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

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ABSTRACT

Objective: Vitamin B\textsubscript{12} deficiency is common in older adults, and may increase the risk of cognitive impairment. The distribution of vitamin B\textsubscript{12} insufficiency in younger age groups is less studied. This study aims to assess the prevalence of vitamin B\textsubscript{12} deficiency (<156 pmol/L) and subclinical low-normal levels (156-250 pmol/L) in a large, random sample of the Australian population across the adult life span. Methods: We examined serum vitamin B\textsubscript{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\textsubscript{12} deficiency in this cohort of men and women was 3.6%. Subclinical low-normal vitamin B\textsubscript{12} levels (156-250 pmol/L) were found in 26%. Serum vitamin B\textsubscript{12} levels declined with age among men ($P < 0.001$) and were lower in men than women ($P < 0.001$). Vitamin B\textsubscript{12} levels were higher among supplements users (8.0% of the cohort). Conclusions: Vitamin B\textsubscript{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 B\textsubscript{12} 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\textsubscript{12} levels.

KEY WORDS: Ageing, epidemiology, public health, vitamin B\textsubscript{12}, vitamin B\textsubscript{12} deficiency

INTRODUCTION

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

Vitamin B₁₂ 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 B₁₂ in older adults despite
adequate dietary intake:

- Infection with Helicobacter pylori was associated with lower
  serum vitamin B₁₂, and an increased risk of deficiency [7],
- Proton pump inhibitor (PPI) use for more than 12 months
  significantly increased the risk of developing vitamin B₁₂
  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
  B₁₂ 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 B₁₂ deficiency have been
in selected populations. Vitamin B₁₂ levels < 185 pmoL/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 B₁₂ levels (<258 pmoL/L) [11]. Elevated
homocysteine level is a non-specific marker for vitamin B₁₂
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 B₁₂ levels (up to 250 pmoL/L) are common in younger
adult Australians, or are a feature of ageing. In the Australian
population, low vitamin B₁₂ 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 B₁₂ deficiency and
subclinical low-normal vitamin B₁₂ 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 B₁₂ 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 1,133
men (mean age, 59.8 years; range 20.7-96.7 years). Serum
vitamin B₁₂ 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 B₁₂ 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
B₁₂ supplements, and 2034 participants who did not use
vitamin B₁₂ 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 B₁₂ 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 B₁₂ were determined using
the Roche vitamin B₁₂ 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 pmoL/L, 5.6% at 427.4
pmoL/L, and 4.8% at 779.2 pmoL/L. The lower reference
value for this assay was 156 pmoL/L. The vitamin B₁₂ analyses
were performed in the Alfred pathology service, clinical
biochemistry unit.

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**Statistical Analysis**

The vitamin B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ Levels**

One hundred and five participants had serum vitamin B₁₂ level in the deficient range (<156 \( \mu \text{mol/L} \)). The age-standardized prevalence of deficiency in the whole cohort was 3.6% (95% CI: 3.0-4.8%). Vitamin B₁₂ 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 B₁₂ levels (156-250 \( \mu \text{mol/L} \)) was 25.8% [Table 1].

**Vitamin B₁₂ Supplements Improve Serum Vitamin B₁₂ Levels**

The median serum vitamin B₁₂ levels of 176 participants who were on vitamin B₁₂ supplements were 36% higher than other participants (Mann–Whitney test, \( P < 0.001 \)). The median for supplement users was 404 \( \mu \text{mol/L} \), with range 158 to \( >1476 \mu \text{mol/L} \); whereas, the median for participants who did not use supplements was 297 \( \mu \text{mol/L} \), with range 66-777 \( \mu \text{mol/L} \). Ten-supplement users (5.7%) had serum vitamin B₁₂ level of 1476 \( \mu \text{mol/L} \) or greater. This is the upper limit of quantitation for the instrument used. Supplement use was a significant predictor of vitamin B₁₂ levels after adjusting for both gender and age (\( P < 0.001 \)). None of the supplement users had deficient serum vitamin B₁₂ levels. Thirteen percent of supplement users had subclinical low-normal serum vitamin B₁₂ levels. There were proportionally more women who took vitamin B₁₂ supplements than men (10.8% vs. 6.5%, \( P = 0.002 \)). Only five participants received intramuscular vitamin B₁₂ injections, so there were insufficient numbers to compare between supplementation types.

**Gender and Age Charts for Serum Vitamin B₁₂ Levels in the Barwon Statistical Division**

Among participants who did not use supplements, serum vitamin B₁₂ levels were lower in men than women (age-adjusted, \( P < 0.001 \)). The median for men was 284 \( \mu \text{mol/L} \) (range 129-564 \( \mu \text{mol/L} \)); whereas the median for women was 308 \( \mu \text{mol/L} \) (range 142-632 \( \mu \text{mol/L} \)). There was a decline of vitamin B₁₂ levels with age in males but not in females [Figure 1].

**Table 1: Characteristics of cohort**

<table>
<thead>
<tr>
<th>Characteristics of cohort</th>
<th>Males</th>
<th>Females</th>
<th>Whole cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of participants</td>
<td>1085</td>
<td>1125</td>
<td>2210</td>
</tr>
<tr>
<td>Number of vitamin B₁₂ supplement users (%)</td>
<td>66 (6.1)</td>
<td>110 (9.8)</td>
<td>176 (8.0)</td>
</tr>
<tr>
<td>Total number of participants who did not use supplements</td>
<td>1019</td>
<td>1015</td>
<td>2034</td>
</tr>
<tr>
<td>Observed prevalences in participants who did not use supplements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient (vitamin B₁₂&lt;156 ( \mu \text{mol/L} )) %</td>
<td>6.3</td>
<td>4.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Subclinical low-normal levels (vitamin B₁₂: 156-250 ( \mu \text{mol/L} )) %</td>
<td>32.2</td>
<td>24.0</td>
<td>28.1</td>
</tr>
</tbody>
</table>

Age-standardized prevalences in participants who did not use supplements:

| Deficient (vitamin B₁₂<156 \( \mu \text{mol/L} \)) % | 3.6   | 3.0     | 3.6          |
| Subclinical low-normal levels (vitamin B₁₂: 156-250 \( \mu \text{mol/L} \)) (%) | 28.4  | 23.4    | 25.8         |

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

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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 \( \mu \text{mol/L} \)) and subclinical low-normal levels (156-250 \( \mu \text{mol/L} \)). 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 pmol/L). Furthermore, 54% of males aged 80 years or over had a serum vitamin B$_{12}$ level < 250 pmol/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 28.3%.

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 pmol/L). Furthermore, 54% of males aged <50 years had a serum vitamin B$_{12}$ level < 250 pmol/L. This is consistent with other data from older studies using less 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 pmol/L). 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 pmol/L). Furthermore, 54% of males aged 80 years or over had a serum vitamin B$_{12}$ level < 250 pmol/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 28.3%.

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

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Year</td>
<td>This study*</td>
<td>2006</td>
<td>2000</td>
<td>2003</td>
<td>2007</td>
<td>2002</td>
<td>1998</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>50-83</td>
<td>&gt;65</td>
<td>65-100</td>
<td>&gt;65</td>
<td>74-80</td>
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<tr>
<td>Number of participants</td>
<td>1237</td>
<td>2901</td>
<td>2015</td>
<td>1271</td>
<td>1048</td>
<td>240</td>
<td>105</td>
</tr>
<tr>
<td>Reference limit for deficiency (pmol/L)</td>
<td>&lt;156</td>
<td>&lt;125</td>
<td>&lt;148</td>
<td>&lt;147</td>
<td>&lt;150</td>
<td>&lt;165</td>
<td>&lt;150</td>
</tr>
<tr>
<td>% Deficient</td>
<td>6.9</td>
<td>6.3</td>
<td>8.8</td>
<td>7.8</td>
<td>6.1</td>
<td>15.3</td>
<td>24.8</td>
</tr>
<tr>
<td>Low-normal range (pmol/L)</td>
<td>156-250</td>
<td>125-220</td>
<td>148-258</td>
<td>-</td>
<td>150-250</td>
<td>165-250</td>
<td>150-260</td>
</tr>
<tr>
<td>% Low-normal</td>
<td>30.2</td>
<td>29.0</td>
<td>30.5</td>
<td>31.9</td>
<td>24.0</td>
<td>35.3</td>
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<tr>
<td>Instrument for measuring B$_{12}$ level</td>
<td>Roche analyzer</td>
<td>Beckman-Access analyzer</td>
<td>Biorad quantaphase II</td>
<td>Baxter fluorometric</td>
<td>AutoDelfia</td>
<td>Beckman DXI analyzer</td>
<td>Dualcount solid phase</td>
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<tr>
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<td>Competitive-binding</td>
<td>Competitive-binding</td>
<td>Competitive-binding</td>
<td>Competitive-binding</td>
<td>Competitive-binding</td>
<td>Competitive-binding</td>
</tr>
</tbody>
</table>

*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

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 pmol/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 pmol/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],
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 B₁₂ supplementation has been shown to improve cognition only in those already experiencing deficiency [27]. If vitamin B₁₂ deficiency or subclinical low-normal levels play a role in cognitive decline, then prophylaxis may be more effective than vitamin B₁₂ replacement after irreversible neuronal damage has occurred. Intervention for those with low levels (156-250 pmol/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 B₁₂ supplements are effective in raising serum vitamin B₁₂ 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 B₁₂ levels where dietary intervention has been ineffective or is inappropriate. Vitamin B₁₂ 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 B₆, and 20 mg vitamin B₁₂) slowed progression of cognitive decline [29]. These findings offer hope that supplementation may be effective in preserving cognitive function.

Vitamin B₁₂ 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 B₁₂ levels are readily assayed, and supplementation is inexpensive and readily available. Associations between low-vitamin B₁₂ levels and many disease states exist, including AD and other neurodegenerative diseases. Associations between low-vitamin B₁₂ 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.

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Moore, et al.: Vitamin B12 deficiency in Australia


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