Original Article

Vitamin B_{12} and Folate status of older New Zealand women

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The primary aim of this study was to assess the biochemical vitamin B_{12} and folate status of a representative group of elderly women (70-80 y) living in Dunedin, New Zealand. A second aim was to determine the prevalence of hyperhomocysteinaemia and to explore the determinants of homocysteine (hcy) concentration in this population. A cross-sectional study was carried out between June and August of 2000. Two hundred and fifty women were randomly selected from the 1998 electoral roll. Fasting blood samples were analysed for folate, vitamin B_{12}, total hcy, creatinine, and haematological parameters. Of the women selected, 87 did not respond, 37 were not traceable, 23 were not eligible or had died, and 103 agreed to participate. The overall response rate was 46%. Based on a cut-off of 150 pmol/L for serum B_{12}, 13 % of participants would be classified as having sub-optimal vitamin B_{12} status. Of the women, 3 and 5 %, respectively, had low serum (<6.6 nmol/L) and erythrocyte folate (<317 nmol/L) concentrations. No participant had megaloblastic anaemia. The prevalence of hyperhomocysteinaemia (>15 μmol/L) in this population was 18%. Hyperhomocysteinaemia in this group may be partly explained by renal insufficiency because there was a significant association between serum creatinine and plasma hcy (P<0.001). Blood folate levels but not serum B_{12} were significantly inversely associated with hcy. In conclusion, there was a moderately high prevalence of hyperhomocysteinaemia and suboptimal plasma vitamin B_{12} concentrations but not low blood folate concentrations in this elderly female population.

Key Words: elderly women, vitamin B_{12}, cobalamin, folate, homocysteine, creatinine

Introduction

There is little data regarding the vitamin B_{12} status of New Zealand elderly. Two earlier reports indicate that the prevalence of sub-optimal vitamin B_{12} status among the elderly in this country is between 7-23%, based on low serum B_{12} concentrations.[1,2] Although these studies suggest a high prevalence of low serum B_{12} concentrations among New Zealand elderly, both were carried out over a decade ago, and neither study used representative sampling techniques. Recently, there has been increased concern about sub-optimal vitamin B_{12} status in the elderly. Evidence is accumulating that even in the absence of anaemia sub-optimal B_{12} status may exist which places elderly at increased risk of neurological abnormalities.[3]

An elevated blood total homocysteine (hcy) concentration, common in older persons, is associated with an increased risk of several health outcomes in the elderly including ischaemic heart[4] and Alzheimer's disease.[5] Blood hcy concentration is inversely correlated with blood folate and vitamin B_{12} and to a lesser extent vitamin B_6 concentrations.[6] Low folate intakes are often implicated in the etiology of hyperhomocysteinaemia in the elderly,[7] and folic acid given as a tablet[8] or added to food[9] has been shown to lower hcy concentrations in the elderly. There are other non-nutritional determinants of hcy concentration such as enzyme defects, impaired renal function, and lifestyle factors such as alcohol consumption and cigarette smoking.[10] The reported prevalence of hyperhomocysteinaemia in a self-selected group of older persons (60+ years) from Dunedin was 6%.[11] There is no published data on the determinants of hcy in a representative group of elderly New Zealanders.

The primary aim of this study, therefore, was to provide up-to-date information on the vitamin B_{12} and folate status of a representative sample of free-living Dunedin women between 70-80 years. Further aims were to determine the prevalence of hyperhomocysteinaemia and to explore the determinants of hcy concentration in this population.

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Materials and methods

The study was carried out between June and August of 2000. The University of Otago Human Ethics Committee approved the study. Two hundred and fifty women, aged 70-80 years, living in the Dunedin, New Zealand, urban area were randomly selected from the 1998 electoral roll and contacted by telephone. Women who were institutionalised, non-ambulatory, or reported the presence of a terminal illness were excluded. Women who agreed to participate were asked to attend an early morning clinic during which fasting blood samples were collected by venepuncture into tubes with and without EDTA and immediately put on ice. A third fresh sample of EDTA-containing whole blood was used for a complete blood count. Within 2 hours, plasma from EDTA tubes and serum from tubes without anticoagulant were separated from whole blood by centrifugation. At a subsequent home visit a questionnaire was administered to obtain information on socio-demographic and health issues. Data on tobacco use was obtained through a self-administered questionnaire previously described by researchers of the European elderly SENECA study.[12]

Table 1. General characteristics of the study population and non-responders*

Parameter	Study population (N=103)		Non-responders (N=64)	
	% (N)	Median (1^{st}, 3^{rd})	% (N)	Median (1^{st}, 3^{rd})
Age (y)		74.3 (72.6, 78.3)		75.2 (72.1,78.1)
Participants living alone	50 (51)		52 (33)	
Subjective health†		8 (7, 9)		8 (7,9)
Subjective appetite		6 (8, 10)		
Participants reporting >1 disease	88 (91)		83 (53)	
No. of prescribed medicines		3 (1,5)		3 (1, 5)
Smoking				
Never	51 (53)			
Previous	41(42)			
Current	8 (8)			
Socio-Economic Index‡				
High	32 (32)			
Medium	53 (55)			
Low	15 (16)			
Weight (kg)		66 (58, 75)		
BMI (kg/m^2)				
<18.5	2 (2)			
		26 (23, 30)		
>30	21 (22)			

*There were no significant differences between the study participants and non-responders; † Range: 1-10; 1 = poor, 10 = excellent; ‡ Based on Elley WB, Irving JC. Revised socio-economic index for New Zealand. N J Educational Studies 1976;11:25-36.

Alcohol intake was determined using a validated self-administered food frequency questionnaire designed to assess intake over the previous year.[13] Serum vitamin B_{12} was determined with an Abbott IMX analyzer, reagents, and calibrators (Abbott Laboratories, Abbott Park, IL). Serum folate and whole blood folate were determined using the microtiter technique exactly as described by O'Broin and Kelleher[14] with Cloramphenicol resistant Lactobacillus casei as the test microorganism. The CV for the folate assay was 11.8%. Erythrocyte folate was calculated from whole blood folate by subtracting serum folate and correcting for haematocrit. The between-run coefficient of variation (CV) was 7.2% based on the controls provided by the manufacturer. Total plasma hcy concentrations were measured by HPLC according to the fluorometric method of Ubbink *et al*.[15] The between-run CV was 2.5%. Serum creatinine was measured on a Cobas Fara Autoanalyser (Roche Diagnostic. Somerville, NJ). The interpretive value for 'at risk' used for serum B_{12} was <150 pmol/L.[16] Interpretive values for serum folate,[17] erythrocyte folate,[17] and plasma hcy were,[6,18,19] < 6.6 nmol/L, < 317 nmol/L, and > 15 μmol/L, respectively.

Statistical analysis

Statistical analyses were performed using SPSS for windows, version 9.0 (SPSS Inc., Chicago, Illinois). The natural log transformation was used to normalize the biochemical variables for the multiple regression analysis. The following steps were used to develop a multiple regression model to explore the determinants of plasma hcy.[20] The following predictor variables which have been shown to be predictors of hcy in other studies were forced into the model: cigarette smoking, alcohol intake, vitamin supplement use (vitamin B_{12}, folic acid, and/or vitamin B_6 ; yes/no), age, serum folate, serum B_{12}, and serum creatinine.[21] The exponential of the beta coefficients from the regression analysis with the natural log transformed outcome data provide comparisons between levels of predictor variables on a ratio scale.

Results

Of the 250 women who were posted an information letter, 103 agreed to participate and met the inclusion criteria. Eighty-seven women were classified as non-responders. Thirty-seven women were not traceable and 23 were not eligible or had died. The overall response rate was 46% for those who were eligible and traceable. Characteristics of the participants and non-responders are given in Table 1. There were no significant differences between the study participants and 64 non-responders who agreed to answer a short questionnaire with respect to age, subjective health and appetite, living arrangement, presence of disease, or number of prescribed medicines.

The median age of the participants was 74.3 years. With the exception of one participant who reported being New Zealand Maori all participants were Caucasian. Common disease conditions reported by the participants included: arthritis (45%), hypertension (21%), osteoporosis (17%), as

well as cardiovascular disease, prior stroke, and angina (20%). Over 20% of the participants had a BMI (kg/m^2) greater than 30 and are classified as obese.[22] Two percent of women had a BMI less than 18.5 and are classified as underweight.[22] Vitamin preparations containing vitamin B_{12}, folic acid, and/or vitamin B_6 were used by 16% of participants. Forty-one percent of participants had smoked at some time during their life but less than 10% were current smokers. Alcohol use was common: almost 60 % of participants claimed to have used alcohol in the previous month.

The distributions of serum vitamin B_{12} concentrations and plasma hcy are given in Figure 1. The median (1^{st}, 3^{rd} quartiles) of vitamin B_{12} and plasma hcy were 273 (194, 380) pmol/L and 11.6 (9.0, 13.8) μmol/L, respectively. Thirteen percent of women had sub-optimal serum B_{12} concentrations (< 150 pmol/L). The prevalence of hyperhomocysteinaemia was 18% in this population based on a cut-off of 15 μmol/L. A summary of biochemical indices is given in Table 2. Based on interpretive values of 6.7 nmol/L for serum folate and 317 nmol/L for RBC folate, 3% and 5% of women, respectively, had low blood folate levels. Based on an interpretive value of 118 g/L, 6.8% of women had haemoglobin concentrations indicative of anaemia.[23] However, there were no cases of megaloblastic anaemia. One woman had an elevated MCV (i.e. >100 fL)[24,25] but her haemoglobin was not low (124 g/L). Further, her serum B_{12}, plasma and RBC folate concentrations were normal (185 pmol/L, 7.8 nmol/L, and 888 nmol/L, respectively). Six participants had a serum creatinine indicative of impaired renal function (>106 μmol/L).[26]

Multiple regression analysis revealed a significant inverse relationship between hcy and serum folate (Table 3). Each 10 nmol/L increase in serum folate was associated with an estimated 7% lower plasma hcy. Despite a narrow range age, was significantly associated with hcy. Serum creatinine, a marker of renal insufficiency, was positively and strongly associated with plasma hcy.

Smoking status, serum B_{12}, and use of supplements (vitamin B_{12}, folic acid, and/or vitamin B_6) were not significantly associated with hcy. Alcohol use was weakly associated with hcy such that each 15 g increase (equivalent to one standard drink) in ethanol intake was associated with a 0.7 % increase in hcy concentration.

Table 2. Biochemical indices in the study sample

Parameter	Median (1^{st}, 3rd)	Interpretive values for 'at risk'	% at risk
Serum B_{12} (pmol/L)	273 (194, 380)	<150	13
Serum folate (nmol/L)	16.4 (11.6, 25.6)	< 6.7	4
Erythrocyte folate (nmol/L)	627 (456, 886)	< 315	5
Plasma homocysteine (μmol/L)	11.6 (9.0, 13.8)	> 15	18
Mean corpuscular vol. (fl)	90.8 (87.8, 93.5)	> 100	1
Haemoglobin (g/L)	134 (128, 142)	< 118	7
Serum creatinine (μmol/L)	65 (55,74)	> 106	6

Table 3. Estimated changes in plasma homocysteine(umol/L) according to selected variables.*

Variable	Estimated % Change	Confidence Interval	*P*
Age (5-y increase)	-7.1	-0.2, -13.4	0.044
Smokers vs. Nonsmokers†	-8.3	-41.5, 43.5	0.557
BMI (kg/m^2) (1-point increase)	0.9	-0.1, 1.9	0.074
Supplement users vs. nonusers‡	-3.7	-39.5, 53.4	0.854
Alcohol (15 g ethanol intake per day increase)	0.7	0.1,1.4	0.047
Serum folate (10-nmol/L increase)	-7.0	-10, -3.7	<0.001
Serum B_{12} (10-pmol/L increase)	-0.2	-0.4, 0.1	0.197
Creatinine (10-umol/L increase)	8.0	5.2, 10.8	<0.000

*Estimated in a regression model with the natural log of homocysteine as the dependent variable. n=103, r^2=0.52; † Current Smoker, n=9; ‡ Folic acid, B_{12}, or Vitamin B_6 supplement user, n=16.

Discussion

Over 10% of the elderly women in the present study had sub-optimal vitamin B_{12} status based on serum vitamin B_{12} (<150 pmol/L). Internationally the prevalence of sub-optimal vitamin B_{12} status in the elderly based on low blood B_{12} levels has varied considerably by study from 1.0% to 40.5 % depending largely on the cutoff used.[27] In the US National Health and Nutrition Examination Survey (NHANES III, 1988-94), which provides the most up-to-date data on the prevalence of sub-optimal vitamin B_{12} status based on serum B_{12} in a nationally representative sample, the prevalence of low serum B_{12} (<150 pmol/L) was 6 % in women 70 years or older (n=883).[28] No similar data exist for New Zealand elderly. Perhaps not surprisingly our reported prevalence in a free-living population of elderly women is much lower than that reported in residents of rest homes and geriatric wards in the Auckland region (n=100 men and women)[2] where nearly one-quarter of residents had serum B_{12} levels indicative of sub-optimal vitamin B_{12} status (<135 pmol/L).

Our estimate is higher than that reported in a community-based sample of elderly men and women over 65 years from a medical centre in Christchurch (n=257 men and women) in the early 1990s[1] where 7.3% of patients had serum vitamin B_{12} levels indicative of sub-optimal vitamin B_{12} status. In the Christchurch study, however, a lower cut-off for serum B_{12} of < 114 pmol/L was used to define sub-optimal vitamin B_{12} status. If a cut-off of 114 pmol/L is applied in the present study a similar 6.9 % of elderly women have sub-optimal vitamin B_{12} status.

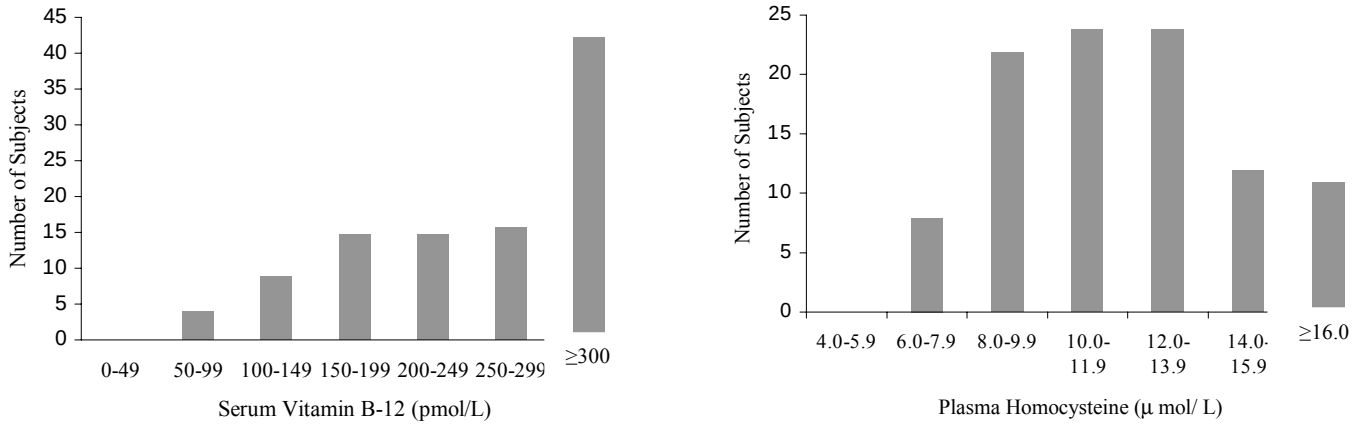

Figure 1. Frequency distribution of plasma homocysteine and serum vitamin B12 among elderly (70-80 y) participants (n=103)

Debate exists about the most appropriate cut-off for serum B_{12} and cutoffs from 70 pmol/L to greater than 200 pmol/L have been suggested.[9,24,29] In our study, as in others,[27] low serum cobalamin concentrations (<150 pmol/L) were not accompanied by megaloblastic anaemia. Anaemia, induced by depletion of vitamin B_{12} occurs only at the far end of the vitamin deficiency spectrum. As a consequence, it is difficult to find abnormal hematological indexes in people who may be mildly deficient.[30] Therefore, the presence of megaloblastic anaemia should not be the sole criteria for the diagnosis of B_{12} deficiency. It is now recognised that significant neurological disturbances can occur in elderly patients, that improve with vitamin B_{12} supplementation, in the absence of haematological abnormalities.[3] Some researchers have even argued that a higher cutoff, as high as 258 pmol/L for serum cobalamin should be established; a cut-off figure that has been based on elevated levels of serum metabolites such as methylmalonic acid and hcy that respond to vitamin B_{12} therapy.[24,29,31-33] Based on a cut-off of 200 pmol/L nearly 50% of participants in the present study would be classified 'at risk' for vitamin B_{12} deficiency. Recent evidence, however, suggests that methylmalonic concentrations need to be interpreted with caution in the elderly because they are often elevated due to renal insufficiency.[34]

In contrast to vitamin B_{12}, the elderly women in our study generally did not appear to have a poor folate status. Very few women had levels of serum or erythrocyte folates indicative of deficiency (3 and 5%, respectively). As with vitamin B_{12}, no national prevalence data are available for folate deficiency in New Zealand seniors. However, our findings are in agreement with the Christchurch researchers where 1 and 3.3 % of elderly persons (>65 years), respectively, had low levels of serum and erythrocyte folate.[1]

In the US NHANES III, however, 7% of elderly women (n=1658) had serum folate levels less than 6.7 nmol/L and over 20% had erythrocyte folate levels (n=1496) less than 317 nmol/L.[28] The radio-isotopic method used in the NHANES study for measuring folate is known to underestimate folate levels, particularly in erythrocyte, relative to the microbiological method, used in our study.[28]

Almost 20% of participants in our study had elevated hcy levels (>15μmol/L). The prevalence of hyperhomocysteinaemia in our study was much higher than the 6% reported in a sub-sample of male and female participants over 60 years age in a recent Dunedin study.[11] This study consisted of adult volunteers and, therefore, may have included healthier individuals. Moreover participants in that study were younger than in our study. Age has been associated with hcy in several studies[35] and was positively associated with hcy in the present study despite an age range of just ten years. In the US NHANES III, nearly 50% of women over 60 years (n=1136) were classified as having high hcy concentrations based on a cut-off of >10.4 μmol/L.[35] Using this cut-off, the prevalence of hyperhomocysteinaemia would be over 40% in our study. As with serum vitamin B_{12} there is considerable debate about the definition of hyperhomocysteinaemia. Cut-offs ranging from 9 to over 20μmol/L have been suggested [36] with 15 μmol/L being the most frequently used.[6,18,19] However, there is evidence that a lower cut-off may be more appropriate.[36] First, in a meta-analyses of observational studies, which included many older participants, it was predicted that each 1 μmol/L decrease in hcy was associated with a 10% reduction in risk of coronary artery disease.[4] This risk reduction was based on a hcy concentration of between 10-15μmol/L. Also the cardiovascular benefit of lowering hcy concentrations in high-risk individuals has recently been demonstrated.[37] Restenosis rates after coronary angioplasty were reduced from 39% to 26% in patients who took a daily folic acid, B_{12}, and B_6 supplement. These patients had a mean baseline hcy concentration of only 11.1 μmol/L.

Despite the general absence of low blood folate concentrations in our study, hcy was inversely correlated with both serum folate and erythrocyte folate. This finding is consistent with several overseas studies where hcy has been shown to be inversely correlated with folate across the whole

range of blood folate concentrations.[10] Further, folic acid has been shown to effectively lower hcy concentrations in populations with adequate biochemical folate status and normal hcy concentrations.[38] It must be remembered that 'cut-off' values for blood folate indices are for megaloblastic anaemia and not for the optimization of hcy concentrations. Given the high prevalence of low serum B_{12} in our study it was somewhat surprising that serum B_{12} was not a significant determinant of plasma hcy. Other investigators report an inverse relationship between hcy and serum B_{12} especially in the elderly[39, 40] but this finding has been less consistent than for folate. Moreover, vitamin B_{12} supplements are generally not as effective as folic acid in lowering hcy.[8] A potential weakness of our study is that we did not consider the effect of vitamin B_6 on hcy concentrations. Vitamin B_6 is involved in the metabolism of hcy. However, it has been shown in several populations, including the elderly, that vitamin B_6 is not an important determinant of hcy.[6,9] Further, in a recent meta-analysis of hcy lowering trials it was established that folic acid reduces blood homocysteine concentrations by 25% and that vitamin B_{12} produces an additional 7% reduction. However, no additional hcy lowering effect could be attributed to vitamin B_6.[8]

The finding that creatinine was the greatest determinant of hcy in our elderly population is an important finding and is consistent with the findings of others.[21,41,42] The relation between circulating concentrations of hcy and serum creatinine may reflect the effect of renal function on hcy concentrations but could also reflect increased hcy production during creatine metabolism.[41,43] Renal failure is frequently accompanied by elevated hcy concentrations.[44] However, urinary clearance of hcy is low and the importance of renal uptake and metabolism of hcy in hcy elimination remains controversial. Homocysteine has been used a functional indicator of folate and to a lesser extent vitamin B_{12} status. The high correlation between creatinine and hcy means that hcy needs to be interpreted with caution as a functional indicator of folate and B_{12} status in the elderly.

Smoking and alcohol use have been associated with hcy in several studies of the elderly.[45,46] The failure to find a significant association between smoking and hcy in our study is likely explained by the low number of current smokers (n=9). The finding that folate, B_{12}, or B_6 supplement use was not associated with lower hcy was unexpected and not consistent with the findings of other investigators. Possible explanations include the low number of vitamin supplement users in the present study (16%), our insensitive measure of vitamin B_{12}, folic acid, and/or vitamin B_6 supplement use (yes/no); or irregular use of supplements by the supplement users.

We aimed to recruit a representative sample of non-institutionalised elderly Dunedin women between 70-80 years. There was no evidence that responders differed markedly from non-responders relative to age, subjective health, and living arrangements. There is the potential, however, that responders differed in unmeasured variables such as biochemical measures. The findings should not be considered representative of the New Zealand elderly female population. Our study was carried out in an urban centre. Further, we did not include institutionalised or non-ambulatory elderly women who would be expected to have a poorer vitamin status than these women.[1] Our results probably underestimate the prevalence of sub-clinical vitamin B_{12} for the general elderly population.

New Zealand health authorities are considering a mandatory food folic acid fortification policy to reduce the incidence of neural tube defects (NZ Ministry of Health, Personal Communications). However, there is a concern that folic acid added to food could mask (i.e. correct) the anaemia of B_{12} deficiency allowing the neurological damage that B_{12} deficiency can cause to progress undetected.[47] We did not find a single case of megaloblastic anaemia in the 103 participants surveyed suggesting the risk of 'masking' is low in this population. Further, hcy was inversely associated with folate blood concentration suggesting that this population might benefit from folic acid fortification through a reduction in hcy. Folic acid fortification in the United States was associated with a fall in hcy concentrations in the elderly.[48]

In conclusion, we have shown a moderately high prevalence of sub-optimal plasma vitamin B_{12} concentrations among non-institutionalised senior women in Dunedin. However, low plasma B_{12} concentrations were not associated with megaloblastic anaemia. There was little evidence of folate deficiency in this group. However, the prevalence of hyperhomocysteinaemia was high and hcy was inversely associated with folate blood concentration.

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