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Serum vitamin B12 status and multi-vitamin use: An association and the relation to the prevalence of macrocytosis, anemia, and cognitive impairment in the REGARDS cohort

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Serum vitamin B12 status and multi-vitamin use: An association and the relation to the prevalence of macrocytosis, anemia, and cognitive impairment in the REGARDS cohort

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B.S.
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2007

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Abstract

Serum vitamin B12 status and multi-vitamin use: An association and the relation to the prevalence of macrocytosis, anemia, and cognitive impairment in the REGARDS cohort
By Rebecca S. Williamson

PURPOSE: Recent studies have provided mixed results in describing the association between cognitive impairment and high serum folate among those with vitamin B12 deficiency. Data has also shown that those in the highest quintile of serum folate levels are those who are taking a multi-vitamin. Since multi-vitamins typically contain 6-25 μg of vitamin B12, these patients are likely those who suffer from an underlying vitamin B12 absorption problem. The objective of this analysis is to examine the association between serum vitamin B12 status and multi-vitamin use. Additionally, this analysis will determine whether there is an increased prevalence of macrocytosis, anemia, and cognitive impairment among multi-vitamin users with low serum B12 concentrations.

METHODS: The REasons for Geographic and Racial Differences in Stroke cohort is a national sample of approximately 30,000 African-American and white participants. We sampled 2,531 REAGRDS participants who were ≥ 50 years with baseline mean corpuscular volume and hemoglobin measurements, complete medical inventory, and a six-item screener score to measure serum vitamin B12 concentration. The sample included 1,000 multi-vitamin users, as defined with the medical inventory. Serum vitamin B12 status was defined as deficient (≤148 pmol/L), borderline deficient (148 – 221 pmol/L), or normal (>221 pmol/L).

RESULTS: Overall, 2.1% of the sample was deficient and 6.3% was borderline deficient. Among multi-vitamin users, the prevalence of deficiency was lower than the prevalence among non-users (1.3% vs. 3.2%). After controlling for age, race, and gender, multi-vitamin was associated with a ~70% reduction in deficiency [OR: 0.28 (95% CI: 0.20, 0.39)]. Multi-vitamin use or B12 status was not associated with anemia, or cognitive impairment. There was no significant association between B12 status and macrocytosis; however, multi-vitamin use was significantly associated with macrocytosis [OR: 1.25 (95% CI: 1.01, 154)].

CONCLUSIONS: Use of multi-vitamin supplements reduces B12 deficiency and B12 borderline serum levels by two-thirds. However, multi-vitamin use and vitamin B12 status does not consistently predict anemia, macrocytosis or cognitive impairment. Further research including serum folate levels and presence of intrinsic factor antibodies is merited to elucidate the inconsistent associations between serum vitamin B12 and macrocytosis, anemia, or cognitive impairment when stratified by multi-vitamin use.

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BACKGROUND

Since the fortification of "enriched" cereal grains with folic acid began in 1996, concern has been raised over the impact of excess folate in the diets of the elderly. Particularly, recent studies have focused on the implications of high folate among those with vitamin B12 deficiency on the risk of macrocytosis, anemia, and cognitive impairment; but the results of these cross-sectional studies have been inconsistent (1-3). A majority of those with high serum folate concentrations consume multi-vitamins, which contain 6-25 µg of vitamin B12 as well (4). Thus, their uncorrected vitamin B12 deficiency supports the idea that they are unable to absorb vitamin B12 and may have an underlying illness such as early or pre-clinical pernicious anemia. While treatment is clear for those with vitamin B12 deficiency and clinical symptoms such as megaloblastic anemia and neuropathy, the evidence for treatment of vitamin B12 deficiency in the absence of these clinical signs is uncertain (5). It is important for public health and clinical practice to know whether or not the cognitive impairment among those on low dose vitamin B12 supplements is due to undiagnosed pernicious anemia or from some other possible cause such as high folate serum concentrations.

Vitamin B12 Deficiency

Vitamin B12 is a water-soluble vitamin that is required for DNA synthesis, red blood cell formation, and neurological function (6). There are two vitamin B12 dependent reactions in humans: 1) re-methylation of homocysteine to yield methionine and 2) conversion of propionyl-coenzyme A (CoA) to succinyl-CoA (through methylmalonyl-CoA). Vitamin B12 is also a co-factor that is necessary to convert 5-

methyltetrahydrofolate to tetrahydrofolate, a reaction that is necessary to covert folate to its usable form (7-9).

Vitamin B12 is found only in animal-source foods; however foods can be fortified with vitamin B12 and most multi-vitamin supplements contain 6-25 μg of vitamin B12 (6). The Institute of Medicine recommends that all persons aged ≥50 years consume 2.4 μg of synthetic vitamin B12 daily from fortified foods or from oral vitamin supplements (10). Inadequate dietary intake or malabsorption from food can result in vitamin B12 deficiency. However, it takes several years for the store of vitamin B12 in the liver to become depleted and serious clinical symptoms to develop (11). Low serum vitamin B12 concentrations are common among the elderly and the prevalence increases with age because as age increases there is a decrease in the production of acid and active intrinsic factor necessary for the release of vitamin B12 from food (5, 12-14).

A diagnosis of vitamin B12 deficiency is typically made on the basis of serum B12 concentration, with <148 pmol/L indicative of deficiency and 148 – 221 pmol/L of marginal deficiency (11). There are also several biological markers that have been proposed to be used as a marker of vitamin B12 deficiency, including elevated methylmalonic acid (MMA), elevated homocysteine, and decreased holotranscobalamin. Homocysteine and MMA concentrations will increase when vitamin B12 concentrations are low because the two vitamin B12 dependent reactions breakdown. When there is a decrease in vitamin B12, methylmalonyl-CoA is unable to be converted to succinyl-CoA in the citric acid cycle (9). Instead, methylmalonyl-CoA is converted to MMA. In the second reaction vitamin B12 is a co-factor in the re-methylation of homocysteine to yield methionine. Thus, when vitamin B12 levels are low, homocysteine is not converted to

methionine, increasing the concentration of homocysteine (9). The use of MMA and homocysteine levels for vitamin B12 deficiency diagnosis is limited because many other factors can impede the usefulness of these markers. Measurement of MMA is a costly analysis that requires mass spectrometry, and the levels of MMA can be increased due to bacterial growth (11). Elevated homocysteine has low specificity for vitamin B12 deficiency as it can also be caused by deficiencies of folate (vitamin B9), riboflavin (vitamin B2), and pyridoxine (vitamin B6) (11). It has also been proposed to measure the concentrations of holotranscobalamin, the transcobalamin-bound B12, as a marker of the biologically active vitamin B12. However, the literature on the usefulness of this test in the clinical setting is limited (8). Moreover, there is a lack of a consistent relationship among these metabolic indicators of vitamin B12 deficiency and serum vitamin B12 concentrations. An analysis of the Framingham cohort found only 27.9% (62/222) classified as low serum B12 concentrations (<258 pmol/L) had elevated serum MMA concentrations and 14.9% (31/222) had elevated serum homocysteine. While 6.1% (20/326) and 2.5% (8/326) of those classified as normal serum B12 concentrations (≥258 pmol/L) had elevated serum MMA and homocysteine concentrations, respectively (15). With multiple ways to measure vitamin B12 deficiency, studies analyzing the association of vitamin B12 with clinical outcomes, such as anemia, macrocytosis, and cognitive impairment, have utilized inconsistent definitions of vitamin B12 deficiency ranging from the clinical cut point of <148 pmol/L to quartiles to a combination of serum B12 and MMA concentrations (1-3).

Pernicious Anemia

Pernicious anemia is an autoimmune disease where the body cannot properly absorb vitamin B12 from the gastrointestinal tract because it fails to produce intrinsic factor, a gastric protein necessary for the absorption of vitamin B12. Patients with pernicious anemia often develop antibodies against intrinsic factor. Since it takes years to deplete the stores of vitamin B12, few have a deficiency severe enough to show the hematological and/or neurological signs characteristic of clinically apparent pernicious anemia. Most patients are asymptomatic but may show subtle clinical signs, such as mild asymptomatic anemia, slight cognitive impairment, and pre-clinical metabolic abnormalities, including decreased serum B12 levels or mildly elevated MMA or homocysteine levels that easily go undiagnosed during a routine medical exam (16). If these patients with asymptomatic pernicious anemia are left untreated, megaloblastic anemia and/or irreversible neurological dysfunction, including cognitive impairment and dementia, may develop. While treatment is clear for those with vitamin B12 deficiency, megaloblastic anemia, and neuropathy, the evidence for treatment of vitamin B12 deficiency in the absence of these clinical signs is uncertain (5).

Vitamin B12 and cognitive impairment

With life expectancy improving, it is estimated there will be 71.5 million people in the United States >65 years by 2030 (17). Thus, the burden of dementia, an age-related public health problem, will be increasing with the increasing elderly populations. However, it has been estimated that approximately 9% of dementias are potentially reversible, including those caused by vitamin B12 deficiency (18, 19).

Two reviews, one conducted by the Agency for Healthcare Research and Quality (AHRQ) and one by the Cochrane Review, provide a comprehensive overview of the

literature on the use of B-vitamins to prevent dementia (20, 21). The AHRQ review was a systematic review which included 25 systematic reviews and 250 primary studies on the prevention of Alzheimer's disease and cognitive decline. There were no studies that used B vitamins or folate in a randomized controlled trial setting to examine the prevention of Alzheimer's disease (20). There were two studies that examined the effect of B vitamins on maintenance of cognitive function and one RCT of micronutrient supplement that contained B vitamins. Overall, B vitamins were found to have no beneficial effect (20). The Cochrane review looked specifically at the effect of vitamin B12 supplementation on preventing onset or progression of cognitive impairment or dementia (21). The review included three trials; however the authors concluded that evidence of vitamin B12 improving cognitive function of people with dementia and low serum B12 levels is insufficient. The included trials were restricted to a small number of patients with Alzheimer's disease and other types of cognitive impairment. No trials involving people without dementia or using other definitions of vitamin B12 deficiency were found (21).

Overall, these negative studies were conducted in the general population including all who had low serum concentrations of vitamin B12. However, older subjects with high serum folates and/or consumers of multi-vitamin supplements with vitamin B12 who also have low serum B12 concentrations may be those at an increased risk for cognitive impairment that could be cured and/or prevented as these are the patients who are able to absorb folic acid from their supplements, but not vitamin B12. Specifically, these patients may have early or pre-clinical pernicious anemia that can be treated with large doses of vitamin B12.

INTRODUCTION

In 1996, the United States Food and Drug Administration (FDA) required "enriched" cereal grain products to have 140 μg of synthetic folic acid per 100 g of grain (22). Since folic acid fortification has been implemented, there has been significant reduction in the number of neural tube defects in the United States. Cases of spina bifida have decreased 31-36% and cases of anencephaly have decreased 16-17% from pre-fortification rates (23-25). Moreover, fortification has resulted in a significant reduction of other birth defects, including anencephaly (20%), transposition of the great arteries (12%), cleft palate only (12%), pyloric stenosis (5%), upper limb reduction defects (11%), and omphalocele (21%) (26). Similar significant reductions in neural tube defects have been seen in other countries that have implemented folic acid fortification. In 1998, Canada implemented fortification. Overall, there was a 46% reduction in the prevalence of neural tube defects in Canada, with a 53% reduction in spina bifida, 38% reduction in anencephaly, and 31% reduction in encephalocele (27).

However, recent studies have raised concern over the impact of increased folate intake on the cognitive function in the elderly with vitamin B12 deficiency. In a 2007 analysis of the National Health and Nutrition Examination Survey (NHANES) 1999-2002, high serum folate among American seniors with low vitamin B12 status was associated with cognitive impairment and anemia, while high serum folate among those with normal vitamin B12 levels was associated with better cognitive function (3). Data from the Nurses' Health Study showed that while multi-vitamin users in the highest plasma vitamin B12 quartile performed better on cognitive tests than their non-using counterparts, multi-vitamin users in the lowest plasma vitamin B12 quartile did worse

compared with the non-multi-vitamin users in the lowest plasma vitamin B12 quartile (1). However, in a study using data from the Sacramento Area Latino Study on Aging (SALSA), researchers found no association between cognitive impairment and high serum folate among Latino seniors with vitamin B12 deficiency (2). Despite dissimilarities in the study populations and the measures of cognitive function, the inconsistent results reported among these studies suggest more research is needed to clarify this issue.

Furthermore, in the 2007 NHANES study, the authors suggested that the adverse outcomes observed among seniors with high folate and low vitamin B12 status were likely due to an exacerbation of vitamin B12 deficiency from excess folate, such as that from folic acid fortification (3). However, subsequent analyses revealed that most seniors in the group with high folate and low vitamin B12 took daily multi-vitamins that contained not only folic acid but also 6-25μg of vitamin B12 (4). Because these seniors are multi-vitamin users who regularly consume a moderate daily dose of vitamin B12, their uncorrected vitamin B12 deficiency supports the idea that they are unable to absorb vitamin B12 and may have an underlying illness such as early or pre-clinical pernicious anemia. Thus, the cognitive impairment and anemia observed among these multi-vitamin users with low serum vitamin B12 are likely the result of early or pre-clinical pernicious anemia and not excess folate from folic acid fortification. In fact, Carmel has estimated 2-4% of Americans aged ≥60 years have undiagnosed, early or pre-clinical pernicious anemia (28). Identifying multi-vitamin users with low serum vitamin B12 concentrations may provide a cost-effective screening test for early intervention and treatment with

sufficient amounts of vitamin B12 to prevent pernicious anemia-related morbidity for at-risk seniors.

This study is the first in a series of studies that will determine if high serum folate levels and low serum vitamin B12 levels predict for pernicious anemia within the REGARDS cohort. Since it has been found that 92% and 74% of those in the highest quintiles of serum folate are multi-vitamin users, multi-vitamin use will be used as a proxy for serum folate levels in this study (4). The objective of this study is to examine the association between serum vitamin B12 status and multi-vitamin use in a sample of REGARDS participants. Additionally, this analysis will determine whether there is an increased prevalence of macrocytosis, anemia, and cognitive impairment among multi-vitamin users with low serum B12 concentrations.

METHODS

Study Population

The REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study is a prospective, population-based, longitudinal study with a primary objective to better understand geographic and racial variation in stroke incidence (www.regardsstudy.org). Study design and inclusion criteria for REGARDS were previously published (29). Briefly, participants were identified from commercially available lists of residents and contacted via an initial mailing followed by a telephone contact. The cooperation rate was 64.6% and the participation rate is 44.7%, which is comparable to rates in other cohort studies (30, 31). As of October 2007, 30,239 participants had been recruited. Data was collected from two sources, a computer assisted telephone interview (CATI) and an in-home examination conducted by a nurse or other health professional. During

the CATI, patients reported demographic characteristics, health conditions, and medication use. During the in-home visit, physical measurements, a resting electrocardiogram, medication inventory, phlebotomy and urine collection were performed and participants completed self-administered questionnaires, including a Food Frequency Questionnaire (FFQ). For this analysis, a sample of 2,531 REGARDS participants age ≥ 50 years with baseline six-item screener scores and MCV and hemoglobin measurements were selected. Specifically, this sample was contained 1,500 REGARDS participants and 1,000 REGARDS participants who were multi-vitamin users.

Serum vitamin B12 concentrations

There is no gold standard test or definition for vitamin B12 deficiency (32). Serum vitamin B12 concentrations were measured to determine vitamin B12 deficiency. Serum vitamin B12 concentrations were measured on the stored serum samples collected at baseline using the Elecsys Vitamin B12 assay (Roche Diagnostics, Indianapolis, IN) at the University of Vermont laboratory. Serum vitamin B12 concentrations ≤148 pmol/L were considered to be indicative of vitamin B12 deficiency and concentrations between 148-221 pmol/L were considered to be borderline deficient.

Multi-vitamin use

During the in-home visit, a medication inventory was taken. Participants provided all medications (prescription and over the counter) and supplements taken within the past two weeks. The interviewer recorded the name of the product. No additional information on the dosage or duration of treatment was obtained. Participants

who presented a multi-vitamin supplement during the medication inventory were classified as multi-vitamin supplement users.

Macrocytosis and Anemia

The hemogram was performed the day after sample collection by automated cell counting on a Beckman Coulter LH 755 Hematology Workcell (Beckman Coulter, Inc. Fullerton, CA). Macrocytosis was defined as MCV ≥95 fL. Anemia was defined in accordance with the World Health Organization (WHO) criteria, hemoglobin <12 g/dL for women and <13 g/dL for men (33).

Cognitive Impairment

On January 2, 2004, the six-item screener was added to the CATI. The six-item screener is a measure of global cognitive function that is derived from the Mini-Mental State Exam. The screener includes both recall and temporal orientation items. Scores range from 0 to 6, with scores ≤4 indicating cognitive impairment. The six-item screener has been shown to have a sensitivity of 74.2% to 84.0% and a specificity of 80.2% to 85.3% in community and clinical samples.(34)

Statistical Analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, NC). For all analyses, p-values <0.05 were considered to be statistically significant. Standard descriptive statistics were used to describe the baseline characteristics and to test for differences among those with vitamin B12 deficiency, borderline deficiency, and normal serum B12 levels. Associations between baseline characteristics and vitamin B12 status were evaluated using chi-squared tests for categorical variables and F-tests for continuous variables.

The crude associate between multi-vitamin use and vitamin B12 status was determined using a logistic regression model. Vitamin B12 status is a three-level exposure variable and was included in the model as two dummy variables. This model was re-run, adjusting for age, race, and gender. This process was repeated to determine the crude and age, race, gender, and education (cognitive impairment only) adjusted logistic regression models for the association between multi-vitamin use and the outcomes (macrocytosis, anemia, and cognitive impairment) as well as vitamin B12 status and the outcomes. Finally, the association between vitamin B12 status and the outcomes stratified by multi-vitamin use was determined using logistic regression, adjusting for age, race, gender, and education (cognitive impairment only). Due to small counts after stratification, no interaction assessment or additional confounders were assessed.

Sensitivity analyses were run using quartiles of serum B12 concentrations to define vitamin B12 status, multi-vitamin use as defined by the intersection of the medicine inventory and the FFQ, anemia according to the Beutler thresholds,(35) and a higher cut-point for macrocytosis (≥99 fL).

RESULTS

A total of 2,531 REGARDS participants were included in the analysis, of which 1,544 were multi-vitamin users and 987 were multi-vitamin non-users. As shown in Table 1, the mean age was 65.7 ± 8.8 years and the sample was 64.8% white and 63.9% female. The geometric mean serum vitamin B12 concentration was 440.97 pmol/L. Overall, 2.1% of the sample (52/2,531) was deficient and 6.3% was borderline deficient (160/2,531). The vitamin B12 status groups were comparable in all baseline

characteristics except current tobacco use. Those who were classified as deficient or borderline deficient were more likely to be smokers (p-value = 0.0232).

Prevalence of outcomes

The three outcomes of interest in this analysis were macrocytosis, anemia, and cognitive impairment. In this sample of REGARDS participants, 20.2% had macrocytosis, 14.2% had anemia, 2.3% had both macrocytosis and anemia, and 6.4% had cognitive impairment.

Association of vitamin B12 status and multi-vitamin use

There was a statistically significant association between vitamin B12 status and multi-vitamin use (Tables 1 and 2). Multi-vitamin use was most prevalent among those with normal vitamin B12 status [63.4% (normal), 38.5% (deficient), 34.4% (borderline); overall p-value <0.0001]. Among multi-vitamin users, there was a lower prevalence of vitamin B12 deficiency and borderline vitamin B12 deficiency as compared to non-users (Multi-vitamin user vs. non-user (deficient): 1.3% vs. 3.2%; Multi-vitamin user vs. non-user (borderline deficient): 3.6% vs. 10.6%; overall p-value <0.0001). When controlling for age, race, and gender, the odds of serum vitamin B12 deficiency and borderline deficiency was decreased by two-thirds among multi-vitamin users as compared to non-users [OR: Multi-vitamin user vs. non-user (deficient): 0.28 (95% CI: 0.20, 0.39); Multi-vitamin user vs. non-user (borderline deficient): 0.34 (0.19, 0.60)].

Association of multi-vitamin use and outcomes

Within this sample of REGARDS participants, macrocytosis was more prevalent in multi-vitamin users than non-users (21.7% vs. 17.2%, p-value = 0.006; Table 2). When controlling for age, race, and gender, multi-vitamin users were at an increased odds

of macrocytosis (OR: 1.25 (1.01, 1.54); p-value = 0.0364). However, anemia prevalence was not significantly different in multi-vitamin users than non-users after controlling for age, race, and gender (13.0% vs. 16.1%, respectively). Furthermore, there was no difference in prevalence of macrocytosis and anemia between multi-vitamin users and non-users (2.1% vs. 2.2%, respectively). There was also no difference in the prevalence of cognitive impairment between multi-vitamin users and non-users (6.4% vs. 6.3%, respectively).

Association of vitamin B12 status and outcomes

As shown in Table 3, 23.1% of those with vitamin B12 deficiency and 21.9% of those with borderline B12 deficiency had macrocytosis as compared to 19.7% of those with normal vitamin B12 levels (p-value = 0.6881). While there was a trend for an increase of macrocytosis as vitamin B12 status decreased, there was no statistically significant association when controlling for age, race, and gender [OR: deficiency vs. normal: 1.24 (0.64, 2.41); borderline deficiency vs. normal 1.05 (0.70,1.55)].

Anemia was most prevalent among those with borderline B12 deficiency (18.1%). Those with borderline B12 deficiency were at increased odds of anemia as compared to those with normal B12 levels when controlling for age, race, and gender (OR: 1.54 (0.99, 2.40); p-value = 0.053). However, similar levels of anemia were seen among those with normal B12 levels and B12 deficiency (13.9% and 13.5%, respectively). There were no participants with vitamin B12 deficiency who had both macrocytosis and anemia.

There were no clear trends seen in the association between serum B12 status and cognitive impairment.

Association of vitamin B12 status and outcomes, stratified by Multi-vitamin use

Among multi-vitamin users, there is an increase in the odds of macrocytosis as the serum vitamin B12 status decreases (Table 4). A similar trend was seen among non-users; however the magnitude of the odds was greater among multi-vitamin users, showing multi-vitamin use results in increase odds of macrocytosis across all vitamin B12 status levels.

The use of a multi-vitamin was not associated with anemia among those with normal serum vitamin B12 status [OR: 0.94 (0.74, 1.19)]. Among multi-vitamin users, the prevalence of anemia was highest in those with borderline deficiency (deficient: 5.0%, borderline deficient: 20.0%, normal: 12.8%). However, among the multi-vitamin non-users, the prevalence of anemia increased as the vitamin B12 status decreases (deficient: 18.8%, borderline deficient: 17.1%, normal: 15.9%).

Regardless of multi-vitamin use, cognitive impairment has lower prevalence among those vitamin B12 deficiency as compared to those with normal vitamin B12 status [Deficient: 5.0% (multi-vitamin user) 3.1% (non-user); Normal: 6.5% (multi-vitamin user) 6.5% (non-user); overall p-value = 0.6323]. Among those with borderline deficiency, multi-vitamin users had a lower prevalence of cognitive impairment than non-users (5.5% vs. 8.6%, overall p-value = 0.6323).

DISCUSSION

In this analysis of 2,531 REGARDS participants, the use of multi-vitamins reduced vitamin B12 deficiency and borderline deficiency by two-thirds. However, multi-vitamin use and vitamin B12 status was not consistently associated anemia, macrocytosis or cognitive impairment. The results show that those with borderline deficiency have a higher prevalence of anemia and cognitive impairment than those who

are B12 deficient. Thus, there is a potential that those who are B12 deficient have been identified and treated by their doctors whereas those who are borderline deficient have yet to be identified by their doctor as needing treatment. If this is the case, this analysis provides preliminary evidence suggesting that a screening method where all those aged 50 years and older are fed 6-25 µg of vitamin B12 may be a feasible screening method for identifying those at high risk for preventable dementia caused by pernicious anemia. Further, having such a screening method could provide some clarity on how to treat those with evidence of vitamin B12 deficiency without clinical symptoms.

The prevalence of vitamin B12 deficiency (≤148 pmol/L) in this sample of REGARDS participants (2.1%) is comparable to the prevalence reported in other cohorts: 5.3% in Framingham, just under 3% in NHANES, and 6.5% in SALSA (2, 3, 15). Moreover, the reduction in vitamin B12 deficiency among multi-vitamin users as compared to non-users has been seen in both the Framingham study and NHANES. In an analysis of 401 members of the original Framingham cohort with multi-vitamin use data, 49.3% (141/286) of those who did not report taking a multi-vitamin use had vitamin B12 <258 pmol/L as compared to 16.5% (19/115) who reported taking a multi-vitamin (15). Similarly, an analysis of NHANES data found the prevalence of vitamin B12 deficiency was reduced by 50% among those taking multi-vitamins as compared to non-users, using definitions of both serum B12 concentration and biochemical markers (32). However, our results measuring the association between vitamin B12 status and multi-vitamin use and the presence of macrocytosis, anemia, and cognitive impairment are inconsistent with what is expected from the published literature. Perhaps this is a consequence of multi-vitamin use being used as a proxy for serum folate levels. While it has been found that a

high proportion of those in the highest quintile of serum folate levels are multi-vitamin users(4), measuring serum folate concentrations are the only definitive method to determine if there is an association between low serum B12 concentrations and high serum folate concentrations and the presence of anemia, macrocytosis, and cognitive impairment. Thus, when serum folate levels are available for this sample, the analysis will be re-conducted. Our analysis has a sample size comparable to similar analyses conducted in the Nurses' study (n = 635) (1), NHANES (n = 1,459) (3), and SALSA (n = 1,535) (2), and thus there was comparable power. The inconsistencies with what was expected could also be a consequence of the fact that each study utilized their own definition of the variables of interest.

This analysis categorized participants as macrocytic, anemic, or vitamin B12 deficient based on cut-points as defined by clinical practice or in peer-review literature; however, improper cut-points can result in misclassification of both the outcomes and the exposures. Alternative cut-points could have been used to define the outcomes of macrocytosis and anemia, as well as vitamin B12 deficiency. Those with a MCV ≥95 fL were classified as macrocytic. However, MCV >99 fL and >100 fL have also been suggested and utilized. If the NHANES definition of macrocytosis, MCV ≥99fL, had been used as the definition of macrocytosis, the prevalence of macrocytosis would have decreased from 20.0% to 4.6%. There would be no macrocytosis present among those with B12 deficiency and 4.4% of the borderline deficient and 4.7% of the normal B12 levels would have been classified as macrocytic. These results are reflective of the findings of Wyckoff and Ganji, who found that subjects with low serum vitamin B12 were likely to be without macrocytosis in the post-fortification era (36). Further, the

significant difference in the prevalence of macrocytosis between multi-vitamin users and non-users would not been seen (5.0% vs. 4.0%, p-value = 0.2243). To classify patients as anemic, this analysis utilized the standard WHO definition of anemia (33). However, age, race, and gender adjusted cut-points based on the Scripps-Kaiser database and confirmed in NHANES have been proposed by Beutler et al (35). A sensitivity analysis was conducted using the Beutler cut-points. While there was a slightly lower prevalence of anemia (12.2%), similar trends in the prevalence of anemia were seen in both the vitamin B12 status groups and multi-vitamin use groups as with the WHO definition. Finally, a sensitivity analysis was conducted using serum B12 concentrations categorized into quartiles. As expected, multi-vitamin use decreased from the highest to the lowest quartile (highest quartile: 74.3%, 3rd: 65.8%, 2nd: 59.6%, and lowest quartile: 44.3%, p-value <0.0001); however there were no clear trends in macrocytosis, anemia, and cognitive impairment across the quartiles.

Beyond the potential biases introduced by using categorical variables, misclassification could result from insensitive measurement methods. For example, there are a multitude of cognitive tests available to measure general cognitive function. Morris et al conducted a similar analysis using NHANES data, which utilizes the Digit Symbol-Coding subtest of the Wechsler Adult Intelligence Scale III as an assessment of cognitive function (3). This assessment must be conducted in-person and thus is not conducive to the follow-up design of the REGARDS study. The cognitive assessment used in the analysis of folate, vitamin B12, and cognitive impairment in the SALSA study, the Modified Mini Mental State Examination (3MSE), must also be administered in person (2). The Nurses' Health Study utilized the Telephone Interview for Cognitive

Status (TICS) in the assessment of folate, vitamin B12, and cognition (1). Similar to the six-item screener collected in REGARDS, this assessment of cognitive function is designed to be administered over the telephone, but is more comprehensive. Thus, certain cognitive tests may be more sensitive to cognitive impairment caused by vitamin B12 deficiency and the six-item may not be the appropriate screener for cognitive impairment caused by vitamin B12 deficiency. Over the course of the REGARDS study, additional cognitive tests that measure executive function have been added, including the CERAD Word List Learning and Recall (WLL) and the Animal Fluency Test (AFT). Future analyses should utilize these tests to determine if measures of executive function are the preferred markers of cognitive impairment resulting from vitamin B12 deficiency.

There are also multiple methods for determining multi-vitamin use within REGARDS. The analyses presented use the medication inventory to classify participants as multi-vitamin users or non-users. However, REGARDS participants were also asked to complete a FFQ. In the REGARDS cohort, the FFQ may be a more sensitive marker of who took multi-vitamins as patients may have only brought what they considered to be medications to the medication inventory, neglecting to bring any multi-vitamin supplement. However, nearly 35% of our sample did not return the questionnaire, similar to the completion rate in the entire REGARDS cohort. Perhaps the most definitive marker of multi-vitamin use would use the intersection of med inventory and the FFQ. However, multi-vitamin use reported on the FFQ is discordant with what was presented at the medicine inventory in approximately 20% of the sample. Use of this definitive definition of multi-vitamin use would limit the sample size by nearly 50%, reducing the power and possibly biasing the results. Nonetheless, sensitivity analyses using the

intersection of the FFQ and medicine inventory were conducted. The results were not significantly different from those presented.

When interpreting the presented results, several limitations should be taken into consideration. First, this analysis utilized REGARDS baseline data cross-sectionally. As with all cross-sectional data, only associations can be determined, causality cannot. Secondly, the REGARDS cohort is nationally representative of the black and white populations in the United States as the primary objective of the REGARDS cohort is to determine differences in incident stroke between blacks and whites; thus this analysis contains no additional race and ethnicities. This sample was also enriched with 1,000 multi-vitamin users, thus national level projections for blacks and whites cannot be extrapolated from this convenient sample. Thirdly, the results of this analysis are not directly comparable to other studies as different definitions of the variables are used. In addition to the various cut-points for continuous variables and methods for measuring cognitive impairment discussed, there is no gold standard to define vitamin B12 deficiency. This analysis used the standard clinical definition. In the NHANES analysis, those with serum vitamin B12 concentrations <148 pmol/L or an MMA >210 nmol/L were defined as having low serum B12, while the Nurses' study analysis utilized quartiles (1, 2). Fourthly, since the medicine inventory was used to define multi-vitamin use, the daily intake of B12 and the duration of supplement use were not controlled for in the analysis. Including the daily intake of vitamin B12 in the analysis would identify those who have an inadequate intake of vitamin B12 versus those who have an inadequate uptake of vitamin B12. Finally, data on gastrointestinal diseases, such as celiac disease,

that impact the absorption of vitamin B12 from food was not collected at baseline and therefore could not be controlled for in the analysis.

CONCLUSION

In summary, this analysis sought to determine the association between serum vitamin B12 status and multi-vitamin use in a sample of 2,531 REGARDS participants. The prevalence of vitamin B12 deficiency was reduced 72% through the consumption of a multi-vitamin, the prevalence of vitamin B12 deficiency decreased from 3.2% among multi-vitamin non-users to 1.3% among multi-vitamin users (p-value <0.0001). Similarly, the prevalence of borderline deficiency was reduced by 66% (multi-vitamin user vs. non-user: 3.6% vs. 10.6%, p-value <0.0001). However, multi-vitamin use and vitamin B12 status did not consistently predict anemia, macrocytosis or cognitive impairment. Further research including serum folate levels and presence of intrinsic factor antibodies is merited to elucidate the inconsistent associations between serum vitamin B12 and macrocytosis, anemia, or cognitive impairment when stratified by multi-vitamin use. Additionally, these analyses should be conducted utilizing the additional cognitive tests collected by REGARDS investigators. This analysis serves as a basis for determining if high serum folate levels and low serum vitamin B12 levels predict for pernicious anemia within the REGARDS cohort.

PUBLIC HEALTH IMPLICATIONS

Cognitive impairment is an important problem for the aging population as it contributes to decreased quality of life, increased neuro-psychiatric symptoms, increased disability, and increased healthcare costs (37-40). Although it is well known that cognitive impairment can be a part of clinically apparent pernicious anemia, recent evidence suggests that undiagnosed and untreated pernicious anemia may be causing curable/preventable cognitive impairment. Thus, examining the association between undiagnosed and untreated pernicious anemia and cognitive impairment and identifying an efficient and effective screening and treatment method are important public health concerns.

The literature of the last 20 years about vitamin B12 deficiency has resulted in confusion and lack of clarity for clinicians. Population screens of older individuals unselected for signs and symptoms of pernicious anemia have identified significant proportions of patients with low serum vitamin B12 concentration, whom have yet to develop neurological or hematological signs and symptoms (15). Some of these individuals have metabolic indicators of vitamin B12 deficiency including raised serum MMA and/or homocysteine concentration while others do not (15). Moreover, subjects with normal serum vitamin B12 concentrations have also been found to have increased serum MMA and/or homocysteine concentrations making it unclear who, if anyone, should be treated and whether or not treatment would benefit the subject (15). However, treatment or voluntary consumption of vitamin supplements with 6-25 micrograms of vitamin B12 will normalize about two-thirds of those with low serum vitamin B12, as seen in this analysis, and about two-thirds of those with abnormal metabolic indicators

(15, 32, 41). The Institute of Medicine used such evidence to recommend that all persons aged ≥50 years consume 2.4 μg of synthetic vitamin B12 from foods fortified with synthetic vitamin B12 or from oral vitamin supplements (10). Even if all those aged ≥50 years consumed 2.4 μg of synthetic vitamin B12 through, for example, FDA required fortification of flour with vitamin B12, there would remain the issue of how to deal with undiagnosed and untreated pernicious anemia for which such a low dose of vitamin B12 would not be sufficient to prevent or cure the hematological and/or neurological disease, including cognitive impairment.

Analyses of NHANES, the Nurses' study, and SALSA suggest that it may be feasible and effective to screen all those 50 and older for preclinical pernicious anemia by feeding 6-25 micrograms of vitamin B12 and identifying those with low serum vitamin B12 concentrations as at high risk for untreated pernicious anemia. Accordingly their current cognitive problems may be reversed and more serious neurological or hematological problems prevented from a much higher dose of vitamin B12. This analysis was the first in a series that will determine if high serum folate levels and low serum vitamin B12 levels predict for pernicious anemia within the REGARDS cohort.

REFERENCES

1. Kang JH, Irizarry MC, Grodstein F. Prospective study of plasma folate, vitamin B12, and cognitive function and decline. *Epidemiology* 2006;17(6):650-7.
2. Miller JW, Garrod MG, Allen LH, et al. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. *Am J Clin Nutr* 2009;90(6):1586-92.
3. Morris MS, Jacques PF, Rosenberg IH, et al. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85(1):193-200.
4. Berry RJ, Carter HK, Yang Q. Cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;86(1):265-7; author reply 7-9.
5. Dangour AD, Allen E, Clarke R, et al. A randomised controlled trial investigating the effect of vitamin B12 supplementation on neurological function in healthy older people: the Older People and Enhanced Neurological function (OPEN) study protocol [ISRCTN54195799]. *Nutr J* 2011;10:22.
6. National Institutes of Health - Office of Dietary Supplements. Dietary Supplement Fact Sheet: Vitamin B12. 2010,
7. Carmel R. Current concepts in cobalamin deficiency. *Annu Rev Med* 2000;51:357-75.
8. Herrmann W, Obeid R, Schorr H, et al. The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. *Curr Drug Metab* 2005;6(1):47-53.

9. RK. Murray, DA Bender, KM. Botham, et al. *Haper's Illustrated Biochemistry, 28th Edition*. The McGraw-Hill Companies, Inc.; 2009.

10. Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington DC: National Academy Press; 1998. (http://www.nap.edu/openbook.php?record_id=6015&page=R1#). (Accessed).

11. Allen LH. How common is vitamin B-12 deficiency? *Am J Clin Nutr* 2009;89(2):693S-6S.

12. Andres E, Loukili NH, Noel E, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. *CMAJ* 2004;171(3):251-9.

13. Clarke R, Grimley Evans J, Schneede J, et al. Vitamin B12 and folate deficiency in later life. *Age Ageing* 2004;33(1):34-41.

14. Suter PM, Golner BB, Goldin BR, et al. Reversal of protein-bound vitamin B12 malabsorption with antibiotics in atrophic gastritis. *Gastroenterology* 1991;101(4):1039-45.

15. Lindenbaum J, Rosenberg IH, Wilson PW, et al. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994;60(1):2-11.

16. Carmel R, Green R, Rosenblatt DS, et al. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program* 2003:62-81.

17. Federal Interagency Forum on Age-Related Statistics. Older Americans 2008: Key Indicators of Well-Being. Washington, DC: US Government Printing Office, 2008,

18. Clarfield AM. The decreasing prevalence of reversible dementias: an updated meta-analysis. *Arch Intern Med* 2003;163(18):2219-29.

19. Ladika DJ, Gurevitz SL. Identifying the most common causes of reversible dementias: a review. *JAAPA* 2011;24(3):28-31, 57.

20. Williams J, Plassman B, Burke J, et al. Preventing Alzheimer's Disease and Cognitive Decline. Rockville, MD, 2010, (U.S. Department of Health and Human Services - Agency for Healthcare Research and Quality

21. Malouf R, Areosa Sastre A. Vitamin B12 for cognition (Review). *The Cochrane Library* 2009(1).

22. US Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid (final rule - 21 CFR Part 101). *Fed Reg* 1996;61:8781-97.

23. Spina bifida and anencephaly before and after folic acid mandate--United States, 1995-1996 and 1999-2000. *MMWR Morb Mortal Wkly Rep* 2004;53(17):362-5.

24. Williams LJ, Mai CT, Edmonds LD, et al. Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. *Teratology* 2002;66(1):33-9.

25. Williams LJ, Rasmussen SA, Flores A, et al. Decline in the prevalence of spina bifida and anencephaly by race/ethnicity: 1995-2002. *Pediatrics* 2005;116(3):580-6.

26. Canfield MA, Collins JS, Botto LD, et al. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States:

findings from a multi-state population-based study. *Birth Defects Res A Clin Mol Teratol* 2005;73(10):679-89.

27. De Wals P, Tairou F, Van Allen MI, et al. Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med* 2007;357(2):135-42.

28. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch Intern Med* 1996;156(10):1097-100.

29. Howard VJ, Cushman M, Pulley L, et al. The reasons for geographic and racial differences in stroke study: objectives and design. *Neuroepidemiology* 2005;25(3):135-43.

30. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989;129(4):687-702.

31. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991;1(3):263-76.

32. Evatt ML, Terry PD, Ziegler TR, et al. Association between vitamin B12-containing supplement consumption and prevalence of biochemically defined B12 deficiency in adults in NHANES III (third national health and nutrition examination survey). *Public Health Nutr* 2010;13(1):25-31.

33. WHO/UNICEF/UNU. Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers. Geneva, Switerland: World Health Organization, 2001,

34. Callahan CM, Unverzagt FW, Hui SL, et al. Six-item screener to identify cognitive impairment among potential subjects for clinical research. *Med Care* 2002;40(9):771-81.

35. Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood* 2006;107(5):1747-50.

36. 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(4):1187-92.

37. Albert SM, Glied S, Andrews H, et al. Primary care expenditures before the onset of Alzheimer's disease. *Neurology* 2002;59(4):573-8.

38. Ernst RL, Hay JW. Economic research on Alzheimer disease: a review of the literature. *Alzheimer Dis Assoc Disord* 1997;11 Suppl 6:135-45.

39. Lyketsos CG, Lopez O, Jones B, et al. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* 2002;288(12):1475-83.

40. Tabert MH, Albert SM, Borukhova-Milov L, et al. Functional deficits in patients with mild cognitive impairment: prediction of AD. *Neurology* 2002;58(5):758-64.

41. Winkels RM, Brouwer IA, Clarke R, et al. Bread cofortified with folic acid and vitamin B-12 improves the folate and vitamin B-12 status of healthy older people: a randomized controlled trial. *Am J Clin Nutr* 2008;88(2):348-55.

TABLES

Table 1. Characteristics of study population by vitamin B12 status

Descriptive Statistics	Total Sample (n = 2531)	Deficient (n = 52)	Borderline (n = 160)	Normal (n = 2319)	p-value
Age, mean (SD)	65.7 (8.8)	64.1 (9.3)	66.7 (9.5)	65.7 (8.7)	0.9657
White, n (%)	1639 (64.8%)	37 (71.2%)	116 (72.5%)	1486 (64.0%)	0.0607
Female, n (%)	1617 (63.9%)	35 (67.3%)	94 (58.8%)	1488 (64.8%)	0.3376
Multi-vitamin user, n (%)	1544 (61.0%)	20 (38.5%)	55 (34.4%)	1469 (63.4%)	<0.0001
Cognitive Impairment, n (%)	161 (6.4%)	2 (3.8%)	12 (7.5%)	147 (6.3%)	0.6368
Macrocytosis					
Mean MCV (SD)	90.2 (5.7)	90.9 (4.4)	90.7 (4.8)	90.1 (5.8)	0.1568
Macrocytic, n (%)	505 (20.0%)	12 (23.1%)	35 (21.9%)	458 (19.7%)	0.6881
Anemia					
Mean Hgb (SD)	13.6 (1.4)	13.6 (1.3)	13.6 (1.4)	13.6 (1.4)	0.6852
Anemic, n (%)	359 (14.2%)	7 (13.5%)	29 (18.1%)	323 (13.9%)	0.3348
Macrocytic and Anemic, n (%)	55 (2.3%)	0 (0.0%)	6 (3.8%)	49 (2.1%)	0.2159
Education					
Less than HS	254 (10.1%)	5 (9.6%)	21 (13.1%)	228 (9.8%)	0.2994
High School	632 (25.0%)	20 (38.5%)	38 (23.8%)	574 (24.8%)	
Some College	682 (27.0%)	10 (19.2%)	43 (26.9%)	629 (27.2%)	
College	959 (38.0%)	17 (32.7%)	58 (36.3%)	884 (38.2%)	
Income, n (%)					
<$20K	400 (15.8%)	7 (13.5%)	32 (20.0%)	361 (15.6%)	0.3359
$20K – $34K	589 (23.3%)	16 (30.8%)	43 (26.9%)	560 (22.9%)	
$35K - $74K	777 (30.7%)	12 (23.1%)	46 (28.8%)	719 (31.0%)	
>$75K	408 (16.2%)	7 (13.5%)	19 (11.9%)	16.5%)	

Descriptive Statistics	Total Sample (n = 2531)	Deficient (n = 52)	Borderline (n = 160)	Normal (n = 2319)	p-value
Not reported	357 (14.1%)	10 (19.2%)	20 (12.5%)	327 (14.1%)	
Self-reported General Health, n (%)					
Poor	79 (3.1%)	3 (5.8%)	7 (4.4%)	69 (3%)	0.7483
Fair	343 (13.6%)	9 (17.3%)	24 (15.1%)	310 (13.4%)	
Good	885 (35%)	20 (38.5%)	57 (35.8%)	808 (34.9%)	
Very Good	802 (31.7%)	12 (23.1%)	47 (29.6%)	743 (32.1%)	
Excellent	420 (16.6%)	8 (15.4%)	24 (15.1%)	388 (16.7%)	
Medications, n (%)					
Metformin	222 (8.8%)	5 (9.6%)	18 (11.3%)	199 (8.6%)	0.5017
PPI	436 (17.3%)	12 (23.1%)	21 (13.1%)	403 (17.4%)	0.2046
H2-receptor antagonist	104 (4.1%)	2 (3.9%)	4 (2.5%)	98 (4.2%)	0.5653
B12 Injections	6 (0.24%)	0 (0.0%)	0 (0.0%)	6 (0.3%)	0.7596
Comorbidity, n (%)					
Current Alcohol User	1326 (52.4%)	25 (48.1%)	78 (48.8%)	1223 (52.7%)	0.4907
Coronary Heart Disease	413 (16.6%)	5 (9.8%)	28 (18.0%)	380 (16.7%)	0.3836
Diabetes	543 (21.6%)	9 (17.7%)	32 (20.0%)	502 (21.8%)	0.6902
Hypertension	1458 (57.9%)	30 (57.7%)	100 (62.5%)	1328 (57.6%)	0.4803
Elevated Cholesterol	1471 (58.8%)	31 (59.6%)	98 (61.6%)	1342 (58.6%)	0.7450
Current Tobacco Use	310 (12.3%)	9 (17.3%)	32 (20.1%)	269 (11.6%)	0.0232
Obese	951 (37.9%)	17 (33.3%)	63 (40.7%)	871 (37.8%)	0.7783
Left ventricular hypertrophy	122 (4.9%)	1 (1.9%)	9 (5.7%)	112 (4.9%)	0.5430

Descriptive Statistics	Total Sample (n = 2531)	Deficient (n = 52)	Borderline (n = 160)	Normal (n = 2319)	p-value
Atrial fibrillation	228 (9.2%)	8 (15.7%)	12 (7.6%)	208 (9.2%)	0.2220
Serum creatinine (mg/dL), mean (SD)	0.88 (0.4)	0.88 (0.3)	0.88 (0.3)	0.88 (0.4)	0.9856
Estimated GFR, mL/min/1.73 square meters, mean (SD)	84.6 (22.6)	81.3 (20.2)	85.3 (26.4)	84.7 (22.4)	0.5599
Cancer diagnosis	166 (14.8%)	0 (0.0%)	11 (19.3%)	155 (14.8%)	0.1449
Prior Stroke	147 (5.8%)	4 (7.7%)	12 (7.7%)	131 (5.7%)	0.4901
Prior TIA	99 (4.2%)	4 (8.3%)	5 (3.5%)	90 (4.1%)	0.3231

Table 2. Association of multi-vitamin use and vitamin B12 deficiency, macrocytosis, anemia, and cognitive impairment

Outcome	MV Status	No. of Subjects, n (%)	Crude OR (95% CI)	Logistic Regression OR (95% CI)[a]
B12 deficient	No	32 (3.2%)	Reference	Reference
	Yes	20 (1.3%)	0.36 (0.21, 0.63)[b]	0.28 (0.20, 0.39)[b]
Borderline B12 deficient	No	105 (10.6%)	Reference	Reference
	Yes	55 (3.6%)	0.30 (0.22, 0.43)[b]	0.34 (0.19, 0.60)[c]
Macrocytosis	No	170 (17.2%)	Reference	Reference
	Yes	335 (21.7%)	1.3 (1.09, 1.63)[d]	1.25 (1.01, 1.54)[e]
Anemia	No	159 (16.1%)	Reference	Reference
	Yes	200 (13.0%)	0.77 (0.62, 0.97)[f]	0.91 (0.72, 1.16)
Macrocytosis and Anemia	No	22 (2.2%)	Reference	Reference
	Yes	33 (2.1%)	0.98 (0.56, 1.65)	0.98 (0.56, 1.71)
Cognitive Impairment	No	62 (6.3%)	Reference	Reference
	Yes	99 (6.4%)	1.02 (0.74, 1.42)	1.16 (0.82, 1.63)

a Basic logistic model adjusted for age, sex, race, and education (cognitive impairment only).
b p-value <0.0001
c p-value = 0.0002
d p-value = 0.0061
e p-value = 0.0364
f p-value = 0.0267

Table 3. Association of serum vitamin B12 concentrations and cognitive impairment, anemia, and macrocytosis.

Outcome	B12 status	No. of Subjects, n (%)	Crude OR (95% CI)	Logistic Regression OR (95% CI)[a]
Macrocytosis	Normal	458 (19.7%)	Reference	Reference
	Borderline	35 (21.9%)	1.14 (0.77, 1.68)	1.05 (0.70,1.55)
	Low	12 (23.1%)	1.22 (0.63, 2.34)	1.24 (0.64, 2.41)
Anemia	Normal	323 (13.9%)	Reference	Reference
	Borderline	29 (18.1%)	1.37 (0.90, 2.08)	1.54 (0.99, 2.40)[b]
	Low	7 (13.5%)	0.96 (0.43, 2.15)	1.14 (0.49, 2.61)
Macrocytosis and Anemia	Normal	49 (2.1%)	Reference	Reference
	Borderline	6 (3.8%)	1.80 (0.76, 4.28)	1.65 (0.68, 3.99)
	Low	0 (0.0%)	Undefined	Undefined
Cognitive Impairment	Normal	147 (6.3%)	Reference	Reference
	Borderline	12 (7.5%)	1.20 (0.65, 2.21)	1.18 (0.64, 2.21)
	Low	2 (3.8%)	0.59 (0.14, 2.45)	0.65 (0.16, 2.73)

a Basic logistic model adjusted for age, sex, race, and education (cognitive impairment only).
b p-value = 0.053

Table 4. Association of serum vitamin B12 concentrations and multi-vitamin use with cognitive impairment, anemia, and macrocytosis.

	Deficient (n = 52)	Borderline (n = 160)	Normal (n = 2319)
Macrocytosis			
% with outcome, n (%)			
MV non-user	6 (18.8%)	20 (19.0%)	144 (16.9%)
MV user	6 (30.0%)	15 (27.3%)	314 (21.4%)
OR (95% CI) [a]			
MV non-user	1.33 (0.68, 2.58)	1.13 (0.76, 1.68)	Reference
MV user	1.69 (0.82 3.46)	1.43 (0.88, 2.33)	1.27 (1.03, 1.58)[b]
Anemia			
% with outcome, n (%)			
MV non-user	6 (18.8%)	18 (17.1%)	135 (15.9%)
MV user	1 (5.0%)	11 (20.0%)	188 (12.8%)
OR (95% CI) [a]			
MV non-user	1.12 (0.48, 2.58)	1.52 (0.97, 2.37)[c]	Reference
MV user	1.05 (0.43, 2.55)	1.43 (0.84, 2.43)	0.94 (0.74, 1.19)
Macrocytosis and Anemia			
% with outcome, n (%)			
MV non-user	0 (0.0%)	2 (1.9%)	20 (2.4%)
MV user	0 (0.0%)	4 (7.3%)	29 (2.0%)
OR (95% CI) [a]			
MV non-user	Undefined	1.65 (0.66, 4.03)	Reference
MV user	Undefined	1.65 (0.53, 5.09)	1.00 (0.57, 1.76)
Cognitive Impairment			
% with outcome, n (%)			
MV non-user	1 (3.1%)	9 (8.6%)	52 (6.1%)
MV user	1 (5.0%)	3 (5.5%)	95 (6.5%)
OR (95% CI)[a]			
MV non-user	0.68 (0.16, 2.85)	1.23 (0.66, 2.31)	Reference
MV user	0.79 (0.18, 3.54)	1.44 (0.67, 3.07)	1.17 (0.83, 1.66)

a Basic logistic model adjusted for age, sex, race, and education (cognitive impairment only).

b p-value = 0.0271

c p-value = 0.0648

APPENDIX. IRB Letter of Exemption

EMORY UNIVERSITY

Institutional Review Board

August 6, 2010

Godfrey Oakley, Jr., MD
Epidemiology

RE: Determination: Not Engaged in Human Subjects Research; IRB Review Not Required
IRB00045213 ; *Cognitive impairment and early/pre-clinical pernicious anemia in relation to serum B12 and folate status among older adults in REGARDS 2003-2007*
PI: Godfrey Oakley, Jr.

Dear Dr. Oakley:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because you and Emory will not be "engaged" in research with human subjects, you will not be receiving identifiable private information about the subjects of the research. To reach this conclusion we consulted the current guidance on engagement issued by the U.S. Office for Human Research Protections.

Specifically, in this project, you will be provided the results in a de-identified manner from UAB, who has performed that data analysis from data obtained at Fletcher Allen Health Care (the teaching hospital of the University of Vermont) and Quest Diagnostics Nichols Institute in Chantilly, Virginia.

This determination could be affected by substantive changes in your role or Emory's role in the project. If such changes occur, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Michael Deryck, BS, CIP
Senior Research Protocol Analyst
This letter has been digitally signed

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