Published in final edited form as:
J Nutr Health Aging. 2011 ; 15(9): 744–750.

The Oldest Old: Red blood cell and plasma folate in African American and White Octogenarians and Centenarians in Georgia

Dorothy B. Hausman[1], **Mary Ann Johnson**[1], **Adam Davey**[2], **John L. Woodard**[3], **Leonard W. Poon**[4], **Robert H. Allen**[5], and **Sally P. Stabler**[5] **for the Georgia Centenarian Study**

Dorothy B. Hausman: dhausman@uga.edu; Mary Ann Johnson: drmaryannjohnson@gmail.com; Adam Davey: adavey@temple.edu; John L. Woodard: John.Woodard@wayne.edu; Leonard W. Poon: lpoon@geron.uga.edu; Robert H. Allen: Robert.Allen@ucdenver.edu; Sally P. Stabler: Sally.Stabler@ucdenver.edu

[1]Department of Foods and Nutrition, University of Georgia, Athens, GA, USA

[2]College of Health Professions, Temple University, Philadelphia, PA, USA

[3]Department of Psychology, Wayne State University, Detroit, MI, USA

[4]Institute of Gerontology, University of Georgia, Athens, GA, USA

[5]University of Colorado Denver Health Sciences Center, Aurora, CO, USA

Abstract

Objective—To determine the overall folate status of a population-based multi-ethnic sample of octogenarians and centenarians and the specific dietary, demographic and physiological factors associated with observed abnormalities.

Design—Population-based multiethnic sample of adults aged 80 to 89 and 98 and above. Setting: Northern Georgia, USA

Setting—Northern Georgia, USA

Participants—Men and women aged 80 to 89 (octogenarians, n = 77) and 98 and older (centenarians, n = 198)

Analyses—Wilcoxon rank sum tests, and Chi square and logistic regression analyses were used to examine associations of low and high folate status with hematological indicators and other variables of interest.

Results—The prevalence of low red blood cell (RBC) folate was low overall, but tended to be higher in centenarians than in octogenarians (6.5% vs. 1.3%, p = 0.058; defined as RBC folate < 317 nmol/L). The risk of having lower RBC folate (< 25th vs. ≥25th percentile for RBC folate for 60yr+ in NHANES 1999–2000) was greater in association with vitamin B12 deficiency (OR =

Corresponding author: Dorothy B. Hausman, Ph.D., The University of Georgia, Department of Foods and Nutrition, 280 Dawson Hall, Athens, GA 30602 USA, Phone: 706-542-4871, FAX: 706-542-5059, dhausman@uga.edu.

1. The Georgia Centenarian Study (Leonard W. Poon, PI) is funded by 1P01-AG17553 from the National Institute on Aging.

2. Two of the authors (SPS and RHA) and the University of Colorado hold patents on the use of assays for total homocysteine and other metabolites to diagnose vitamin B12 and folate deficiencies, and a company has been formed at the University of Colorado to perform such assays.

3. Additional investigators in the Georgia Centenarian Study include Jonathan Arnold (University of Georgia), Marla Gearing (Emory University School of Medicine), Robert C. Green (Boston University School of Medicine), S. Michal Jazwinski (Tulane University Health Sciences Center), Peter Martin (Iowa State University), Maurice MacDonald (Iowa State University), William R. Markesbery (deceased), Willard L. Rodgers (University of Michigan), Christopher Rott (University of Heidelberg), Ilene C. Siegler (Duke University), and J. Lisa Tenover (Palo Alto VA Health Care System).

5.36; 95%CI: 2.87–10.01), African American race (OR = 4.29; 95%CI: 2.08–8.83), and residence in a skilled nursing facility (OR = 3.25; 95%CI: 1.56–6.78) but was not influenced by age, gender, B-vitamin supplement use, high/low food score or presence of atrophic gastritis. Combined high plasma folate and low vitamin B12 status was present in some individuals (n=11), but was not associated with increased prevalence of anemia or cognitive impairment in this study.

Conclusions—Low RBC folate status (< 317 nmol/L) was rare in this post folic acid fortification sample of octogenarians and centenarians. RBC folate status (< 25th percentile) was strongly associated with 1) vitamin B12 deficiency, which has strong implications for vitamin treatment, and 2) with being African American, suggesting racial disparities exist even in the oldest old.

Introduction

Our previous studies showed a high prevalence of both vitamin D insufficiency (1) and vitamin B12 deficiency (2) in a population-based study of older adults, with the prevalence of both conditions over two-fold greater in centenarians as compared with octogenarians. Deficiencies of vitamin B12 and folate are associated with elevated blood homocysteine levels, a risk factor for many chronic conditions. Altered homocysteine and folate status have been implicated as independent risk factors for cardiovascular disease, cognitive impairment, dementia, Alzheimer's Disease and depression in some studies (3–8), but not in others (9). Conversely, it has been suggested that old age and diseases such as Alzheimer's may increase the need for folate (10).

Folate status has been widely studied in the US since the required fortification of all enriched cereal-grain products beginning in 1998. Recent studies report an improvement in folate status from pre-fortification values across all ages, including older adults (11–14). These and other studies (15–16) have examined folate status in older adults (aged 60+) collectively, with no further demarcation within this age classification. Our finding of two-fold higher prevalence of both vitamin D insufficiency (1) and vitamin B12 deficiency (2) in centenarians as compared with octogenarians, suggests that there is considerable heterogeneity in nutrient status in the 'older adult' age group. To our knowledge, measures of folate status have not been examined specifically in the 'oldest old' population groups in the US post-folic acid fortification.

The primary objective of this study was to determine measures of folate status and predictors of high and low folate status in a population based multi-ethnic sample of adults aged 80 to 89 and 98 and above from northern Georgia in the US. In addition, since several studies have reported potential detrimental effects of high serum folate in older individuals with low vitamin B12 (15–21) status, the associations between vitamin B12 and folate status and selected measures of physical and cognitive health were also examined.

Methods

Study Population

Study participants were part of the Georgia Centenarian Study, a population-based multidisciplinary study conducted in 44 counties in northern Georgia (USA) from 2002 to 2005. The original study included 244 centenarians (defined as age 98 and older) and 80 octogenarians. The sampling procedures and data collection methods have been described elsewhere (1,22). Briefly, recruitment of participants from skilled nursing facilities was based on estimates of the 'institutionalized' population of the area according to the 2000 U.S. Census tabulations. The 'community dwelling' participants resided in private residences and personal care homes and were recruited from voter registration lists.

Participants were recruited to match census figures for gender and race/ethnicity (white or black; all were non-Hispanic) and were interviewed by trained personnel in their place of residence. All questionnaires and procedures were approved by the University of Georgia Institutional Review Board on Human Subjects.

Folate, Homocysteine and Other Biochemical Indices

Non-fasting blood samples were collected as previously described (1,22). Plasma folate and vitamin B12 and RBC folate were analyzed using Quantaphase II Vitamin B12/Folate Radioassay (Bio-Rad, Richmond, CA). Serum total homocysteine, methylmalonic acid (MMA) and 2-methylcitric acid were analyzed by capillary gas chromatography-mass spectrometry (23–25). Low RBC folate was defined as < 317 nmol/L based on the criteria used by the National Health and Nutrition Examination Survey (NHANES; 12). The 25^{th} percentile for RBC folate for combined racial/ethnic groups ≥ 60 yr from NHANES 1999–2000 (564 nmol/L and 616 nmol/L for men and women, respectively; 11) defined those of 'lower' (< 25^{th} percentile) vs. 'higher' (≥ 25^{th} percentile) RBC folate status. High to Folate Status folate status was defined as plasma folate > 45.3 nmol/L (20 ng/mL; 12,17). Vitamin B12 deficiency was defined as plasma vitamin B12 < 258 pmol/L, serum MMA > 271 nmol/L, and MMA > 2-methylcitric acid (25,26). Hyperhomocysteinemia was defined as serum homocysteine >13.9 μmol/L (27). Serum pepsinogen I was analyzed by enzyme immunoassay (Pepsinogen I ELISA: ALPCO Diagnostics, Windham, NH) and a value of < 60 ng/mL was used as an indicator of moderate to severe atrophic gastritis (28). Hemoglobin and serum creatinine were assessed by a clinical diagnostic laboratory (LabCorp, Inc., Burlington, NC). A value of > 127 umol/L was used as an indicator of poor renal function (26). Anemia was defined as hemoglobin < 12 g/dL for females or < 13 g/dL for males (29).

Demographic, Nutrition and Health Information

Information regarding age, gender, race/ethnicity, living arrangements, health conditions (cardiovascular disease, diabetes, hypertension, etc) and behaviors (including tobacco and nutritional supplement use) were obtained from each participant (or his/her caregiver) by self-report. Participants were classified as taking a B-vitamin supplement if they reported using a multivitamin, or vitamin B12, folic acid and/or other B-vitamin-containing supplement. Participants who had missing data for supplement use were categorized as not receiving supplements. Questions regarding food intake were adapted from the Mini-Nutritional Assessment (30,31) and the response categories represented current frequency of consumption of food groups, including dairy products (milk, yogurt, and cheese); meat, fish, or poultry; orange/yellow vegetables; green vegetables; and citrus and non-citrus fruit and juice. The total food score, ranging from 0 to 5, was based on comparisons with the Dietary Guidelines for Americans (32) 1,600-calorie meal pattern for sedentary older adults, as previously described (31).

Body weight and height were measured by interviewers, obtained from charts or via self-report. Body mass index (BMI) was calculated as weight (pounds)/height (inches)^2 × 703. BMI categories were based on the National Institutes of Health (33) guidelines, and focused on underweight defined as < 18.5 kg/m^2 and overweight/obese defined as ≥ 25 kg/m^2.

Cognitive performance was assessed using the Mini-Mental State Examination (MMSE; 34). Exam scores are based on a 30-point scale, with a higher score indicative of a higher degree of cognitive performance. For the purpose of this study, MMSE scores ≤ 23 were indicative of cognitive impairment and those 24 or above indicative of no impairment (35).

Exclusions from Data Analysis

Participants missing data for primary variables of interest or receiving vitamin B12 injections were excluded from the present analyses. From the original sample of octogenarians (n = 80), two individuals were excluded due to missing blood values (n = 1) or receiving vitamin B12 injections (n = 1). From the original sample of centenarians, 45 individuals were excluded due to missing data for one or more key blood values (n = 27) or receiving vitamin B12 injections (n = 17). Compared to the included centenarians (n = 199), the excluded centenarians did not differ in gender (85.4% vs. 82.2% female), race/ethnicity (71.1% vs. 80.4% white), place of residence (48.9% vs. 41.7% skilled nursing facility), use of B-vitamin containing supplements (33.3% vs. 34.2%), prevalence of low RBC folate (<317 nmol/L; 6.7% vs. 6.5%), or concomitant high folate/low B12 status (2.2% vs. 4.0%). Those excluded, however, were more likely to meet the criteria for lower RBC folate status (66.7% vs. 37.7%, $p < 0.001$).

Statistical Analyses

As is typical of B-vitamin related metabolites, the data were not normally distributed and non-parametric methods were used. Frequencies, means, standard deviations, medians and range of values are reported. Bivariate analyses of covariates within the octogenarian and centenarian samples were conducted separately. Group differences were assessed with the Wilcoxon rank sum test for continuous variables and Chi square analysis for categorical variables (Table 1 and Table 2). Probabilities reported are unadjusted for multiple tests.

Multivariate logistic regression analyses were used to examine associations of low or high folate status with age, gender, race/ethnicity, living arrangements, use of a B-vitamin-containing supplement, total food score, vitamin B12 deficiency, serum pepsinogen, BMI, tobacco use ever, current tobacco use and MMSE. Analyses were conducted using SAS 9.2 and Stata 11.1.

Results

Compared to octogenarians, the centenarians in this study were more likely to be women, live in a skilled nursing facility, have a higher food score, have a lower MMSE score, have a lower BMI, and were less likely to have ever used tobacco products (Table 1). The older group also had significantly lower plasma folate and higher serum homocysteine, MMA and creatinine. The two age groups did not differ in race/ethnicity, use of B-vitamin-containing supplements, plasma vitamin B12, serum pepsinogen, or current tobacco use (Table 1) or in the prevalence of cardiovascular disease (55.8% vs 61.5%, for octogenarians and centenarians, respectively), diabetes (15.8% vs. 9.2%) or hypertension (42.9% vs. 45.1%). However, 6.5% of centenarians as compared with 1.3% of octogenarians had low RBC folate ($p = 0.058$).

To determine potential predictors of those at risk for low RBC folate status, the sample was dichotomized based on the 25^{th} percentile of RBC folate from NHANES 1999–2000 (11). As such, more centenarians than octogenarians had ‘lower’ RBC folate status (37.7% vs. 24.4%, respectively; $p < 0.05$). Among octogenarians ‘lower’ RBC folate status (<25^{th} percentile) was more prevalent in women vs. men, African Americans vs. whites, skilled nursing facility residents vs. community dwellers and in those with lower serum pepsinogen and MMSE scores (Table 2). Octogenarians with ‘lower’ and higher (>25^{th} percentile) RBC folate status did not differ in age, use of B-vitamin containing supplements, total food score, serum creatinine, BMI or tobacco use. Among centenarians, lower RBC folate status (< 25^{th} percentile) was more prevalent in African Americans vs. whites and in those not using B-vitamin containing supplements (Table 2). Centenarians did not differ by folate status in age,

gender, place of residence, total food score, serum pepsinogen or creatinine, BMI, tobacco use or MMSE score. Regardless of age group, those with 'lower' RBC folate status had higher serum homocysteine and MMA, lower plasma vitamin B12, and a greater prevalence of vitamin B12 deficiency as compared to those of 'higher' RBC folate status.

Logistic regression analysis of the total analytical sample indicated that when controlled for other risk factors the odds of having 'lower' RBC folate status were about five times higher in vitamin B12 deficient vs. vitamin B12 adequate participants, four times higher in African Americans vs. whites, and three times higher for those living in skilled nursing facilities vs. in the community (Table 3). Age, gender, B-vitamin supplement use, food score and presence of atrophic gastritis were not related to the probability of being of 'lower' RBC folate status (Table 3). Similar analysis of the centenarians only indicated vitamin B12 deficiency (OR = 4.53; 95% CI: 2.26, 9.08; $p < 0.0001$), African American race/ethnicity (OR = 4.11; 95% CI: 1.78, 9.52; $p = 0.001$) and skilled nursing facility residence (OR = 2.45; 95% CI: 1.05, 5.68; $p = 0.037$) as the primary predictors of 'lower' RBC folate status for this age group (Full model not shown).

Analyses exploring high plasma folate status (> 45.3 nmol/L) indicated an overall prevalence of 35% which is comparable to the 32% prevalence in high folate concentrations reported for older adults in NHANES 2003–2004 (12). However, there was a greater prevalence of high folate status in our octogenarians as compared with centenarians (46.1% vs. 30.7%; $p = 0.015$). Logistic regression analysis of the total sample indicated that when controlled for demographics, diet patterns, supplement use and vitamin B12 status, the probability of high folate status was predicted by vitamin B12 adequacy (OR = 6.22; 95% CI: 2.96, 13.06; $p < 0.0001$), being white (OR = 2.98; 95% CI: 1.28, 6.93; $p = 0.011$), using B-vitamin containing supplements (OR = 2.71; 95% CI: 1.47, 4.99; $p = 0.001$), and living in the community (OR = 2.42; 95% CI: 1.16, 5.06; $p = 0.019$). Similar predictors were observed for centenarians only (data not shown).

Overall, only 3.8% (3 of 78) of the octogenarians and 4.0% (8 of 199) of the centenarians met the criteria for having both high plasma folate status and vitamin B12 deficiency. In the total analytic sample the only predictor of concurrent high plasma folate status and vitamin B12 deficiency was use of B-vitamin containing supplements (OR = 3.54; 95% CI: 1.01, 12.43; $p = 0.048$). In this study, concurrent high plasma folate status and vitamin B12 deficiency was not an independent risk factor for anemia (OR = 0.52; 95% CI: 0.1, 2.4; $p = 0.40$; reference group =low B12, normal folate) or cognitive impairment (MMSE ≤ 23; OR = 0.48; 95% CI: 0.1, 2.4; $p = 0.37$). Use of alternate cut-offs for defining low B12 status (plasma B12 < 148 pmol/L and MMA > 271 [assay reference value; 27] or > 370 nmol/L [12] and high folate status (59 nmol/L; 15–16) resulted in higher proportions of individuals with low B12/high status (6–19%) and associations between vitamin B12 status and anemia for all low vitamin B12 groups, but not specifically for those of low vitamin B12/high folate status (data not shown).

Discussion

To our knowledge, this is the first study to report folate status specifically in the oldest old segment of the U.S. population in the post-fortification period. As expected, the prevalence of low RBC folate (<317 nmol/L) was overall very low, but tended to be higher for centenarians than octogenarians. Strong associations were observed between both low and high folate status and race/ethnicity, living arrangements and vitamin B12 status, albeit in opposite directions. High folate status was also associated with the use of B-vitamin containing supplements. Similar to other recent studies (15,17), we found a small percentage of participants with concurrent high folate status and vitamin B12 deficiency, which was

associated with the use of B-vitamin containing supplements. However, in contrast to previous research (15,20), these individuals did not appear to be at greater risk for anemia or cognitive impairment.

The prevalence of low RBC folate (<317 nmol/L) in this population-based study of centenarians was 6.5% which, not surprisingly, is considerably lower than the prevalence (19–21%) reported for Italian centenarians not exposed to mandatory folic acid fortification (36,37). Nonetheless, the prevalence in Georgia centenarians is higher than for octogenarians and slightly higher than the prevalence of 2.1 to 2.3% for those ≥ 60 yr and 4.0% for those ≥ 70 yr. reported in recent NHANES studies (12,13). Low RBC folate is seen in megaloblastic anemia due to B12 deficiency and thus is not specific to folate status alone. Very few subjects in this study had elevated homocysteine, low serum folate and normal MMA as would be expected in a subject with pure folate deficiency. Most subjects with low RBC folate also had elevated MMA and nine of the 14 participants (64%) with RBC folate < 317 nmol/L were also vitamin B12 deficient. Thus, choosing folate therapy alone for a centenarian with low RBC folate could leave vitamin B12-deficient participants untreated for B12 deficiency.

As previously reported, vitamin B12 deficiency is highly prevalent in this population, particularly among centenarians (2), and vitamin B12 deficiency emerged as a primary predictor of ‘lower’ RBC folate in this study. This is consistent with previous findings in older adults (26) and likely reflects the closely linked intermediary metabolism of folate and vitamin B12 (38,39) as well as dietary and nutrient supplement consumption patterns. Elevated MMA and homocysteine, biochemical markers of vitamin B12 deficiency (40), were also associated with lower RBC folate status. The relationship between folate and homocysteine status in this population is being further explored in our on-going studies.

Race/ethnicity emerged as a primary predictor of folate status, similar to our previous findings regarding vitamin D (1) and vitamin B12 status (2) in this cohort. African Americans were found to have lower folate status, but better vitamin B12 status (2) than whites. This is consistent with the findings of Stabler et al. (27) which indicated that cobalamin deficiency with elevated MMA was more prevalent in elderly white as compared with African American disabled women and that folate deficiency was more prevalent in the African Americans. That study, conducted prior to folic acid fortification in the US, suggested that mandatory food fortification would be important for increasing folic acid intake and improving folate status of African Americans. More recent data indicates that while both serum and RBC folate levels have increased and the prevalence of at risk for low folate has decreased in both whites and blacks since folic acid fortification, differences between blacks and whites persist (13). Our finding of a greater than 4-fold greater odds ratio for lower folate status in African Americans vs. whites, confirms that racial/ethnic disparities extend to even the oldest old and support the contention that specific targeting of high risk populations, rather than food fortification alone, may be needed to eliminate the remaining disparities in folate status (41).

Folic acid fortification, though successful in its original intent of decreasing neural tube defects, has led to concern regarding potential masking of vitamin B12 deficiency and other adverse effects of high folate intake (15–21). Associations between combined low vitamin B12/high folate status and increased circulating concentrations of homocysteine and MMA (16) and a higher prevalence of both anemia and cognitive impairment (15) have been detected among adult participants of recent NHANES studies. In this study, we found a 4% prevalence of vitamin B12 deficiency combined with high folate in Georgia octogenarians and centenarians but no associations of such with anemia or cognitive impairment. The failure to detect specific associations between low vitamin B12/high folate status and these

conditions as had been observed in previous studies conducted post-fortification is likely due not only to the small sample size of the present study but also to the high overall prevalence of both anemia (~45% overall, > 50% for centenarians) and moderate to severe cognitive impairment (18% for octogenarians, 73.4% for centenarians) in the study population. Furthermore, at these advanced ages both conditions are likely of complex and/or non-nutritional etiology as evidenced by a higher prevalence of anemia of chronic disease in centenarian as compared with octogenarian participants of the Georgia Centenarian Study (Haslam et al., in preparation).

In summary, this study examined measures of folate status in a unique population-based sample of older age groups not frequently studied. Strengths of the study are the inclusion of centenarians with diversity in race and place of residence and the comparison group of octogenarians. Limitations of the study include the relatively small sample size, lack of detailed information on folic acid intake from foods and supplements, and possible underreporting of supplement intake. Measurement of these indices in future studies are needed to verify associations, or lack thereof, between intake and supplement use and folate status in this population group.

Acknowledgments

Authors acknowledge the valuable recruitment and data acquisition effort from M. Burgess, K. Grier, E. McCarthy, L. Strong and S. Reynolds, data acquisition team manager; S. Anderson, M. Janke, and T. Savla, data management; M. Poon for project fiscal management and H. Hamner for insightful comment and review of the manuscript.

References

1. Johnson MA, Davey A, Park S, Hausman DB, Poon LW. for the Georgia Centenarian Study. Age, race and season predict vitamin D status in African American and White octogenarians and centenarians. J Nutr Health Aging. 2008; 12:690–695. [PubMed: 19043643]
2. Johnson MA, Hausman DB, Davey A, Poon LW, Allen RH. Stabler SP for the Georgia Centenarian Study. Vitamin B12 dificiency in African American and white octogenarians and centenarians in Georgia. J Nutr Health Aging. 2010; 14:339–345. [PubMed: 20424799]
3. Quadri P, Fragiacomo C, Pezzati R, Zanda E, Forloni G, Tettamanti M, Lucca U. Homocysteine, folate and vitamin B-12 in mild cognitive impairment, Alzheimer disease and vascular dementia. Am J Clin Nutr. 2004; 80:114–122. [PubMed: 15213037]
4. Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, Porcellini E, Licastro F. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr. 2005; 82:636–643. [PubMed: 16155278]
5. Selhub J. The many facets of hyperhomocysteinemia: studies from the Framingham cohorts. J Nutr. 2006; 136:1726S–1730S. [PubMed: 16702347]
6. McCully KS. Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr. 2007; 86 supple:1563S–1568S. [PubMed: 17991676]
7. McNulty H, Pentieva K, Hoey L, Ward M. Homocysteine, b-vitamins and CVD. Proc Nut Soc. 2008; 67:232–237.
8. Forti P, Rietti E, Pisacane N, Olivelli V, Dalmonte E, Mecocci P, Ravaglia G. Blood homocysteine and risk of depression in the elderly. Arch Gerontol Geriatr. 2010; 51:21–25. [PubMed: 19646770]
9. Nilsson K, Gustafson L, Hutberg B. Plasma homocysteine, cobalamin/folate status, and vascular disease in a large population of psychogeriatric patients. Dement Geriatr Cogn Disord. 2006; 22:358–366. [PubMed: 16954692]
10. Snowdon DA, Tully CL, Smith CD, Perez Riley K, Markesbery WR. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun Study. Am J Clin Nutr. 2000; 71:993–998. [PubMed: 10731508]
11. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and

Nutrition Examination Survey 1999–2000. Am J Clin Nutr. 2005; 82:442–450. [PubMed: 16087991]

12. Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, Fisher KD, Mulinare J, Osterloh JD. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988–2004. Am J Clin Nutr. 2007; 86:718–727. [PubMed: 17823438]
13. Ganji V, Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: Analysis of data from National Health and Nutrition Examination Surveys, 1988–1994, 1999–2000 and 2001–2002. J Nutr. 2006; 136:153–158. [PubMed: 16365075]
14. McDowell MA, Lacher DA, Pfeiffer CM, Mulinare J, Picciano MF, Rader JI, Yetley EA, Kennedy-Stephenson J, Johnson CL. Blood folate levels: the latest NHANES results. NCHS Date Brief. 2008 May.:1–8.
15. Morris MS, Jacques PF, Rosenberg IH, Selhub. Folate and vitaminB-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of golic acid fortification. Am J Clin Nutr. 2007; 85:193–200. [PubMed: 17209196]
16. Selhub J, Morris MS, Jacques PF. In vitamin B-12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. PNAS. 2007; 104:19995–20000. [PubMed: 18056804]
17. Ray JG, Vermeulen MJ, Langman LJ, Boss SC, Cole DEC. Persistence of vitamin B12 insufficiency among elderly women after folic acid fortification. Clin Biochem. 2003; 36:386–391.
18. Brouwer I, Verhoef P. Folic acid fortification: is masking of vitamin B-12 deficiency what we should really worry about? Am J Clin Nutr. 2007; 86:897–898. [PubMed: 17921362]
19. Wyckoff KF, Ganji V. Proportion of individuals with low serum vitamin B-12 concentrations without macrocytosis is higher in the post-folic acid fortification period than in the pre-folic acid fortification period. Am J Clin Nutr. 2007; 86:1187–1192. [PubMed: 17921401]
20. Selhub J, Morris MS, Jacques PF, Rosenberg IH. Folate-vitamin B-12 interaction in relation to cognitive impairment, anemia and biochemical indicators of vitamin B-12 deficiency. Am J Clin Nutr. 2009; 89:702–706S.
21. Miller JW, Garrod MG, Allen LH, Haan MN, Green R. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methlymalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. Am J Clin Nutr. 2009; 90:1586–1592. [PubMed: 19726595]
22. Poon LW, Jazwinski SM, Green RC, Woodard JL, Martin P, Rodgers WL, Johnson MA, Hausman DB, Arnold J, et al. Methodological considerations in studying centenarians: lessons learned from the Georgia centenarian studies. Ann Rev Gerontology and Geriatrics. 2007; 27:213–264.
23. Stabler SP, Marcell PD, Podell ER, Allen RH, Lindenbaum J. Assay of methylmalonic acid in the serum of patients with cobalamin deficiency using capillary gas chromatography-mass spectrometry. J Clin Invest. 1986; 77:1610–1612.
24. Stabler SP, Marcell PD, Podell ER, Allen RH, Savage DG, Lindenbaum J. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. J Clin Invest. 1988; 81:466–474. [PubMed: 3339129]
25. Allen RH, Stabler SP, Savage DG, Ldenbaum J. Elevation of 2-methylcitric acid I and II levels in serum, urine, and cerebrospinal fluid of patients with cobalamin deficiency. Metabolism. 1993; 42:978–988. [PubMed: 8345822]
26. Johnson MA, Hawthorne NA, Brackett WR, Fischer JG, Gunter EW, Allen RH, Stabler SP. Hyperhomocysteinemia and vitamin B-12 deficiency in elderly using Title IIIc nutrition services. Am J Clin Nutr. 2003; 77:211–220. [PubMed: 12499344]
27. Stabler SP, Allen RH, Fried LP, Pahor M, Kittner SJ, Penninx BWJH, Gurlanik JM. Racial differences in prevalence of cobalamin and folate deficiencies in disabled elderly women. Am J Clin Nutr. 1999; 70:911–919. [PubMed: 10539754]
28. Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relations among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology: a study in relatives of patients with pernicious anemia. Gastroenterology. 1982; 83:204–209. [PubMed: 7084603]

29. Blanc B, Finch CA, Hallberg L, et al. Nutritional anaemias. Report of a WHO Scientific Group. WHO Tech Rep Ser. 1968; 405:1–40.
30. Guigoz Y, Vellas B, Garry PJ. Assessing the nutritional status of the elderly: the Mini-Nutritional Assessment as part of the geriatric evaluation. Nutr Rev. 1996; 54:59–65.
31. Johnson MA, Davey A, Hausman DB, Park S, Poon LW. for the Georgia Centenarian Study. Dietary differences between centenarians residing in communities and in skilled nursing facilities:the Georgia Centenarian Study. Age. 2006; 28:333–341.
32. US Department of Health and Human Services and US Department of Agriculture (2005). Dietary Guidelines for Americans. 2005. Available online at http://www.health.gov/dietaryguidelines/dga2005/document/pdf/DGA2005.pdf
33. National Institutes of Health: National Heart Lung and Blood Institute, North American Association for the Study of Obesity. Practical Guide to the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. 2000 [[cited; 1–80]]. Available from: http://www.nhlbi.nih.gov/guidelines/obesity/prctgd_c.pdf
34. Folstein MF, Folstein SE, McHugh PR. “Mini-Mental State”: a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12:189–198. [PubMed: 1202204]
35. Folstein, MF.; Folstein, SE.; McHugh, PR.; Fanjiang, G. Mini-Mental State Examination user’s guide. Odessa, FL: Psychological Assessment Resources; 2001.
36. Ravaglia G, Forti P, Maioli F, Vettori C, Grossi G, Bargossi AM, Caldarera M, Franceschi C, Facchini A, Mariani E, Cavalli G. Elevated plasma homocysteine levels in centenarians are not associated with cognitive impairment. Mech Ageing Dev. 2000; 121:251–261. [PubMed: 11164478]
37. Malaguarnera M, Pistone G, Motta M, Vinci E, Oreste G, Avellone G, Musumeci S. Elevated plasma total homocysteine in centenarians. Clin Chem Lab Med. 2004; 42:307–310. [PubMed: 15080565]
38. Institute of Medicine. Food and Nutrition Board. National Academy of Sciences. Washington DC: National Academy Press; 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline.
39. Stover PJ. One-carbon metabolism-genome interactions in folate-associated pathologies. J Nutr. 2009; 139:2402–2405. [PubMed: 19812215]
40. Stabler, SP. Vitamin B12. In: Bowan, BA.; Russell, RM., editors. Present Knowledge in Nutrition. 9th edition. Vol. Volume II. Washington, DC: International Life Sciences Institute; 2006.
41. Dowd JB, Aiello AE. Did national folic acid fortification reduce socioeconomic and racial disparities in folate status in the US? Int J Epidemiol. 2008; 37:1059–1066. [PubMed: 18456713]

Table 1

Demographics, B-vitamin Supplements, Dietary Patterns, Biochemical Indices and Other Factors Related to Folate Status in Octogenarians and Centenarians: The Georgia Centenarian Study[1]

	Octogenarians Median, range, Mean ± SD, or % [n=78]	**Centenarians Median, range, mean ± SD, or % [n=199]**
Age	83.6, 80.5–90.0 84.4 ± 2.8	100.1, 98.1–108.5 100.5 ± 2.0***
Gender		
Women	65.4 [51]	85.4 [170]***
Men	34.6 [27]	14.6 [29]
Race		
White	82.0 [64]	80.4 [160]
African American	18.0 [14]	19.6 [39]
Living arrangements		
Skilled nursing facility	14.1 [11]	41.7 [83]***
Community	85.9 [67]	58.3 [116]
B-vitamin supplements[2]		
No	68.0 [53]	65.8 [131]
Yes	32.0 [25]	34.2 [68]
Total food score[3]	2.0, 0–5 2.4 ± 1.1	3.0, 0–5 3.1 ± 1.6***
< 3	65.4 [51]	47.5 [94]*
≥ 3	34.6 [27]	52.5 [104]
Plasma folate (nmol/L)	43.3, 10.7–160 52.2 ± 31.5	29.2, 4.1–156 38.7 ± 26.1***
< 6.8	0	1.0 [2]
Red cell folate (nmol/L)	868, 267–2088 915 ± 400	776, 146–2558 848 ± 456
< 317 nmol/L	1.3 [1]	6.53 [13]
Lowest quartile (*based on NHANES-folate*)		
≤ 25%tile	24.4 [19]	37.7 [75] *
> 25%tile	75.6 [59]	62.3 [124]
Homocysteine (μmol/L)	10.4, 6.2–29.1 10.9 ± 3.7	13.6, 6.4–72.3 14.6 ± 6.9***
> 13.9	15.4 [12]	46.7 [93]***
Vitamin B12 (pmol/L)	290, 74–847 315 ± 140	314, 74–2285 361 ± 257
Vitamin B12 deficient[4]		
Yes	23.1 [18]	36.2 [72]*
No	76.9 [60]	63.8 [127]
Methylmalonic acid (nmol/L)	284, 144–1261	384, 163–8078

	Octogenarians Median, range, Mean ± SD, or % [n=78]	**Centenarians Median, range, mean ± SD, or % [n=199]**
	351 ± 216	524 ± 644***
> 271 nmol/L	57.7 [45]	79.9 [159]***
Pepsinogen (ng/ml)	78.3, 1.29–296 99.2 ± 72.4	78.4, 1.2–300 92.2 ± 69.1 [192]
< 10 (severe atrophic gastritis)	7.8 [6]	5.6 [11]
10 to < 60 (moderate atrophic gastritis)	19.5 [15]	29.6 [58]
≥ 60 (atrophic gastritis not present)	72.7 [56]	64.8 [127]
Creatinine (μmol/L)	79.6, 53.0–186 86 .0 ± 24.8	88.4, 35.4–486 95.8 ± 42.9*
> 127 μmol/L	6.4 [5]	15.8 [31]*
BMI, kg/m^2	26.0, 17.4–39.7 26.0 ± 4.3 [75]	22.4, 14.6–35.3 22.7 ± 4.3 [188]***
≥ 25	58.7 [44]	26.3 [51]***
Tobacco use, ever	57.7 [45]	28.6 [56]***
Tobacco use, current	6.4 [5]	2.6 [5]
MMSE score	27, 0–30 24.6 ± 7.8	17, 0–30 16.7 ± 8.6 ***
< 23	18.0 [14]	73.9 [147]***
≥ 23	82.0 [64]	26.1 [52]

*1*Participants receiving vitamin B12 injections or missing data for plasma folate, RBC folate, serum vitamin B12, serum homocysteine, serum methylmalonic acid or supplement intake were excluded. Differences between octogenarians and centenarians that were statistically significant are noted as follows:

*$p < 0.05$,

**$p < 0.01$,

***$p < 0.001$.

*2*B-vitamin supplements included multivitamin/mineral, B-vitamins, or single oral supplements of vitamin B12.

*3*Total food score ranged from 0 to 5 and one point was given for each of the following five food groups: two or more servings of meat, poultry, or fish daily, two or more servings of dairy foods daily, three or more servings of fruit daily, three or more servings of orange or yellow vegetables weekly, and four or more servings of green vegetables weekly.

*4*Vitamin B12 deficiency defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > serum 2-methylcitric acid.

Table 2

Relationships with Demographics, B-vitamin Supplements, Dietary Patterns, Biochemical and Hemotological Indices, and Cognitive Status in Octogenarians and Centenarians: The Georgia Centenarian Study − 1st vs. all other quartiles of RBC folate [1,2]

	Octogenarians Median, range, mean ± SD, or % [n]		Centenarians Median, range, mean ± SD, or % [n]	
	1st quartile folate	All Others	1st quartile folate	All Others
Age	83.2, 80.8–90.0 84.6 ± 3.1 [19]	83.6, 80.5–90.0 84.3 ± 2.7 [59]	100.3, 98.1–106.0 100.6 ± 2.0 [75]	100.1, 98.1–108.5 100.4 ± 2.0 [124]
Gender				
Women	31.4 [16] *	68.3 [35]	36.4 [62]	63.5 [108]
Men	11.1[3]	88.9 [24]	44.8 [13]	55.2 [16]
Race				
White	18.8 [12] *	81.2 [52]	33.1 [53] **	66.9 [107]
African American	50.0 [7]	50.0 [7]	56.4 [22]	43.6 [17]
Living arrangements				
Skilled nursing facility	63.6 [7] **	36.4 [4]	43.4 [36]	56.6 [47]
Community	17.9 [12]	82.1 [55]	33.6 [39]	66.4 [77]
B-vitamin supplements[3]				
No	73.7 [14]	66.1 [39]	76.0 [57] *	59.7 [74]
Yes	26.3 [5]	33.9 [20]	24.0 [18]	40.3 [50]
Total food score[4]	2, 0–5 2.5 ± 1.3	2, 1–5 2.3 ± 1.1	3, 0–5 3.1 ± 1.6	3, 0–5 3.1 ± 1.5
< 3	63.2 [12]	66.1 [39]	48.0 [36]	47.2 [58]
≥ 3	36.9 [7]	33.9 [20]	52.0 [39]	52.8 [65]
Plasma folate (nmol/L)	21.4, 10.7–114.5 27.7 ± 22.1***	52.2, 21.9–160.0 60.6 ± 30.0	19.4, 4.1–65.9 20.4 ± 9.2***	43.5, 10.9–156.1 49.9 ± 26.8
< 6.8	0 [0]]	0 [0]	2.7 [2]	0 [0]
Red cell folate (nmol/L)	479, 267–612 463 ± 102***	996, 571–2088 1061 ± 348	441, 146–610 435 ± 113***	1046, 597–2558 1097 ± 400
< 317 nmol/L	5.3 [1]	0 [0]	17.3 [13] ***	0 [0]
Homocysteine (nmol/L)	11.3, 8.4–29.1 13.5 ± 5.3**	9.8, 6.2–17.4 10.0 ± 2.5	15.9, 8.0–72.3 17.6 ± 9.0***	11.8, 6.4–28.8 12.8 ± 4.5
> 13.9	36.8 [7] **	8.5 [5]	69.3 [52] ***	33.1 [41]
Vitamin B12 (pmol/L)	231, 74–385 225 ± 97**	327, 136–847 344 ± 141	235, 74–2286 291 ± 283***	364, 74–1288 404 ± 230
Vitamin B12 deficient[5]				
Yes	52.6 [10] ***	13.6 [8]	53.3 [40] ***	25.8 [32]
No	47.4 [9]	86.4 [51]	46.7 [35]	74.2 [92]
Methylmalonic acid (nmol/L)	338, 154–1130 469 ± 307*	271, 144–1261 313 ± 164	423, 174–8078 678 ± 981**	359, 163–1975 431 ± 254

	Octogenarians Median, range, mean ± SD, or % [n]		Centenarians Median, range, mean ± SD, or % [n]	
	1st quartile folate	All Others	1st quartile folate	All Others
> 271 nmol/L	79.0 [15] *	50.8 [30]	84.0 [63]	77.4 [96]
Pepsinogen (ng/ml)	61.5, 1.3–296 72.1 ± 69.8*	85.4, 9.2–278 107.5 ± 71.7	81.3, 1.2–300 93.2 ± 67.7	74.4, 3.2–264 91.5 ± 70.3
< 10 (severe atrophic gastritis)	16.7 [3] *	5.1 [3]	5.3 [4]	5.8 [7]
10 to < 60 (mod atrophic gastritis)	33.3 [6]	15.2 [9]	26.7 [20]	31.4 [38]
≥ 60 (atroph gastritis not present)	50.0 [9]	79.7[47]	68.0 [51]	62.8 [76]
Creatinine (μmol/L)	79.6, 53.0–185.6 91.2 ± 35.0	88.4, 53.0–167.9 84.4 ± 20.6	88.4, 35.4–185.6 93.8 ± 32.7	88.4, 44.2–486.2 97.0 ± 47.9
> 127 μmol/L	15.8 [3]	3.4 [2]	18.1 [13]	14.5 [18]
BMI, kg/m^2	26.6, 21.9–34.7 26.9 ± 3.2	25.7, 17.4–39.7 25.7 ± 4.6	22.5, 15.1–35.3 22.9 ± 4.7	22.3, 14.6–35.0 22.5 ± 4.1
≥ 25	76.5 [13]	54.4 [31]	31.1 [23]	23.3 [28]
Tobacco use, ever	47.4 [9]	61.0 [36]	31.1 [23]	27.0 [33]
Tobacco use, current	5.3 [1]	6.8 [4]	2.7 [2]	2.5 [3]
MMSE score	27, 0–30 21.6 ± 9.5	28, 0–30 25.5 ± 7.0	17, 0–30 16.1 ± 8.9	18, 0–30 17.0 ± 8.4
≤ 23	36.8 [7] *	11.9 [7]	74.7 [56]	73.4 [91]
> 23	63.2 [12]	88.1 [52]	25.3 [19]	26.6 [33]

1 Participants receiving vitamin B12 injections or missing data for plasma folate, RBC folate, serum vitamin B12, serum homocysteine, serum methylmalonic acid or supplement intake were excluded.

2 Comparisons were made within each age group between individuals below or above the 25th percentile for RBC folate for participants 60 and over in NHANES 1999–2000 (564 nmol/L and 616 nmol/L for men and women, respectively; Pfeiffer et al., 2005). Differences between folate status groups within an age were determined by Wilcoxon rank sum test and Chi square analysis for continuous and categorical data, respectively and statistical significane is noted as follows:

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$.

3 B-vitamin supplements included multivitamin/mineral, B-vitamins, or single oral supplements of vitamin B12.

4 Total food score ranged from 0 to 5 and one point was given for each of the following five food groups: two or more servings of meat, poultry, or fish daily, two or more servings of dairy foods daily, three or more servings of fruit daily, three or more servings of orange or yellow vegetables weekly, and four or more servings of green vegetables weekly.

5 Vitamin B12 deficiency is defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > serum 2-methylcitric acid.

Table 3

Logistic Regression Model Predicting 'Lower' RBC Folate Status in Octogenarians and Centenarians: The Georgia Centenarian Study[1,2]

Variable	b	LR χ^2	p(χ^2)	Odds Ratio	95% CI	
Centenarians vs. octogenarians	0.36	1.02	0.3120	1.43	0.71	2.90
Female vs. male	0.13	0.11	0.7407	1.13	0.54	2.40
African American vs. White	1.46	16.32	0.0001	4.29	2.08	8.83
Skilled nursing facility vs. community	1.18	10.28	0.0013	3.25	1.56	6.78
B-vitamin supplements: yes vs. no[3]	−.56	2.84	0.0920	0.57	0.30	1.10
Food score: high vs. low[4]	−.43	1.48	0.2232	0.65	0.32	1.31
B12 deficient vs. B12 adequate[5]	1.68	30.27	< 0.0001	5.36	2.87	10.01
Atrophic gastritis: not present vs. moderate or severe[6]	−.25	0.61	0.4333	0.78	0.42	1.45
Intercept	−1.94					
LR $\chi^2(8)$	62.66					

[1] Participants receiving vitamin B12 injections or missing data for plasma folate, RBC folate, serum vitamin B12, serum homocysteine, serum methylmalonic acid or supplement intake were excluded.

[2] 'Lower' folate status was defined as having an RBC folate below the 25th percentile for participants 60 and over in NHANES 1999–2000 (564 nmol/L and 616 nmol/L for men and women, respectively; Pfeiffer et al., 2005).

[3] B-vitamin supplements included multivitamin/mineral, B-vitamins, or single oral supplements of vitamin B12.

[4] For determination of total food score one point was given for each of the following five food groups: two or more servings of meat, poultry, or fish daily, two or more servings of dairy foods daily, three or more servings of fruit daily, three or more servings of orange or yellow vegetables weekly, and four or more servings of green vegetables weekly. Values ranged from 0 to 5 and were dichotomized as < 3 (low) vs. ≥ 3 (high).

[5] Vitamin B12 deficiency is defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > serum 2-methylcitric acid.

[6] Severity of atrophic gastritis was assessed from serum pepsinogen I concentrations, defined as not present (≥ 60 ng/ml), moderate (10 to < 60 ng/ml), or severe (< 10 ng/ml) and coded as 0 (present) or 1 (moderate or severe).