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VITAMIN B12 DEFICIENCY IN AFRICAN AMERICAN AND WHITE OCTOGENARIANS AND CENTENARIANS IN GEORGIA

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Abstract

Objective—Test the hypotheses that vitamin B12 deficiency would be prevalent in octogenarians and centenarians and associated with age, gender, race/ethnicity, living arrangements (community or skilled nursing facility), animal food intake, B-vitamin supplement use, atrophic gastritis, folate status, and hematological indicators.

Design—Population-based multi-ethnic sample of adults aged 80 to 89 and 98 and above.

Setting—Northern Georgia in the United States.

Participants—Men and women aged 80 to 89 (octogenarians, n = 80) and 98 and older (centenarians, n = 231).

Measurements—Wilcoxon signed-rank tests, Fisher's exact tests, and logistic regression analysis was used to examine the associations of vitamin B12 status with the variables of interest.

Results—After excluding participants receiving vitamin B12 injections (n = 17), the prevalence of vitamin B12 deficiency was higher in centenarians than in octogenarians (35.3% vs. 22.8%, $p < 0.05$, defined as plasma vitamin B12 < 258 pmol/L and serum methylmalonic acid > 271 nmol/L and methylmalonic acid > serum 2-methylcitrate) and in both age groups was correlated with significantly higher homocysteine ($p < 0.05$) and lower plasma and red cell folate ($p < 0.01$), but was not related to hemoglobin, anemia, mean cell volume, or macrocytosis. In logistic regression analysis, the probability of being vitamin B12-deficient was significantly increased by being a centenarian vs.

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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.

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 (University of Kentucky School of Medicine), William L. Rodgers (University of Michigan), Christopher Rott (University of Heidelberg), Ilene C. Siegler (Duke University), J. Lisa Tenover (Emory University School of Medicine), and John L. Woodard (Wayne State University).

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octogenarian ($p < 0.03$), by being white vs. African American ($p < 0.02$), by increasing severity of atrophic gastritis ($p < 0.001$), and by not taking oral B-vitamin supplements ($p < 0.01$), but was not related to gender, living arrangements, or animal food intake.

Conclusions—Centenarians and octogenarians are at high risk for vitamin B12 deficiency for many of the same reasons identified in other older adult populations. Given the numerous potential adverse consequences of poor vitamin B12 status, efforts are needed to ensure vitamin B12 adequacy in these older adults.

Introduction

Centenarians are a growing population, but little is known about their nutritional status (1,2). Nutrition related factors that have been explored in centenarians include low energy intakes (3), antioxidant status (4–6), and cardiovascular diseases in centenarians and their offspring (7–10). We reported in a population-based study of centenarians that the prevalence of vitamin D deficiency was 8.9% (< 25 nmol/L 25-hydroxyvitamin D) and of vitamin D insufficiency was 36.7% (< 50 nmol/L) (2). Poor vitamin D status was associated with being African American vs. white, not taking vitamin D-containing supplements, and having vitamin D status measured in the spring vs. other seasons (2). In contrast to our findings, the vast majority of Italian centenarians were vitamin D deficient (11), suggesting that dietary and/or environmental factors may contribute to differences in micronutrient status in the oldest old from different countries.

Little attention has been given in centenarians to other common nutritional problems, such as poor vitamin B12 status, which affects about 10% to 15% of older adults (12,13). Risk factors for vitamin B12 deficiency are related to several demographic, dietary, and age-related health factors, and may be under diagnosed (12–26). Neurologic and/or hematologic disorders are the most well known signs of vitamin B12 deficiency (12,13). As reviewed by Baik and Russell (13), the likelihood of vitamin B12-related neurologic disorders increases with age and can involve the spinal cord, peripheral nerves, optic nerves, and cerebrum and lead to sensory disturbances in the extremities (paresthesia or numbness), gait ataxia, cognitive impairment, and mood changes; treatment delay decreases the likelihood that the neurologic symptoms can be reversed. Hematologic disorders include macrocytic anemia that may be accompanied by neutropenia and thrombocytopenia, because vitamin B12 deficiency affects these rapidly dividing cells in the bone marrow (13); the hematologic complications of vitamin B12 deficiency are reversed with vitamin B12 treatment. There is concern that diagnosis based on hematologic abnormalities alone could lead clinicians to miss some cases of deficiency, as anemia and macrocytosis are not reliable indicators of vitamin B12 deficiency (19–21,26). Markers of vitamin B12 status are serum or plasma vitamin B12 and methylmalonic acid. Total homocysteine is also a marker for vitamin B12 as well as folate status. Elevated methylmalonic acid is specifically associated with vitamin B12 deficiency, while elevated homocysteine is associated with poor folate as well as poor vitamin B12 status (12,13,20). In addition, serum pepsinogen can be used as a biomarker of atrophic gastritis (27). Atrophic gastritis decreases the production of the acid and digestive enzymes needed to cleave protein-bound vitamin B12 from the natural chemical form of the vitamin found in food (12,13). Atrophic gastritis affects up to 30% of older adults and is the most common cause of vitamin B12 deficiency (12,13).

The primary objective of this study was to determine the 1) prevalence of poor vitamin B12 status and 2) factors associated with poor vitamin B12 status in a population based multi-ethnic sample of adults aged 80 to 89 and 98 and above from northern Georgia in the US. It was hypothesized that 1) vitamin B12 deficiency would be prevalent and 2) older age, female gender, white race/ethnicity, living arrangements (skilled nursing facility), lower animal food intake, non-use of B-vitamin supplements, presence of atrophic gastritis, lower plasma folate

and red cell folate, and abnormal hematological indicators would be associated with vitamin B12 status.

Methods

Study Population

Study participants were part of the Georgia Centenarian Study, a representative population-based multidisciplinary study of centenarians conducted in 44 counties in northern Georgia (USA) from 2002 to 2005. The study included 244 centenarians (defined in this study as age 98 and older) and 80 octogenarians recruited from the community, personal care homes and skilled nursing facilities. The sampling procedures and data collection methods have been described elsewhere (1,2). Briefly, the proportion of participants from each age group recruited from skilled nursing facilities was based on estimates of the “institutionalized” population of the study area according to the 2000 U.S. Census figures. The remaining “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 non-Latino) 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.

Vitamin B12, Folate, and Other Biochemical Indices

Non-fasting blood samples were collected as previously described (1,2). Plasma vitamin B12, plasma folate, and red blood cell folate were analyzed using a radioassay (Quantaphase II Vitamin B12/Folate Radioassay; Bio-Rad, Richmond, CA). Serum methylmalonic acid, total homocysteine, 2-methylcitric acid, and cystathionine were analyzed by capillary gas chromatography-mass spectrometry (29–30). Vitamin B12 deficiency was defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > 2-methylcitric acid (23,30). Serum pepsinogen I was analyzed by an enzyme immunoassay (Pepsinogen I ELISA; ALPCO Diagnostics, Windham, NH) and used to define the severity of atrophic gastritis: not present (≥ 60 ng/ml), moderate (10 to < 60 ng/ml), and severe (< 10 ng/ml) (27). Hematological indicators (hemoglobin and mean cell volume), ferritin, and creatinine were assessed by a clinical diagnostic laboratory (LabCorp, Inc., Burlington, NC). Anemia was defined as hemoglobin < 120 g/L in women and < 130 g/L in men (31), and macrocytosis as MCV > 100 fl (23).

Demographic, Nutrition, and Health Information

Participants (or the caregivers of cognitively impaired participants) were asked to provide information about age, gender, race/ethnicity, living arrangements (community or skilled nursing facility), health conditions, activities of daily living, and the names of their current prescription and non-prescription medications, which were used to determine whether participants received injections of vitamin B12 or used a B-vitamin supplement (defined for the purposes of this study as using a vitamin B12- or folate-containing supplement such as a multivitamin and/or other B-vitamin-containing supplement) (1). The frequency of use of supplements and the amount of vitamin B12, folate, and other nutrients in supplements were not determined. Questions regarding food intake were adapted from the Mini-Nutritional Assessment (2,32) and were “How many servings of milk, yogurt, and cheese does this individual usually consume?” and “How many servings of meat, poultry, or fish does this individual usually consume?” Response categories were frequency per day or per week; responses from the two questions were summed to create the variable “animal food intake” and dichotomized (0 is < 2 servings daily, 1 is ≥ 2 servings daily).

Exclusions from Data Analysis

Missing data in the original sample of octogenarians (n = 80) were n = 1 methylmalonic acid, 1 2-methylcitrate, 1 cystathionine, 1 pepsinogen, 1 serum creatinine, and 1 ferritin. Because the 1 individual missing both methylmalonic acid and 2-methylcitrate had a plasma vitamin B12 concentration of 605 pmol/L, they were categorized as vitamin B12-adequate. One participant receiving vitamin B12 injections was excluded from most analyses, yielding an analytic sample of 79 participants (of whom 4 were missing data for at least one variable of interest).

Missing data in the total sample of centenarians (n = 244) were n = 10 plasma vitamin B12, 20 serum methylmalonic acid, 19 2-methylcitrate, 19 cystathionine, 19 homocysteine, 14 plasma folate, 16 red cell folate, 18 pepsinogen, 17 creatinine, 21 ferritin, 10 hemoglobin, 10 MCV, and 10 animal foods. The 12 participants who provided plasma vitamin B12 but who were missing methylmalonic acid and/or 2-methylcitrate were categorized as follows: vitamin B12-deficient if plasma vitamin B12 = 73.7 pmol/L (n = 1) or vitamin B12 < 258 pmol/L and pepsinogen = 15 ng/ml (n = 1); vitamin B12-adequate if vitamin B12 > 258 pmol/L (n = 7); or not categorized if vitamin B12 ranged from 188 to 226 pmol/L (n=3). Participants (n = 13) that could not be categorized as either vitamin B12-deficient or adequate were excluded from all analyses yielding a sample of 231. Unless otherwise stated, centenarians that received vitamin B12 injections (n = 16) were excluded yielding an analytic sample of 215 (of whom 30 were missing data for at least one variable of interest). Compared to the included participants (n = 215), the excluded participants (n = 29) did not differ in gender or race/ethnicity, but were more likely to reside in a skilled nursing facility (40% vs. 66%, $p < 0.01$).

Statistical Analyses

As is typical of B-vitamin related metabolites, the data were not normally distributed and non-parametric methods were used. Because there is no consensus on the definition of vitamin B12 deficiency or for abnormal concentrations of pepsinogen for assessment of atrophic gastritis, methylmalonic acid, homocysteine, and related metabolites, several cut-offs were reported as proposed by others (12–20,23,26,27). Frequencies, means, standard deviations, medians and ranges are reported. Spearman correlation analyses were used to explore the relationship of plasma vitamin B12 status with plasma and red cell folate. Because of the unique nature of the centenarian sample, bivariate analyses of the relationships of the covariates within the octogenarian and within the centenarian samples were conducted separately (Tables 1 and 2). Group differences were assessed with the Wilcoxon rank sum test for continuous variables and with Fischer's exact test for categorical variables. Probabilities reported are unadjusted for multiple tests. Logistic regression analysis was used to explore potential predictors of vitamin B12 deficiency that included age, gender, race/ethnicity, living arrangements, using a B-vitamin supplement, animal food intake, and severity of atrophic gastritis (Table 3). Analyses were conducted with the SAS statistical software (version 9.1, SAS Institute, Cary, NC). $P \leq 0.05$ was considered statistically significant.

Results

Unless otherwise stated, the results refer to participants not receiving vitamin B12 injections. With respect to general indicators of health in these octogenarians and centenarians [sample size], 57% [79] and 61% [215], respectively, reported a history of cardiovascular disease, 28% [79] and 29% [215] reported a history of cancer, 18% [79] and 15% [215] reported a history of depression, median physical activities of daily living scores were 14 [78] and 13 [196] [15 point scale], median instrumental activities of daily living scores were 13.5 [78] and 10 [196] [14 point scale], median Mini-mental Status Exam scores were 28 [79] and 17 [215] [30 point scale (33)], and median body mass index was 25.9 kg/m² [76] and 22.4 kg/m² [217];

centenarians receiving [n = 215] vs. not receiving vitamin B12 injections [n = 16] did not differ significantly in these health indices.

Characteristics that are the focus of this study of the octogenarians and centenarians are shown in Tables 1 and 2. The two age groups did not differ in race/ethnicity, their use of B-vitamin supplements, animal food intake, plasma vitamin B12, serum pepsinogen, serum ferritin, or mean cell volume (MCV) (Table 1). However, compared to octogenarians the centenarians were significantly more likely to be women and to reside in a skilled nursing facility. The older age group also had significantly higher serum methylmalonic acid, 2-methylcitric acid, cystathionine, homocysteine, creatinine, and prevalence of anemia, and significantly lower plasma and red cell folate, and hemoglobin.

Differences in the variables of interest between the vitamin B12-deficient and vitamin B12-adequate participants within each age group were assessed (Table 2). The prevalence of vitamin B12 deficiency was significantly higher in centenarians than in octogenarians (35.3% vs. 22.8%, $p < 0.05$, Table 2). Within each age group the vitamin B12-deficient group compared to the adequate group had significantly lower plasma vitamin B12, plasma folate, red cell folate, and pepsinogen, along with higher serum methylmalonic acid, 2-methylcitric acid, and homocysteine. Within the octogenarians, but not the centenarians, the vitamin B12-deficient group had significantly lower ferritin. Within the centenarians, but not the octogenarians, the vitamin B12-deficient group had significantly higher cystathionine, were more likely to be white than African American, and were less likely to use B-vitamin supplements. Serum creatinine, hemoglobin, anemia, MCV, and macrocytosis were not significantly different between the vitamin B12-deficient and the vitamin B12-adequate individuals in either age group. Hematological status remained unassociated with vitamin B12 deficiency in analyses controlled for potentially confounding factors (age, gender, race/ethnicity, living arrangements, ferritin, and creatinine, data not shown).

Plasma vitamin B12 was strongly correlated with plasma and red cell folate in the octogenarians ($\rho = 0.40$ and $\rho = 0.45$, respectively, $p < 0.001$) and in the centenarians ($\rho = 0.36$ and $\rho = 0.47$, respectively, $p < 0.001$).

In logistic regression analysis, gender, living arrangements, and animal food intake were not related to the probability of vitamin B12 deficiency. However, when controlled for other risk factors, the probability of being vitamin B12-deficient was 2.6 times higher for each category of increased severity of atrophic gastritis, and was about two times higher in centenarians vs. octogenarians, and about two times higher in whites vs. African Americans, but was about 50% less likely in those taking B-vitamin supplements (Table 3).

Among centenarians reporting vitamin B12 injections (n = 16, 7 community dwelling, 9 skilled nursing facility), 15 were vitamin B12-adequate (> 258 pmol/L, median: 503, mean $\pm$ SD: 1250 $\pm$ 1309, range 270–4830) and 1 remained vitamin B12-deficient. Among the 15 participants who received vitamin B12 injections and met our criteria of biochemical vitamin B12 adequacy, there were 7 with abnormally high methylmalonic acid (> 271 nmol/L), 2 with elevated homocysteine (> 13.9 mol/L), 11 with anemia, and 2 with macrocytosis.

If the centenarians receiving vitamin B12 injections (n = 16) are included, then the prevalence of vitamin B12 deficiency was 39.8% assuming that all had been deficient based on a probable previous diagnosis of vitamin B12 deficiency ($16+76/231 = 39.8\%$) and the prevalence was significantly higher than in the octogenarians (1 received injections; $1+18/80$, 23.8%); the prevalence of vitamin B12 deficiency was still higher in centenarians than in octogenarians ($p < 0.05$). Alternatively, including those receiving vitamin B12 injections and using our biochemical definition of vitamin B12 status led to a prevalence of vitamin B12 deficiency of 33.3% in centenarians (1 of 16 was biochemically deficient: $1+76/231 = 33.3\%$), which was

significantly higher than in the octogenarians (1 received injections and was biochemically adequate; 18/80, 22.5%) ($p < 0.05$).

Discussion

To our knowledge, this is the first reported study of the vitamin B12 status of centenarians in the US. Although the prevalence of vitamin B12 deficiency in these centenarians was higher than the octogenarians and many other population based samples of older people (13), we found no evidence that vitamin B12 status was associated with anemia in either age group. Other findings include the strong association of vitamin B12 status with race/ethnicity, B-vitamin supplement use, and atrophic gastritis, but not with gender, living arrangements, or animal food intake.

The prevalence of vitamin B12 deficiency seen in this population-based study of centenarians – 35.3% – is among the highest reported among older adults (13). Using similar methodology in other vulnerable populations, the prevalence of vitamin B12 deficiency was 23% in a multi-ethnic convenience sample of older adults receiving home delivered or congregate meals in Georgia (mean age = 76.4) (23) and was 19% and 8% in white and African American, respectively, disabled older women in the Women's Health and Aging Study (18). The prevalence was even higher in centenarians when it was assumed that those receiving vitamin B12 injections were being treated for vitamin B12 deficiency (39.8%). However, it is not known whether or not a diagnosis of vitamin B12 deficiency preceded the use of vitamin B12 injections. Thus, assuming these individuals had a previous diagnosis of vitamin B12 deficiency may overestimate the prevalence of deficiency. Nevertheless, our findings suggest that screening of vitamin B12 status is warranted in centenarians who reside in either community settings or skilled nursing facilities. Among the 16 centenarians reporting vitamin B12 injections, one met our criteria of vitamin B12 deficiency and two had macrocytosis, suggesting that those receiving treatment may need additional monitoring and/or adjustments in their treatment to ensure vitamin B12 adequacy or that the macrocytosis may have been related to some other health condition (22).

Hemoglobin concentrations, anemia, and macrocytosis were not related to vitamin B12 status in the octogenarians or the centenarians. Similarly, it has been recognized recently, e.g., population-based study of older Finnish adults (26), and historically (19,20) that hematological disorders are not consistently associated with vitamin B12 deficiency. The inconsistent associations of vitamin B12 status with hematological factors may be related to the presence or absence of other nutrition and health related conditions related to anemia such as folate deficiency, iron deficiency, renal disease, chronic inflammation or other conditions, as well as to the cause, duration, and degree of vitamin B12 deficiency (e.g., atrophic gastritis or pernicious anemia) (22,34). However, folate deficiency was rare in this sample. Based on plasma folate, only 2 participants had folate deficiency (< 6.8 nmol/L, as defined by Pfeiffer et al., 2007, 35), both were centenarians, and one was anemic and one was not. A complete examination of the causes of anemia was beyond the scope of the present study.

The primary predictors of vitamin B12 status in this study were race/ethnicity, B-vitamin supplement use, and atrophic gastritis as assessed by serum pepsinogen and were similar to those seen in relatively younger samples of older people (18,23). Similar to our findings, others have reported that African Americans have better vitamin B12 status than whites (18,21,23), but the reasons are unknown. Those with atrophic gastritis have limited ability to cleave protein-bound vitamin B12 from animal foods (12). Therefore, consumption of added vitamin B12 in supplements and fortified foods is recommended, because this chemical form apparently does not require gastric acid and/or enzymes (e.g., pepsinogen) for initial digestion (12). When controlled for other factors, the use of B-vitamin supplements decreased the probability of

being vitamin B12-deficient by about 50% (Table 3). It is not clear why there was not a protective effect of animal food intake on vitamin B12 status in the present study, which we observed in our previous study of somewhat younger elders aged in their mid-seventies (23). Perhaps atrophic gastritis may have been present for many more years in the octogenarians and centenarians compared to younger samples of older adults resulting in a more extensive depletion of body stores of vitamin B12 (22).

Vitamin B12 status and folate status were positively associated in this study, as we have seen previously in other older adults (23). Contributors to this positive association probably include consumption of dietary supplements that contain both vitamin B12 and folate, as well as their links in intermediary metabolism (12,13). In contrast to others (36), we found no evidence that high plasma folate status combined with low vitamin B12 status was associated with any of the hematological indicators when controlled for potential confounders (e.g., age, gender, race/ethnicity). Additional studies of anemia, folate, and homocysteine are ongoing in this cohort and will be reported elsewhere.

Limitations of this study include the relatively small sample size and lack of information concerning the amount of vitamin B12 and folic acid ingested from supplements, fortified foods, and other foods. Also, we had insufficient serum or plasma to measure other important markers of vitamin B12 status such as holotranscobalamin and antibodies to intrinsic factor that are seen in pernicious anemia. Measurement of these indices is needed in future studies.

In summary, in this cross-sectional population-based study there was a high prevalence of vitamin B12 deficiency among centenarians and octogenarians, with a higher prevalence in centenarians than in octogenarians. Age, race/ethnicity, atrophic gastritis, and B-vitamin supplement use emerged as strong predictors of vitamin B12 status. Additional research is needed to develop protocols for screening, prevention, and treatment of vitamin B12 deficiency in the very old. Given concerns about the negative impact of poor vitamin B12 status on health and well being, especially related to cognition, depression, and frailty (13,16,17,25), studies are in progress with this cohort to examine the relationships of vitamin B12 status with mental and physical health. Lastly, other researchers using this data set to explore relationships of vitamin B12 status and health should consider limiting their analyses to the subset of participants who did not report receiving vitamin B12 injections.

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Table 1

Demographics, B-vitamin Supplements, Animal Food Intake, and Biochemical and Hematological Indices Related to Vitamin B12 Status in Octogenarians and Centenarians: The Georgia Centenarian Study^a

	Octogenarians	Centenarians
	Median, range, Mean $\pm$ SD, or % [n]	Median, range, mean $\pm$ SD, or % [n]
Gender		
Women	65.8 [52]	85.1 *** [183]
Men	34.2 [27]	14.9 [32]
Race		
White	82.3 [65]	80.0 [172]
African American	17.7 [14]	20.0 [43]
Living arrangements		
Skilled nursing facility	13.9 [11]	40.0 *** [86]
Community	86.1 [68]	60.0 [129]
B-vitamin supplements ^b		
No	68.4 [54]	65.1 [140]
Yes	31.6 [25]	34.9 [75]
Animal food intake (servings daily) ^c		
< 2	29.1 [23]	30.4 [65]
≥ 2	70.9 [56]	69.6 [149]
Vitamin B12-deficient ^d		
Yes	22.8 [18]	35.3 * [76]
No	77.2 [61]	64.7 [139]
Vitamin B12 (pmol/L)	290, 74–847	317, 74–2286
	319 $\pm$ 143 [79]	362 $\pm$ 251 [215]
< 74	3.8 [3]	3.3 [7]
< 148	7.6 [6]	11.6 [25]
< 150	7.6 [6]	12.1 [26]
< 185	11.4 [9]	22.8 * [49]
< 258	38.0 [30]	39.1 [84]
Methylmalonic acid (nmol/L)	284, 144–1261	384, 163–8078 ***
	351 $\pm$ 216 [78]	525 $\pm$ 635 [207]
> 271	57.7 [45]	80.2 *** [166]
> 370	21.8 [17]	53.6 *** [111]
2-methylcitric acid (nmol/L)	203, 112–416	246, 97–813 **
	220 $\pm$ 71 [78]	269 $\pm$ 106 [201]
Cystathionine (nmol/L)	273, 116–974	430, 136–1428 ***
	307 $\pm$ 144 [79]	475 $\pm$ 222 [208]
Homocysteine (μ mol/L)	10.3, 6.2–29.1	13.6, 6.4–72.3 ***
	10.9 $\pm$ 3.7 [79]	14.5 $\pm$ 6.8 [208]

	Octogenarians	Centenarians
	Median, range, Mean $\pm$ SD, or % [n]	Median, range, mean $\pm$ SD, or % [n]
> 9	69.6 [55]	87.5*** [182]
> 13	17.7 [14]	53.8*** [112]
> 13.9	15.2 [12]	46.6*** [97]
Plasma folate (nmol/L)	43.8, 10.7–160	29.2, 4.1–156 ***
	53.1 $\pm$ 31.6 [79]	38.7 $\pm$ 25.9 [211]
Red cell folate (nmol/L)	874, 267–2088	773, 146–2558 *
	917 $\pm$ 398 [79]	851 $\pm$ 459 [208]
Pepsinogen (ng/ml)	79.0, 1.3–296	77.0, 1.2–300
	99.2 $\pm$ 71.9 [78]	92.2 $\pm$ 69.5 [208]
< 10 (severe atrophic gastritis)	7.7 [6]	5.8 [12]
10 to < 60 (moderate atrophic gastritis)	19.2 [15]	29.8 [62]
$\geq$ 60 (atrophic gastritis not present)	73.1 [57]	64.4 [134]
Creatinine (μ mol/L)	79.6, 53.0–186	88.4, 35.4–486 *
	86.0 $\pm$ 24.8 [78]	95.8 $\pm$ 42.1 [210]
> 127 μ mol/L	6.4 [5]	15.7* [33]
Ferritin (μ g/L)	68.0, 9.0–1257	76.0, 1.6–134
	105 $\pm$ 152 [78]	110 $\pm$ 110 [204]
Hemoglobin (g/L)	134, 97–165	120, 75–167 ***
	133 $\pm$ 16 [79]	121 $\pm$ 14 [211]
Anemic ^e	25.3 [20]	50.2*** [106]
Mean cell volume (fl)	91, 74–103	92, 71–135
	91.3 $\pm$ 5.1 [79]	91.7 $\pm$ 6.5 [211]
$\geq$ 100 (macrocytosis)	1.3 [1]	3.3 [7]

^a Participants receiving vitamin B12 injections were excluded. Differences between octogenarians and centenarians that were statistically significant are noted as follows:

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$;

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

^c Animal food intake included milk, cheese, yogurt, meat, fish, and poultry (0 is < 2 servings daily, 1 is $\geq$ 2 servings daily).

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

^e Anemic defined as hemoglobin < 120 g/L in women and < 130 g/L in men.

Table 2

Vitamin B12 Deficiency Relationships with Demographics, B-vitamin Supplements, Animal Food Intake, and Biochemical and Hematological Indices in Octogenarians and Centenarians: The Georgia Centenarian Study^a

	Octogenarians		Centenarians	
	Median, range, mean $\pm$ SD, or % [n]		Median, range, mean $\pm$ SD, or % [n]	
	B12-deficient	B12-adequate ^e	B12-deficient	B12-adequate ^f
All	22.8 [18]	77.2 [61]	35.3 ^e [76]	64.7 [139]
Gender				
Women	21.2 [11]	78.8 [41]	34.4 [63]	65.6 [120]
Men	25.9 [7]	74.1 [20]	40.6 [13]	59.4 [19]
Race				
White	23.1 [15]	76.9 [50]	39.0 [67]	61.1 [*] [105]
African American	21.4 [3]	78.6 [11]	20.9 [9]	79.1 [34]
Living arrangements				
Skilled nursing facility	27.3 [3]	72.7 [8]	30.2 [26]	69.8 [60]
Community	22.1 [15]	77.9 [53]	38.8 [50]	61.2 [79]
B-vitamin supplements ^b				
No	72.2 [13]	67.2 [41]	75.0 [57]	59.7 [83]
Yes	27.8 [5]	32.8 [20]	25.0 [19]	40.3 [*] [56]
Animal food intake (servings daily) ^c				
< 2	44.4 [8]	24.6 [15]	36.8 [28]	26.8 [37]
$\geq$ 2	55.6 [10]	75.4 [46]	63.2 [48]	73.2 [101]
Vitamin B12 (pmol/L)	212, 74–254	348, 141–847 ^{***}	178, 74–256	396, 134–2286 ^{***}
	186 $\pm$ 66 [18]	358 $\pm$ 136 [61]	170 $\pm$ 56 [76]	467 $\pm$ 255 [139]
< 74	16.7 [3]	0 ^{**} [0]	9.2 [7]	0 ^{***} [0]
< 148	27.8 [5]	1.6 ^{***} [1]	31.6 [24]	0.7 ^{***} [1]
< 150	27.8 [5]	1.6 ^{***} [1]	32.9 [25]	0.7 ^{***} [1]
< 185	27.8 [5]	6.6 [*] [4]	60.5 [46]	2.2 ^{***} [3]
< 258	100 [18]	19.7 ^{***} [12]	100 [76]	5.8 ^{***} [8]
Methylmalonic acid (nmol/L)	401, 272–1130	267, 144–1261 ^{***}	534, 273–8078	327, 163–804 ^{***}
	509 $\pm$ 280 [18]	304 $\pm$ 169 [60]	821 $\pm$ 982 [74]	360 $\pm$ 138 [133]
> 271	100 [18]	45.0 ^{***} [27]	100 [74]	69.2 ^{***} [92]
> 370	55.6 [10]	11.7 ^{***} [7]	82.4 [61]	37.6 ^{***} [50]
2-methylcitric acid (nmol/L)	240, 136–377	185, 112–416 ^{***}	262, 140–813	234, 97–530 [*]
	250 $\pm$ 64 [18]	211 $\pm$ 71 [60]	299 $\pm$ 127 [74]	253 $\pm$ 88 [134]
Cystathionine (nmol/L)	326, 116–573	255, 138–974	528, 225–1107	388, 136–1428 ^{***}
	335 $\pm$ 129 [18]	299 $\pm$ 148 [61]	561 $\pm$ 220 [74]	428 $\pm$ 210 [134]
Homocysteine (μ mol/L)	11.4, 8.3–29.1	9.7, 6.2–16.9 [*]	15.9, 8.3–72.3	11.8, 6.4–24.9 ^{***}
	13.5 $\pm$ 5.4 [18]	10.1 $\pm$ 2.5 [61]	18.2 $\pm$ 9.1 [74]	12.5 $\pm$ 3.9 [134]

	Octogenarians		Centenarians	
	Median, range, mean $\pm$ SD, or % [n]		Median, range, mean $\pm$ SD, or % [n]	
	B12-deficient	B12-adequate ^e	B12-deficient	B12-adequate ^f
> 9	94.4 [17]	62.3 ** [38]	98.6 [73]	81.3 ** [109]
> 13	33.3 [6]	13.1 * [8]	78.4 [58]	40.3 *** [54]
> 13.9	27.8 [5]	11.5 [7]	73.0 [54]	32.1 *** [43]
Plasma folate (nmol/L)	31.2, 13.2–114	52.0, 10.7–160 **	23.7, 7.5–156	37.5, 4.1–152 ***
	37.4 $\pm$ 24.3 [18]	57.7 $\pm$ 32.1 [61]	29.4 $\pm$ 22.5 [75]	43.9 $\pm$ 26.2 [136]
Red cell folate (nmol/L)	599, 267–1424	957, 350–2088 **	570, 146–2558	944, 192–2546 ***
	690 $\pm$ 329 [18]	984 $\pm$ 394 [61]	664 $\pm$ 380 [75]	956 $\pm$ 467 [133]
Pepsinogen (ng/ml)	59.7, 1.3–253	85.6, 9.4–296 ***	67.5, 1.2–300	80.5, 5.8–262 *
	57.5 $\pm$ 60.2 [17]	111 $\pm$ 71 [61]	85.4 $\pm$ 74.5 [75]	96.1 $\pm$ 66.4 [133]
< 10 (severe atrophic gastritis)	29.4 [5]	1.6 *** [1]	13.3 [10]	1.5 ** [2]
10 to < 60 (moderate atrophic gastritis)	23.5 [4]	18.0 [11]	29.3 [22]	30.1 [40]
$\geq$ 60 (atrophic gastritis not present)	47.1 [8]	80.3 [49]	57.3 [43]	68.4 [91]
Creatinine (μ mol/L)	79.6, 53.0–186	84.0, 53.0–133	88.4, 35.4–486	88.4, 44.2–221
	92.3 $\pm$ 36.8 [18]	84.1 $\pm$ 19.9 [60]	101 $\pm$ 55 [74]	93.1 $\pm$ 32.8 [136]
> 127 μ mol/L	11.1 [2]	5.0 [3]	18.9 [14]	14.0 [19]
Ferritin (μ g/L)	38.5, 11.0–297	79.0, 9.0–1257 **	73.5, 7.0–531	77.5, 1.6–634
	59.2 $\pm$ 68.6 [18]	118 $\pm$ 167 [60]	104 $\pm$ 103 [72]	114 $\pm$ 114 [132]
Hemoglobin (g/L)	133, 101–161	135, 97–166	121, 75–150	120, 85–167
	132 $\pm$ 1.6 [18]	133 $\pm$ 1.6 [61]	121 $\pm$ 1.3 [75]	121 $\pm$ 1.5 [136]
Anemic ^d	27.8 [5]	24.6 [15]	48.0 [36]	51.5 [70]
Mean cell volume (fl)	90, 74–99	92.0, 82–103	91.0, 78–135	92, 71–108
	89.4 $\pm$ 6.1 [18]	91.9 $\pm$ 4.6 [61]	91.6 $\pm$ 7.2 [75]	91.7 $\pm$ 6.1 [136]
$\geq$ 100 (macrocytosis)	0 [0]	1.6 [1]	4.0 [3]	2.9 [4]

^a Participants receiving vitamin B12 injections were excluded. Vitamin B12 deficiency defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > serum 2-methylcitric acid;

^b B-vitamin supplements included multivitamin/mineral, B-vitamins, or single oral supplements of vitamin B12;

^c Animal food intake included milk, cheese, yogurt, meat, fish, and poultry (0 is < 2 servings daily, 1 is $\geq$ 2 servings daily);

^d Anemic defined as hemoglobin < 120 g/L in women and < 130 g/L in men;

^e Differences between vitamin B12-deficient versus adequate within the octogenarians that were statistically significant are noted as follows:

* p < 0.05,

** p < 0.01,

*** p < 0.001;

^f Differences between vitamin B12-deficient versus adequate within the centenarians that were statistically significant are noted as follows:

* p < 0.05,

** p < 0.01,

 $p < 0.001$.

Table 3Predictors of Vitamin B12 Deficiency in Octogenarians and Centenarians: The Georgia Centenarian Study^a

Variable	b	Odds Ratio (95% CI)	p-value
Centenarians vs. octogenarians	0.74	2.10 (1.08, 4.12)	0.03
Female vs. male	-0.17	0.84 (0.42, 1.67)	0.62
White vs. African American	0.96	2.60 (1.22, 5.53)	0.02
Skilled nursing facility vs. community	0.12	1.12 (0.60, 2.09)	0.71
B-vitamin supplements: yes vs. no	-0.81	0.45 (0.24, 0.82)	0.01
Animal food intake: ≥ 2 vs. < 2 ^b	-0.43	0.65 (0.36, 1.18)	0.16
Atrophic gastritis: not present vs. moderate severity vs. severe ^c	0.96	2.61 (1.67, 4.07)	0.001
Intercept	-1.46		0.001

^a n = 285. Participants receiving vitamin B12 injections were excluded. Vitamin B12 deficiency defined as plasma vitamin B12 < 258 pmol/L, serum methylmalonic acid > 271 nmol/L, and methylmalonic acid > 2-methylcitric acid;

^b Animal food intake included milk, cheese, yogurt, meat, fish, and poultry (0 is < 2 servings daily, 1 is ≥ 2 servings daily);

^c Severity of atrophic gastritis was assessed from serum pepsinogen I concentrations and defined as not present (≥ 60 ng/ml), moderate (10 to < 60 ng/ml), or severe (< 10 ng/ml) and coded as 0, 1, and 2, respectively.