Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20140716041521

Original Research

GESDAV

The prevalence of vitamin B_{12} deficiency in a random sample from the Australian population

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Received: September 14, 2013
Accepted: July 16, 2014
Published: October 02, 2014

ABSTRACT

Objective: Vitamin B_{12} deficiency is common in older adults, and may increase the risk of cognitive impairment. The distribution of vitamin B_{12} insufficiency in younger age groups is less studied. This study aims to assess the prevalence of vitamin B_{12} deficiency (<156 ρmol/L) and subclinical low-normal levels (156-250 ρmol/L) in a large, random sample of the Australian population across the adult life span. **Methods:** We examined serum vitamin B_{12} levels in a random sample of 1085 men and 1125 women aged 20-97 years between 1994 and 2006; in the Barwon statistical division, a regional area in southeastern Australia that is representative of the socioeconomic status of the Australian population. **Results:** The age-standardized prevalence of vitamin B_{12} deficiency in this cohort of men and women was 3.6%. Subclinical low-normal vitamin B_{12} levels (156-250 ρmol/L) were found in 26%. Serum vitamin B_{12} levels declined with age among men ($P < 0.001$) and were lower in men than women ($P < 0.001$). Vitamin B_{12} levels were higher among supplements users (8.0% of the cohort). **Conclusions:** Vitamin B_{12} levels decline with age, and have been associated with neurodegenerative diseases and cognitive decline. Early intervention by diet education or supplement use to address this age-associated decline in vitamin levels may be an effective strategy to prevent cognitive decline in a significant segment of the population. Such intervention may need to start in mid-life (from 50-years of age) before the onset of age-related decline in vitamin B_{12} levels.

KEY WORDS: Ageing, epidemiology, public health, vitamin B_{12}, vitamin B_{12} deficiency

INTRODUCTION

Vitamin B_{12} is an enzyme co-factor in two pathways in humans, namely (i) the regeneration of methionine from homocysteine in the cytoplasm, and (ii) the re-arrangement of methylmalonic acid (MMA)-Coenzyme A (CoA) to succinyl-CoA in the mitochondria. These reactions support DNA synthesis and lipid metabolism, and also detoxify the substrates homocysteine and MMA. Low-serum vitamin B_{12} levels have been associated with cognitive decline in Alzheimer's disease (AD) [1], Parkinson's disease [2], and vascular dementia [3].

The lower reference value for serum vitamin B_{12} varies with each method of assay and population sampled, but approximates to 150 ρmol/L. Metabolic abnormalities, such as elevated homocysteine or MMA levels, altered deoxyuridine suppression, or hematological changes frequently occur with low-normal B12 levels (~150-250 ρmol/L) [4]. Laboratory indicators of vitamin B_{12} deficiency, including hyperhomocysteinemia, neutrophil hypersegmentation, and macrocytosis may also be seen at vitamin B_{12} concentrations up to 250 ρmol/L [5]. In addition, serum vitamin B_{12} levels < 308 ρmol/L were associated with increased brain atrophy in a survey of 107 over-60 year olds [6]. The findings are of particularly concern for the ageing population as the conditions causing low vitamin B_{12} levels occur more frequently with increasing age.

Vitamin B_{12} is obtained from sea-food, animal products, eggs and dairy, but is lacking from strict vegetarian diets. Older adults may consume less animal source foods due to an inability to prepare meals, poor teeth, or reduced income. A number of conditions can disrupt absorption of vitamin B_{12} in older adults despite adequate dietary intake:

- Infection with *Helicobacter pylori* was associated with lower serum vitamin B_{12} and an increased risk of deficiency [7],
- Proton pump inhibitor (PPI) use for more than 12 months significantly increased the risk of developing vitamin B_{12} deficiency (odds ratio 4.45; 95% confidence interval [CI]: 1.47-13.34) in those aged 65 years or older [8],
- Pernicious anemia, an autoimmune reaction to intrinsic factor or the parietal cells, experienced by 15% of vitamin B_{12} deficient over-65 year olds [7],
- An interaction between the cubilin receptor and the anti-diabetic drug metformin inhibits adequate uptake in up to 30% of metformin users [9],
- Resection or disease of the absorbing surface at the distal ileum.

Previous Australian studies of vitamin B_{12} deficiency have been in selected populations. Vitamin B_{12} levels < 185 ρmol/L were seen in 22.9% of participants in the study of 2901 over-50 year olds in Sydney [10]. In a study of 299 over-75 year olds in Perth, 55% had low-vitamin B_{12} levels (<258 ρmol/L) [11]. Elevated homocysteine level is a non-specific marker for vitamin B_{12} deficiency. Homocysteine levels were elevated in 24% of men and women aged 70 years and older in Perth [12].

It is unclear from these previous studies whether low-normal vitamin B_{12} levels (up to 250 ρmol/L) are common in younger adult Australians, or are a feature of ageing. In the Australian population, low vitamin B_{12} levels was reported to increase the risk of cognitive impairment, and this risk was exacerbated when folate levels were high[13] and also amongst patients with diabetes who used metformin [14].

We studied the prevalence of vitamin B_{12} deficiency and subclinical low-normal vitamin B_{12} levels in a population-based, random sample of Australian men and women aged 20-years and older. The efficacy of commercially-available supplements to prevent or treat low-normal vitamin B_{12} levels was assessed.

METHODS

Participants and Setting

An age-stratified random sample (n = 3,034) was drawn from the commonwealth electoral rolls for the region defined as the Barwon statistical division in Victoria, Australia [15]. This sample was drawn initially to investigate the epidemiology of osteoporosis in Australian women as part of the Geelong osteoporosis study (GOS). Data generated on this cohort was used to establish the reference ranges used in bone densitometers in Australia, initially for women [16], and later for men [17].

Fasting serum samples were obtained between 1994 and 1997 from 1,244 women (mean age, 52.6 years; range 20.3-93.1 years), and between 2001 and 2006 from 1133 men (mean age, 59.8 years; range 20.7-96.7 years). Serum vitamin B_{12} assays were performed in the same laboratory using the same instrumentation and type of assay, for the duration of the study. A number of participants did not have samples taken for vitamin B_{12} measurement (n = 657). A further 165 participants did not adequately complete questionnaires, so were excluded. The final response fraction was 72.9%, which included 176 participants who reported using vitamin B_{12} supplements, and 2034 participants who did not use vitamin B_{12} supplements.

Ethical Approval

This study was approved by the Barwon Health Human Research Ethics Committee, under reference number 09/12. Written, informed consent was obtained from all participants.

Self-Reported Questionnaires

Details of all medications used and of all current and past medical conditions were self-reported by participants and were documented by questionnaire. Participants were asked to record both current and previous use of supplements and to define the usage period, as well as the route of administration. The supplements used were those typically available over-the-counter. Vitamin B_{12} supplement use included multi-vitamins and low-dose formulations.

Biochemical Analysis

Blood samples were collected after an overnight fast and were centrifuged, separated and stored at-80°C. Serum concentrations of vitamin B_{12} were determined using the Roche vitamin B_{12} reagent kit (Roche Diagnostics, Mannheim, Germany) on a Roche modular analytics E170 analyzer (Electro-Chemiluminescence Immunoassay) with inter-assay precision of 5.9% at 183.7 ρmol/L, 5.6% at 427.4 ρmol/L, and 4.8% at 779.2 ρmol/L. The lower reference value for this assay was 156 ρmol/L. The vitamin B_{12} analyses were performed in the Alfred pathology service, clinical biochemistry unit.

Statistical Analysis

The vitamin B_{12} levels of supplement user-and non-supplement user-groups, and between men and women, were compared using the Mann–Whitney test. A linear regression model was formed to investigate the association between serum vitamin B_{12} levels (response) and supplement use (predictor), with age and gender as adjusters. Differences at the $P = 0.05$ level were considered significant. Age-standardized estimates for the prevalence of deficiency and of subclinical low-normal vitamin B_{12} levels in the Australian population were derived using the 2006 census data collected by the Australian Bureau of Statistics [18]. All statistical analyses were performed using Minitab® Version 15.1.1.0 (Minitab Inc., Pennsylvania State College, Pennsylvania, USA).

RESULTS

The Prevalence of Deficiency and Subclinical Low-normal Serum Vitamin B_{12} Levels

One hundred and five participants had serum vitamin B_{12} level in the deficient range (<156 ρmol/L). The age-standardized prevalence of deficiency in the whole cohort was 3.6% (95% CI: 3.0-4.8%). Vitamin B_{12} deficiency was rarer in those under the age of 50 years (2.2%); whereas 5.2% (95% CI: 4.2-6.9%) of over 50 years old were deficient. This number increased to 8.5% (95% CI: 6.9-11.3%) in over 65 years old. The age-standardized prevalence of low-normal vitamin B_{12} levels (156-250 ρmol/L) was 25.8% [Table 1].

Vitamin B_{12} Supplements Improve Serum Vitamin B_{12} Levels

The median serum vitamin B_{12} levels of 176 participants who were on vitamin B_{12} supplements were 36% higher than other participants (Mann–Whitney test, $P < 0.001$). The median for supplement users was 404 ρmol/L, with range 158 to > 1476 ρmol/L; whereas, the median for participants who did not use supplements was 297 ρmol/L, with range 66-777 ρmol/L. Ten-supplement users (5.7%) had serum vitamin B_{12} level of 1476 ρmol/L or greater. This is the upper limit of quantitation for the instrument used. Supplement use was a significant predictor of vitamin B_{12} levels after adjusting for both gender and age ($P < 0.001$). None of the supplement users had deficient serum vitamin B_{12} levels. Thirteen percent of supplement users had subclinical low-normal serum vitamin B_{12} levels. There were proportionally more women who took vitamin B_{12} supplements than men (10.8% vs. 6.5%, $P = 0.002$). Only five participants received intramuscular vitamin B_{12} injections, so there were insufficient numbers to compare between supplementation types.

Gender and Age Charts for Serum Vitamin B_{12} Levels in the Barwon Statistical Division

Among participants who did not use supplements, serum vitamin B_{12} levels were lower in men than women (age-adjusted, $P < 0.001$). The median for men was 284 ρmol/L (range 129-564 ρmol/L); whereas the median for women was 308 ρmol/L (range 142-632 ρmol/L). There was a decline of vitamin B_{12} levels with age in males but not in females [Figure 1].

Table 1: Characteristics of cohort

Characteristics of cohort	Males	Females	Whole cohort
Total number of participants	1085	1125	2210
Number of vitamin B_{12} supplement users (%)	66 (6.1)	110 (9.8)	176 (8.0)
Total number of participants who did not use supplements	1019	1015	2034
Observed prevalences in participants who did not use supplements			
Deficient (vitamin B_{12}<156 ρmol/L) %	6.3	4.0	5.2
Subclinical low-normal levels (vitamin B_{12}: 156-250 ρmol/L) %	32.2	24.0	28.1
Age-standardized prevalences in participants who did not use supplements[a]			
Deficient (vitamin B_{12}<156 ρmol/L) %	3.6	3.0	3.6
Subclinical low-normal levels (vitamin B_{12}: 156-250 ρmol/L) (%)	28.4	23.4	25.8

[a]Age-standardized values have been derived using the 2006 census data collected by the Australian Bureau of Statistics [19]

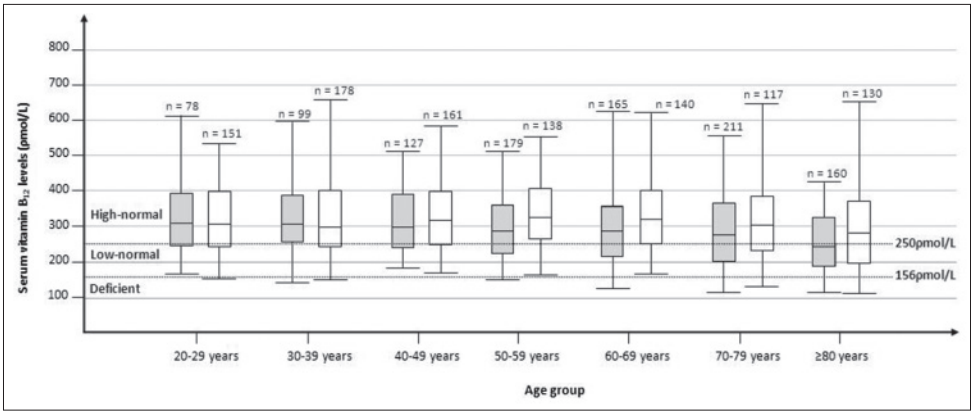

Figure 1: Age stratified serum vitamin B12 levels of men and women across the adult lifespan. Box-and-whisker charts showing the median (line inside box), inter-quartile range (boxed region), and the 2.5th and 97.5th centiles for vitamin B12 levels. The marked regions indicate the ranges for deficiency (<156 ρmol/L) and subclinical low-normal levels (156-250 ρmol/L). Results for males are shaded, results for females are not shaded

Excluded Patients were Older and Included a Higher Proportion of Men

In total, 822 participants originally recruited to the GOS study (27.1%) were not included in this investigation of vitamin B_{12} levels. The proportion of vitamin B_{12} supplement users in the excluded group was not different to that in the included group (7.4% vs. 8.0%, Chi-square, $P = 0.6753$). The excluded group were older (mean difference 2.4 years, $P = 0.004$) and included a higher proportion of men (55% vs. 49%, $P < 0.004$). Age and gender standardization of our prevalence data would counteract the lower response fraction, increasing confidence in the results obtained.

DISCUSSION

In our cohort, the age-standardized prevalence of deficiency was 3.6%. This is consistent with other data from older studies using strict definitions of pernicious anemia [20]. The serum vitamin B_{12} levels of just over one-quarter of our cohort were in the subclinical low-normal range (156-250 ρmol/L). Furthermore, 54% of males aged 80 years or over had a serum vitamin B_{12} level < 250 ρmol/L. Our findings in this age group are similar to figures reported earlier in Australia [10], and in other countries, with deficiency in over 50 years old of 5.2% and subclinical low-normal levels in 28.5%.

The majority of studies investigating the prevalence of deficiency and subclinical low-normal values have been conducted in older adults. Table 2 compares our findings with those from a Sydney study, and other countries, showing a similar prevalence of deficiency in the Australian population to the USA [18], Israel [21], and Finland [22]. Subclinical low-normal serum vitamin B_{12} levels are high in each of the older populations surveyed, and range from 24.0% to 35.3%. For comparison, only data from over 50 years old in the GOS cohort is shown in Table 2.

The clinical reference value used to define vitamin B_{12} deficiency was that provided by the assay kit manufacturer (<156 ρmol/L; Roche Diagnostics, Mannheim, Germany). This value was originally derived from a survey of 178 healthy US adults with no signs of vitamin B_{12} deficiency. Recently, attention was drawn to the failure of vitamin B_{12} assays to identify patients with pernicious anemia which will respond to B_{12} supplementation [25]. The presence of anti-intrinsic factor antibodies may interfere with competitive-binding assays that are currently available, such that false-normal or even very high levels (>1000 ρmol/L) are measured. Carmel and Agrawal estimate failure rates (false-normal cobalamin levels) are between 22% and 35% [26].

Pernicious anemia was self-reported in questionnaires by 11 participants (0.5%); of whom, none had a serum vitamin B_{12} measurement that was in the deficient range (<156 ρmol/L). This was despite that only three participants with pernicious anemia also reported using vitamin B_{12} supplement. One limitation of the current study is that anti-intrinsic factor antibodies were not measured, so it is not possible to estimate the actual number that may have false-normal results due to interference by anti-intrinsic factor antibodies in the serum, though this number is likely to be small.

Twenty-six percent of participants who did not use supplements had a serum vitamin B_{12} measurement in the subclinical low-normal range. This is the first assessment of subclinical low-normal serum vitamin B_{12} levels in a large random sample of the adult Australian population. Flood *et al.* similarly reported a high prevalence of subclinical low-normal serum vitamin B_{12} levels, though their study was restricted to those aged over 50 years. Our findings demonstrate that a high prevalence in subclinical low-normal serum vitamin B_{12} levels (156-250 ρmol/L) is common in adults in Australia, and not confined to older people.

Vitamin B_{12} levels were lower in men and declined with increasing age in males but not in females. The median of serum vitamin B_{12} levels in men aged 80 years or older was in the subclinical low-normal range. As low vitamin B_{12} levels has been associated with cognitive impairment and AD [1,3],

Table 2: The prevalence of deficiency and subclinical low-normal serum vitamin B_{12} levels in over-50 year olds

Country	Australia	Australia [10]	USA [20]	Israel [21]	Finland [22]	Canada [23]	The Netherlands [24]
Year	This study[a]	2006	2000	2003	2007	2002	1998
Age range (years)	>50	>50	50-83	>65	65-100	>65	74-80
Number of participants	1237	2901	2015	1271	1048	240	105
Reference limit for deficiency (ρmol/L)	<156	<125	<148	<147	<150	<165	<150
% Deficient	6.9	6.3	8.8	7.8	6.1	15.3	24.8
Low-normal range (ρmol/L)	156-250	125-220	148-258	-	150-250	165-250	150-260
% Low-normal	30.2	29.0	30.5	-	31.9	24.0	35.3
Design	Cross-sectional study	Cross-sectional study	Cross-sectional study	Cross-sectional study	Cross-sectional study	Cross-sectional study	Cross-sectional study
Setting	Community	Community	Community	Community, day centers and aged care homes	Community	Community	Community
Instrument for measuring B_{12} level	Roche analyzer	Beckman-access analyzer	Biorad quanta-phase II	Baxter fluorometric	AutoDelfia	Beckman DXI analyzer	Dualcount solid phase
Method of assay	Competitive-binding	Competitive-binding	Competitive-binding	Competitive-binding	Competitive-binding	Competitive-binding	Competitive-binding

[a]A sub-sample of the cohort are shown here for comparison to other published studies in over-50 year olds, excludes results on 973 participants aged<50 years

dietary intervention studies should be considered in those with declining levels. Men and women were recruited at different times, therefore there is potential for assay shifts (for instance, due to lot-to-lot reagent shifts) to influence results.

In clinical trials, vitamin B_{12} supplementation has been shown to improve cognition only in those already experiencing deficiency [27]. If vitamin B_{12} deficiency or subclinical low-normal levels play a role in cognitive decline, then prophylaxis may be more effective than vitamin B_{12} replacement after irreversible neuronal damage has occurred. Intervention for those with low levels (156-250 ρmol/L) may need to be started early at a time when cognition is normal, then continued for many years or decades to see an effect. Such long-term studies of supplementation have not been carried out.

Vitamin B_{12} supplements are effective in raising serum vitamin B_{12} levels [11], our data confirms supplements that are available commercially (including low-dose formulations) may be effective since there were no supplement users who were deficient. Supplements should be considered for those with declining vitamin B_{12} levels where dietary intervention has been ineffective or is inappropriate. Vitamin B_{12} replacement therapy is safe and effective by way of injection or oral preparations [28].

The vitamins in cognitive clinical trial, which included 266 participants followed for 2 years, reported that homocysteine-lowering B-vitamins (0.8 mg folic acid, 0.5 mg vitamin B_{12}, and 20 mg vitamin B_6) slowed progression of cognitive decline [29]. These findings offer hope that supplementation may be effective in preserving cognitive function.

Vitamin B_{12} deficiency and subclinical low-normal levels are common in a representative Australian adult population, are lower in men, decline with age, and improve with supplementation. Vitamin B_{12} levels are readily assayed, and supplementation is inexpensive and readily available. Associations between low-vitamin B_{12} levels and many disease states exist, including AD and other neurodegenerative diseases. Associations between low-vitamin B_{12} levels also exist with commonly used medications, such as PPIs or metformin. Further study to gauge the effects of early or prophylactic supplementation in these areas will be important given the prevalences of each of these associations in the ageing population.

ACKNOWLEDGMENTS

The study investigators would like to extend their thanks to current and past staff of the clinical trials unit (The Geelong Hospital), for recruiting the cohort, and also to the study participants for sharing their time and information.

The GOS was supported by funding from the Victorian Health Promotion Foundation (ID 91–0095); the National Medical Research Council (ID 251638, ID 299831, ID 628582); the Geelong Region Medical Research Foundation; Barwon Health, Perpetual Trustees; the Dairy Research and Development Corporation; the University of Melbourne; the Ronald Geoffrey Arnott Foundation; ANZ Charitable Trust; the American Society for Bone and Mineral Research, and Amgen (Europe) GmBH. The B_{12} measurements were supported by the special purpose funds of the Alfred Pathology Service. The study financial sponsors did not play any role in the design, execution, analysis and interpretation of data, or writing, editing or decision to publish the manuscript.

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Source of Support: The GOS was supported by funding from the Victorian Health Promotion Foundation (ID 91–0095); the National Medical Research Council (ID 251638, ID 299831, ID 628582); the Geelong Region Medical Research Foundation; Barwon Health, Perpetual Trustees; the Dairy Research and Development Corporation; the University of Melbourne; the Ronald Geoffrey Arnott Foundation; ANZ Charitable Trust; the American Society for Bone and Mineral Research, and Amgen (Europe) GmbH. The B12 measurements were supported by the special purpose funds of the Alfred Pathology Service, **Conflict of Interest:** None declared.