van Dongen, Dirk Lemaître)

g Nutrition, Vieillissement et Maladies Cardiovasculaires (NVMCV), UFR de Médecine, Domaine de la Merci, 38056 La Tronche, France (Michel de Lorgeril)

h Nutritional Epidemiology Unit, National Cancer Institute, Milan, Italy (Vittorio Krogh, Sabrina Sieri, Manuela Bellegotti, Daniela Del Sette Cerulli)

i Unit of Epidemiogy & Population Genetics, Institute of Food Sciences CNR, Avellino, Italy (Alfonso Siani, Gianvincenzo Barba, Paola Russo, Antonella Venezia)