Clinical Nutrition xxx (2015) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

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

Kevin J. Rycyna [a], Dean J. Bacich [b, *, 1], Denise S. O'Keefe [b, 1]

[a] Department of Urology, University of Pittsburgh Medical Center, United States
[b] Department of Urology, University of Texas Health Science Center at San Antonio, United States

ARTICLE INFO

Article history:
Received 28 January 2015
Accepted 6 July 2015

Keywords:
Folate
Folic acid
Fortification
Divergence
Serum
Dietary

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.

* Corresponding author. Department of Urology, 7703 Floyd Curl Drive, Mail Code 7845, San Antonio, TX 78229-3900, United States. Tel.: +1 210 562 4099.
E-mail address: bacich@uthscsa.edu (D.J. Bacich).

[1] Equal senior author.

http://dx.doi.org/10.1016/j.clnu.2015.07.002
0261-5614/

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

Please cite this article in press as: Rycyna KJ, et al., Divergence between dietary folate intake and concentrations in the serum and red blood cells of aging males in the United States, Clinical Nutrition (2015), http://dx.doi.org/10.1016/j.clnu.2015.07.002