FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

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Efeitos da Suplementação de Vitamina B12 em Pacientes Idosos

REVISÃO DE ARTIGOS

ÁREA CIENTÍFICA DE GERIATRIA

Trabalho realizado sob a orientação de:

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03/2018

The Effects of Vitamin B12 Supplementation in Elderly Patients

A Systematic Review

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Abbreviations

AD – Alzheimer's disease

ADAS-Cog – Alzheimer's Disease Assessment Scale - Cognitive section

ADMA - asymmetric dimethylarginine

BMD – bone mineral density

B-PROOF – B-vitamins for the prevention of osteoporotic fractures

BUA – broadband ultrasound attenuation

cB12 – composite indicator of vitamin B12 status

CFR – coronary flow reserve

CIMT – carotid intima-media thickness

CNS – central nervous system

CoA – Coenzyme A

CVD – cardiovascular disease

DGS – Direção Geral de Saúde

DNA – deoxyribonucleic acid

eNOS – endogenous nitric oxide synthase

FRS – Framingham risk score

GI – gastrointestinal

HC – haptocorrin

HCl – hydrochloric acid

HDL – high density lipoprotein

Holo-TCII – holo-transcobalamin II

HRV – heart rate variability

IF – intrinsic factor

IM - intramuscular

INE – Instituto Nacional de Estatística

ITT – insulin tolerance test

IV – intravenous

MCI – mild cognitive impairment

MCV – mean corpuscular volume

MI – myocardial infarction

MMA – methylmalonic acid

MMSE – Mini-Mental State Examination

N_2O – nitrous oxide

NO – nitric oxide

OPEN – Older People and Enhanced Neurological Function

PA – pernicious anemia

PWV – pulse wave velocity

QALY – quality-adjusted life years

RBC – red blood cell

RCT – randomized control trial

RDA – recommended dietary allowance

RNA – ribonucleic acid

SAM – S-adenosylmethionine

TC II – transcobalamin II

TICS-M – Telephone Interview for Cognitive Status – Modified

TTDE – transthoracic Doppler echocardiography

VITATOPS – vitamins to prevent stroke

WHO – world health organization

Resumo

A tendência para o envelhecimento da população é uma realidade e só irá aumentar com o tempo. Assim, encontrar métodos seguros e económicos que melhorem a qualidade de vida nas idades mais avançadas é de grande importância. A nutrição tem um papel fundamental na saúde em todas as faixas etárias e regiões demográficas. As alterações fisiológicas inerentes ao envelhecimento aumentam o risco de desnutrição no idoso.

Entre os vários micronutrientes essenciais ao corpo humano, a vitamina B12 – também conhecida por cobalamina – tem um papel significativo. O seu défice pode levar a disfunção hematológica e neurológica, e pode ter outras consequências ainda não estabelecidas. O aumento da homocisteína, frequentemente causado por um défice de vitamina B12 é, também, considerado um fator de risco para múltiplas doenças. Pode ser um desafio diagnosticar este défice, visto que os biomarcadores atualmente disponíveis, como os níveis séricos de vitamina B12 e as concentrações de homocisteína, podem não ser tão sensíveis como se espera.

A suplementação com vitamina B12 oral ou intramuscular é uma prática comum quando o défice está confirmado ou na presença de sintomas. Entretanto, propôs-se que pode haver benefícios em suplementar vitamina B12 em idosos assintomáticos com níveis séricos normais, visto que pode haver uma deficiência funcional subjacente. Poderá haver benefícios em diminuir as concentrações de homocisteína com vitamina B12. Os grupos de maior risco, como os idosos, vegans e diabéticos medicados com metformina, devem ser os que mais beneficiam com suplementação de forma preventiva.

É claro que, na grande maioria dos estudos com suplementação de vitamina B12, se verifica um aumento dos níveis séricos de vitamina B12 e uma diminuição da homocisteína sérica, mesmo em doentes com anemia perniciosa ou a realizar hemodiálise. Entretanto, outros biomarcadores, como a holotranscobalamina II e o ácido metilmalónico, parecem ser mais sensíveis em detetar alterações nos níveis de vitamina B12.

Entre os estudos que relacionam a ocorrência ou risco de doença com suplementação, os resultados são contraditórios. As melhorias mais consistentes com a suplementação são no domínio cardiovascular, que analisam fatores de risco quantitativos para avaliar melhorias. Outras alterações, como função cognitiva, depressão, função neurológica e risco de fratura dão conclusões mais contraditórias, apesar da correlação positiva se verificar nos estudos mais pequenos e menos fiáveis.

No espetro dos efeitos negativos, o mais notável é a associação entre a suplementação de vitamina B12 e o risco de neoplasia. Entretanto, nenhum destes estudos recorre apenas à suplementação exclusiva com vitamina B12, envolvendo outras vitaminas como a vitamina B6 e o ácido fólico. Isto salienta a necessidade de novos estudos que relacionem a suplementação de vitamina B12 com o risco de cancro.

Em conclusão, a suplementação de vitamina B12 aumenta a sua concentração e baixa os níveis séricos de homocisteína, apesar dos benefícios associados não serem claros. São necessários mais estudo em idosos assintomáticos e, antes da suplementação neste grupo de risco ser recomendada de forma universal, a associação entre a suplementação de vitamina B12 e o risco de cancro precisa de ser aprofundada.

Palavras-chave: vitamina B12; suplementos nutricionais; idoso; idoso de 80 anos ou mais; sistema cardiovascular; disfunção cognitiva; depressão; neoplasias

Abstract

The trend of an aging population is already a reality, and will only increase with time. Therefore, finding safe and inexpensive methods to increase quality of life in later years is of great importance. Nutrition plays a vital role in maintaining health in all ages and demographics, and the physiological aging process places the elderly at a greater risk of undernutrition.

Among the many micronutrients essential to the human body, vitamin B12 – also known as cobalamin – plays a significant role. Deficiency can lead to haematological and neurological dysfunction, and may have other consequences not yet established. The increase in serum homocysteine, often caused by a vitamin B12 deficiency, is also considered a risk factor for several diseases. Yet it can be a challenge to diagnose said deficiency, as biomarkers currently in use, including serum vitamin B12 levels and homocysteine concentrations, may not be as sensitive in detecting a functional deficiency as may be currently expected.

Supplementation with oral or intramuscular vitamin B12 is common practice when a vitamin B12 deficiency is confirmed, or symptoms have already begun to manifest. However, it was proposed that there may be benefits to supplementing vitamin B12 in asymptomatic elderly individuals with normal serum levels, as there may be an underlying functional deficiency. There may also be some advantages to lowering homocysteine concentrations with vitamin B12. Those at most risk, such as the elderly, vegans, and diabetics medicated with metformin, should benefit the most from this preventative supplementation.

It is clear in almost all studies involving vitamin B12 supplementation that there is a significant increase in serum vitamin B12 and decrease in serum homocysteine, even in patients with pernicious anaemia or undergoing hemodialysis. Yet other biomarkers, such as holotranscobalamin II and methylmalonic acid, appear to be more sensitive in detecting changes in vitamin B12 levels.

Among the studies relating disease occurrence or risk with supplementation, results are often contradictory. The most consistent improvements with supplementation are in the cardiovascular trials, which use quantitative risk factors to detect improvements. Other changes, such as cognitive function, depression, neurological function, and fracture risk produce mixed results, although positive results are more commonly seen among the smaller, less reliable trials.

In terms of negative effects, the most notable results are among those investigating an association between vitamin B12 supplementation and cancer risk. However, none of these trials involve vitamin B12 monotherapy, involving other vitamins such as vitamin B6 and folic acid. This underlines the need for trials investigating the risk of cancer with exclusive vitamin B12 supplementation.

In conclusion, vitamin B12 supplementation clearly lowers serum homocysteine, although the benefits provided from improved vitamin B12 status and lowered homocysteine are not completely clear. Further study is needed into the benefits in asymptomatic elderly, and before universal supplementation among this high-risk demographic can be recommended, the association between cancer and vitamin B12 supplementation needs to be analysed.

Keywords: vitamin B 12; dietary supplements; aged; aged, 80 and over; cardiovascular system; cognitive dysfunction; depression; neoplasms

Materials and Methods

A literature search was performed to find systematic reviews and original studies relating vitamin B12 status and/or supplementation to different outcomes in elderly patients. 116 articles published between 2007 and 2017 were originally found; after removing duplicates and those considered inadequate in terms of relevance or quality, the total number of articles included was reduced to 68. Databases such as PubMed, B-on, Web of Science and the Cochrane Library were used to find these articles, with combinations of the following search terms: *"Vitamin B 12", "Vitamin B 12/therapy", "Vitamin B 12/therapeutic use", "Dietary Supplements", "Aged", "Aged, 80 and over", "Cost-Benefit Analysis", "Cognitive Decline", "Cardiovascular System", "Neoplasms", and "Depression"*.

Background information regarding theoretical topics important to this review were found in several books, including "Krause's Food & the Nutrition Care Process", "Handbook of Clinical Nutrition and Aging", and "Henry's Clinical Diagnosis and Management by Laboratory Methods".

Epidemiological and demographic data was obtained from sources such as "An Aging World: 2015 International Population Reports" and a report from Portugal's Direção Geral de Saúde (DGS) "DGS – Idade Maior em Números".

1 – Introduction

The number of senior citizens is on the rise.[1,2] In 2015, the percentage of the world population over 65 years of age was roughly 8.5%; however, by 2050 it is estimated that this will increase to 16.7%. Yet only when we look at the geographical distribution of the elderly population do we truly start to see how prominent this demographic can be. In Europe, 17.4% of the population was aged over 65 years old in 2015, and this is predicted to reach 27.8% by 2050.[2] These statistics highlight the importance of geriatric medicine.

Advanced age is a risk factor for many chronic diseases and general health concerns, including cardiovascular disease, cancer, and respiratory disease,[3] yet many live consistently healthy lives well into their later years while remaining independent. It is important to identify differences that are present in the lives of these healthy elderly individuals, and which of these we can apply to the general population. One such factor is nutrition.[1] From basic energy needs to micronutrients, food can help prevent, alleviate or even cause illness, being linked to a myriad of pathological processes in the human body.[1,3,4]

One of the potential problems associated with nutrition and aging in general is undernutrition, which may be caused by malabsorption (due to age, disease or gastrointestinal (GI) tract interventions), dietary choices (veganism, vegetarianism, etc.),[5] food insecurity, or conditions that impede eating or seeking food (e.g. anorexia, cognitive decline, dysphagia, poor dentition etc.)[1,4], to name a few. Many of these can be avoided, namely by identifying those at risk of undernutrition and correcting these shortages through fortification (having shown positive results in correcting deficiencies in countries such as the United States and Chile with folic acid fortification of wheat flour[6,7]) or supplementation.[4]

Micronutrient deficiencies are an important subset of undernutrition. These molecules are essential to healthy bodily function, and most can only be obtained through dietary sources. When these are not available in adequate quantities, important chemical reactions in many

metabolic pathways can be compromised, leading to ill health.[7]

One example of such a deficiency is that of vitamin B12 (cobalamin). This micronutrient acts as a coenzyme in one carbon metabolism, as well as the formation of methionine from homocysteine (a risk factor for cardiovascular disease (CVD)[8]), deoxyribonucleic acid (DNA) synthesis and cellular energy production.[9] These reactions lead to the maintenance and production of healthy brain tissue (through the production of myelin),[10] and red blood cells,[9] making vitamin B12 an essential micronutrient in a healthy, balanced diet. However, vitamin B12 can only be obtained through animal products, such as meat, eggs and milk, and although micronutrients are usually more easily obtained through whole foods, the process of absorbing vitamin B12 is more easily achieved through synthetic forms, such as cyanocobalamin, found in supplements. These factors, combined with the mechanism of absorption becoming less efficient with age, imply that a substantial portion of the population may be at risk of vitamin B12 deficiency.[1,6,9,11]

The focus of this work is whether the supplementation of vitamin B12 could be a useful tool for the prevention of undiagnosed vitamin B12 deficiency and subsequent symptoms, whether the lowering of homocysteine through vitamin B12 supplementation can improve morbidity and/or mortality, and whether supplementation should be universal among the elderly, or limited to high-risk groups and symptomatic patients.

2 – Nutrition and the elderly

Aging causes many changes in human physiology as it progresses; while not all are bad, it is the negative changes we must focus on when it comes to geriatric medicine. From intrinsic to environmental causes, there are a myriad of factors related to death and disease that are not well understood, and must be the focus of study if we are to alleviate their burden.[12]

Among the many changes to the aging body are those of the GI tract. Dysphagia caused by neurological diseases or dementia, achlorhydria, an increase in the prevalence of atrophic gastritis, decreased calcium absorption in the small intestine, higher incidence of constipation and an increase of bacterial overgrowth are but a few of the changes that can occur.[4,12] While no single theory can fully explain all changes related to aging,[4] these frequent alterations can place the elderly at risk for nutritional imbalance.[4,12]

Nutrition plays a significant role in disease, morbidity and mortality in all demographics, and the elderly are no exception. As the proportion of those over 65 years old increases, so too does the importance of maintaining their health, both through the treatment of pre-existing disease, but also by prevention;[1,4,12] in nutrition, this includes primary prevention (with a focus on disease prevention and health promotion through healthy eating and exercise), secondary prevention (risk reduction and the slowing of progression of an already existing disease) and tertiary prevention (avoiding issues related to functional limitations, such as chewing and appetite).[4] Identifying signs and symptoms, or those that are most at risk, and using these preventative strategies to reduce obstacles in treatment can benefit both the individual patient, and society as a whole by reducing dependency and cost-of-care.[12]

When it comes to treatment of nutritional deficits, such as those caused by a lack in the local food supply, a dietary or religious restriction, or through the body's inability to obtain nutrients from ingested food, there are several approaches available.[1,4,5,9] Supplementation is one such tool, used by the general population and medical professionals to help bridge the gap

between the body's necessity for one or more nutrients and the inability for the patient or their body to obtain it.[1,4,12] Usually reserved for situations in which food intake or absorption is inadequate (such as alcohol abuse, atrophic gastritis, interaction with medication), supplements are generally inexpensive and safe, and can be an effective method to increase the intake of many micronutrients.[1] Identifying those at risk is critical for choosing who to screen or prescribe preventative supplementation. For example, there is a general decrease in micronutrient reserves in the elderly, and in osteoporosis there may be an increase in the requirements of vitamin D and calcium.[4,12] Yet the majority of supplement use appears to be from those seeking to "improve" or "maintain" their health, as opposed to increasing the intake of specific micronutrients that are known to be deficient, and prescribed supplement use generally has poor adherence outside of a controlled clinical setting.[1]

3 – Vitamin B12: role

Vitamin B12 belongs to the water-soluble B complex, which also includes B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), and folate (B9).[4,7] It consists of two major components: a ring structure surrounding a cobalt molecule, and a nucleotide group.[13] While vitamin B12 is referred to as "cobalamin", it can be found in several forms, which include the bioactive forms found primarily in food, known as methylcobalamin and 5-deoxyadenosylcobalamin, and the synthetic form cyanocobalamin. The former are primarily in animal products, such as meat, eggs and dairy products,[4,5] while cyanocobalamin is the form most often found in supplements. Any form is valuable, as it is possible to convert vitamin B12 from one arrangement to another, but it is important to note that some forms are more easily absorbed in the GI tract.[1,4]

The cobalamin molecule contains cobalt,[4,13] and is used as a cofactor for methionine synthase and methylmalonyl Coenzyme A (CoA) mutase.[4,10,14] These enzymes are involved in the production of methionine from homocysteine, and succinyl CoA from methylmalonyl CoA, respectively.[4,9] The methionine-homocysteine cycle, responsible for the transfer of one-carbon (methyl) groups to proteins and DNA, relies on methylcobalamin and folate for the initial conversion of homocysteine to methionine, permitting the subsequent synthesis of S-adenosylmethionine (SAM) from methionine. SAM is then converted to S-adenosylhomocysteine via donation of a methyl group, which in turn becomes homocysteine, returning to the "start" of the cycle (Figure 1). The methyl group being transferred originates from a folate molecule (5-methyltetrahydrofolate), making both vitamin B12 and folate essential to the process.[4,13] Once homocysteine has been formed in this cycle, it can be converted via two routes: by repeating the methionine cycle again, or by being converted to cysteine through a vitamin B6-dependent pathway.[4,9] However, if either vitamin B12 or folate are deficient, the vitamin B6-dependent pathway is not sufficient to eliminate homocysteine

completely, and there will be an increase of serum homocysteine levels.[4,13]

The methyl group transfer is essential for the formation of several critical compounds in the human body, including DNA, ribonucleic acid (RNA), myelin and catecholamines, making vitamin B12 essential for almost every tissue in the human body,[15] although those most frequently affected by a deficiency of vitamin B12 are erythrocytes and myelinated neurons.[7,12]

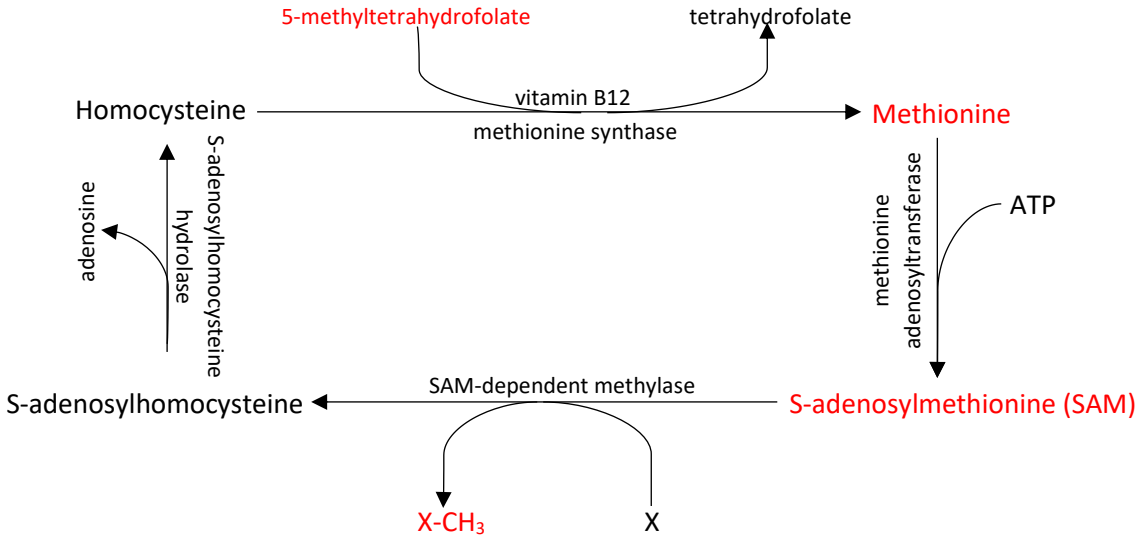

Figure 1: Diagram summarizing the homocysteine-methionine cycle. Molecules containing the transferred methyl group are red.

The methylmalonyl CoA reaction, producing succinyl CoA, is dependent on adenosylcobalamin.[13,16] While succinyl CoA is involved in the Krebs cycle, the importance of this reaction lies in the reagent, as a deficiency of vitamin B12 will lead an accumulation of methylmalonic acid (MMA), resulting in methylmalonate acidaemia and aciduria.[13,16,17] The significance of this will be discussed in conjunction with vitamin B12 deficiency further ahead.

4 – Vitamin B12 absorption, transport and reservoirs

The absorption of vitamin B12 begins in the mouth, where cobalamin molecules from food or supplements bind to "R-binders" found in saliva.[13] Once the bolus is in the stomach it mixes with gastric secretions, consisting of hydrochloric acid (HCl), pepsinogen, pepsin (the activated form of pepsinogen), gastric lipase, gastrin, mucus and intrinsic factor.[4] The pepsin, which is converted from its inactive form pepsinogen by hydrochloric acid, separates the salivary R-binders from the vitamin B12 molecule, which re-binds to a second type of R-binder at the stomach's acidic pH.[13] This mixture continues into the duodenum, where pancreatic enzymes separate cobalamin from the R-binders once again, and in the newly alkaline atmosphere cobalamin molecules finally combine with intrinsic factor.[4,13,17]

Intrinsic factor (IF) is a glycoprotein produced and secreted by gastric parietal cells,[4,16] and is essential to the absorption process of vitamin B12, as only 1% of ingested cobalamin is absorbed through passive diffusion.[18] Once bonded, the cobalamin-IF complex becomes very resistant to digestion, and in the presence of ionic calcium can connect to specialized receptors in the epithelial cells of the ileum, facilitating the absorption of vitamin B12.[4,13,16] These receptors become more frequent further along the small intestine, reaching maximum density around the terminal ileum (Figure 2). Once inside the epithelial cells of the ileum, the bond is cleaved, resulting in free cobalamin and IF, the latter of which is destroyed.[13]

While in the epithelial cells of the ileum, or after being absorbed into the bloodstream, cobalamin can connect to two types of transport proteins: transcobalamin II (TCII) or haptocorrin (HC), also known as transcobalamin I and transcobalamin III.[4,13] Once cobalamin attaches to TCII it is known as holo-transcobalamin II (holo-TCII). Although only around 20-25% of cobalamin in the blood is connected to TCII,[4,9] it is the most important transport protein for vitamin B12 as it is the only form that can actually deliver vitamin B12 to cells, since there are specific receptors for holo-TC on cell surfaces that permit their endocytosis into the

cytosol.[4,9,13,16]

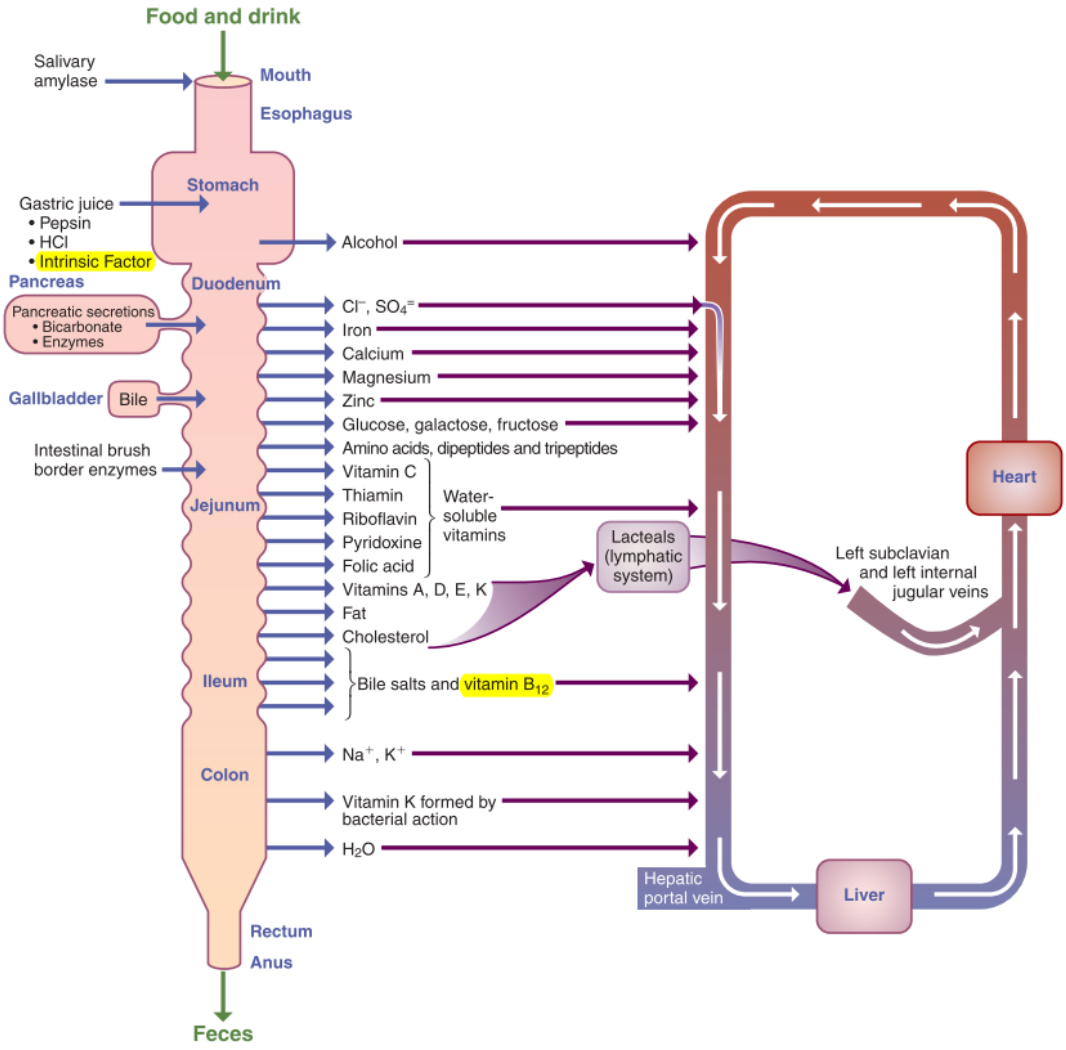

Figure 2: Simplified diagram of the GI tract production of intrinsic factor and absorption of vitamin B12 (highlighted), along with many other nutrients. Adapted from: Mahan L, Raymond J. Krause's food & the nutrition care process. Fourteenth edition.

On the other hand, although haptocorrin binds up to 80% of vitamin B12 in the bloodstream, the haptocorrin proteins are much larger and do not possess the ability to deliver cobalamin to cells that perform DNA synthesis,[4,13] only being available to storage liver cells.[19] Therefore, haptocorrin acts as a circulating reservoir for cobalamin, in addition to the amount stored in the liver (around 1µg of adenosylcobalamin per 1g of liver tissue[13]), and prevents the removal of large quantities of B12 from circulation.[4,9,13] However, this dynamic implies that

while serum B12 may be reduced due to decreased levels of haptocorrin, there may not be any symptoms of vitamin B12 deficiency as long as holo-TCII levels remain normal and cells continue to receive regular amounts of vitamin B12.[4,13] Alternatively, if it is TCII that is decreased and HC is normal there is very little vitamin B12 being delivered to cells but a large amount in circulation, resulting in a state of vitamin B12 deficiency with normal serum B12. This is known as a functional vitamin B12 deficiency.[13]

5 – Vitamin B12 deficiency

5.1 – Causes

The causes for a decrease in functional cobalamin are varied and not mutually exclusive, but some are more frequently encountered in elderly populations, with a prevalence around 10-15% in those over 65 years of age.[11,13]

The first step to obtaining vitamin B12 is through ingestion, and so it is important to consider if there is adequate intake of vitamin B12 in the food a patient is eating.[4,11,13] However, pure food deficiencies in developed countries are extremely rare, excluding populations that do not ingest any animal products, such as strict vegans, or groups at higher risk such as institutionalized elderly.[5,11,13,20] While this is not the most likely cause of a vitamin B12 deficiency, the socioeconomic status of the patient is important to bear in mind.[12,20]

Since hydrochloric acid is essential for the separation of cobalamin from salivary R-binder proteins, and achlorhydria affects as many as 30% of those over 50 years of age due to a physiological decrease in the production of HCl,[4] a high stomach pH is another possible cause of deficiency. Protein pump inhibitors and histamine H_2-receptor antagonists also decrease HCl production and can be a risk factor for vitamin B12 deficiency.[4,13]

Another vital step for absorption of cobalamin is binding with IF.[13] Whether through a decreased production of IF in stomach parietal cells, antibodies that prevent the binding of IF to cobalamin,[13,17] or the attachment of antibodies to the cobalamin-IF complex that prevent its absorption in the ileum, any disruption of the process can lead to a severe vitamin B12 deficiency.[4,13,17] Chronic atrophic gastritis is one such cause, often a result of a chronic *Helicobacter pylori* infection, and prevalence tends to increase with age.[12] A decrease in IF production due to autoimmune or chronic atrophic gastritis is known as pernicious anemia.[4,12]

Pernicious anemia (PA) usually manifests in later life (over 40 years of age[4,13]), has a positive family history in around 30% of diagnoses,[13] and over 90% of those diagnosed have

parietal cell antibodies in circulation.[4,13] It is usually concomitant with atrophic gastritis or gastric atrophy, with an absence of IF production, and it is not unusual to also find a decrease in the production of HCl and gastric juice. Anti-intrinsic factor antibodies are also a frequent find in patients with PA, positive in up to 75% of patients.[13]

Patients that have undergone GI surgery may be at risk as well. Total gastrectomy will completely remove all gastric parietal cells, leaving patients incapable of producing HCl or IF and greatly reducing their ability to absorb cobalamin.[4,13] Terminal ileum inflammation or resection, such as in Crohn's disease, will also compromise the intestines ability to absorb vitamin B12, as most cobalamin-IF receptors are localized here.[4,13]

Pharmacological interactions are an important aspect to bear in mind when prescribing medication to patients.[4,15] Metformin (frequently prescribed in type II diabetics), antiretrovirals, colchicine and nitrous oxide (N_2O) can all cause interactions in the absorption, transport or metabolism of vitamin B12, and can further aggravate deficiencies caused by other diseases such as PA.[4,13,15]

It is important to note that stores of vitamin B12 in the liver and in circulation can last several years after absorption stops.[4]

5.2 – Consequences

A vitamin B12 deficiency can have an impact on many different systems in the human body.[12,13] Since the methylation process is essential to the formation of many tissues and molecules, there can be wide ranging repercussions from deficiency.[1,4,11,21]

An established consequence of cobalamin deficiency is megaloblastic anemia, with a mean corpuscular volume (MCV) usually between 100-110fL.[4,12,16] It is caused by an alteration in the synthesis of DNA and results in immature red blood cell (RBC) progenitors in circulation and bone marrow.[4] Vitamin B12 or folate deficiency is the cause in 95% of cases of megaloblastic anemia and is reversible with treatment; however it is favourable to differentiate

which is lacking and correct it exclusively.[4]

Neurological symptoms are another frequent finding in those with a vitamin B12 deficiency.[4,12,13,15] Both central and peripheral nerves can be affected due to the involvement of vitamin B12 in the production of myelin.[4,13] Spinal cord degradation is frequently observed in patients with severe vitamin B12 deficiencies.[19] Some potential signs and symptoms include numbness, tingling, loss of position sensations, loss of vibratory sensations, weakness, spasticity, irritability, confusion, disorientation, emotional instability, and psychosis,[4,7,12,13,15] and any may be present, with or without simultaneous haematological alterations.[13] The progression of neurological symptoms is usually halted with repletion of cobalamin, but the degree of recovery of symptoms depends on how long the deficiency has existed and how severe it was.[12]

Other symptoms, such as glossitis and constipation, may be associated with vitamin B12 deficiency, but can also be present in a multitude of other deficiencies (such as iron or other B complex vitamins).[4,13,17]

Vitamin B12 deficiency can also have indirect cardiovascular or musculoskeletal consequences. A deficiency in B12 can cause hyperhomocystinemia which has been established as a direct risk factor for atherosclerosis and thrombosis.[13] Studies also suggest that there may be an influence on cognitive decline, as well as bone metabolism and quality, and subsequently fracture risk.[22,23] These effects, among others, will be analysed by this review.

5.3 – Diagnosis

How vitamin B12 deficiency is defined remains somewhat controversial. Although the cut-offs for deficiency can vary geographically, these are usually defined as around <200ng/L of serum vitamin B12.[11,12] Yet serum B12 is not the only marker for deficiency, nor is it the most sensitive.[4] As mentioned previously, vitamin B12 status can be normal while there is a severe deficiency on a functional level.[4,13] Therefore, it may be more pertinent to check other

indicators such as MMA and homocysteine, biomarkers that will accumulate when their metabolic pathways do not have enough cobalamin to function at an appropriate rate.[4,13,16]

One problem with using homocysteine is that it can be eliminated through an alternate pathway using vitamin B6.[4,9,13] While it will still be increased if there is a vitamin B12 shortage, this alternate elimination method makes it less sensitive to small changes of B12. Additionally, as the pathway is also dependent on folate, a folate deficiency can also increase homocysteine, regardless of vitamin B12 levels.[4,13]

On the other hand, an increase of serum MMA is also caused by vitamin B12 deficiency and is more sensitive than homocysteine because it does not depend on other nutrients. Yet impaired renal function can cause an increase of MMA, and so these values should be interpreted with caution.[13] Combined with how expensive assays for homocysteine or MMA can be means the value of using these to evaluate the existence or severity of a vitamin B12 deficiency is still not clear.[11] MMA may assist in differentiating between a vitamin B12 or folate deficiency when symptoms are the only indication.[11,13]

Another potential marker for identifying a functional cobalamin deficiency is directly measuring holo-TCII, since it has a half-life of around six minutes[19] and its levels fall below the normal range before total serum B12 due to the high proportion of vitamin B12 connected to HC.[13] However, it has not been shown to be clinically useful in comparison with serum vitamin B12 concentrations,[11,13,17] unless they are used in conjunction, in which case there may be a benefit.[9]

Signs and symptoms can also be used to detect vitamin B12 deficiency. Megaloblastic anemia is a strong sign that there is either a vitamin B12 or folate deficiency, and neurological symptoms without a clear cause should include undernutrition in the differential diagnoses.[4,7,12] Additionally, as vitamin B12 toxicity is not considered a risk,[4] therapeutic trials should be considered, as opposed to using biomarkers, to confirm the suspicion of a deficiency.[13]

6 – Vitamin B12: Supplementation

Before considering the potential benefits of vitamin B12, it is important to know whether supplements improve vitamin B12 status in patients, with or without deficiency. Fortunately, this correlation is clear in any number of studies that evaluate vitamin B12 supplementation, either through multivitamins, specific B12 supplements or intramuscular (IM) injections.[18,20,24–26] This includes patients with risk factors, such as metformin use.[24] Meta-analysis shows that for every doubling of vitamin B12 intake, serum levels increase by around 11%.[25]

Despite the recommended dietary allowance (RDA) being around 2.4µg/day,[18] the adjusted dosage for vitamin B12 supplements in any given individual are not clear; however, since high serum cobalamin concentrations are not considered dangerous,[4] a frequent approach is a megadose to correct deficiencies, with one popular regimen for oral cyanocobalamin being:[20]

1. 1000µg/day for 15 days, followed by;
2. 1000µg/10 days for 1 month, followed by;
3. 1000µg/month.

In cases with a decreased or absent production of IF, such as in PA, only around 1% of ingested cobalamin is absorbed.[18] In these situations, a dosage of 1000µg/day will equate to 10µg/day of bioavailable cobalamin, which is still higher than the RDA, and this dosage is considered to be sufficient to meet the needs of PA patients.[27]

Vitamin B12 supplementation provides an effective and relatively inexpensive method to revert vitamin B12 deficiencies.[4] Usually consisting of cyanocobalamin, supplements can be administered via injection or orally, but the effectiveness of each is still a subject of discussion.[4,28] While intramuscular or intravenous (IV)[29] vitamin B12 is generally available to the body much faster, allowing a rapid reversion of symptoms, oral administration has been

proven to be equally as effective while not subjecting the patient to injections (notable, since a relatively large portion of the elderly population is medicated with antiaggregant and anticoagulant therapy[20]). This results in fewer healthcare costs, and is generally preferred by patients.[18,20,26] If doubts remain regarding the effectiveness of oral supplementation, alternate strategies may be implemented, such as an initial IM dose and subsequent oral supplementation, or repeated serum vitamin B12 measurements during treatment.[18]

As it can be difficult for the elderly, institutionalized or otherwise, to meet their general nutritional needs, a supplementation method with various nutrients may be needed. In these cases, a multivitamin may be considered.[30] These supplements can contain a combination of many micronutrients[30] or be tailored to a patient's specific needs[31] and can be useful for ensuring that any undiagnosed deficiencies do not go untreated. However, it is important to bear in mind that there may be problems associated with supplementing multiple micronutrients simultaneously, such as vitamin B12 with folic acid. Brito et al. suggest that high serum folate may be an obstacle to improving vitamin B12 status, since subjects with a higher serum folate had a lower increase in serum cobalamin after an intramuscular injection with 10mg cyanocobalamin ($p<0.001$).[6] A Cochrane review also suggests that supplementing folic acid while vitamin B12 levels are low could worsen cognitive function compared to baseline,[32] and other sources attribute this to the delay in treatment caused by correcting haematological alterations (megaloblastic anaemia) with folic acid while vitamin B12 deficiencies continue to worsen.[33] These results appear to suggest that it may be more prudent to supplement vitamin B12 before initiating folic acid supplementation, but further study may be needed in this area.

As a side-note, an alternate method to supplement vitamin B12 is through food fortification. This method implies adding quantities of cobalamin to certain foods at an industrial level, before they are processed.[34] Folic acid fortification of flour is already practiced in many countries, including the United States, Canada, and Chili, with clear beneficial effects

on folate status.[6,34,35] However, this method is not a possibility in a clinical setting and will therefore not be the focus of this review.

7 – Vitamin B12 supplementation and effects on biomarkers

While homocysteine and MMA are useful tools for evaluating vitamin B12 deficiencies,[4,13,16] they may also have a role in predicting disease. Hyperhomocystinemia appears to be a risk factor for cognitive deficits and cardiovascular disease, especially in the oldest old,[36,37] and its involvement in depression has been a subject of recent investigation.[38]

Most studies involving supplementation of vitamin B12 over any period show a decrease in related metabolites, namely homocysteine and MMA, and even seemingly unrelated risk biomarkers such as asymmetric dimethylarginine (ADMA) after acute ischemic stroke.[6,8,22,31,39–43] There are significant responses with doses of oral vitamin B12 as low as 500µ/day, though biomarker values may not always be completely normalized.[8,37,44] Even in patients with impaired renal function and undergoing hemodialysis, supplementation with vitamin B12 successfully reduces homocysteine levels.[8]

Serum holo-TCII status, another useful measure of a functional vitamin B12 deficiency, is also improved by supplementation.[6,45,46] This can be observed in both oral and intramuscular supplementation,[6,45] implying that either can be effective at re-establishing a supply of vitamin B12 to cells for use in metabolic processes.

As supplementation clearly has a statistically significant effect on serum levels of metabolites, the question remains whether elevated homocysteine is the direct cause of disease, or simply an early warning sign. This is an important conclusion to reach, as treatment may either benefit the patient, by lowering homocysteine levels and alleviating disease, or obscure an early warning sign that could be useful for deciding to initiate other treatments.

8 – Vitamin B12 supplementation and cardiovascular disease

Despite a percentage decrease in cardiovascular deaths in Portugal as of 2012, CVD remains the highest cause of mortality in the over 65 year old population according to Statistics Portugal (Instituto Nacional de Estatística, INE).[3] Therefore, any reliable indication of high cardiovascular risk is of great value to the medical community in reducing morbidity and mortality, by identifying those most susceptible and applying preventative strategies.

The relationship between homocysteine and cardiovascular risk has been reasonably established in the past, although defining if the relationship is causal or correlative has not been straightforward, as there has been conflicting evidence.[11,36,47] High serum homocysteine is routinely observed in patients with CVD, type 2 diabetes mellitus and hypertension.[40] Since serum homocysteine can be lowered with vitamin B12 supplementation, one approach is to use vitamin B12 to reduce homocysteine and then analyse other well established risk factors, such as coronary flow reserve (CFR).[47]

CFR represents the response of coronary blood flow after administration of adenosine and is generally abnormal when there are alterations in the heart's blood vessels. Kurt et al., in a randomized, double-blind, placebo-controlled trial involving 44 elderly patients with serum vitamin B12 <180mg/dL, measured CFR from the left anterior descending coronary artery using transthoracic Doppler echocardiography (TTDE). After 8 weeks of supplementation with oral vitamin B12 (500µg) and folic acid (5mg), CFR measurements were repeated, showing a statistically significant improvement in the treatment group. An improvement in total cholesterol was also observed. Although the number of subjects was considered low, this result is consistent with similar studies performed in the past.[47]

Similarly, carotid intima-media thickness (CIMT) has also been of use in establishing homocysteine's effect on cardiovascular health as a measure of arterial stiffness, which is a risk factor for CVD. The "B-vitamins for the Prevention of Osteoporotic Fractures" (B-PROOF)

study was a multicentre, randomized, placebo-controlled intervention study with 2919 participants over 65 years old that were supplemented with vitamin B12 (500µg) and folic acid (400µg) or placebo for 2 years. While the focus was fracture risk, other parameters were also analysed; namely CIMT and aortic-femoral pulse wave velocity (PWV), another measure of arterial stiffness. A sub-study performed by Dijk et al. took these into account, and found that while homocysteine was reduced with supplementation, there was no significant effect on CIMT or PWV in 497 participants that met the inclusion criteria. There was a reduction in cerebrovascular events in the treatment group, although it was only significant among women, and there was no difference in cardiac events, despite an increase in aortic pulse pressure.[36] Potter et al. also analysed CIMT in a single-centre sub-study of the Vitamins to Prevent Stroke (VITATOPS) trial, which was a randomised, double-blind, parallel, placebo-controlled trial involving 123 medical centres in 20 countries. 8164 patients with previous stroke or transient ischaemic event were given either a B vitamin supplement (consisting of 25mg vitamin B6, 500µg vitamin B12 and 2mg folic acid) or placebo for a median duration of 3.4 years. It was observed that, among the 173 participants that met the sub-study inclusion criteria, CIMT was not reduced, and flow-mediated dilation was not increased in the long-term, implying no improvement in cardiovascular morbidity or mortality risk.[48]

The VITATOPS trial itself also studied cardiovascular outcomes by analysing if supplementation with B vitamins reduced cardiovascular events, rather than effects on risk factors. The primary outcome was the occurrence of non-fatal stroke or MI, or death from vascular cause, whichever occurred first, while the secondary outcomes were any vascular event or revascularization procedure. The results showed an improvement in the relative risk of stroke in the treatment group between 0-18%, although this was not considered statistically significant, although there was a statistically significant reduction in vascular death in the treatment group ($p<0.04$). The authors note that sub-group analysis shows that the risk reduction with

supplementation is more notable in patients with symptomatic small vessel disease. Regarding the study's limitations, it is important to note that only the first vascular event was considered in the primary outcome, so participants with multiple vascular events only counted as a single positive result. It is also noted that the relative risk of the primary outcome would suggest a significant benefit in the treatment group if those lost to follow-up maintained the results of the overall trial population.[49]

Wang et al. used the Framingham risk score (FRS - calculated using various risk factors) to evaluate cardiovascular risk in a double-blind, randomized, placebo-controlled trial involving 390 healthy elderly participants. Vitamin C (50mg) was used in both the treatment and control groups, with 400µg folic acid, 2mg B6 and 10µg B12 in the treatment arm. Supplementation lasted 12 months, with follow-up 6 months after cessation. Despite the low dose of vitamin B12, there was an 80% increase in serum levels. There was a significant reduction in adjusted FRS at 12 months, particularly in participants with folate deficiencies, as well as a significant increase in high density lipoprotein (HDL), also at 12 months. Both effects reverted 6 months after cessation and coincided with a return to baseline of folate and vitamin B12 levels, implying a need for consistent supplementation in order to maintain a long-term effect.[50]

Finally, as previously mentioned, there appears to be a correlation between vitamin B12 and other risk biomarkers, namely ADMA. An increase of ADMA has been observed in many diseases and is associated with endothelial disfunction and atherosclerosis due to its inhibitory effects in nitric oxide (NO) production by endogenous nitric oxide synthase (eNOS). Xia et al. investigated the association of ADMA with acute ischaemic stroke in 72 patients compared to healthy controls and found that levels of both ADMA and homocysteine were increased in stroke patients. No participants had other diseases associated with these markers, such as coronary heart disease, atrial fibrillation and kidney dysfunction. After supplementation with

folic acid and vitamin B12, there was a significant decrease in ADMA and homocysteine, as well as an increase in NO and eNOS, implying an improvement in endothelial function.[39]

9 – Vitamin B12 supplementation and cognitive function

The cognitive effects of vitamin B12 deficiency and supplementation have been a focus of recent study. The effects on the central nervous system may manifest as a cognitive decline in elderly patients, even if serum levels of cobalamin are low-normal. Despite the cognitive effects of deficiency, patients may also suffer from dementia or mild cognitive impairment (MCI), an intermediate step between the normal cognitive decline associated with aging and more serious forms of cognitive and memory decline such as Alzheimer's disease (AD).[4] Patients in these categories may benefit from alleviating a vitamin B12 deficiency that may be further contributing to their condition.[19,29] It is also important to study if there is a curative or protective effect of supplementing vitamin B12 in patients without a deficiency.

Hyperhomocystinemia is considered a risk factor for cognitive decline, as well as neurodegenerative diseases like AD, for which the risk is doubled if homocysteine levels are high.[51] Studies have shown that this effect also applies to other cognitive aspects, such as spatial copying skills, although once again it is unclear if homocysteine is simply an indicator of disease or a direct cause of these alterations.[29]

Several studies in recent years have attempted to find a link between vitamin B12 deficiency and an increased chance of developing cognitive impairment, with or without dementia. Kim et al. and Nelson et al. both performed analyses comparing dietary intake of vitamins and cognitive decline among the elderly population, although Kim et al. observed any change in cognition through the Mini-Mental State Examination (MMSE) and cognitive testing, while Nelson et al. focussed on new cases of dementia or AD during their study. Kim et al. concluded that, among 321 adults with MCI (n=100), AD (n=100), or no cognitive impairment (n=121), normal vitamin B12 intake only had a positive cognitive effect in patients with pre-existing AD and had no effect on healthy patients or those with MCI.[52] Nelson et al., in the Cache County Memory, Health and Aging study, analysed the incidence of new cases of AD

or dementia among 5092 participants over 65 years old. No difference was found in the risk of developing AD or dementia compared to the normal population, regardless of vitamin B12 intake.[53] However, both these studies relied on reported dietary intake rather than measuring serum levels of cobalamin or markers of functional deficiency. Siswanto et al. conducted a similar study, albeit retrospective and investigating serum vitamin B12 levels in 399 patients with dementia. No association was found between deficiency and dementia risk. It is important to note that patients under 65 years old were included in the study; however, since dementia is more common in the elderly, the results may still be relevant.[54]

Many recent studies have investigated if supplementation with vitamin B12 influences cognitive function in the elderly, but the method to evaluate cognition varied between them. Cognitive tests, both in person and through telephone calls, were popular methods among the studies, including MMSE, verbal memory tests and the Alzheimer's Disease Assessment Scale - Cognitive section (ADAS-Cog).

Kang et al. performed a sub-study involving 2009 women aged 65 years or older taking a multivitamin with vitamin B6, folic acid, and 1mg of vitamin B12, or placebo, for 7 years. All participants had elevated cardiovascular risk but no cognitive deficit at baseline. Cognitive function was evaluated using 5 tests administered via telephone 1.2 years and 6.6 years after treatment began. General cognition (via MMSE), verbal memory, and category fluency were tested. No change in cognitive function was observed between the placebo or treatment groups at any point in the study. However, sub-group analysis showed the greatest cognitive benefit in participants with low intakes of vitamin B12 (<2.4µg/day) versus vitamin B6 or folate.[35]

Dangour et al. analysed the benefits of vitamin B12 supplementation in those over 75 years old with a moderate but asymptomatic vitamin B12 deficiency. 191 participants without cognitive impairment (verified by MMSE >24) took a vitamin B12 supplement (1mg) or placebo for 12 months. Cognitive function was measured with the California Verbal Learning

Test at baseline and at 12 months. No improvement in cognitive function was observed by correcting a moderate, asymptomatic vitamin B12 deficiency. This study also evaluated neurological function, but those results will be discussed further ahead.[45]

Several more studies used the MMSE as a basis for evaluating cognitive function. Hankey et al. performed a sub-study of the VITATOPS trial, using a cut-off score of <24 on the MMSE to define cognitive impairment, and analysed 3089 unimpaired participants with a history of stroke or transient ischemic attack at least 6 months before the trial began. While homocysteine was lowered by treatment with a multivitamin (500µg/day of vitamin B12), there was no significant effect on the incidence of cognitive impairment or decline during the study's 2.8 year duration.[37] Lee, Kim and Sok performed a similar study using an adapted version of the MMSE in Korean, but where participants had previously diagnosed MCI. 48 participants ≥65 years old took a multivitamin containing vitamins B6, B12 and folic acid over 12 weeks and showed a statistically significant improvement in cognitive function.[31]

Kwok et al. also used the MMSE, together with the Marris Dementia Rating Scale, Category Verbal Fluency Test, the Neuropsychiatric Inventory and the Delirium Rating Scale. 30 participants with mild to moderate vitamin B12 deficiency were supplemented exclusively with methylcobalamin (1mg) by alternating intramuscular injection (3 times in the first week; once a month from 16 weeks to 40 weeks) and oral therapy (daily from the 2nd to the 16th week). No difference in cognition was observed during the trial, although there was a significant improvement in delirium. In terms of criticism, the number of participants was low, and there was no placebo-controlled group to compare with, so while cognitive function may not have improved, there may have been a protective effect with supplementation. Overall, the study quality was poor, and conclusions should be made with care.[55]

A randomized control trial (RCT) by Walker et al., involving 900 elderly adults with elevated psychological distress, studied the effect on cognition of supplementation with folic

acid (400µg/day) and vitamin B12 (100 µg) for 2 years. The Telephone Interview for Cognitive Status – Modified (TICS-M) was used. There was a significant improvement at 24 months in the treatment arm, particularly in immediate and delayed memory performance, although no changes occurred in orientation, calculation, semantic memory or processing speed. No changes were significant at 12 months of treatment. It is noted that higher homocysteine levels at baseline corresponded with a lesser improvement of cognitive function at 24 months.[56]

The ADAS-Cog is another method of evaluating cognitive function in older adults, and was used by Ford et al. to study the effect of a multivitamin supplement (25mg B6, 2mg folic acid, 400µg B12) versus placebo in 299 hypertensive men over 2 years in a RCT. While there was no statistically significant effect on either ADAS-Cog scores or incidence of cognitive impairment, the latter was only analysed in 73 participants, yet showed a nonsignificant 28% decrease.[57] Further analysis with a larger population may show statistically significant results. Aisen et al. performed a similar study with 409 participants with mild to moderate AD and normal B12 and homocysteine levels: no change was observed between placebo or multivitamin groups after 18 months.[58]

Looking at systematic reviews, we see comparable results and conclusions. Moore et al. focussed on studies that used vitamin B12 monotherapy or vitamin B12 plus folic acid, due to their similar roles in the methylation process. The review found that despite MCI being associated more frequently with lower serum cobalamin levels, treatment with supplements did not appear to improve cognition unless symptoms of deficiency were already present. Some criticisms put forward by the authors include that the MMSE was by far the most frequent method of assessment of cognitive deficit and subsequent changes, despite the existence of more robust and specific tests for these types of symptoms, such as the ADAS-Cog. Other criticisms included the lack of trials involving B12 supplements in conjunction with medication, to check for synergistic effects, and the lack of screening of depression, as this may influence

cognitive tests without a direct neurological cause. It was also mentioned that high folate with low vitamin B12 may cause neurological symptoms to manifest more easily. Whether this was due to the masking of a deficiency by preventing megaloblastic anaemia (an early warning sign) or by the overutilization of vitamin B12 due to the excess of folic acid driving the metabolic pathways is not clear. This may be an issue in countries with folate fortification of food and should prompt further study.[19]

A systematic review conducted by Krause and Roupas concluded that supplementation of vitamin B12 showed no clear benefit in cognition or dementia outcomes in healthy elderly. However, when using markers such as MMA and holo-TCII to analyse B12 status, those that showed a functional deficiency had a significant association with cognitive decline and dementia risk.[59] Zhang et al. performed a similar review, but only considered studies that included elderly patients with pre-existing cognitive-related diseases. The relationship between high homocysteine and cognitive impairments was clear; however, treatment with B-vitamins did not appear to influence cognition in elderly patients already suffering from neurodegenerative diseases such as AD or other dementias.[51]

Other studies looked at the indirect effect of vitamin B12 supplementation on cognitive function. One such study, a RCT by Sun et al., looked at how effective B-vitamin supplementation can be as an adjunctive treatment with a cholinesterase inhibitor, but no benefit was found in the supplement group.[60]

One study in development by Sanchez et al. is looking into whether the method of supplementation can have an effect on cognitive and neurological impairment; namely comparing milk fortification with vitamin B12 or an oral supplement, but results are not yet available.[61]

A summary of prospective trials and reviews involving vitamin B12 supplementation's effect on cognition can be found in table 1.

Authors	*Review*	*Vitamin B12 Monotherapy*	*Cognitive test used*	*Previous deficiency*	*No. of subjects*	*Result*
Kang et al.			MMSE		2009, ♀	Ø
Dangour et al.		✓	MMSE	✓	191	Ø
Hankey et al.			MMSE		3089	Ø
Lee, Kim & Sok			MMSE		48	+
Kwok et al.		✓	MMSE	✓	30	+*
Walker et al.			TICS-M		900	+
Ford et al.			ADAS-Cog		299, ♂	Ø
Aisen et al.			ADAS-Cog		409	Ø
Moore et al.	✓	✓	N/A	N/A	N/A	+**
Krause & Roupas	✓		N/A	N/A	N/A	Ø
Zhang et al.	✓		N/A	N/A	N/A	Ø

Table 1: Summarised methods and outcomes of vitamin B12 supplementation studies.

Legend: ✓: applies; MMSE: Mini-Mental State Examination; TICS-M: Telephone Interview for Cognitive Status- Modified; ADAS-Cog: Alzheimer's Disease Assessment Scale – Cognitive section; N/A: Not Applicable; ♀: only women; ♂: only men; Ø: No improvement or change; +: significant improvement; *: improvement in secondary outcome: delirium; **: improvement only if symptoms were already present before treatment.

10 – Vitamin B12 supplementation and depression

Vitamin B12's psychological effects have also been a focal point of recent trials. There has been evidence of an improved response to antidepressant medication if administered together with a vitamin B12 supplement, although the mechanism behind this association is not understood.[4]

Before observing the effects of supplementation, it may be important to see if there is an association between low vitamin B12 status and depression. Noronha et al. and Kim et al. both analysed the predictive value of vitamin B12 deficiency with regards to depression. Noronha et al. examined serum cobalamin and folic acid in 84 older adults living in care homes and used questionnaires to assess depressive symptoms. There was an association between general undernutrition and depressive symptoms, but this was independent of vitamin B12 status.[62] Kim et al.'s study was similar, but also analysed serum homocysteine concentrations in 631 Korean elderly, and found a significantly higher risk of depressive symptoms in those with poorer vitamin B12 status and higher homocysteine concentrations.[38] These contradictory results may be due to the statistical power offered by the larger population in the Kim et al. study, or due to physiological characteristics of the populations themselves. Skarupski et al. performed a similar investigation but utilized a food-frequency questionnaire to estimate vitamin intakes, and the Center for Epidemiologic Studies Depression Scale to analyse depressive symptoms. Among 3503 elderly participants, it was found that those with higher intakes of vitamins B6 and B12 – through diet or supplements – had a decreased probability of developing depression in up to 12 years of follow-up. Statistically, every 10µg/day extra of vitamin B12 translated to a 2% decrease in the chance of developing depressive symptoms per year. However, it is noted that despite the positive results, there may be an alternate cause of these results, such as healthy overall diet being the cause of decreased depression.[63]

Walker et al., Okereke et al., de Koning et al. and Lee, Kim and Sok all performed RCTs where the occurrence of depression or depressive symptoms was analysed following treatment with oral tablet multivitamin supplements.

The population in Walker et al.'s trial consisted of 909 community-dwelling older adults with elevated psychological distress. A tablet with either folic acid and 100µg of vitamin B12 or placebo was administered daily for 24 months, and depressive symptoms were assessed via the Patient Health Questionnaire at 6, 12 and 24 months. No effect was observed in the incidence of depressive symptoms when compared with placebo, although the authors do note that the dosage may have been too low to show a significant effect. This is reflected in the serum concentration of homocysteine, that did not lower during the trial (although there was an increase in the placebo group).[64]

Okereke et al. focussed on the association between lowering homocysteine and preventing depression in 1993 women over 65 but found no significant difference in incident cases between the treatment (folic acid, vitamin B6, and 1mg/day of vitamin B12) and placebo groups after 7 years. The primary outcome was confirmed by self-reported clinician-reported depression or clinically significant depressive symptoms.[65] The trial by de Koning et al. was similar, but involved 2919 participants with elevated homocysteine concentrations and the outcome was the reduction of depressive symptoms. No such reduction was observed after treatment with folic acid and 500µg/day vitamin B12 or placebo for 2 years. However, a secondary outcome was health-related quality of life, and among the four measures used, one showed a significant benefit in the treatment groups, implying a benefit with supplementation.[21]

Lee, Kim and Sok, previously mentioned for their study on the effect on cognitive function, had a secondary outcome regarding depression, and showed a significant improvement in depression with supplementation.[31]

11 – Vitamin B12 supplementation and neurological effects

The neurological effects of vitamin B12 deficiency can, in theory, affect any myelinated neuron, and this is reflected in the variety of methods used to study such effects.

Solomon performed a retrospective analysis of neuropathies that were suspected to be related to vitamin B12 deficiencies. The cases were between 1993 and 2005. For the first 6 years, only patients with laboratory results consistent with B12 deficiency – such as altered B12, MMA or homocysteine values – were treated with supplementation. After this point, all patients with signs or symptoms were treated, regardless of metabolic evidence. Treatment consisted of cyanocobalamin, administered either via a 2mg/ day oral supplement or a 1mg intramuscular injection six times in the first 2 weeks, weekly for another 8 weeks, and monthly thereafter. In 65 of the 78 subjects, there was a significant clinical improvement with treatment, and it is notable that haematological abnormalities (such as megaloblastic anaemia) were rare in those that responded. 72% of patients had B12 values in the normal reference range, and metabolic marker values were not always indicative of deficiency. Responses were positive even in cases where there was another clear cause for neuropathy.[66] It is important to note that subjects were not blinded and there no control for comparison.

Unlike cognition or depression, neurological effects can also be studied using more quantitative methods, such as electrophysiological measures. Nerve conduction can be measured relatively accurately, and measurements can be repeated, permitting the calculation of an average conduction velocity or amplitude. This reduces confounders, increases the reliability of results and permits a more objective outcome – one not dependant on participants' subjective responses. A decrease in nerve conduction is often caused by demyelination, and as myelin production is dependent on vitamin B12 there may be an association.[67]

Miles et al. performed two sub-analyses of an RCT performed on 191 elderly participants. The first sub-analysis was regarding the baseline data of the subjects, and whether

there was an association with baseline serum vitamin B12 and neurological function. 19 nerve conduction outcomes were measured, as well as 4 clinical signs of neurological alterations. No association was found between vitamin B12 status and neurological function. One strength of this sub-study was the use of a composite indicator of vitamin B12 status (cB12) calculated by considering serum cobalamin, holo-TCII and homocysteine; however, MMA was not included, which serves the same role and would increase reliability further.[67] The other sub-analysis performed by Miles et al. was based on the same RCT, but also included some results of said trial. It was found that baseline vitamin B12 status did not appear to impact the degree of improvement offered by supplementation – participants with the greatest improvements in neurological function could just as easily have normal or deficient cobalamin status at baseline. These results indicate that vitamin B12 status may not be a good indicator of a need for supplementation in older adults, and that there may be a benefit regardless of serum markers. The limitation of not measuring MMA remains in this sub-analysis.[46]

The RCT these analyses were based on, the Older People and Enhanced Neurological Function (OPEN) study by Dangour et al., included 191 participants over 75 years of age with a moderate vitamin B12 deficiency. A daily tablet with 1mg of vitamin B12 or placebo was administered for 12 months, after which no effect was observed on any of the 19 nerve conduction measures.[45] A pre-treatment and post-treatment study by Brito et al. gave contradictory results: a single 10mg intramuscular injection of vitamin B12 appears to significantly improve cB12 and myelinated neuron conductivity in elderly with poor vitamin B12 status. However, the number of participants were lower (51 vs 191) and there was no placebo control in this study, making the results obtained from the OPEN study more reliable.[6]

A study by Sucharita et al. used heart rate variability (HRV) as a measure for neurological function, as myelinated sympathetic and parasympathetic neurons are responsible for a change in heart rate. A lower HRV is common in the elderly, and is also associated to

higher mortality, so a method of reversing this decrease could prove effective in reducing cardiovascular risk; however, this value remains tied to neurological function and not cardiovascular function. In the trial, 47 subjects were categorized into low vitamin B12 and normal vitamin B12 status, and it was found that those with worse B12 status at baseline had lower HRV. However, all subjects were supplemented, and all showed a significant improvement in HRV from baseline. The authors note that vitamin B12 deficiency may cause a reduction in sympathetic nervous activity, and this has been implicated as a risk factor for falls. These results indicate that vitamin B12 supplementation may reduce such incidents by improving HRV.[68] Once again, it is important to note that this trial did not include a placebo controlled group, and therefore the reliability of these results may be reduced.

Other studies investigated the efficacy of vitamin B12 as an adjuvant treatment, such as those for painful diabetic neuropathies in a trial by Alvarado and Navarro. Vitamin B12 with Gabapentin showed promising results, as only 50% of the normal Gabapentin dose was needed to achieve the same reduction in pain as previous trials; however, study participants were not elderly and so results may not be applicable.[69] It may be valuable to perform similar studies in those aged over 65, especially considering the prevalence of Diabetes Mellitus type 2 in this demographic.

12 – Vitamin B12 supplementation and bone quality

The mechanisms relating bone metabolism to vitamin B12 are not fully understood. Several studies in recent years have attempted to investigate this association, with mixed results.

Serum homocysteine is considered a risk factor for osteoporotic fracture and lowering homocysteine via vitamin B12 supplementation is hypothesised to decrease the risk. The largest recent trial to test this is the B-PROOF trial, with 2919 participants over 65 years of age undergoing treatment with folic acid and vitamin B12 (400µg/day and 500µg/day, respectively) or placebo for 2 years. Both intervention and placebo tablets contained vitamin D_3 in equal doses. Despite a significant decrease in serum homocysteine in the treatment group, the time for first fracture was not significantly different compared to placebo. When an exploratory analysis was performed, a significant improvement in fracture risk was observed in those over 80 years of age ($p=0.020$ for osteoporotic fracture and $p=0.018$ for any type of fracture). Despite this, the authors do not recommend vitamin B12 and folic acid supplementation for the prevention of osteoporotic fracture in the elderly.[70] Sub-studies within the B-PROOF trial used other measures to judge bone quality, such as Enneman et al. using imagological parameters, namely:

- Femoral neck bone mineral density (BMD);
- Lumbar spine BMD;
- Calcaneal broadband ultrasound attenuation (BUA);
- Calcaneal speed of sound.

Once again there was a significant improvement in one parameter (calcaneal BUA) in those over 80, but overall analysis showed no significant difference between treatment and placebo groups.[71]

Other similar studies reached equivalent conclusions. Keser et al. performed a randomized, double-blind, placebo-controlled trial where 31 women over 65 years old with

elevated homocysteine were supplemented with 800µg/day of folic acid and 1000µg/day of vitamin B12 or placebo for 4 months. Serum bone turnover markers were used to assess the effect of supplementation on bone quality. No difference was found between the treatment and placebo groups.[22]

A RCT by Grieger et al. used a multivitamin containing vitamin B12 as the intervention; however, as the multivitamin also contained vitamin D, and its effects on bone quality have been well documented, this trial was not included in this review as any benefits of B12 would likely be obscured by those of vitamin D.[30]

13 – Vitamin B12 and other effects

Vitamin B12 deficiency and supplementation may have systemic effects not considered in the previous categories; however, as these other areas are not a major focus of study, they have been divided into the following sub-groups.

13.1 – Vitamin B12 supplementation and hypoglycaemia awareness

A case study of a single patient with type 1 diabetes showed a significant improvement in the subject's awareness of hypoglycaemia. Baseline levels of serum vitamin B12 were low (132pg/mL), and homocysteine was high (15.7nmol/mL). An insulin tolerance test (ITT) before treatment showed a lack of awareness of hypoglycaemia despite low glucose levels. After 4 weeks of treatment – 2 weeks of 1mg/day intramuscular vitamin B12, followed by 2 weeks of 1.5mg/day oral vitamin B12 – a repeated ITT manifested symptoms such as palpitations and cold sweats at higher blood glucose concentrations than the previous asymptomatic test. Plasma regulatory hormone levels also improved during hypoglycaemia: adrenaline increased from 1910pmol/L to 2730pmol/L, and adrenocorticotropic hormone (ACTH) and cortisol also improved substantially.[72] While single case studies such as these provide little in the way of actual evidence to the scientific community, they do provide examples of what to study in the future. It may prove useful to focus on such effects of vitamin B12 supplementation in the future and see if this relationship is maintained in large RCTs.

13.2 – Vitamin B12 supplementation and glossitis

Despite a study by Sun et al. focussing on homocysteine reduction in patients with atrophic glossitis, one conclusion was the reduction of symptoms after supplementation with B-vitamins. All groups had complete remission of symptoms within 1 year of start of supplementation. However, this trial was not exclusive to participants over the age of 65, and contained vitamins B1, B2, B6, B12, calcium pantothenate, nicotinamide and calcium.[73] More

exclusive studies, such as B12 monotherapy and only including elderly participants, may provide a benefit in the future.

13.3 – Vitamin B12 deficiency and all-cause mortality

A prospective study by Jia, Aucott and McNeill compared serum micronutrient levels to all-cause mortality. However, no association was found between serum vitamin B12 concentrations and mortality.[74] This study did not measure any markers for functional deficiency such as holo-TCII or MMA, so there may still be value in repeating this study with these assays or studying the effect of supplementation on mortality. During the B-PROOF trial, no effect was observed on all-cause mortality between treatment and placebo groups.[70]

13.4 – Vitamin B12 supplementation and physical performance, strength and falling

A secondary analysis of the B-PROOF trial by Swart et al. examined the effects of supplementation on homocysteine levels and subsequent physical activity and fall likelihood. There was a general decline in physical performance and handgrip strength in both the treatment and placebo groups, with no significant difference between them, and there was no advantage in preventing falls. However, there was an overall positive effect on gait, and in a sub-group analysis it was shown that those over 80 years old showed a benefit in physical performance with supplementation.

13.5 – Vitamin B12 supplementation and cataract

In a large randomised, double-masked, placebo-controlled trial by Christen et al. involving 3925 women over 40 years old, supplementation with 2.5mg/day of folic acid, 50mg/day of vitamin B6 and 1mg/day of vitamin B12 showed no benefit in preventing cataract when compared to placebo. On the contrary, there was a significant increase in cataract extraction in the treatment group, particularly in those aged over 65 years old; however, cataract incidence was self-reported, so results may not be reliable. Further study may be warranted into

this area, as it is unclear if vitamin B12 is the cause. One of the other vitamins, or another unidentified factor may be responsible for the increase in cataract extraction.[75]

13.6 – Vitamin B12 supplementation and cancer risk

The B-PROOF trial found a significant association between cancer risk and supplementation with folic acid and vitamin B12. The cancers associated with supplementation were mainly localised in the GI tract and had a more pronounced effect in those over 80 years of age and women. The authors note that the study was not powered to detect cancer risk, and so that may affect the reliability of this association.[70]

Other trials supplementing folic acid, vitamin B6 and vitamin B12 have also shown a clear association between these micronutrients and cancer risk. Brasky et al. evaluated 77118 participants from a cohort trial and concluded that there was a significant association in men between use of vitamin B6 and B12 supplements and cancer diagnoses, estimating a 30-100% increase in lung cancer risk, depending on dosage of supplements used. Among smokers this risk was increased. Women did not appear to be affected.[76] Similarly, a RCT by Ebbing et al. found an increase in cancer risk in 6837 participants treated with folic acid (800µg/day) and vitamin B12 (400µg/day) for 38 months, compared to placebo.[77] While neither of these trials were exclusive to elderly patients, mean age at baseline was over 60 in both cases, although additional trials with participants exclusively over 65 years of age would provide more reliable results for geriatric medicine.

14 – Cost-effectiveness of vitamin B12 diagnosis and supplementation

Whether vitamin B12 supplementation is cost-effective is also an important aspect if we are to consider universal supplementation. Several studies have investigated whether this is the case.

Mnatzaganian et al. compared the cost-effectiveness in quality-adjusted life years (QALY) gained, and compared 5 possible treatment approaches in individuals with undiagnosed fatigue:

1. Order serum cobalamin tests and treat accordingly with IM hydroxocobalamin injections;
2. Order serum cobalamin tests and treat accordingly with oral cyanocobalamin tablets;
3. Do not order tests, but treat with IM hydroxocobalamin injections;
4. Do not order tests, but treat with oral cyanocobalamin tablets;
5. Do not test and do not treat – re-evaluate after 3 months.

Option 5 was used as a reference point, as it would provide the lowest cost and lowest QALY gained. Overall, option 4 was the most cost-effective option, and the authors note that the excessive requesting of serum cobalamin tests may not be expensive individually but can accumulate greatly over time.[78]

Regarding the differences between oral and IM vitamin B12, Masucci and Goeree also state that oral supplementation is much more cost-effective than IM injection, although it is assumed that adherence to treatment would be similar for both methods.[79] This is further reinforced by Kolber and Houle, as they also state that oral therapy is equally as effective and preferred by patients.[18]

While fortification may more cost-effective, the safety of long-term vitamin B12 supplementation remains controversial, and those most at need of vitamin B12 may have absorption problems that prevent any benefit that the low doses that fortified food can provide.[34]

15 – Conclusions

Vitamin B12 is important to most tissues of the human body, and the repercussions of its deficiency can affect several systems. The demographic most at risk of a cobalamin deficiency is the elderly, and this risk is compounded among those with gastritis and diabetes medicated with metformin, both of which become more common with age.

Results clearly show that supplementation has a significant effect on levels of relevant biomarkers: serum B12, holo-TCII, homocysteine and MMA. While homocysteine is considered a risk factor for several pathological processes in the human body, the question remains whether reducing serum homocysteine prevents disease. Results are contradictory at best, and even when they are positive, it is not clear if the benefits are from reducing homocysteine or from supplying more vitamin B12 to cells.

On the other hand, those with symptoms of deficiency can benefit from treatment. Atrophic glossitis and cognitive deficits caused directly by deficiency generally respond favourably to supplementation. However, it remains unclear if there is a benefit in healthy individuals, or those with an asymptomatic deficiency. There is a lack of trials that use holo-TCII and MMA as criteria for defining a vitamin B12 deficiency, so further study may be needed into the benefits of supplementation in those that have normal serum B12 despite biomarkers that indicate a functional deficiency.

Regarding cardiovascular outcomes, most studies shared a limitation in not using exclusive vitamin B12 supplementation, although ethical issues regarding treatment may be a factor when dealing with elderly populations that may be deficient in other micronutrients. Results were generally positive, and these improvements in cardiovascular risk markers imply that homocysteine may have a direct role in pathophysiological mechanisms and not just screening for cardiovascular risk. There were improvements in healthy patients without signs

of cardiovascular disease, although homocysteine was usually elevated in such cases. However, whether improvements in these values improved morbidity or mortality was not clear.

While results from trials attempting to improve cognitive function with supplementation may not be promising, there appears to be an association between cognitive impairment, elevated homocysteine, and functional vitamin B12 deficiencies. However, the relationship can be justified in several ways, such as:

- Low vitamin B12 levels contributing directly to cognitive impairment;
- Cognitive impairment decreasing the patient's ability to meet nutritional needs;
- Other factors that lead to cognitive impairment also leading to vitamin B12 deficiency.

It is also important to note that there is no gold standard test to analyse cognitive function. While MMSE remains the most common, more specialised tools are already available, and should be the focus of further refinement in the future.

With psychological symptoms, results are once again contradictory, although the studies involving larger populations (and therefore more reliability) tend to have more positive results, implying a protective effect of normal vitamin B12 status. Whether this is through the higher bioavailability of vitamin B12 or through the lowering of homocysteine remains unclear.

Neurological symptoms gave contradictory results with supplementation. One clear trend was the lack of improvement in all RCTs, whereas weaker studies without controls or adequate blinding resulted in universally positive results. Seeing as the former provide greater scientific evidence, this suggests that supplementation with vitamin B12 does not benefit neurological function when compared with placebo.

The B-PROOF trial, being the largest study into fracture risk among the elderly, does seem to imply some benefit with supplementation. Those over 80 years of age seem to benefit from lowering homocysteine and increasing availability of vitamin B12, which is consistent

with the decrease in absorption of vitamin B12 with age. Other trials also show positive results, although these are generally among the secondary outcomes.

One particularly important factor to mention would be the apparent increased risk of cancer with supplementation of B-vitamins. Although no study that showed this association used vitamin B12 exclusively, this increase in cancer risk appears to be confirmed by multiple high-quality trials. It is essential to investigate this matter further before considering universal supplementation of vitamin B12 in the elderly.

In summary, vitamin B12 supplementation could be of benefit to elderly patients, particularly those already showing signs of deficiency. The physiological aging process tends towards a decrease in absorption, making old age a risk factor for deficiency. There also appears to be some benefit to lowering homocysteine, although results are contradictory, and distinguishing whether vitamin status or homocysteine concentrations are responsible remains a challenge. When considering the cost-effectiveness of supplementation, as well as the impracticality of screening for a functional deficiency through MMA and holo-TCII in every asymptomatic patient, a case can be made for universal supplementation in the elderly; however, further study into the safety of long-term vitamin B12 supplementation is necessary. Therapeutic trials in those suspected of deficiency may be the most cost-effective option, although investigations refining the groups most at risk would also be of value. Poor adherence can prevent widespread improvements in vitamin B12 status, and so alternate methods of administration may be beneficial – food fortification may be a possibility in the future, but the viability of such a program would require analysis beyond the scope of this review.

16 – References

1. Bales CW, Locher JL, Saltzman E, editors. Handbook of Clinical Nutrition and Aging [Internet]. Third edit. New York, NY: Springer New York; 2015. Available from: http://link.springer.com/10.1007/978-1-4939-1929-1
2. He W, Goodkind D, Kowal P. An Aging World : 2015 International Population Reports. Aging (Albany NY). 2016;(March):165.
3. Nogueira P, Afonso D, Alves MI, Vicêncio PO, Silva J da, Rosa MV, et al. DGS - Idade Maior Em Números. Direção Geral de Saúde. 2014;18–25.
4. Mahan LK, Raymond JL. Krause's food & the nutrition care process [Internet]. Fourteenth. St. Louis, Missouri: Elsevier Inc.; 2016. Available from: https://www.elsevier.com/books/krauses-food-and-the-nutrition-care-process/mahan/978-0-323-34075-5
5. Sobiecki JG, Appleby PN, Bradbury KE, Key TJ. High compliance with dietary recommendations in a cohort of meat eaters, fish eaters, vegetarians, and vegans: Results from the European Prospective Investigation into Cancer and Nutrition-Oxford study. Nutr Res [Internet]. 2016;36(5):464–77. Available from: http://dx.doi.org/10.1016/j.nutres.2015.12.016
6. Brito A, Verdugo R, Hertrampf E, Miller JW, Green R, Fedosov SN, et al. Vitamin B-12 treatment of asymptomatic, deficient, elderly Chileans improves conductivity in myelinated peripheral nerves, but high serum folate impairs Vitamin B-12 status response assessed by the combined indicator of Vitamin B-12 status. Am J Clin Nutr. 2016;103(1):250–7.
7. Shergill-Bonner R. Micronutrients. Paediatr Child Heal (United Kingdom). 2017;27(8):357–62.
8. Tayebi A, Biniaz V, Savari S, Ebadi A, Shermeh MS, Einollahi B, et al. Effect of

Vitamin B 12 Supplementation on Serum Homocysteine in Patients Undergoing Hemodialysis: A Randomized Controlled Trial. Saudi J Kidney Dis Transpl. 2016;27(2):256–62.

9. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010;2(3):299–316.

10. Selhub J, Troen A, Rosenberg IH. B vitamins and the aging brain. Nutr Rev. 2010;68(SUPPL. 2).

11. de Benoist B. Conclusions of a WHO Technical Consultation on Folate and Vitamin B $_{12}$ Deficiencies. Food Nutr Bull [Internet]. 2008;29(2_suppl1):S238–44. Available from: http://journals.sagepub.com/doi/10.1177/15648265080292S129

12. Durso S, Bowker L, Price J, Smith S. Oxford Handbook of Geriatric Medicine [Internet]. New York, NY: Oxford University Press; 2010. Available from: http://www.oxfordmedicine.com/view/10.1093/med/9780199586097.001.0001/med-9780199586097

13. Elghetany MT, Schexneider KI, Banki K. Chapter 32 - Erythrocytic Disorders [Internet]. Twenty Thi. Vol. 1, Henry's Clinical Diagnosis and Management by Laboratory Methods. Elsevier Inc.; 2016. 559-605.e2 p. Available from: http://dx.doi.org/10.1016/B978-0-323-29568-0.00032-2

14. MacFarlane AJ, Shi Y, Greene-Finestone LS. High-Dose Compared with Low-Dose Vitamin B-12 Supplement Use Is Not Associated with Higher Vitamin B-12 Status in Children, Adolescents, and Older Adults1,2. J Nutr [Internet]. 2014 Jun 1;144(6):915–20. Available from: https://academic.oup.com/jn/article/144/6/915-920/4616023

15. Forman SA, Ishizawa Y. Chapter 26 - Inhaled Anesthetic Pharmacokinetics: Uptake, Distribution, Metabolism, and Toxicity. Miller's Anesth 2-Volume Set. 2015;638–669.e5.

16. Disorders M. Chapter 85 – Defects in Metabolism of Amino Acids. Nelson Textb Pediatr [Internet]. 2016;636–678.e1. Available from: http://www.sciencedirect.com/science/article/pii/B9781455775668000855

17. Högenauer C, Hammer HF. Chapter 104 – Maldigestion and Malabsorption. Sleisenger Fordtran's Gastrointest Liver Dis [Internet]. 2016;1788–1823.e9. Available from: http://www.sciencedirect.com/science/article/pii/B9781455746927001046

18. Kolber MR, Houle SKD. Oral vitamin B12: a cost-effective alternative. Can Fam Physician [Internet]. 2014;60(2):111–2. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3922551&tool=pmcentrez&rendertype=abstract

19. Moore E, Mander A, Ames D, Carne R, Sanders K, Watters D. Cognitive impairment and vitamin B12: A review. Vol. 24, International Psychogeriatrics. 2012. p. 541–56.

20. Couderc AL, Camalet J, Schneider S, Turpin JM, Bereder I, Boulahssass R, et al. Cobalamin deficiency in the elderly: Aetiology and management. A study of 125 patients in a geriatric hospital. J Nutr Heal Aging. 2015;19(2):234–9.

21. de Koning EJ, van der Zwaluw NL, van Wijngaarden JP, Sohl E, Brouwer-Brolsma EM, van Marwijk HWJ, et al. Effects of two-year vitamin B12 and folic acid supplementation on depressive symptoms and quality of life in older adults with elevated homocysteine concentrations: Additional results from the B-Proof study, an RCT. Nutrients. 2016;8(11).

22. Keser I, Ilich JZ, Vrkić N, Giljević Z, Colić Barić I. Folic acid and vitamin B12 supplementation lowers plasma homocysteine but has no effect on serum bone turnover markers in elderly women: A randomized, double-blind, placebo-controlled trial. Nutr Res. 2013;33(3):211–9.

23. Zhang H, Tao X, Wu J. Association of homocysteine, vitamin B12, and folate with

bone mineral density in postmenopausal women: a meta-analysis. Arch Gynecol Obs. 2014;289(5):1003–9.

24. Kancherla V, Garn J V., Zakai NA, Williamson RS, Cashion WT, Odewole O, et al. Multivitamin use and serum vitamin B12 concentrations in older-adult metformin users in REGARDS, 2003-2007. PLoS One. 2016;11(8):2003–7.

25. Dullemeijer C, Souverein OW, Doets EL, Van Der Voet H, Van Wijngaarden JP, De Boer WJ, et al. Systematic review with dose-response meta-analyses between vitamin B-12 intake and European Micronutrient Recommendations Aligned's prioritized biomarkers of vitamin B-12 including randomized controlled trials and observational studies in adults and elder. Am J Clin Nutr. 2013;97(2):390–402.

26. Bnf K. Oral or intramuscular vitamin B12? Drug Ther Bull. 2009;47(2):19–21.

27. Andrès E, Fothergill H, Mecili M. Efficacy of oral cobalamin (vitamin B12) therapy. Expert Opin Pharmacother [Internet]. 2010;11(2):249–56. Available from: http://www.tandfonline.com/doi/full/10.1517/14656560903456053

28. Sanz-Cuesta T, González-Escobar P, Riesgo-Fuertes R, Garrido-Elustondo S, del Cura-González I, Martín-Fernández J, et al. Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). BMC Public Health [Internet]. 2012;12(1):394. Available from: http://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-12-394

29. Reay JL, Smith MA, Riby LM, Reay JL, Smith MA, Riby LM. B Vitamins and Cognitive Performance in Older Adults: Review. ISRN Nutr [Internet]. 2013;2013:1–7. Available from: http://www.hindawi.com/journals/isrn.nutrition/2013/650983/

30. Grieger J a, Nowson C a, Jarman HF, Malon R, Ackland LM. Multivitamin

supplementation improves nutritional status and bone quality in aged care residents. Eur J Clin Nutr. 2009;63:558–65.

31. Lee HK, Kim SY, Sok SR. Effects of Multivitamin Supplements on Cognitive Function, Serum Homocysteine Level, and Depression of Korean Older Adults With Mild Cognitive Impairment in Care Facilities. J Nurs Scholarsh. 2016;48(3):223–31.

32. Malouf R, J GE, Malouf R, Evans JG. Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people (Review) Folic acid with or without vitamin B12 for the prevention and treatment of healthy elderly and demented people. 2009;(4):2008–10.

33. De Lourdes Samaniego-Vaesken M, Alonso-Aperte E, Varela-Moreiras G. Vitamin food fortification today. Food Nutr Res. 2012;56:1–9.

34. Green R. Is it time for vitamin B-12 forti cation? What are the questions? Am J Clin Nutr. 2009;89(2):712S–716S.

35. Kang JH, Cook N, Manson J, Buring JE, Albert CM, Grodstein F. A trial of B vitamins and cognitive function among women at high risk of cardiovascular disease. Am J Clin Nutr [Internet]. 2008;88(6):1602–10. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-57449117345&partnerID=tZOtx3y1

36. Van Dijk SC, Enneman AW, Swart KMA, Van Wijngaarden JP, Ham AC, Brouwer-Brolsma EM, et al. Effects of 2-year vitamin B12 and folic acid supplementation in hyperhomocysteinemic elderly on arterial stiffness and cardiovascular outcomes within the B-PROOF trial. J Hypertens. 2015;33(9):1897–906.

37. Hankey GJ, Ford AH, Yi Q, Eikelboom JW, Lees KR, Chen C, et al. Effect of B vitamins and lowering homocysteine on cognitive impairment in patients with previous stroke or transient ischemic attack: A prespecified secondary analysis of a randomized,

placebo-controlled trial and meta-analysis. Stroke. 2013;44(8):2232–9.

38. Kim J-M, Stewart R, Kim S-W, Yang S-J, Shin I-S, Yoon J-S. Predictive value of folate, vitamin B12 and homocysteine levels in late-life depression. Br J Psychiatry [Internet]. 2008;192(4):268–74. Available from: http://bjp.rcpsych.org/cgi/doi/10.1192/bjp.bp.107.039511

39. Xia X-S, Li X, Wang L, Wang J-Z, Ma J-P, Wu C-J. Supplementation of folic acid and vitamin B12 reduces plasma levels of asymmetric dimethylarginine in patients with acute ischemic stroke. J Clin Neurosci [Internet]. 2014;21(9):1586–90. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0967586814001088

40. Mao X, Xing X, Xu R, Gong Q, He Y, Li S, et al. Folic Acid and Vitamins D and B12 Correlate With Homocysteine in Chinese Patients With Type-2 Diabetes Mellitus, Hypertension, or Cardiovascular Disease. Medicine (Baltimore) [Internet]. 2016;95(6):e2652. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00005792-201602090-00032

41. Vineeta Shobha SDTRGSPSUSUKSAVK. Vitamin B12 deficiency & levels of metabolites in an apparently normal urban south Indian elderly population. Indian J Med Res. 2011;134(4):432.

42. Favrat B, Vaucher P, Herzig L, Burnand B, Ali G, Boulat O, et al. Oral vitamin B12 for patients suspected of subtle cobalamin deficiency: A multicentre pragmatic randomised controlled trial. BMC Fam Pract [Internet]. 2011;12(1):2. Available from: http://www.biomedcentral.com/1471-2296/12/2

43. Solomon LR. Functional vitamin B12 deficiency in advanced malignancy: implications for the management of neuropathy and neuropathic pain. Support Care Cancer [Internet]. 2016;24(8):3489–94. Available from: http://dx.doi.org/10.1007/s00520-016-

3175-5

44. Hill MH, Flatley JE, Barker ME, Garner CM, Manning NJ, Olpin SE, et al. A Vitamin B-12 Supplement of 500 g/d for Eight Weeks Does Not Normalize Urinary Methylmalonic Acid or Other Biomarkers of Vitamin B-12 Status in Elderly People with Moderately Poor Vitamin B-12 Status. J Nutr [Internet]. 2013;143(2):142–7. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.112.169193

45. Dangour AD, Allen E, Clarke R, Elbourne D, Fletcher AE, Letley L, et al. Effects of vitamin B-12 supplementation on neurologic and cognitive function in older people: a randomized controlled trial. Am J Clin Nutr [Internet]. 2015;102(3):639–47. Available from: http://ajcn.nutrition.org/cgi/doi/10.3945/ajcn.115.110775

46. Miles LM, Allen E, Clarke R, Mills K, Uauy R, Dangour AD. Impact of baseline vitamin B12 status on the effect of vitamin B12 supplementation on neurologic function in older people: Secondary analysis of data from the OPEN randomised controlled trial. Eur J Clin Nutr [Internet]. 2017;71(10):1166–72. Available from: http://dx.doi.org/10.1038/ejcn.2017.7

47. Kurt R, Yilmaz Y, Ermis F, Kalayoglu Besisik S, Polat N, Elitok A, et al. Folic Acid and Vitamin B12 Supplementation Improves Coronary Flow Reserve in Elderly Subjects with Vitamin B12 Deficiency. Arch Med Res [Internet]. 2010;41(5):369–72. Available from: http://dx.doi.org/10.1016/j.arcmed.2010.07.007

48. Potter K, Hankey GJ, Green DJ, Eikelboom J, Jamrozik K, Arnolda LF. The effect of long-term homocysteine-lowering on carotid intima-media thickness and flow-mediated vasodilation in stroke patients: a randomized controlled trial and meta-analysis. BMC Cardiovasc Disord [Internet]. 2008;8(1):24. Available from: http://bmccardiovascdisord.biomedcentral.com/articles/10.1186/1471-2261-8-24

49. Hankey GJ, Eikelboom JW, Baker RI, Gelavis A, Hickling SC, Jamrozik K, et al. B

vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins to prevent stroke (VITATOPS) trial: A randomised, double-blind, parallel, placebo-controlled trial. Lancet Neurol [Internet]. 2010;9(9):855–65. Available from: http://dx.doi.org/10.1016/S1474-4422(10)70187-3

50. Wang L, Li H, Zhou Y, Jin L, Liu J. Low-dose B vitamins supplementation ameliorates cardiovascular risk: a double-blind randomized controlled trial in healthy Chinese elderly. Eur J Nutr. 2015;54(3):455–64.

51. Zhang DM, Ye JX, Mu JS, Cui XP. Efficacy of Vitamin B Supplementation on Cognition in Elderly Patients with Cognitive-Related Diseases: A Systematic Review and Meta-Analysis. J Geriatr Psychiatry Neurol. 2017;30(1):50–9.

52. Kim H, Kim G, Jang W, Kim SY, Chang N. Association between intake of B vitamins and cognitive function in elderly Koreans with cognitive impairment. Nutr J [Internet]. 2014;13(1):118. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25516359%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4290102

53. C. N, H.J. W, R.G. M, Nelson C, Wengreen HJ, Munger RG, et al. Dietary folate, vitamin B-12, vitamin B-6 and incident alzheimer's disease: The cache county memory, health, and aging study. J Nutr Health Aging [Internet]. 2009;13(10):899–905. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed12&NEWS=N&AN=358102365%5Cnhttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medc&NEWS=N&AN=19924351

54. Siswanto O, Smeall K, Watson T, Donnelly-Vanderloo M, O'Connor C, Foley N, et al. Examining the association between vitamin B12 deficiency and dementia in high-risk hospitalized patients. J Nutr Heal Aging. 2015;19(10):1003–8.

55. Kwok T, Lee J, Lam L, Woo J. Vitamin B12 supplementation did not improve cognition but reduced delirium in demented patients with vitamin B12 deficiency. Arch Gerontol Geriatr. 2008;46(3):273–82.

56. Walker JG, Batterham PJ, Mackinnon AJ, Jorm AF, Hickie I, Fenech M, et al. Oral folic acid and vitamin B-12 supplementation to prevent cognitive decline in community-dwelling older adults with depressive symptoms—the Beyond Ageing Project: a randomized controlled trial. Am J Clin Nutr [Internet]. 2012 Jan 1;95(1):194–203. Available from: https://academic.oup.com/ajcn/article/95/1/194/4576568

57. Ford AH, Flicker L, Alfonso H, Thomas J, Clarnette R, Martins R, et al. Vitamins B_{12}, B_6, and folic acid for cognition in older men. Neurology [Internet]. 2007;64(17):1540–7. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med5&NEWS=N&AN=17210813%5Cnhttp://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2010-22713-010&site=ehost-live%5Cnandrew.ford@uwa.edu.au

58. Aisen PS, Schneider LS, Sano M, Diaz-arrastia R, Stokes KT, Thomas RG, et al. High Dose B Vitamin Supplementation and Cognitive Decline in Alzheimer's Disease: A Randomized Controlled Trial. Jama. 2009;300(15):1774–83.

59. Krause D, Roupas P. Effect of vitamin intake on cognitive decline in older adults: Evaluation of the evidence. J Nutr Health Aging [Internet]. 2015;19(7):745–53. Available from: http://link.springer.com/10.1007/s12603-015-0539-3

60. Sun Y, Lu CJ, Chien KL, Chen ST, Chen RC. Efficacy of Multivitamin Supplementation Containing Vitamins B6 and B12 and Folic Acid as Adjunctive Treatment with a Cholinesterase Inhibitor in Alzheimer's Disease: A 26-Week, Randomized, Double-Blind, Placebo-Controlled Study in Taiwanese Patients. Clin

Ther. 2007;29(10):2204–14.

61. Sánchez H, Albala C, Lera L, Castillo JL, Verdugo R, Lavados M, et al. Comparison of two modes of vitamin B12 supplementation on neuroconduction and cognitive function among older people living in Santiago, Chile: a cluster randomized controlled trial. a study protocol [ISRCTN 02694183]. Nutr J [Internet]. 2011;10(1):100. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-80053365302&partnerID=tZOtx3y1

62. Noronha MB, Cunha NA, Araújo DA, Abrunhosa SF, Rocha AN, Amaral TF. Undernutrition, Serum Vitamin B12, Folic Acid and Depressive Symptoms in Older Adults. Nutr Hosp [Internet]. 2015;32(1):354–61. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-84936772039&partnerID=tZOtx3y1

63. Skarupski KA, Tangney C, Li H, Ouyang B, Evans DA, Morris MC. Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. Am J Clin Nutr [Internet]. 2010 Aug 1;92(2):330–5. Available from: http://jn.nutrition.org/content/140/1/103.full.pdf

64. Walker JG, Mackinnon AJ, Batterham P, Jorm AF, Hickie I, McCarthy A, et al. Mental health literacy, folic acid and vitamin B12, and physical activity for the prevention of depression in older adults: Randomised controlled trial. Br J Psychiatry. 2010;197(1):45–54.

65. Okereke OI, Cook NR, Albert CM, Van Denburgh M, Buring JE, Manson JAE. Effect of long-term supplementation with folic acid and B vitamins on risk of depression in older women. Br J Psychiatry. 2015;206(4):324–31.

66. Solomon LR. Vitamin B $_{12}$ -responsive neuropathies: A case series. Nutr Neurosci [Internet]. 2016;19(4):162–8. Available from:

http://www.tandfonline.com/doi/full/10.1179/1476830515Y.0000000006

67. Miles LM, Allen E, Mills K, Clarke R, Uauy R, Dangour AD. Vitamin B-12 status and neurologic function in older people: A cross-sectional analysis of baseline trial data from the Older People and Enhanced Neurological Function (OPEN) study. Am J Clin Nutr. 2016;104(3):790–6.

68. Sucharita S, Thomas T, Antony B, Vaz M. Vitamin B12 supplementation improves heart rate variability in healthy elderly Indian subjects. Auton Neurosci [Internet]. 2012;168(1–2):66–71. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1566070211005145

69. Alvarado AM, Navarro SA. Clinical trial assessing the efficacy of gabapentin plus B complex (B1/B12) versus pregabalin for treating painful diabetic neuropathy. J Diabetes Res. 2016;2016.

70. van Wijngaarden JP, Swart KM, Enneman AW, Dhonukshe-Rutten RA, van Dijk SC, Ham AC, et al. Effect of daily vitamin B-12 and folic acid supplementation on fracture incidence in elderly individuals with an elevated plasma homocysteine concentration: B-PROOF, a randomized controlled trial. Am J Clin Nutr [Internet]. 2014 Dec 1;100(6):1578–86. Available from: https://academic.oup.com/ajcn/article/100/6/1578/4576655

71. Enneman AW, Swart KMA, van Wijngaarden JP, van Dijk SC, Ham AC, Brouwer-Brolsma EM, et al. Effect of Vitamin B12 and Folic Acid Supplementation on Bone Mineral Density and Quantitative Ultrasound Parameters in Older People with an Elevated Plasma Homocysteine Level: B-PROOF, a Randomized Controlled Trial. Calcif Tissue Int. 2015;96(5):401–9.

72. Fujita S, Kozawa J, Ishibashi C, Kimura T, Kitamura T, Fukui K, et al. An Impaired Awareness of Hypoglycemia Improved After Vitamin B₁₂

Treatment in a Type 1 Diabetic Patient. Intern Med [Internet]. 2017;56(11):1383–5. Available from: https://www.jstage.jst.go.jp/article/internalmedicine/56/11/56_56.8489/_article

73. Sun A, Wang YP, Lin HP, Chen HM, Cheng SJ, Chiang CP. Significant reduction of homocysteine level with multiple B vitamins in atrophic glossitis patients. Oral Dis. 2013;19(5):519–24.

74. Jia X, Aucott LS, McNeill G. Nutritional status and subsequent all-cause mortality in men and women aged 75 years or over living in the community. Br J Nutr. 2007;98(2007):593–9.

75. Christen WG, Glynn RJ, Chew EY, Albert CM, Manson JE. Folic Acid, Vitamin B 6 , and Vitamin B 12 in Combination and Age-Related Cataract in a Randomized Trial of Women. Ophthalmic Epidemiol [Internet]. 2016 Jan 2;23(1):32–9. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0161642007007476

76. Brasky TM, White E, Chen CL. Long-term, supplemental, one-carbon metabolism–Related Vitamin B use in relation to lung cancer risk in the vitamins and lifestyle (VITAL) cohort. J Clin Oncol [Internet]. 2017;35(30):3440–8. Available from: http://dx.doi.org/10.1200/JCO.2017.72.7735

77. Ebbing M, Bonaa KH, Nygard O, Arnesen E, Ueland PM, Nordrehaug JE, et al. Cancer Incidence and Mortality After Treatment With Folic Acid and Vitamin B12. JAMA [Internet]. 2009 Nov 18;302(19):2119. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2009.1622

78. Mnatzaganian G, Karnon J, Moss JR, Elshaug AG, Metz M, Frank OR, et al. Informing disinvestment with limited evidence: Cobalamin deficiency in the fatigued. Int J Technol Assess Health Care. 2015;31(3):188–96.

79. Masucci L, Goeree R. Vitamin B12 intramuscular injections versus oral supplements:

A budget impact analysis. Ont Health Technol Assess Ser [Internet]. 2013;13(24):1–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24379898