Report to

Food Standards Australia and New Zealand

Assessment of Risk of Masking Vitamin B12 Deficiency from an Increase in Folic Acid Intake

From

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Executive Summary

The objective of this report was to conduct a review to determine:

1. The current teaching on recognition and diagnosis of vitamin B12 deficiency in Australia and New Zealand medical schools. If this differs from current practice, describe the current diagnostic approach used by the medical profession.
2. the appropriate criteria for determination of vitamin B12 deficiency in Australia and New Zealand
3. the prevalence of those at risk of vitamin B12 deficiency in Australia and New Zealand, including identified subgroups such as those over 50 years, vegans etc
4. whether folic acid intakes up to 1.0mg/day improve the haematological sequelae of vitamin B12 deficiency.

The method to determine current teaching in Australian and New Zealand medical schools used was the most methodologically sound given the short time frame for the completion of the report. The remaining parts were approached by systematic reviews of the literature, hand searching, email contacts and approaching key informants for unpublished data.

No standard approach to teaching medical students could be located, given the number of problem based learning programs. There is variation in the levels of serum B12 taken as indicative of B12 deficiency, and laboratory derived standards depend on machines and reagents used, often being supplied by manufacturers.

The prevalence of B12 deficiency and insufficiency is variable but may be up to 25% of elderly people and higher among vegetarians who do not take supplements.

It is recommended that more appropriate points for the determination of deficiency be identified and that this be from epidemiological studies where increased risk is identified.

There is no evidence that at intake levels of 1mg of dietary folate equivalents, that masking of B12 will occur.

Introduction

Food Standards Australia and New Zealand (FSANZ) are currently investigating mandatory fortification with folic acid. The aim of mandatory fortification with folic acid is to reduce the incidence of neural tube defects. Additionally there are several other heath benefits that increased intake of folic acid may produce, this includes its positive effects on homocysteine and cardiovascular disease, along with cancer (1).

Despite these encouraging outcomes, the issue of the risks associated with mandatory fortification with folic acid must also be explored. One such risk is the suggestion that high levels of folic acid intake may resolve the anaemia associated with vitamin B12 deficiency (2). This is referred to as the 'masking' of vitamin B12 deficiency. This is of particular concern in population groups at risk of vitamin B12 deficiency such as those over 50 years of age and vegans (2). Reviews have generally concluded that folic acid intakes up to greater than 5mg/day improve the anaemia of vitamin B12 deficiency, where as at levels up to 1mg/day no such effect can be concluded (2-4).

The draft for the Nutrient Reference Values for Australia and New Zealand has suggested an upper intake limit for folate from fortified foods or supplements of up to 1mg/day for adults aged 19 years and over, along with those who are pregnant or lactating (5). Therefore with respect to the masking of vitamin B12 deficiency by folic acid the issue is whether intakes up to 1mg/day will cause this suggested masking effect.

Flood et al in a study of 2895 people aged over 49 years found that at current levels of voluntary folic fortification in Australia 0.4% of the study population were consuming over 1mg/day of folic acid. It was estimated that this would increased to 0.5% with mandatory fortification (6). This provides evidence which suggests that subsets of the Australian and New Zealand population will be consuming up to and greater than 1mg of folic acid per day.

Therefore, firstly evidence is required to support or refute the hypothesis that folic acid intakes up to 1mg/day improve the haematological sequelae of vitamin B12 deficiency. Secondly, to assess the effect of masking of vitamin B12 deficiency caused by folic acid on the population, prevalence of deficiency within the Australian and New Zealand population must first be evaluated, particularly among the at risk groups previously mentioned. In doing so the appropriate criteria for definition of vitamin B12 deficiency must be determined, to ensure prevalence data has been assessed correctly. Finally given that the diagnosis of vitamin B12 deficiency requires further investigation than solely the associated anaemia, current teaching in

Australian and New Zealand medical schools will be reviewed to show the extent to which reliance is placed on the haematological sequelae of vitamin B12 deficiency for diagnosis.

Objectives

To conduct a review to determine:

1. The current teaching on recognition and diagnosis of vitamin B12 deficiency in Australia and New Zealand medical schools. If this differs from current practice, describe the current diagnostic approach used by the medical profession.
2. the appropriate criteria for determination of vitamin B12 deficiency in Australia and New Zealand
3. the prevalence of those at risk of vitamin B12 deficiency in Australia and New Zealand, including identified subgroups such as those over 50 years, vegans etc
4. whether folic acid intakes up to 1.0mg/day improve the haematological sequelae of vitamin B12 deficiency.

Part One

The current teaching on recognition and diagnosis of vitamin B12 deficiency in Australia and New Zealand medical schools. If this differs from current practice, describe the current diagnostic approach used by the medical profession.

Method

To determine the current teaching on recognition and diagnosis of vitamin B12 deficiency in Australia and New Zealand medical schools the following steps were taken;

1. The Committee of Deans of Australian Medical Schools, the Australian Medical Students Association and the Australian Medical Council were contacted regarding curriculum content in Australian and New Zealand medical schools relevant to vitamin B12 deficiency.

2. A survey was sent to senior academics of all Australian and New Zealand medical schools who are members of the Committee of Deans of Australian Medical Schools. Additionally specific teaching staff from the disciplines of geriatrics, haematology and neurology who were nominated by members of the Hunter Ageing Research Network (a network of clinicians and researchers interested in ageing) were also surveyed. The survey aimed to determine current teaching practices.

3. Common medical textbooks used in some Australian and New Zealand medical schools, which were made available from information provided by medical librarians, were reviewed for relevant information regarding vitamin B12 deficiency.

4. Two journals; the Medical Journal of Australia and Australian Family Physician were searched online from 1996 and 2002 respectively. The search terms; vitamin B12 and vitamin B12 deficiency were used. These two journals were chosen as they are routinely available to medical students and commonly include summary articles on diagnosis

5. The internet search engine Google, was used to search the World Wide Web, using the search term 'vitamin B12 deficiency. The first twenty results were reviewed; those which were relevant to the recognition and diagnosis of vitamin B12 deficiency and from sources which students would consider as reputable and authoritative were retrieved and data extracted. Such a search was conducted as it shows with information is readily accessible by students.

All information provided from the five sources will be presented to highlight the current information a medical student may be presented concerning vitamin B12 deficiency.

Results

1. The curriculum content regarding vitamin B12 deficiency from Australian and New Zealand medical schools was not available from the Committee of Deans of Australian Medical Schools, the Australian Medical Students Association and the Australian Medical Council . It was found however that the majority (at least 11/20) of the medical schools in Australia and New Zealand are now using problem based learning, at the very least as a component of their program. Therefore it is unlikely that there is strict curriculum that exists regarding 'how to' diagnose vitamin B12 deficiency.

2. The survey of deans had a response rate of 11% (2/18). One university which replied was unable to provide specific information as its program was solely problem based learning. The other stated the topic of B12 deficiency is taught through out the course, but specifically with nutritional assessment. As part the teaching of nutritional assessment the specific vitamin deficiencies discussed would rely on the questions of the students. Otherwise students would become aware of diagnosis in clinical cases, should they encounter patients or cases with vitamin B12 deficiency.

3. Textbooks from two universities' medical students reading list were reviewed, of which 12 included information regarding the diagnosis and/or recognition of vitamin B12 deficiency. Of the textbooks 3 were pathology, 1 general practice, 3 haematology, 2 general medical, 1 neurology, 1 medical nutrition and 1 geriatric textbook. The information retrieved from the textbooks is outline in Table 1

4. One article was retrieved from the Australian Family Physician. No relevant articles were found in the Medical Journal of Australia. The data extracted was reviewed with that from medical textbooks (Table 1).

5. The search of the world wide web using the internet search engine google, produced 5 documents which were deemed to be from reputable sources and relevant to the recognition

and diagnosis of vitamin B12 deficiency. Of these 3 focused on the anaemia of vitamin B12 deficiency.

Discussion

The method to determine current teaching in Australian and New Zealand medical schools used was the most methodologically sound given the short time frame for the completion of the report. Ideally information from medical schools regarding their curriculum would have been more highly sought after and not based solely on the response to an email survey. However, given the current trend in the use of problem based learning in both Australian and New Zealand medical, it is possible that exactly what is being 'learnt' by medical students may not have even been available from academics, due to the self-directed nature of this style of learning. Therefore the 'best' approach would have been to contact the students themselves. Such a method has been used previously to discover the knowledge of medical students regarding cancer biology, management and epidemiology. Students were surveyed on the first day of internship with a response rate of 84%

Standard reference and text books, available to medical students, reviewed for this report (n=12) were selected from a range of subject areas. The majority of information regarding vitamin B12 deficiency, including aetiology, diagnosis and management was found in chapters relating to anaemias. All 'anaemia' chapters specifically included vitamin B12 deficiency neurological complications with the exception of the practice text (25). Given that the older population is at greater risk of vitamin B12 deficiency (16, 55) a more extensive search of specialist geriatric texts would have been beneficial. The geriatric text edited by Ratnaike (2002) (26) was selected as it is a current Australian text.

Tables one provides a summary of information regarding vitamin B12 deficiency sourced from the reviewed texts. Traditionally, diagnosis of vitamin B12 deficiency has been based on clinical evidence and serum vitamin B12 results (16). This was found to be the recommendation in 15 references. Caution should be taken when interpreting serum B12 results, as studies have shown that vitamin B12 deficiency neuropsychiatric disorders have been found to occur commonly in individuals whose serum B12 levels were above 150 pmol/L and in whom anaemia and macrocytes were absent (56) (30). In these cases serum MMA, which has a high sensitivity, and serum homocysteine tests were used to diagnose vitamin B12 deficiency (56) (16). These subjects showed neurological disorders associated with B12 deficiency in the absence of anaemia or macrocytes (56). The majority of texts refer to the Schilling test as an option in

diagnosing the cause of the vitamin B12 deficiency. In practice, this test has poor sensitivity and is generally unavailable in Australia (15).

Conclusion

It is not possible to identify specific medical curriculum relating to the diagnosis and recognition of B12 deficiency. There is a range of materials available, but it remains with medical practitioners to be alert to the neurological sequelae of deficiency or early signs of insufficiency.

Table 1. Data extracted from medical textbooks, Australian Family Physician and search of the world wide web by clinical manifestations of vitamin B12 deficiency.

Clinical Manifestation		Reference
Neurological – Symptoms	Peripheral neuropathy	Stabler & Allen (8), Antony AC (9), Hoffbrand, Pettit & Moss (10), Babior BM (11) Kumar V, Cotran RS & Robbins SL (12) CulliganDJ (13) Jeffery DR (14) Charlton KE & Schloss IC(15) Doust J(15) Oh, RC & Brown, DL(16)
	Ataxia	Antony AC (9) Babior BM (11) Kumar V, Cotran RS & Robbins SL (12) CulliganDJ(13) Cotran RS & Kumar V (17) Charlton KE & Schloss IC(14) Andres et al (18) Dharmarajan & Norkus (19)
	Weakness and paraesthesiae of lower limbs	Ironside JW (17) Cotran RS, Kumar V (16) Chanarin I[21] Jeffery DR (14) Charlton KE & Schloss IC (15) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19) Ezra (22) Anderson (23)
	Stiffness of extremities	Antony AC (9)

	Diminished proprioception	Stabler & Allen(8) Hoffbrand, Pettit & Moss (10) Babior BM (11) Chanarin I[1] Jeffery DR (14) Doust J(15)
	Decreased or increased deep tendon reflexes	Antony AC (9) Chanarin (21) Dharmarajan & Norkus (19)
	Spastic paraparesis	Cotran RS, Kumar V (17) Chanarin I[21] Jeffery DR(14)
	Urinary or faecal incontinence	Antony AC (9) Jeffery DR(14) Chanarin (21) Andres et al (18) Dharmarajan & Norkus (19)
	Altered mentation	Stabler & Allen (8) Chanarin (21) Jeffery DR (14)
	Perversion of taste and smell	Babior BM (11)
	Depression	Stabler & Allen (8) Antony AC (9) Babior BM (11) Chanarin (21) Goh & Dhillon (24) Charlton KE & Schloss IC(14) Dharmarajan & Norkus (19) Oh, RC & Brown, DL(16)

	Psychoses	Stabler & Allen (8) Hoffbrand, Pettit & Moss (10) Babior BM (11) CulliganDJ (13) Jeffery DR (14) Charlton KE & Schloss IC (15) Dharmarajan & Norkus (19) Oh, RC & Brown, DL(16)
	Optic atrophy	Hoffbrand, Pettit & Moss (10) Babior BM(11) CulliganDJ (13) Jeffery DR (13) Andres et al (18) Dharmarajan & Norkus (19)
	Irritability	Antony AC(9) Chanarin(21) Oh, RC & Brown, DL(16)
	Vertigo	Antony AC(9)
	Dementia	Babior BM (11) Culligan DJ (13) Jeffery DR (14) Goh WMH, Dhillon (24) Charlton KE & Schloss IC(14) Andres et al (18) Oh, RC & Brown, DL(16) Dharmarajan & Norkus (19) Ezra (22)
	Mild confusion	Charlton KE & Schloss IC(15)
	Apathy	Jeffery DR (14) Charlton KE & Schloss IC(15)
	Sub-acute combined systems disease	Oh, RC & Brown, DL(16)
	Impotence	Dharmarajan & Norkus (19)

	Memory loss	Oh, RC & Brown, DL(16) Dharmarajan & Norkus (19)
Symptoms associated with anaemia or B12 deficiency?	Tiredness/fatigue	Murtagh J (25) Kumar V, Cotran RS & Robbins SL (11) Culligan DJ (12) Chanarin (21) Jeffery DR(13) Ratnaike (26) Charlton KE & Schloss IC (14) Unknown (27) Ezra (22)
	Muscle weakness	Murtagh J (25) Chanarin (21) Jeffery DR (13) Charlton KE & Schloss IC(14)

	Headache	Murtagh J (25) Chanarin (21) Unknown (27) Anderson(23)
	Lack of concentration	Murtagh J (25)
	Faintness/dizziness	Murtagh J (25) Unknown (27)
	Dyspnoea on exertion	Murtagh J (25) Kumar V, Cotran RS & Robbins SL (11) Culligan DJ (12) Chanarin (21) Charlton KE & Schloss IC(14) Unknown (27) Ezra (22) Anderson (23)
	Palpitations	Murtagh J (25)
	Angina on effort	Murtagh J (25)
	Intermittent claudication	Murtagh J (25)
	Congestive heart failure	Kumar V, Cotran RS & Robbins SL (11) Culligan DJ (12)
	Sore mouth	Culligan DJ (12) Charlton KE & Schloss IC(14) Ezra (22) Anderson (23)
	Bruising and mucosal haemorrhage	Culligan DJ (12)
	Anorexia	Chanarin (21) Goh & Dhillon (24) Charlton KE & Schloss IC(14) Doust J(15) Ezra (22) Anderson (23)
	Vomiting	Chanarin (21)

	Diarrhoea	Chanarin (21) Ratnaike (26) Charlton KE & Schloss IC(14) Doust J(15) Ezra (22)
	Fever	Charlton KE & Schloss IC(15)
	Megablastic anaemia	Oh, RC & Brown, DL(16) Dharmarajan & Norkus (19)
	Pancytopaenia	Oh, RC & Brown, DL(16)
Haematological tests to establish megaloblastic anaemia	Peripheral blood film and count	Hoffbrand AV (28) Stabler & Allen(7) Chanarin (21) Charlton KE & Schloss IC(14)
	Bone marrow aspiration	Hoffbrand AV (28) Antony (8) Chanarin (21)
	Serum bilirubin, iron, LDH	Hoffbrand AV (28)

Tests to establish B12 deficiency Megaloblastic anaemia is recognized by: (28) i) raised mean corpuscle volume (MCV) >100fL (MCV may be normal if there is associated iron deficiency ii) Oval macrocytes in blood film iii) Poikilocytosis & anisocytosis present in severe cases iv) Hypersegmented neutrophils (5+ lobes) in peripheral blood v) Neutrocyte & lymphocyte count moderately reduced vi) Platelet count may be moderately reduced Bone marrow aspirate is hypercellular with enlarged erythroblasts with morphological abnormalities	Serum vitamin B12 and folate: red cell folate	Hoffbrand AV (28) Stabler & Allen (7) Antony (8) Hoffbrand, Pettit & Moss (9) Hines (29) Babior BM (10) Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Chanarin (21) Jeffery DR (13) (26)Charlton KE & Schloss IC(14) Patten JP (30) Culligan DJ (12) Goh & Dhillon (24) Andres et al (18) Doust J(15) Oh, RC & Brown, DL (16) Anderson (23)
	Serum homocysteine	Hoffbrand AV (28) Stabler & Allen (7) Antony (8) Babior BM (10) Chanarin (21) Charlton KE & Schloss IC(14) Andres et al (18) Oh, RC & Brown, DL (16)

	Serum methylmalonic acid (MMA) levels Considered 'gold standard' in diagnosing vitamin B12 deficiency (Antony AC (9))	Hoffbrand AV (28) Stabler & Allen (7) Antony (8) Hines (29) Babior BM (10) Chanarin (21) Charlton KE & Schloss IC(14) Oh, RC & Brown, DL (16)
	Urinary MMA – the texts indicate the test is available, however at time of their publication there was inadequate clinical data to support its widespread use.	Antony (8) Hines (29) Babior BM (10) Jeffery DR (13) Charlton KE & Schloss IC(14)
	Deoxyuridine suppression test	Hoffbrand AV (28) Hoffbrand, Pettit & Moss(9) Babior BM(10) Chanarin (21) Dharmarajan & Norkus (19)
	Holo-transcobalamin II (holo-TC) –use still under evaluation	Antony (8) Charlton KE & Schloss IC(14) Dharmarajan & Norkus (19)
Tests for cause of B12 deficiency	Serum antibodies to parietal cell, intrinsic factor	Hoffbrand AV (28) Stabler & Allen (7) Antony (8) Hoffbrand, Pettit & Moss (9) Babior BM (10) Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Dixon MF (31) Charlton KE & Schloss IC(14) Doust J(15)
	Serum immunoglobulins	Hoffbrand AV (28) Antony (8)

	Gastric secretion; intrinsic factor, acid	Hoffbrand AV (28) Doust J(15) Antony (8) Hoffbrand, Pettit & Moss (9)
	Endoscopy, gastric deficiency	Hoffbrand AV (28) Hoffbrand, Pettit & Moss (9)
	Barium meal + follow-through	Hoffbrand AV (28) Hoffbrand, Pettit & Moss (9)
	Radioactive B12 absorption tests (alone, with intrinsic factor, after antibiotics, with food)	Hoffbrand AV (28) Hoffbrand, Pettit & Moss (9) Hines (29)
	Proteinuria, fish tapeworm ova, intestinal flora etc	Hoffbrand AV (28)
	Schilling test	Stabler & Allen (7) Antony AC (8) Hoffbrand, Pettit & Moss (9) Hines (29) Babior BM (10) Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Chanarin (21) Jeffery DR (13) Charlton KE & Schloss IC(14) Dharmarajan & Norkus (19) Unknown(27) Anderson (23)
	Diet history	Hoffbrand, Pettit & Moss (10)

Physical presentation that may indicate investigation for vitamin B12 deficiency	Glossitis	Stabler & Allen (7) Antony (8) Hoffbrand, Pettit & Moss (9) Culligan DJ (12) Cotran RS, Kumar V (17) Charlton KE & Schloss IC (14) Andres et al (18) Doust J(15)
	Vitiligo	Antony (8) Hoffbrand, Pettit & Moss (9) Chanarin (21)
	Mild jaundice	Hoffbrand, Pettit & Moss (9) Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Andres et al (18)
	Pallor	Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Chanarin (21) Charlton KE & Schloss IC(14) Unknown (27) Ezra (22)
	Tachycardia	Murtagh J(25) Chanarin (21)
	Systolic flow murmur	Murtagh J(25) Chanarin (21)
	Ankle oedema	Chanarin (21)
	Hypotensive	Murtagh J(25) Chanarin (21)
	Rapid weak pulse rate	Unknown (27) Anderson(23)

Patient History – Where i) disorder that affects vitamin B12 absorption or ii) reduce dietary intake of vitamin B12 or iii) change of weight	Total or partial gastrectomy	Antony (8) Hines (29) Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Culligan DJ (12) Chanarin (21) Charlton KE & Schloss IC(14) Andres et al (18) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Anastomosis	Antony AC (9)
	Fistula	Antony AC (9)
	Bowel resection	Antony (8) Hines (29) Culligan DJ (12) Chanarin (21) Charlton KE & Schloss IC(14) Andres et al (18)
	Family history of pernicious anaemia	Hoffbrand, Pettit & Moss (9) Murtagh J(25) Culligan DJ (12) Charlton KE & Schloss IC[5]
	Weight loss	Antony AC Hoffbrand, Pettit & Moss (9) Babior BM (10) Culligan DJ (12) Chanarin (21) Ratnaike (26) Charlton KE & Schloss IC(14)
	Strict selective diet, especially vegan	Murtagh J(25) Kumar V, Cotran RS & Robbins SL (11) Culligan DJ (12) Chanarin (21) Charlton KE & Schloss IC(14) Andres et al (18) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Strictures	Chanarin (21)

	Chronic gastritis	Dixon MF(31) Chanarin (21) Charlton KE & Schloss IC (14) Andres et al (18) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Crohn's Disease	Culligan DJ (12) Chanarin (21) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Gluten sensitivity	Chanarin (21)
	Poverty	Charlton KE & Schloss IC (14)
	Tropical Sprue	Chanarin (21) Ratnaike (26) Charlton KE & Schloss IC(14)
	Bacterial overgrowth	Charlton KE & Schloss IC(14) Andres et al (18) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Fish tapeworm	Charlton KE & Schloss IC(14) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Haemodiaylysis	Charlton KE & Schloss IC(14)
	Drug interactions	Charlton KE & Schloss IC[5] Andres et al (18) Oh, RC & Brown, DL (16) Dharmarajan & Norkus (19)
	Chronic alcoholism	Andres et al (18)
	Congenital deficiency of transcobalamin II	Andres et al (18)
	Pancreatic disease	Dharmarajan & Norkus (19) Andres et al (18)
	Age >65	Andres et al (18)

		Oh, RC & Brown, DL (16)
	Age > 50	Dharmarajan & Norkus (19)
	Multiple sclerosis	Dharmarajan & Norkus (19)
Vitamin B12 serum level	Normal 170-800 pg/ml	Chanarin (21)
	Low < 170 pg/ml	Chanarin (21)
	Normal >200 pg/ml	Jeffery DR (13)
	Abnormal <200 pg/ml	Jeffery DR (13)
	Association with neurologic abnormalities <100 pg/ml	Jeffery DR(13) Chanarin (21)
	Deficiency < 100pmol/L	Charlton KE & Schloss IC(15)
	Mild deficiency <237pg/ml	Charlton KE & Schloss IC(15)
	Normal 200-900pg/ml	Charlton KE & Schloss IC(15)
	Immunoassay; Normal 145-880 pmol/L Microbiological assay; Normal 115-660 pmol/L	
	Low < 147 pmol/L Borderline (requires further investigation) 147-220 pmol/L	Antony (8)
	Low < 125 pmol/L Normal 147 – 440	Hines (29)
	Normal 147 – 664 pmol/L Requires further investigation - < 258 pmol/L	Stabler (7)
	Low <200 pg/mL (150pmol/L) Deficiency < 100pg/mL (74pmol/L) No Deficiency >400pg/mL Measure serum MMA/homocysteine if 100-400pg/mL (74-295 pmol/L)	Oh, RC & Brown, DL (16)

Table 2. Data extracted from google search results by clinical manifestations of vitamin B12 deficiency

Clinical Manifestation		Reference
Neurological – Symptoms	Paresthesias	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25) Ezra (26) Anderson (27)
	Ataxia	Dharmarajan & Norkus (25)
	Decreased or increased deep tendon reflexes	Dharmarajan & Norkus (25)
	Peripheral neuropathy	Oh, RC & Brown, DL(24)
	Subacute combined systems disease	Oh, RC & Brown, DL(24)
	Urinary or faecal incontinence	Dharmarajan & Norkus (25)
	Optical atrophy	Dharmarajan & Norkus (25)
	Impotence	Dharmarajan & Norkus (25)
Psychiatric	Irritability	Oh, RC & Brown, DL(24)
	Dementia	Oh, RC & Brown, DL(24) Dharmarajan & Norkus (25) Ezra (26)
	Memory Loss	Oh, RC & Brown, DL(24) Dharmarajan & Norkus (25)
	Depression	Oh, RC & Brown, DL(24) Dharmarajan & Norkus (25)
	Psychosis	Oh, RC & Brown, DL(24) Dharmarajan & Norkus (25)
Haematologic	Megablastic anaemia	Oh, RC & Brown, DL(24) Dharmarajan & Norkus (25)
	Pancytopaenia	Oh, RC & Brown, DL(24)
Other Symptoms	Cerebrovascular disease	Dharmarajan & Norkus (25)
Tests to establish B12 deficiency	Serum vitamin B12 and folate: red cell folate	Oh, RC & Brown, DL (24) Anderson (27)

Tests for cause of B12 deficiency	Serum homocysteine	Oh, RC & Brown, DL (24)
	Serum methylmalonic acid (MMA) levels	Oh, RC & Brown, DL (24)
	Serum holotranscobalamin II	Dharmarajan & Norkus (25)
Tests for cause of B12 deficiency	Serum antibodies to parietal cell, intrinsic factor	Dharmarajan & Norkus (25)
	Deoxyuridine suppression test	Dharmarajan & Norkus (25)
	Schilling test	Dharmarajan & Norkus (25) Unknown(28) Anderson (27)
Physical presentation that may indicate investigation for vitamin B12 deficiency	Breathlessness	Unknown (28) Ezra (26) Anderson (27)
	Fatigue	Unknown (28) Ezra (26)
	Dizziness	Unknown (28)
	Rapid weak pulse rate	Unknown (28) Anderson(27)
	headaches	Unknown (28) Anderson (27)
	Pale skin	Unknown (28) Ezra (26)
	Loss of appetite	Ezra (26) Anderson (27)
	Diarrhoea	Ezra (26)
	Sore mouth and tongue	Ezra (26) Anderson (27)
Patient History – Where i) disorder that affects vitamin B12 absorption or ii) reduced dietary intake of vitamin B12 or iii) weight loss indicates investigation into vitamin B12 status	Gastric surgery/Ileal surgery	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	Crohn's Disease	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	Prolonged use of histamine H_2 receptor blockers or proton pump inhibitors	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)

	Chronic gastritis	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	Pancreatic diseases	Dharmarajan & Norkus (25)
	Age >65	Oh, RC & Brown, DL (24)
	Strict selective diet, especially vegan	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	Bacterial overgrowth	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	Tapeworm infestation	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)
	HIV infection	Dharmarajan & Norkus (25)
	Multiple sclerosis	Dharmarajan & Norkus (25)
	Age > 50 years	Dharmarajan & Norkus (25)
Vitamin B12 serum level	Low <200 pg/mL (150pmol/L)	Oh, RC & Brown, DL (24)
	Deficiency < 100 pg/mL (74 pmol/L)	Oh, RC & Brown, DL (24)
	No Deficiency > 400 pg/ml	Oh, RC & Brown, DL (24)
	Measure serum MMA/homocysteine if 100 – 400 pg/ml (74-295 pmol/L)	Oh, RC & Brown, DL (24) Dharmarajan & Norkus (25)

Part Two

The appropriate criteria for determination of vitamin B12 deficiency in Australia and New Zealand

Method

To determine the appropriate criteria for determination of vitamin B12 deficiency in Australia and New Zealand the following websites were searched;

- Royal Australian College of General Practitioners (www.racgp.org.au)
- Royal Australian College of Physicians; Internal Medicine-Adult Medicine Division (www.racp.edu.au) and,
- Royal College of Pathologists Australasia (www.rcpa.edu.au)

The following search terms were used:

- Vitamin B12 deficiency
- Vitamin B12 deficiency and diagnosis
- Vitamin B12 and testing or tests

Additionally data collected in part one regarding the appropriate criteria for determination of vitamin B12 deficiency was also used..

Results

Of the 3 college websites searched only the Royal College of Pathologists of Australasia included information regarding the diagnosis of vitamin B12 deficiency (Figure 1.). Of the two other websites, one could not be accessed by the general public and the other had no information available regarding vitamin B12 deficiency. Notably all websites had members section which may have included more information, but could not be accessed for this report.

Figure 1. Results regarding appropriate diagnosis of vitamin B12 deficiency

Suggested tests for Vitamin B12 deficiency

FBC, blood film, differential WCC, platelet count.

Vitamin B12 and folate assays.

Bone marrow aspiration only occasionally required for diagnosis and/or confirmation.

Review clinical features for likely cause.

Methylmalonate, plasma or urine, has been used to monitor treatment.

Serum vitamin B12 specifically

Reference interval: 120-680pmol/L

Investigation of a patient with high MCV and/or morphological changes suggestive of megaloblastic anaemia

Source: Royal College of Pharmacology Australasia RCPA Manual (available: http://www.rcpamanual.edu.au)

Discussion

For the purpose of this review the serum vitamin B12 reference range of 120 - 680 pmol/L (2), used by The Royal College of Pathologists of Australasia (RCPA) has been assumed to be gold standard. This range is found in the RCPA manual, however the research supporting this range has not been detailed.

There are a variety of methods that can be used when deciding on reference ranges. Namely i) normative values, two standard deviations from the mean using the general population as a reference, ii) aged specific normative values and iii) clinical values from epidemiological studies at below which there are increased risk of neurological sequelae. Further, given the criteria currently used to determine reference ranges for serum B12 estimation none of the published cut-points may be associated with increased risk of significant neurological sequelae. The variations in the range of normal serum vitamin B12 is not surprising given the fact that ranges depend the variables of the make of machine, reagent used and the variations in sample populations used to calculate standard deviations from normal.

There is a wide range of published estimates for the lower limit of normal serum vitamin B12 levels (57). This is due in part to the lack of a consistently defined gold standard for the diagnosis of vitamin B12 deficiency (1). The method of testing can result in different reference ranges. Serum tests done by immunoassay typically giving a higher normal range (eg 146-880pmol/L) than the older microbiological assay method (eg 117-660 pmol/L). Current research indicates that relying on a serum B12 lower limit of 120 pmol/L for diagnosis would result in individuals with clinically significant B12 deficiency being overlooked.

Conclusion

The estimates of the prevalence of B12 deficiency depend on the specific level selected for indication of deficiency. This is variable and depends on laboratory values which in turn can depend on machines and reagents. It would be better to identify the level at which risk is increased and to use this as the defining level.

Part Three

The prevalence of those at risk of vitamin B12 deficiency in Australia and New Zealand, including identified subgroups such as those over 50 years and vegans

Method

To determine the prevalence of vitamin B12 deficiency in Australia and New Zealand a systematic review was conducted.

Criteria for considering studies for this review

1. Types of studies

The review aimed to include random sample surveys, cross-sectional surveys and cohort studies. Along with experimental studies that have measured vitamin B12 deficiency at baseline, prior to intervention.

2. Types of participants

Participants include Australians and New Zealanders with vitamin B12 deficiency. This includes groups at risk of vitamin B12 deficiency including; the elderly and vegetarians.

3. Types of interventions

This review aimed to include non-experimental studies and data from experimental studies prior to intervention, therefore specific interventions need not be specified.

4. Types of outcome measures

Percentage or number of participants with vitamin B12 deficiency.

5. Search strategy for identification of studies

The review consisted of a search of published and unpublished literature in the English language. The following databases were searched: Cochrane, Medline/PubMed/Premedline, Cinahl, Ebsco Megafile Premier, Embase, Science Direct

The following search strategy was used:

- Vitamin B12 deficiency
- Prevalence OR incidence OR rate OR frequency OR proportion
- Australia OR New Zealand
- 1 & 2 & 3

Additionally bibliographies and reference lists of articles retrieved were searched for relevant literature.

Key informants from major longitudinal studies conducted in Australia were contacted regarding relevant data. This included the;

- Hunter Community Study
- Sydney Older Peoples Study
- Blue Mountains Eye Study
- Australian Longitudinal Study of Aging
- Busselton Study

Part Three and Four

For both this and the following part (p38) the following methods were used.

Selection Process

All studies identified from the search of databases were assessed for relevance against the inclusion criteria of the review based on the information contained in the title, abstract and descriptor. Those that were relevant were retrieved.

Methods of the review

1. Critical Appraisal

All studies that meet the inclusion criteria were included in the review. Each study was judged by its level of evidence, based on the National Health and Medical Research Councils Levels of Evidence (7) .

2. Data extraction

Data were extracted regarding the:

- study design,
- existence of randomization,
- existence of blinding,
- date and/or duration of the study/intervention,
- inclusion criteria of participants,
- baseline characteristics of participants,
- setting
- outcome measures and,
- results

3. Data synthesis

Results are recorded in a narrative summary. Results from part four will be classed as either;

- convincing evidence
- probably evidence
- possible evidence
- insufficient evidence

based on the FSANZ guidelines for classifying the likelihood that the assessed evidence is substantiated .

Results

Eleven studies were deemed appropriate for inclusion (Table 3). A total of 8625 participants were included in these studies. Of these 7 were set in Australia and the remaining 4 in New Zealand. Participants included:

- Seventh Day Adventists (n=2) who were studied due to their vegetarian status,
- The at risk age group of >50 years (n=7)
- Indigenous Australians (n= 1), and
- Adults (n=1) with participants aged 20-90 years

The majority of studies were cross-sectional surveys (n=8), with the other data coming from cohort studies (n=2). Vitamin B12 deficiency was assessed using serum vitamin B12 in all studies retrieved. Criteria for identification of vitamin B12 deficiency from serum levels varied between studies with the lower cut off point of reference ranges from 104pmol/L to 221pmol/L.

In the Australia and New Zealand studies retrieved the percentage of participants with vitamin B12 deficiency ranged from 0.4% to 73%. In the 'at risk' population of those aged greater than 50 years deficiency ranged from 7.3% to 53%, using reference ranges from 135 to 185pmol/L to define vitamin B12 deficiency. Notably one of the these studies excluded those taking vitamin B12 supplements (29). Studies in Seventh Day Adventist vegetarians found levels of deficiency at 21.7 and 53 % using reference ranges from 118 to 171pmol/L to define vitamin B12 deficiency. A study of indigenous Australians found levels of vitamin B12 deficiency at 1.2% (n= 365). One study of an adult population found levels of deficiency at 0.4% using a reference range of less than 160pmol to define vitamin B12 deficiency.

Discussion

The systematic review of the prevalence of vitamin B12 deficiency in Australia and New Zealand raised several issues regarding the 'true' prevalence of deficiency. It has been noted that the RCPA recommends a reference range of 120 to 680pmol/L for serum vitamin B12, which suggests a 'deficiency' exists at less than 120pmol/L. However the studies involved in the systematic review included vitamin B12 deficiency existing from serum vitamin B12 concentrations of 104pmol/L to 221pmol/L. This suggests there is a margin of both under and over estimation of prevalence of vitamin B12 deficiency among some study populations. However as previously noted the reference point of <120pmol is low and higher cut off points for serum vitamin B12 have been recommended, particularly for use with the elderly (58)

The results of an unpublished study which used less than 135pmol/L serum vitamin B12 to define deficiency shows that when a reference range of <120pmol is used deficiency would fall from 10.5% to 5.8%. This equates to a 5% decrease in absolute prevalence, for a decrease of only 15pmol/L of serum vitamin B12. Likewise Flood et al show variations in vitamin B12 deficiency in an Australian population aged greater than 49 years of 15.7% with an increase of the reference range for serum vitamin B12 by 60pmol/L. Without nationally accepted diagnostic criteria, the true prevalence of clinical relevant vitamin B12 deficiency will remain unknown.

Despite the limitations of the unavailability of a 'gold standard' to define vitamin B12 deficiency the results regarding the prevalence of vitamin B12 deficiency suggests prevalence levels are relatively high. In the 'at risk' population of those aged greater then 50 years prevalence ranged from 7.3 % to 33%. Similar values have been recorded among the older population throughout the world, with prevalence of deficiency ranging from 3.0 to 40.5% (59). Notably, once again this vast range highlights the inconsistencies in defining 'deficiency'. As serum vitamin B12 levels decrease with age (58) the older population is at increased risk of vitamin B12 deficiency, this coupled with the aging population of Australia and New Zealand, it suggests that the prevalence of deficiency at a population level in both countries will continue to increase.

Vegans are those who consume no animal products and refrain from the use of animal products such as leather or wool. Vegans are at increased risk of vitamin B12 deficiency as dietary vitamin B12 is available only from animal products. Therefore an effort must be made by vegans to ensure adequate vitamin B12 intake (60). The systematic review was unable to retrieve any studies concerning prevalence of vitamin B12 deficiency among vegans in Australia and New Zealand. A search of the literature was also unable to find the prevalence of veganism within the

two countries. The 1995 National Nutrition Survey reported that 3.7% of Australians classify themselves as vegetarian (56). Of which one can assume that a proportion of would be vegans. Although this does not represent a major proportion of the population, the issue of vitamin B12 deficiency among Australian and New Zealand vegans is one that should be explored further(33,39).

It is also important to note that further data regarding the prevalence of vitamin B12 deficiency may be available from longitudinal studies undertaken in Australia including;

- the Sydney Older Peoples Study, where data are available, so contact with chief investigators may be required and,
- Hunter Community Study would be able to provide data from stored bloods if funding was made available for analysis

Conclusion

The prevalence of B12 deficiency relates to the criteria for deficiency selected. However, it can reasonably be expected that about 25% of the older population have at least B12 insufficiency. In the vegetarian population estimates of deficiency as high as 70% have been found.

Table 3. Included studies for part three: Prevalence of vitamin 12 deficiency in Australia and New Zealand

Study	Method	Participants	Outcome measures	Results	Definition of vitamin B12 deficiency (reference range)
Armstrong et al (1974) (33)	*Study design:* cross-sectional survey *Randomisation:* no *Date conducted:* unclear	*Inclusion criteria:* Seventh day Adventists aged 30 or over who were vegetarians, attending a 10 day religious convention *Baseline characteristics:* N= 561 239 men aged 30 to 85 years 322 women aged 26 to 91 years 7th day Adventists for 4 months to 80 years, Median 33 years. *Setting:* Perth, Western Australia	% of n with low serum vitamin B 12 (also divided into men and women	All: 21.7% (122/561) Women 21.1% Men 22.6%	118-646pmol/L
Barber et al (1989) (34)	*Study design:* Cross-sectional survey *Randomisation:* no *Date conducted:* Unclear	*Baseline information:* n=100. 51 male 49 female *Inclusion criteria:* those aged 70 years or older, residing in geriatric wards or rest homes. Participants with identified causes of irregular vitamin B12 and folate concentrations such as those diagnosed with pernicious anaemia, Crohn's disease, myeloproliferative disorders, malnutrition, those who had, had a gastrectomy or who took vitamin B12 supplements, methotrexate, phenytoin, colchine or metformin were excluded. *Setting:* Auckland, New Zealand	% of n with vitamin B12 concentrations of: - <37 - 38-70 - 71-100 - 101-134 - >135pmol/L	<37pmol/L= 3% (3/100) 38-70pmol/L= 1% (1/100) 71-100pmol/L=6% (6/100) 101-134pmol/= 23% (23/100) >135pmol/L= 67% (67/100) i.e. 33% with low B12 concentrations	135-700pmol/L
De jong (2003) (35)	*Study design:* Cross-sectional survey	*Inclusion criteria:* women, aged 70 to 80 years living in Dunedin, New Zealand. Institutionalised, non-ambulatory or presence of terminal illness	% of n with vitamin B12 deficiency	at risk of vitamin B 12 deficiency 13%	<150pmol/L

	Randomisation: Yes- randomly selected from 1998 electoral rolls *Date conducted:* June and August 2000	were excluded *Baseline information:* n= 103/250 (46%) Median age 74.3 years *Setting:* Dunedin, New Zealand			
Flicker et al (2004) (36)	*Study design:* Cross-sectional survey *Randomisation;* no *Date conducted:* unclear	*Baseline information:* n=572 273 female. Mean age 74.8 (4.4) range 70-92 years. 299 male. Mean age 78.9 (2.8) range 68-86 years. *Inclusion criteria:* aged 75 years or older. Males were part of a large population based study for abdominal aortic aneurysm and females were recruited. Participants were excluded if they had significant cognitive impairment, severe physical illness and if they took B-group vitamin supplements. *Setting:* Perth, Western Australia.	% of n with vitamin B12 deficiency	Men 14% Women 6%	140-646pmol/L

Flood et al (2004) (37)	*Study design:* Cohort study *Randomisation:* Unclear *Date conducted:* 1997-2000	*Baseline information:* n=2963/3508 (84%) *Inclusion criteria:* Persons aged 50 years or over residing in two postcode areas of western Sydney *Setting:* Western Sydney Australia	% of n with low serum B12	1) 22.9% 2) 35.3% 3) 6.3%	1) <185pmol/L 2) <220pmol/L 3) <125pmol/L
Green et al 2005 (38)	*Study design:* Prospective cross-sectional survey *Randomisation:* 3 stage stratified design, including randomisation *Date conducted:* 1997	*Baseline information:* n= 466. Median age 72 (interquartile range 68, 77) 228 male 238 female *Inclusion criteria:* those aged 65 years or older who had taken part in the 1996/1997 Health Survey and 1997 National Nutrition Survey for whom enough serum was available for analysis of vitamin B12. Non-institutionalised urban and rural dwelling. *Setting:* New Zealand	% normal, marginal and deficient serum vitamin B12 (95% CI)	Normal 60 (55, 65) Marginal 28 (23,34) Deficient 12 (8,16)	<221pmol/L, marginal 148-221pmol/L, deficient <148pmol/L
Hanger et al 1991 (32)	*Study design:* Prospective cross-sectional survey *Randomisation:* yes but method unclear *Date conducted:* unclear	*Baseline information:* *n*= 204/298 (79.4%) Mean age 74.3 years (+/- 6.9) 131 females and 73 males *Inclusion criteria:* 1) Aged 65 years and older, patient of medical centre and not living in institutional care 2) Additional exclusion (separate results); those taking B12 or folate supplements and those who have conditions known to influence the measurement of vitamin B12 or cause low folate levels *Setting:* Papanui Medical Centre, Christchurch, New Zealand.	% of n with vitamin B12 deficiency	2) 7.3%	Serum vitamin B12 <104pmol/L
Hokin and Butler 1999 (39)	*Study design:* Cross-sectional survey	*Baseline information:* n=245/340 (72%) Mean age 46 years	% of n below laboratory reference range	Control: 21% Vegetarians: 53%	Laboratory reference range for

	Randomised: no *Date conducted:* 1997	Age range 22 to 80 years 331 men and 9 women *Inclusion criteria:* Participants were seventh day adventist ministers, that were lactoovovegetarians or vegans. Those who took vitamin B12 supplements were excluded (n=42) Those who ate flesh products >1 per week were excluded, classed as non vegetarians and used as controls (n=53). *Setting:* Australia	for vitamin B12 concentration % of n below Herbert lower limit for vitamin B 12 concentration	Control: 40% Vegetarians: 73%	vitamin B12 concentration 175-850-pmol Herbert lower limit for vitamin B 12 concentration 221pmol/L
Hung et al 2003 (40)	*Study design:* Cohort study *Randomisation:* Unclear. *Date conducted:* 1969	*Baseline information:* n=2950/3676 (80%) 1419 men. Mean age 48.4 (15.7) 1531 women. Mean age 47.9 (15.3) *Inclusion criteria:* aged 20- 90 years, on Australian electoral role and residing in Busselton. *Setting:* Busselton, Western Australia, Australia.	% of n with reduced vitamin B12 concentration	0.4%	160-850ng/L
			% of n with vitamin B12 concentration in particular range (ng/l) divided into male and female	*0 to 269.9* M=21.6 F= 26.4 *270 to 329.9* M= 23.8 F= 23.3 *330 to 389.9* M=18.5 F= 18.4 *>390* M= 36.1 F= 31.8	

Shaw et al (41)	*Study design:* Cross-sectional survey *Randomisation:* Unclear *Date conducted:* 1997-1998	*Baseline information:* n=365 Mean age 42 (1) years 153 men (42%) and 212 women *Inclusion criteria:* urban indigenous Australians, residing in one of five indigenous communities in south-east Queensland. *Setting:* south-east Queensland Australia.	% of n with vitamin B12 deficiency	1.2%	160-665pmol/L
Byles et al (2003) Unpublished	*Study design:* cross-sectional survey *Randomisation:* yes Date conducted: 2003	*Inclusion criteria:* inpatients at a hospitals aged >65 years *Baseline characteristics* N=86/96 *Setting:* John Hunter Hospital, Newcastle	% of n with vitamin B12 deficiency	10.47%	135-600pmol

Part Four

Whether folic acid intakes up to 1.0mg/day improve the haematological sequelae of vitamin B12 deficiency.

Method

To determine whether folic acid intakes up to 1.0mg/day improve the haematological sequelae of vitamin B12 deficiency a systematic review was conducted.

Criteria for considering studies for this review

1. Types of studies

The review aimed to include randomised control trials (RCT) or systematic reviews which evaluate the impact of folic acid intakes up to 1.0mg/day on the haematological sequelae of vitamin B12 deficiency. In the absence such studies, other research methods such as non-randomised control trials, longitudinal studies, cohort (both retrospective and prospective), case-control studies, time series and case series, were used to evaluate the impact.

2. Type of participants

Any studies involving humans were included, whilst those using animals were excluded.

3. Types of interventions

Interventions of interest were those that include the addition of folic acid to the intakes of participants, whether through direct dietary consumption, fortified foodstuff or supplementation.

4. Types of outcome measures

Outcome measures that were included were; changes in

- no of participants with vitamin B12 deficiency with/without anaemia
- Mean cell volume, haemaglobin, reticulocyte count

5. Search strategy for identification of studies

The review consisted of a search of published and unpublished literature in the English language. The following databases were searched: Cochrane, Medline/PubMed/Premedline, Cinahl, Ebsco megafile Premier, Embase, Science Direct

The following search strategy was used:

- Vitamin B12 deficiency OR vitamin B12
- Folic acid OR folate

- 3 - 1& 2
- Limited to humans

Additionally bibliographies and reference lists of articles retrieved were searched for relevant literature.

Results

A total of 13 studies met the inclusion criteria (Table 4.). The study designs used were; quasi-experimental (time series) (n=4), case studies (n=5), cross-sectional surveys (n=2) and pre-test post-test studies (n=2). No studies of high level evidence (I-III2), based on the criteria of the NHMRC were retrieved.

The studies with interventions (n=11) focused on supplemental folic acid, either orally (n= 7) or intravenously (n=1) or both (n=2), along with the fortification of foods with folate (n=3). Three studies included levels of folic acid ess than 1mg/day, whilst 7 included levels greater than 1mg/day. 2 studies included both treatment with greater than 1mg/day and less than 1mg/day.

Level III-3 evidence from time-series studies raised the following issues:

- Two of the studies used the outcome measures of the existence of haematological remission and/or neurological remission in participants at follow-up following supplementation with folic acid of greater than 1mg/day. Neither study was able to show an insignificant level of haematological relapse among participants. Both studies suggested that neurological relapse can precede haematological remission in B12 deficient subjects consuming folic acid supplements and also that the majority of haematological relapses occur after greater than one year of treatment with folic acid. Additionally some participants were able to be remain in haematological remission for up to seven years (42,43).
- Hansen et al were able to show improvement in the haematological sequale (via increasing reticulocyte count) of vitamin B12 deficiency in participants (n=3) being treated with greater than 1mg/day folic acid. Those who were treated with less than 1mg/day (n=9) did not show a consistent improvement (44)
- Mill et al showed no significant difference between the number of participants (n= 1785) with vitamin B12 deficiency with anaemia, before, during or after fortification of grain with folic acid. The goal for intake of folic acid following fortification was 1mg/day, however intake was not measured as part of the study (45).

Level V evidence from pre-test post-test studies raised the following issues

- Bok et al showed improvements in the haematological sequelae(via improvement in reticulocyte count) of vitamin B12 deficiency following treatment with 15mg/day of folic acid for up to 8 days (46).
- Hirsch et al showed despite significant increases in folate concentrations following fortification of folate there was no significant change in mean cell volume (47, 52)

The included case studies presented results with participants presenting with neurological manifestations of vitamin B12 deficiency with no signs of anaemia following treatment with folic acid supplements and levels greater than 1mg/day (47-51) and less than 1mg/day (47,52)

The two cross-sectional surveys included provide varying results;

- Drazwoski et al studied vitamin B12 deficient women who had taken 4-5mg/day of folate from supplements and showed no signs of anaemia in any of the participants. Notably the survey included four participants.(50)
- Metz was unable to find a significant relationship between low serum vitamin B12 concentrations and high MCV at varying levels of serum folate concentrations (53)

Discussion

Of the studies retrieved there are seven which the NHMRC do not classify as 'evidence'. Of the remaining six studies, there is evidence of level III-3 and IV, the two lowest levels of evidence according to the NHMRC.

As outlined in the results the studies provide varying results. Notably it is mainly the studies which the NHMRC do not class as evidence that suggest an improvement in the haematological sequelae of vitamin B12 deficiency. However these studies, mainly conducted in the 1940s and 1950s, are predominantly case studies, which are unable to show a true association between the 'masking' of vitamin B12 deficiency and folic acid. These studies also predominantly include levels of folic acid greater than 1mg/day so therefore this does not suggest that levels up to 1mg/day will produce the same results.

The studies classified as evidence do not provide consistent results that show that the haematological sequelae of vitamin B12 deficiency is improved by folic acid intakes up to 1mg/day. Whilst two time series studies show existence of haematological remission in

participants with vitamin B12 deficiency, this is at folic acid intake of >1mg per day (42,43). As is the results of Bok et al that show improvement in reticulocyte counts in vitamin B12 deficient participants (46). The two studies which include folic acid intakes of up to 1mg/day do not support the hypothesis that such levels will improve the haematological sequale of vitamin B12 deficiency.

It should be noted that some studies that were found in the review of the literature were unable to be retrieved given the short time frame of the completion of this report. (Appendix I) However it appears, that the majority of these studies were case studies, and therefore would be unable to provide evidence to either support or oppose the hypothesis that folic acid intakes up to 1mg/day improve the haematological sequelae of vitamin B12 deficiency.

In conclusion there is insufficient evidence to suggest that folic acid intakes up to 1.0mg/day improve the haematological sequelae of vitamin B12 deficiency. This is because there is no evidence of an appropriate quality that show an improvement at these levels of folic acid. However it can not be suggested that studies of high level evidence, such as a randomized control study be conducted to discover the extent to which folic acid intake up to 1.0mg/day improves the haematological sequale of vitamin B12 deficiency. It would be unethical to intentionally withhold treatment of vitamin B12 from an individual with deficiency.

Conclusion

There is little or no evidence that masking of B12 deficiency will occur at dietary folate equivalent intake levels of 1mg.

Table 4 Summary of studies

Study	Method	Participants	Intervention	Outcome measures	Results	Level of Evidence
Baldwin and Dalessio (1961) (52)	*Study design:* Case study *Randomisation:* no *Blinding:* no *Study duration/date: 1956 and 1960*	*Baseline characteristics:* Case 1: 61 yo female with anaemia Case 2: 73 year old male with neurological symptoms *Setting:* New York Hospital	Case 1: treated with folic acid (5mg), 0.2 mg combined with 4.2mg vitamin b12, 38g of iron, 50mg vitamin C and 0.3g combined with extract of stomach, 0.12g sulfate. 0.5mg of vitamin B1 and 0.25mg of vitamin B12 (for 5 years) Total of 6.28mg of folic acid/day Case 2: Treated with vitamin tablets containing 0.25mg of folic acid (for 18 months). Total of 0.5mg of folic acid per day.	*Case 1* Anaemia responded to treatment. Patient represented with paresthesias in the legs and feet. Hematocrit, hamoglobin and red cell count within normal range. Schilling tests positive, i.e. patient had pernicious anaemia. *Case 2:* Represented with worsening neurological symptoms. Blood test revealed macrocytosis. Schillings test positive i.e. patient had pernicious anaemia. *Articles conclude that doses of folic acid improved haematological sequelae of vitamin B12 deficiency The lower dose of folic acid to a lesser extent.*		Does not fit into NHMRC levels of evidence
Bok et al (1958) (46)	*Study design:* Pre-test Post-test *Randomisation:* no *Blinding:* no *Study duration/date:* 4-8 days	*Baseline characteristics:* n=13. *Inclusion criteria:* patients with pernicious anaemia without signs of nervous system disease. Age not mentioned	15mg of folic acid/day for 5 days in n=10, 4 days in n=1, 6 days in n=1 and 8 days in n=1	Reticulocyte response	11/13 showed a positive reticulocyte response	IV

Curry Ellison (1960) (47)	*Study design:* Case studies *Randomisation:* no *Blinding:* no *Study duration/date:* 1950 and 1956	*Baseline characteristics:* Case 1: 58 yo female. Presented with numbness and tingling of wrists, feet and lower legs, weakness and fatigue. Patient had been self medicating with vitamins containing up to 2.7mg of folic acid Case 2: 70 yo female. Presented with numbness of the fingers and parethesia of the legs. Taking folic acid supplements from previous admission (up to 1mg/day) *Setting:* Charleston Memorial Hospital	Case 1: 2.7mg folic acid daily for 2 years Case 2: Up to 1.0mg folic acid/day for 3 months	Case 1 & 2: diagnosed with pernicious anaemia. No signs of anaemia	Does not fit into NHMRC levels of evidence

Drazkowski (2002) (50)	*Study design:* Retrospective cross-sectional survey *Randomisation:* no *Blinding:* no *Study duration/date:* unclear	*Baseline characteristics:* n=4 all female Age range 23-36 *Inclusion criteria:* female inpatients with epilepsy and vitamin B12 deficiency taking folate supplements (4-5mg/day for 18-24months) *Setting:* Barrow Neurologic Institute, Epilepsy Specialty Clinic	No intervention	1- Vitamin B12 (pg/mL) 2- Hemoglobin/ hematocrit 3 Mean corpuscular volume (fL)	Participant 1: 1- 133 2- 12.5/44 3- 97 Participant 2: 1- 159 2- 13/43 3- 94 Participant 3 1- 116 2- 13.5/46 3- 91 Participant 4: 1- 73 2- 12.8/46 3- 99 *Concluded that the folic acid alone may have masked the anaemia of vitamin B12 deficiency*	Does not fit into NHMRC levels of evidence
Hansen and Weinfeld (1962) (44)	*Study design:* Time series *Randomisation:* no *Blinding: no* *Study duration/date:* Unclear	*Inclusion criteria:* patients with pernicious anaemia *Baseline characteristics:* n= 12/17. No mention of age *Setting:* Sweden	Group 1: n=9 injected with 0.1-0.4mg folic acid/day then 2-5μg/day vitamin B12 Group 2: n=3 treated with 1-3mg folic acid orally per day.	Reticulocyte response	Group 1: 5/9 had an unchanged reticulocyte count 4/9 increase in count (when on folic acid) Group 2: increased reticulocyte count in all participants	III-3
Hirsch et al (2002) (54)	*Study design:* Pre-test Post-Test *Randomisation:* no	*Baseline characteristics:* *n= 108.* Mean age 74.4 (3.7)	Fortification of wheat flour with 220μg of synthetic folic acid/100g of wheat flour.	Mean serum vitamin B12 (pmol/L), serum folate, packed red cell volume (L),	Vitamin B12 unchanged. Significant increase in folate (P<0.001)	V

	Blinding: no *Study duration/date:* December 1999 to July 2000	*Inclusion criteria:* Free living subjects, aged 70 years or over with low income. *Setting:*Santagio, Chili		mean corpuscular Hb (g/L) and Mean corpuscular volume (fL)	Significant increase in MCV	
Katz (1973) (48)	*Study design:* Case study *Randomisation:* no *Blinding:* no *Study duration/date:* 1972	*Baseline characteristics:* 48 year old male. *Inclusion criteria:* self-medicating with folic acid due to anorexia, fatigue, dizziness and nausea. *Setting:* Royal Victoria Hospital.	8mg of folic acid/day	Patient diagnosed with pernicious anaemia. High serum folate concentration. Macrocytosis. Although appeared to be in haematological remission due to high doses of folic acid.		Does not fit into NHMRC levels of evidence
Metz et al (2004) (53)	*Study design:* cross-sectional survey *Randomisation:* no *Blinding:* no *Study duration/date:* data collected from January 1999 to December 2001	*Baseline information:* n= 63 472 *Inclusion criteria*: blood samples that had been taken on which a full blood examination and assay of serum vitamin B12 and folate were analysed. Age not mentioned *Setting:* Victoria, Australia.	No intervention	Change in mean MCV with decreasing concentrations of serum vitamin B12 at different serum folate concentrations (<15nmol/l, >15 to 30nmol/L, >30 to 45nmol/L and >45nmol/L)	No significant difference in MCV between groups (based on folate concentrations) at any level of vitamin B12	Does not fit into NHMRC levels of evidence

Mills et al (2003) (45)	*Study design*: Time series *Randomisation:* no *Blinding: no* *Study duration/date:* January 1992 and March 2000	*Baseline characteristics:* n= 1785 Median age: 67 years. Age range 53-75 years. 69% African American. *Inclusion criteria:* patients at medical centre who had vitamin B12 concentration measured and it was below 258pmol/L *Setting:* Veterans Affair Medical Centre, Washington DC.	Optional fortification of grain with folic acid began in March 1996 Mandatory fortification of grain with folic acid began in January 1998 Aim for <1000μg folic acid/day	% of n with vitamin B12 deficiency with anaemia. *Vitamin B12 deficiency defined as concentration <258pmol/l.* *Hematocrit <38.6 and MCV > 96.7 fl defined as anaemia.*	Pre: 39.2% (275/702) Optional fortification: 45.5% (198/435) Mandatory fortification: 37.6% (164/436)	III-3
Ross et al (1948) (51)	*Study design:* Case studies *Randomisation:* no *Blinding:* no *Study duration/design:* 1946	*Inclusion criteria:* pernicious anaemia previously treated with liver extract *Baseline characteristics:* n=22 13 male 9 female Age range 43-83 *Setting:* Massachussets Memorial Hospital. US	Oral supplements- 1 -N=5 15mg/day of folic acid for 8-17 months 2- N=2 10mg/day folic acid for 12 months 3- N= 2 5.0mg/ day folic acid for 12 months 4- N= 1 2.5 mg folic acid for 11 months 5- N=5 1.25mg/day folic acid for 9.5 to 11 months 6- 3 of above 5 given 15mg/day for an additional 1.5-2.5 months	Haematological and neurologic status	N= 12/21 showed improvements in haematological status of which n=10 declined after 6 months Of the n=12 4 had definite progression or development of subacute combined degeneration, n=2 had probable progression. Range in onset from 11-16months.	Does not fit into criteria of NHMRC

			Intramuscular injections- 7- n=3 100mg/month folic acid for 7- 10 months 8- Above changed to oral folic acid- n=3 1.25mg/day at 9 and 7 months and 15mg at 10 months. 9- n= 1 40mg/month for 9 months then 1.25mg/day orally for 3 months 10- n=2 30mg/month for 5-6 months changed to 1.25mg/day at 5 months (for 7 months) and 15mg/day at 6 months (for 6 months)			
Shwartz (1950) (42)	*Study design:* Time series *Randomisation:* unclear *Blinding:* no *Study duration/time:* 3.5 years	*Inclusion criteria:* Pernicious anaemia in haematologic and neurologic remission. *Baseline characteristics:* N=98 *Setting:* Cook County Hospital, Chicago, US	5mg orally of folic acid/day for 2.5 years. Seen monthly for interviews and neurologic and haematologic examination.	Number of: - neurologic - haematologic - hematologic and neurologic relapse - Interrupted therapy - Satisfactory maintenance *Neurologic relapse defined as posterior and/or lateral column dysfunction.* *Haematologic relapse defined*	*Neurologic relapse* N=4 in one year N=19 1-2 years *Haematologic relapse* N=4 in <12 months N= 8 1-2years N=11 >2 years *Both* N=1 <12 months N=3 1-2 years N=5 >2 years *Interrupted therapy* N= 26 did not complete intervention *Satisfactory maintenance* N=12	III-3

				as macrocytosis and falling erythrocyte		
Wagley (1948) (49)	*Study design:* Case studies *Randomisation:* no *Blinding:* no *Study duration/date:* unclear	*Inclusion criteria:* pernicious anaemia in hematologic and neurologic relapse. *Baseline characteristics:* N=11 8 female, 3 male Age range 31-74 *Setting:* John Hopkins Hospital Boston,	Case 1: 25mg/d folic acid for 8 days Case 2: 10mg/day for 12 months Case 3 10mg/day for 11 months Case 4 20mg/day for 12 months Case 5 5mg/day for 10 months. Case 6: 30mg/day for 27 months & 10 mg/day for 8 months Case 7: 5mg/day for 9 months Case 8: 20mg/day for 35 days Case 9: 100mg/day (intravenously) for 26 days. 15mg/day for 11 months Case 10 10mg/day for 4 months Case 14: 600mg (intravenously) for 14 days and 300mg (intravenously) for 2 days.	Haematological and neurological response.	No haematological relapse in any cases. 8/11 showed neurological disturbances (varying severity)	Does not fit into NHMRC criteria
Will et al (1959) (43)	*Study design:* Time series *Randomisation:* no	*Inclusion criteria:* pernicious anaemia *Baseline*	Folic acid 30mg taken orally 3 times per week.	Number of participants in - haematological	6months n=1 haematological, neurological and combined relapse	

	Blinding: no *Study duration/date:* 1945-1948 conducted and followed for 1 to 10 years	*characteristics:* n=36 *Setting:* Cincinnati Hospital, Ohio, US		- neurological or - combined relapse at 6 months, 12 months, and 2-10 years	1 year n= 4 neurological relapse n=3 combined relapse 2 year n=2 combined relapse 3 year n=5 haematological relapse 4th year n=2 haematological n=1 neurological n=2 combined 6th year n= 1 neurological and 1 combined. 7th year n= 1 haematological relapse.	

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APPENDIX I

Studies to be retrieved for the systematic review of part four that could not be retrieved within the timeframe

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