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Original article

Divergence between dietary folate intake and concentrations in the serum and red blood cells of aging males in the United States

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SUMMARY

Background & aims: As part of a broader study examining the relationship between serum folate concentrations and prostate cancer progression, we determined if there are age related changes in serum folate concentration compared to folate intake in the U.S. male population.

Methods: Weighted data from the 2007–2008 and 2009–2010 NHANES databases was analyzed. A subpopulation of male participants was selected who were older than one year of age, had completed two days of dietary recall including supplement usage, and had fasted for at least 4 h prior to having their serum folate measured. Total dietary folate equivalent (DFE) intake (mcg) represented the combination of all natural food folate and folic acid from fortification and dietary supplements. Geometric means of serum folate (nM), red blood cell (RBC) folate (nM), and DFE intake were calculated for nine consecutive age groups, with each group generally representing a 10 year span. Analysis was then focused on males older than 20 years of age.

Results: A total of 19,142 subjects were in the initial NHANES population, which represented over 294 million people within the United States. Applying our inclusion criteria created a final subpopulation size of 3775. Subsequent analysis of the age groups for all males older than 20 years found the following: The mean serum folate (nM) with 95% CI levels ranged from 28.2 (26.6, 29.9) to 55.1 (47.5, 63.9). RBC folate (nM) concentrations with 95% CI levels without any fasting exclusions ranged from 795.6 (741.5, 853.7) to 1038.4 (910.7, 1184.2). Serum and RBC folate concentrations were significantly higher with age across these age groups ($p < 0.001$). However, the mean total daily DFE intake did not significantly differ ranging from 640.4 (574.7, 713.7) to 720.2 (665, 780) mcg, ($p = 0.373$). Serum folate concentrations in men with total daily DFE intake of at least 1000 mcg increased more significantly with increasing age than serum folate concentrations in men with less than 400 mcg of total daily DFE intake ($p < 0.001$). There was a similar trend with the RBC folate concentrations ($p = 0.054$).

Conclusions: We observed higher serum and RBC folate concentrations and a divergence between dietary folate intake and these folate concentrations in older males. This phenomenon was evident at total DFE intakes that were significantly less than the 1000 mcg tolerable upper intake level currently recommended by the Institute of Medicine.

1. Introduction

The National Health and Nutrition Examination Survey (NHANES) is a nationally-representative survey that began in 1959 and combines interviews with physical examinations to determine the health and nutritional status of the non-institutionalized United States population [1]. The survey is composed of elements including health status interviews, food frequency questionnaires, up to two 24 h dietary recalls, and physical examinations with associated blood testing. The second and third installments of NHANES, between 1976-1980 and 1988–1994 respectively, demonstrated significant folate deficiencies (serum folate < 6.81 nM) in some populations [2,3]. Due to the findings that folic acid supplementation could help reduce fetal neural tube defects [4], fortification of U.S. cereal-grain products became mandatory in 1998. Population based analysis since that time

Abbreviations: NHANES, National Health and Nutrition Examination Survey; DFE, dietary folate equivalent.

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has shown, on average, an approximately 2.5 fold increase in serum folate concentrations compared to pre-fortification [5].

Folate mediated one carbon metabolism is directly linked to the *de novo* synthesis of purine nucleotides as well as the re-methylation of homocysteine to create methionine [6]. As we have recently reviewed [7], the benefits of folic acid fortification in the U.S. male population, specifically for prostate cancer, remain unclear. Some studies even suggest a detrimental effect with high serum folate concentrations. Unfortunately, there are some common flaws for many of the studies investigating the impact of serum folate and folic acid intake on cancer outcomes. One such flaw is to measure serum folate concentrations once and assume this value to be constant over a period of years. Another commonality is to collect data on total folate intake and assume that these quantities result in the same effect on serum folate concentrations over the years and across patients [7]. It was our hypothesis that equal folate intake may not result in equal serum or RBC folate concentrations across adult men of different ages. If this is in fact true, then the conclusions of previous studies utilizing only dietary intake data could be called into question. Given the need for continued investigation into the potentially detrimental effects of increasing folic acid intakes via fortification and patient self-supplementation, we analyzed the relationship between folate intake and both long and short-term folate status indicators, which are RBC and serum folate concentration respectively, in adult men of different ages using the most recent NHANES data.

2. Methods

2.1. Study population

The NHANES results are currently released to the public in 2 year cycles. Approval from the University of Pittsburgh Institutional Review Board (PRO12080354) was obtained prior to data analysis. Starting in 2007, the method for serum folate measurement was changed to the more accurate microbiologic assay, rather than the Quantaphase II radioassay, which had been used in all previous NHANES installments [8]. We therefore combined the results from the 2007–2008 and 2009–2010 surveys in order to create the largest and most current serum folate data set that did not require any conversion of the data.

A subpopulation of the entire 2007–2010 NHANES cohort was used for analysis. Inclusion criteria were males of all ages greater than one year old, only those subjects who provided two separate 24 h dietary recalls, and who had serum folate measured. Supplement data contained within the two day 24 h recalls was used to estimate daily intakes for all of our analyses. The quantities of folic acid contained within the reported supplements were provided within the NHANES dataset and originally derived from the NHANES Dietary Supplement Database [8].

We primarily analyzed those males who fasted for at least 4 h prior to blood testing, in an attempt to analyze only steady state serum concentrations of folate and not artificial spikes in the folate concentration found after eating. However, we also analyzed all males without any fasting exclusions as well as only those who fasted for at least 8 h in order to ensure our shorter time frame did not skew results.

The final subpopulation was then divided by 10 year increments into nine different age groups. These were 1–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–79, and 80 + years old. One subject in the 21–30 age group was excluded from this final subpopulation due to having an extreme outlaying serum folate of over 500 nM. For completeness, we included the descriptive statistical results for males 20 years of age and younger in the tables. However, the primary objective of this study was to examine trends in adult males, therefore all reported ranges and trend analyses are for only males aged 21 years and older.

2.2. Dietary folate equivalent analysis

The bioavailability of naturally occurring folate in food has traditionally been reported as 50–60%, while the bioavailability of folic acid, which is the synthetic form of folate that is used for fortification and contained in dietary supplements, is approximately 85% [9]. Therefore, in order to combine the two sources and account for the increased absorption of folic acid, the total daily folate and folic acid intake must be converted into dietary folate equivalents (DFE). A conversion factor of 1.7 has been estimated by using the ratio of bioavailabilities of the two sources of folate, 85:50. This is then used to multiply the amount of folic acid contained in fortified food and in dietary supplements [9,10], resulting in the following equation:

Total Daily DFE Intake = μg food folate + (μg folic acid from fortification * 1.7) + (μg folic acid from supplements * 1.7).

The average total daily DFE intake was then calculated by averaging the combined food folate and folic acid from fortification and dietary supplements reported in the two days of dietary recall.

2.3. Statistical methods

All statistical analysis was performed using Stata v12.1 (StatCorp LP, College Station, Tx). The Day 2 dietary recall weights were applied so that the subpopulation still represented the U.S. population as a whole. the variance estimation (VCE) component of the *svy* command was set to *linearized*, as is currently recommended by the National Center for Health Statistics for all NHANES data [11].

All folate concentration and DFE data were log transformed in order to normalize their distributions. The geometric means of serum folate concentration, RBC folate concentration, and total daily DFE intake were then calculated for each age group. All mention of calculated means in this manuscript should therefore be regarded as geometric means.

Due to using linearized variance estimation and the "within person means method" of averaging two days of dietary recall [8,11], exact distributions of intake across each age group cannot be accurately calculated. However, this ultimately doesn't affect the geometric mean of the variable being analyzed [12]. Therefore, we established an estimate of the 25th, 50th, and 75th percentiles of average DFE intake throughout the entire subpopulation and used these estimates to set cutoffs for the top and bottom quartiles of DFE intake. Considering that 400 mcg is the current Recommended Daily Allowance (RDA) for DFE intake [13], and that 1000 mcg was the quantity used in a recent randomized placebo controlled trial investigating folic acid supplementation and the risk of prostate cancer [14], these two amounts were conceptually easy and appropriate to utilize for our quartile cutoffs.

Testing for statistically significant trends for serum and RBC folate concentration and DFE intake across age groups was done using the linear regression followed by an Adjusted Wald test. An interaction test was performed in order to determine if the increase of serum and RBC folate concentrations over age groups differed significantly between those subjects in the top versus the bottom quartiles of DFE intake. A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Serum folate

The subpopulation of males who had a serum folate measured, reported two days of dietary recall and had fasted for at least 4 h, created a final subpopulation size of 3775. This

represented over 69 million males in the United States after appropriate weighting was applied. The results of our analysis can be found in Table 1.

The mean serum folate (nM) for adult men in this population ranged from 28.2 (26.6, 29.9) to 55.1 (47.5, 63.9), and demonstrated a significant increase with age ($p < 0.001$) (Table 1). The mean total daily DFE intake (mcg) for adult men ranged from 640.4 (574.7, 713.7) to 720.2 (665, 780) and did not differ across age groups ($p = 0.373$) (Table 1).

Adult men in the bottom quartile of total daily DFE intake ingested less than 403.4 mcg (391.5, 415.7) while the median intake was 626.4mcg (614, 645.5) per day, and the top quartile of intake was greater than 1053.6mcg (1022.5, 1085.7). Ranges for serum folate for males who fasted for at least 4 h and were within the top and bottom quartiles of total daily DFE intake are shown graphically in Fig. 1 and provided in Table 2.

Mean total daily DFE intakes within the top and bottom DFE quartiles (Table 2) were also calculated. Total daily DFE intakes were significantly lower within the bottom DFE intake quartile in adult males, ($p = 0.027$), while there was no difference in mean total daily DFE intake within the top quartile ($p = 0.102$) between age groups of adult males.

Linear regression of mean total daily DFE intake across all age groups of adult males demonstrated no significant difference ($p = 0.373$). Serum folate was significantly higher in each age group, starting in men at least 20 years old up to those men 80 and above ($p < 0.001$). Similarly, serum folate for men in the lowest and highest quartile of total daily DFE intake was significantly higher ($p = 0.003$ and $p < 0.001$, respectively) across the same age groups. Utilizing a regression interaction test, we confirmed serum folate in the top total daily DFE intake quartile was significantly higher between age groups compared to the bottom quartile ($p < 0.001$), which is shown graphically in Fig. 1.

In order to ensure our results were not artificially skewed due to our 4 h fasting requirement, we ran duplicate analyses on all men with no fasting exclusions and also with a minimum of 8 h fasting cut-off (Supplemental Table 1). There was no difference in the overall trends in serum folate concentrations between age groups and over total daily DFE quartiles that have already been stated. Therefore, the remainder of the analysis involving serum folate is done with the 4 h fasting requirement. As fasting should not affect the RBC folate concentration, all analysis regarding RBCs had no fasting exclusions applied.

Mean daily intake of naturally occurring food folate was calculated (Table 1) and, in adult males, was significantly lower with increasing age ($p = 0.007$). Similar calculations were done for the mean daily intake of folic acid (Table 1), however this was significantly higher across the adult male age groups ($p = 0.045$).

Due to the smaller total change in serum folate in those individuals with a lesser total daily DFE intake, we aimed to find the lower limit of intake that would result in no difference between age groups. In adult men with a total DFE intake of 300 mcg per day or less (Table 3), there was no statistical difference between the age groups ($p = 0.27$). When the population is expanded to include adult men taking up to 350 mcg total DFE intake daily, serum folate (nM) was significantly higher over the age groups ($p = 0.008$), ranging from 23 (19.9, 26.6) to 32.8 (26.8, 40.2). In this NHANES cohort, approximately 85% of the adult men reported total daily DFE intake of over 300 mcg.

In another sub-analysis, the original subpopulation was further narrowed by excluding any person who took a dietary supplement. The remaining subjects therefore had folate intake only from natural food sources or from food that was fortified with folic acid. The mean serum folate concentrations (Table 4) were significantly higher with increasing age ($p < 0.001$), as they were when

Table 1
Geometric means of serum folate (nM), red blood cell (RBC) folate (nM), daily dietary folate equivalent (DFE)[h] intake (mcg), DFE intake from only naturally occurring food folate (mcg), and DFE intake from only folic acid (mcg), stratified by age group, in the United States from 2007 to 2010.

Age group (yrs)	n[a]	Mean serum folate, in nM (95% CI)[b]	n[c]	Mean RBC folate, in nM (95% CI)[d]	Mean daily DFE intake (mcg) (95% CI)[e]	Mean DFE intake, only food folate, in mcg (95% CI)[f]	Mean DFE intake, only folic acid, in mcg (95% CI)[g]
1–10	395	52.0 (50.4, 53.8)	1288	1081.5 (1047.7, 1116.4)	545.4 (498.1, 597.3)	131.6 (126.5, 136.9)	429.9 (373.2, 495.2)
11–20	700	37.6 (35.1, 40.3)	1203	947.7 (897.1, 1001.1)	617.2 (578.6, 658.5)	168.6 (158.2, 179.7)	458.6 (417, 504.4)
21–30	408	28.2 (26.6, 29.9)	644	933.9 (876.8, 994.8)	640.4 (574.7, 713.7)	206.1 (190.1, 223.5)	454.6 (396, 521.9)
31–40	419	29.8 (28, 31.8)	735	970.7 (940.1, 1002.4)	673.6 (627.6, 722.8)	227.9 (214.9, 241.6)	456.8 (407.6, 511.8)
41–50	457	33.7 (31, 36.7)	727	1075.3 (1021.9, 1131.4)	678.7 (614.4, 749.7)	220.2 (207.2, 234)	475.1 (404.1, 558.7)
51–60	493	37.2 (35, 39.6)	768	1142 (1085.7, 1201.1)	720.2 (665, 780)	223.7 (211.6, 236.5)	556.5 (497.2, 622.8)
61–70	450	43 (39.8, 46.4)	717	1250.4 (1193.8, 1309.7)	712.2 (634.2, 799.7)	213.5 (198.5, 229.6)	527.5 (436.7, 637.1)
71–79	283	44.7 (40.6, 49.2)	471	1325.2 (1262.4, 1391.3)	713.8 (648.1, 786.3)	194 (180.2, 208.9)	576.2 (494.2, 671.9)
80+	170	55.1 (47.5, 63.9)	296	1461.7 (1357.1, 1574.3)	669.3 (565.5, 792.1)	180.5 (162.6, 200.3)	545.2 (404.5, 734.8)

[a] Unweighted "n" for each age group in the serum folate analysis. Subpopulation criteria were all men who had serum folate measured, had recorded two 24 h dietary recalls, and had fasted for at least 4 h prior to lab work. All analysis was performed with appropriate weighting applied to each person, as per the NHANES guidelines.
[b] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p < 0.001$.
[c] Unweighted "n" for each age group in the RBC folate analysis. Subpopulation criteria were the same as with serum folate analysis, however there was no fasting exclusion applied.
[d] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p < 0.001$.
[e] No significant difference across adult age groups starting in the third decade of life, $p = 0.373$.
[f] Only naturally occurring food folate without any folic acid. This significantly decreases starting after the third decade of life, $p = 0.007$.
[g] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p = 0.045$. Included folic acid from fortification and supplementation.
[h] Dietary Folate Equivalent (DFE) is the combination of folate derived from natural food sources with folic acid obtained from both food fortification and dietary supplements.

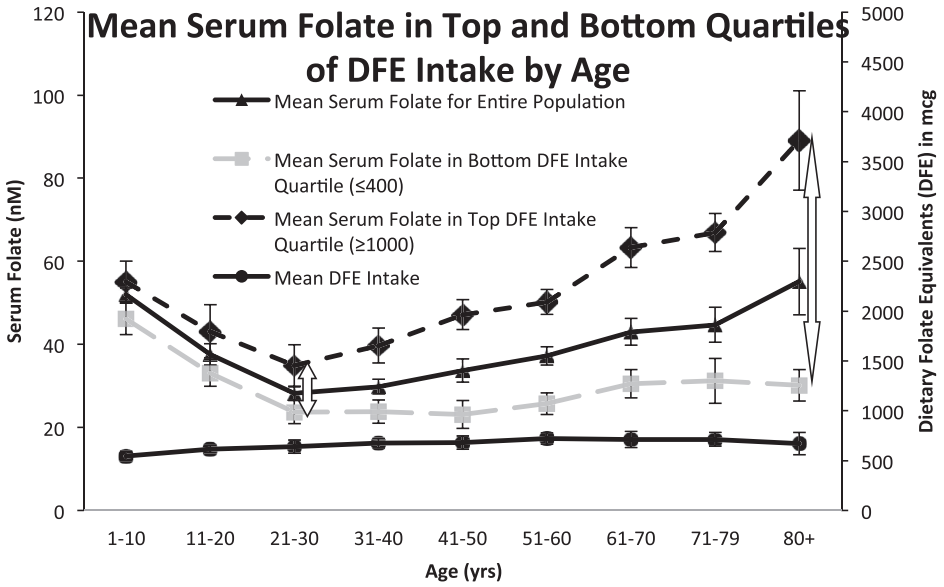

Fig. 1. Geometric mean serum folate (nM) for the entire subpopulation (solid line with triangle markers) compared to mean serum folate (nM) in the subpopulations of males within the top (broken line with diamond markers) and bottom (broken line with square marker) quartiles of mean daily dietary folate equivalent (DFE) intake by age group (≥1000 mcg and ≤400mcg, respectively), with 95% CIs, using the National Health and Nutrition Examination Survey (NHANES) results from 2007 to 2010. Beginning in the third decade of life (21–30), mean serum folate increased significantly over the remaining age groups ($p < 0.001$). There was no difference in mean daily DFE (solid line with circle markers) intake across adult age groups ($p = 0.373$). There was a significant increase in serum folate concentrations beginning in the third decade of life (21–30 yrs) that continues over the remaining age groups in both the top and bottom quartiles of DFE intake ($p < 0.001$ and $p = 0.003$, respectively). Interaction testing between the two linear regressions reveals a significant difference in the rate of increase of serum folate in the top DFE intake quartile compared to the bottom quartile, which is represented graphically by the two arrows ($p < 0.001$). Conversion factor for serum folate concentration is 1 ng/mL = 2.266 nM.

supplement takers were included. The mean total daily DFE intake (mcg) without supplements became significantly lower ($p < 0.001$) as adult male ages increased. This was different from the original subpopulation, which had no significant difference in total daily DFE intake between the age groups.

3.2. RBC folate analysis

Mean RBC folate concentration (Table 5) for adult males without fasting exclusions was significantly ($p < 0.001$) higher with increasing age. Mean RBC folate concentrations for males in the top

Table 2
Geometric means of serum folate and daily DFE[f] intake in males within the bottom quartile of daily DFE intake compared to males in the top quartile of daily DFE intake, stratified by age group, in the United States from 2007 to 2010.[g]

Age group	Bottom daily DFE intake quartile (≤400 mcg)			Top daily DFE intake quartile (≥1000 mcg)		
	n[a]	Mean serum folate, in nM (95% CI)[b]	Mean DFE intake (95% CI)[c]	n[a]	Mean serum folate, in nM (95% CI)[d]	Mean DFE Intake, in mcg (95% CI)[e]
1–10	132	46.1 (42.4, 50.2)	294.3 (276, 313.8)	49	55.0 (50.1, 60.5)	1217.9 (1121.7, 1322.4)
11–20	169	33.1 (30, 36.5)	285.2 (266.6, 305)	141	43.1 (37.1, 50.1)	1316.3 (1234.4, 1403.6)
21–30	108	23.7 (21, 26.9)	287.6 (262, 315.6)	82	34.9 (30.1, 40.4)	1379.1 (1290.1, 1474.2)
31–40	89	23.8 (21.2, 26.8)	279.9 (251, 312)	103	39.7 (35.6, 44.2)	1486.6 (1387.8, 1592.4)
41–50	104	23.1 (19.8, 26.8)	300.9 (285.7, 316.9)	126	47.1 (43.5, 51)	1325.6 (1242.3, 1414.5)
51–60	117	25.7 (23.3, 28.4)	287.9 (268.8, 308.3)	133	50.2 (47.2, 53.4)	1374.2 (1285.1, 1469.5)
61–70	124	30.5 (27.2, 34.3)	290.7 (275.8, 306.4)	141	63.3 (58.5, 68.4)	1488.3 (1414.2, 1566.2)
71–79	66	31.5 (26.5, 37.4)	286.4 (267.9, 306.1)	103	66.9 (62.4, 71.7)	1428.5 (1335, 1528.4)
80+	48	30.1 (26.4, 34.3)	252.5 (229.5, 277.8)	62	89.1 (77.6, 102.2)	1394 (1260.5, 1541.7)

[a] Unweighted "n" for each age group. All analysis was performed with appropriate weighting applied to each person, as per the NHANES guidelines.
[b] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p = 0.003$.
[c] Significantly decreases across age groups starting in the third decade of life (age group 21–30), $p = 0.027$.
[d] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p < 0.001$.
[e] No significant difference across age groups starting in the third decade of life, $p = 0.102$.
[f] Dietary Folate Equivalent (DFE) is the combination of folate derived from natural food sources with folic acid obtained from both food fortification and dietary supplements.
[g] The rate of increase of serum folate in males over age groups within the highest quartile of DFE intake is significantly greater than the rate of increase of serum folate in males within the lowest quartile of DFE intake, $p < 0.001$.

Table 3
Geometric mean serum folate (nM) by age for two quantities of mean daily dietary folate equivalent (DFE)[d] intake (mcg), with 95% CIs, using the National Health and Nutrition Examination Survey (NHANES) results from 2007 to 2010.

Age group (yrs)	Daily DFE intake of ≤300 mcg		Daily DFE intake of ≤350 mcg	
	n[a]	Mean serum folate (nM)[b]	n[a]	Mean serum folate (nM)[c]
21–30	51	22.9 (19.4, 27)	82	23 (19.9, 26.6)
31–40	43	23.8 (20.9, 27.1)	67	24.4 (21.6, 27.6)
41–50	45	23.7 (18.6, 30.3)	69	24.1 (19.9, 29.1)
51–60	70	22.9 (20.2, 26)	90	23.6 (21.3, 26.1)
61–70	62	26.3 (22.6, 30.5)	102	28.5 (24.9, 32.4)
71–79	31	27.6 (22.8, 33.5)	51	32.8 (26.8, 40.2)
80+	28	27.9 (23.3, 33.4)	41	29.8 (26.3, 33.7)

[a] Unweighted "n" for each age group. All analysis was performed with appropriate weighting applied to each person, as per the NHANES guidelines.
[b] No significant difference across age groups starting in the third decade of life (age group 21–30), p = 0.27.
[c] Significantly increases across age groups starting in the third decade of life (age group 21–30), p = 0.008.
[d] Dietary Folate Equivalent (DFE) is the combination of folate derived from natural food sources with folic acid obtained from both food fortification and dietary supplements.

and bottom quartiles of total daily DFE intake were significantly higher (p < 0.001) across all adult age groups.

The overall trends in RBC folate with age were compared between total daily DFE intake quartiles by using a regression interaction test. This analysis trended towards there being a significantly higher RBC folate concentrations between age groups in the top DFE intake quartile compared to the bottom quartile (p = 0.054).

In the sub-analysis of men taking in no more than 300 mcg of total DFE per day, there was no difference in RBC folate across the lifespan of adult men, p = 0.0828. As with serum folate, RBC folate was significantly higher across adult age groups in the subpopulation of men who ingested up to 350 mcg of DFE daily, p < 0.001.

Table 4
Geometric mean serum folate (nM) and daily DFE[d] intake (mcg) in males who do not take dietary supplements, in the United States from 2007 to 2010.

Age group	n[a]	Mean serum folate (nM) in non-users of supplements (95% CI)[b]	Mean daily DFE intake (mcg) in non-users of supplements (95% CI)[c]
1–10	316	50.8 (48.7, 53)	482.7 (451.1, 516.5)
11–20	601	35.4 (32.8, 38.1)	556.6 (523.8, 591.5)
21–30	333	25.8 (24.2, 27.5)	571.8 (518.1, 631.2)
31–40	320	27.4 (25.5, 29.4)	566.2 (515.9, 621.5)
41–50	331	29.7 (27.4, 32.2)	534.8 (481.9, 593.4)
51–60	331	32.6 (29.6, 36)	551.2 (487.6, 623.1)
61–70	292	33.3 (30.8, 36.1)	482.8 (438.7, 531.3)
71–79	161	34.3 (30, 39.3)	476.6 (441.8, 514.1)
80+	101	40.5 (35.4, 46.5)	441.1 (389.2, 500)

[a] Unweighted "n" for each age group. All analysis was performed with appropriate weighting applied to each person, as per the NHANES guidelines.
[b] Significantly increases across age groups starting in the third decade of life (age group 21–30), p < 0.001.
[c] Significantly decreases across age groups starting in the third decade of life (age group 21–30), p < 0.001.
[d] Dietary Folate Equivalent (DFE) is the combination of folate derived from natural food sources with folic acid obtained from food fortification.

4. Discussion

Fortification of the United States diet with folic acid has been mandatory since 1998. This was instituted after previous NHANES installments demonstrated significant deficiencies within the U.S. population and it was discovered that folic acid supplementation could help prevent neural tube defects [2,4]. Since that time, there have been multiple studies that have shown significant increases in overall serum folate concentrations [2,5,15]. To our knowledge, this is the first study that specifically examines the relationship between serum folate and varying levels of DFE intake over the spectrum of ages in United States males. Our group is particularly interested in this area because of the potential relationships between prostate tumor growth and increased intake of DFEs or increased serum folate concentrations that have previously been reported [7,16].

Our analysis of the NHANES data from 2007 to 2010 demonstrates a "U-shaped" distribution of serum folate concentration over the lifespan of males, with the lowest concentrations occurring in the third decade of life (Fig. 1). This overall trend has been previously reported [17], and we confirm the findings of a separate report that this trend is statistically significant in adult men with the most recent NHANES data, defined as between the third and ninth decades of life [5].

The exact etiology of this trend is unknown at this time. Due to the complexity of folate absorption, metabolism, and excretion, there are many different factors that could be contributing to the overall homeostasis of serum folate concentrations in men of different ages.

4.1. DFE intake

One of the major factors that contributes to the concentration of folate in serum and tissue (which in this study was represented by RBC) is the quantity of DFE that is consumed. While we calculated values for males of all ages, we specifically focused our analysis on adult men, starting in the third decade of life. Our analysis reports for the first time that despite equivalent levels of DFE intake, serum folate concentrations are significantly higher as adult men age. In addition to this overall trend, the serum folate in men ingesting over 1000 mcg of total DFE daily is significantly higher between age groups than in men ingesting 400 mcg of total daily DFE or less (Fig. 1). Similar findings were noted with RBC folate concentrations across adult age groups. The degree of increase in RBC folate between age groups for men in the top and bottom quartiles of DFE intake trended towards significance. These findings would suggest that there is a threshold of folate intake at which the regulation of both the serum and tissue folate concentrations changes in older men. Additionally, based on the difference in the degree of increase in serum folate as men age between those ingesting less than 300 mcg and those ingesting more, the threshold at which serum folate and DFE intake diverges appears to be between 300 and 400 mcg of DFE. However the Institute of Medicine recommendations are that 1000 mcg DFE is the tolerable upper limit for adult men, and furthermore, 400 mcg DFE is the recommended daily intake for folate [13]. As greater than 80% of men report regularly consuming more than 300 mcg DFE per day, the implications of these findings

Table 5
Geometric means of red blood cell (RBC) folate (nM) and daily DFE (mcg)[f] intake in males within the bottom quartile of daily DFE intake compared to males in the top quartile of daily DFE intake, stratified by age group, in the United States from 2007 to 2010.[g]

Age group	Bottom daily DFE intake quartile (≤400 mcg)			Top daily DFE intake quartile (≥1000 mcg)		
	n[a]	Mean RBC folate, in nM (95% CI)[b]	Mean DFE intake (95% CI)[c]	n[a]	Mean RBC Folate, in nM (95% CI)[d]	Mean DFE intake, in mcg (95% CI)[e]
1–10	412	991.5 (945.4, 1039.8)	282.3 (272.5, 292.4)	152	1194.2 (1106.3, 1289.1)	1254.5 (1184.3, 1328.9)
11–20	294	871.1 (820.9, 924.3)	282.7 (268.5, 297.6)	238	1007 (929.4, 1091.1)	1338.4 (1281.7, 1397.7)
21–30	164	795.6 (741.5, 853.7)	286.7 (267.8, 306.9)	137	1169.4 (1041.2, 1313.5)	1409.2 (1346.8, 1474.5)
31–40	159	842.7 (764.6, 928.8)	282.6 (262.5, 304.2)	172	1150.2 (1067.7, 1239)	1443.6 (1371.4, 1519.6)
41–50	162	870.7 (793.4, 955.5)	302 (287.7, 317)	213	1326.3 (1240.9, 1417.6)	1339.3 (1271.2, 1410.9)
51–60	163	945.8 (851.3, 1050.7)	289.6 (272, 308.5)	225	1394.9 (1293.2, 1504.5)	1391.6 (1329, 1457.1)
61–70	193	1018.6 (957.4, 1083.8)	295.1 (277.7, 313.6)	237	1571.5 (1470.9, 1679)	1431.7 (1366.2, 1500.4)
71–79	114	1038.8 (921.4, 1171.1)	287.3 (276.6, 298.4)	173	1649 (1556, 1747.4)	1439.4 (1366.6, 1516)
80+	71	1038.4 (910.7, 1184.2)	268.7 (247.8, 291.4)	113	1900.1 (1792.7, 2013.9)	1402.7 (1324.8, 1485.1)

[a] Unweighted "n" for each age group. All analysis was performed with appropriate weighting applied to each person, as per the NHANES guidelines.
[b] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p < 0.001$.
[c] No significant difference across age groups starting in the third decade of life, $p = 0.26$.
[d] Significantly increases across age groups starting in the third decade of life (age group 21–30), $p < 0.001$.
[e] No significant difference across age groups starting in the third decade of life, $p = 0.24$.
[f] Dietary Folate Equivalent (DFE) is the combination of folate derived from natural food sources with folic acid obtained from both food fortification and dietary supplements.
[g] The rate of increase of serum folate in males over age groups within the highest quartile of DFE intake trended towards being significantly greater than the rate of increase of serum folate in males within the lowest quartile of DFE intake, $p = 0.054$.

could be quite significant as the efforts to define safe serum folate concentrations continue.

The finding of significantly less intake of naturally occurring food folate as men age was unexpected. However, since there was overall no difference in total daily DFE intake across the same age groups, it was not surprising to find significantly higher total folic acid intake with increasing age. It is therefore plausible that the higher serum and RBC folate concentrations seen in older adult men are due to intake from folic acid contained within nutritional supplements. This hypothesis was tested by removing supplement users from a separate subgroup analysis, so only natural folate intake from food, and folic acid intake from fortified food were included. Within this subpopulation, there were significantly lower daily DFE intakes as men age. However, despite the overall lower daily DFE intakes, the significantly higher serum folate concentrations over the same age groups remained. Therefore, while it appears that older men derive a larger portion of their DFE intake from supplements compared to younger adult men, this is not the only factor contributing to the higher concentration of folate in the serum. This point is worthy of future consideration and investigation as the difference in metabolism, circulating concentrations, and clinical impact are still not fully understood when comparing naturally occurring folate to synthetic folic acid.

4.2. Overall implications

The global effect of higher serum and RBC folate concentrations despite equivalent amounts of total daily DFE intake in men of different ages is unknown. There have been many studies examining the risks and benefits of folate supplementation, especially as it relates to cancer [7,14,18–20]. However, most of these studies have assumed that ingestion of a certain quantity of folate or folic acid will produce equivalent serum folate concentrations, and thus equal clinical affects. We have demonstrated that the same level of intake may not have the same effect on serum and RBC folate in men of different ages, thus calling into question the applicability of these studies to current practices. In fact, a recent systematic review and meta-analysis investigating folate intake and risk of prostate cancer found no association between folate intake and prostate cancer, however importantly, the authors did find a significant association with high serum folate concentration and prostate cancer, OR 1.14 (95% CI 1.02, 1.28) [21]. Considering our findings, it is possible that the studies using dietary intake of folate did not show an association with prostate cancer due to the improper assumption that equal quantities of DFE intake will result in equal serum and tissue folate concentrations. Furthermore, our results suggest this assumption is especially inaccurate when studying older men, who are also the ones most at risk for developing prostate cancer.

5. Conclusions

Analysis of the most recent four years of NHANES data continues to demonstrate significantly higher serum folate and RBC folate concentrations in older men, despite equivalent levels of folate intake and whether or not they take supplements containing folic acid. Since there are potentially deleterious effects of high serum folate concentrations, and serum folate concentrations appear to be affected by age, further research into the appropriate quantity of folate intake for individuals of different ages and health statuses should be undertaken.

Statement of authorship

The order of authorship is an accurate reflection of the contributions to this work. All authors were involved in the design, implementation, and analysis for this study. Additionally, all authors give their full approval for the current manuscript that is submitted.

Conflict of interest

There are no conflicts of interest for any author to report.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2015.07.002

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