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The Relationship between Cobalamin Deficiency and Neurological Dysfunction in Older Adults

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THE RELATIONSHIP BETWEEN COBALAMIN DEFICIENCY AND NEUROLOGICAL DYSFUNCTION IN OLDER ADULTS

by

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Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the Master of Science in Clinical Nutrition.

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ABSTRACT

THE RELATIONSHIP BETWEEN COBALAMIN DEFICIENCY AND NEUROLOGICAL DYSFUNCTION IN OLDER ADULTS

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The prevalence of cobalamin (Cbl) deficiency among older adults is higher than among younger adults, and is estimated to be between 14% and 23%. Persistent Cbl deficiency can cause a variety of neurological deficits. Neurological dysfunction occurs commonly among older adults, raising the research question whether or not there is a relationship between the high prevalence of Cbl deficiency and neurological dysfunction among older adults.

This case-control study enrolled 120 subjects with and without neurological dysfunction through the Faculty Medical Offices' Internal Medicine and Neurology Outpatient Clinics. All subjects received a neurological and cognitive exam. Blood samples were drawn to assess serum Cbl, methylmalonic acid (MMA), and serum total homocysteine (tHcys) levels. To test the hypothesis whether Cbl deficient subjects consumed less crystalline (free) Cbl, Cbl found in fortified foods and supplements, a food frequency questionnaire was designed.

The prevalence of Cbl deficiency was 16.6% among the "true" controls, and 25% among the cases (Odds Ratio = 1.7, 95% confidence interval = 0.54 - 5.1). "True" controls had perfect neurological scores, while other control subjects had reduced vibration sense. Cbl deficiency in older adults was related to low free Cbl intake, not dietary Cbl intake. Subjects who obtained a daily average of 0 and 1.0 mcg of free Cbl were most likely to be Cbl deficient (41.5%), while those who obtained 2.0 mcg of free Cbl were least likely to be Cbl deficient (13%) (P=.008).

This study was unable to show that older adults with neurological dysfunction are at greater odds of a Cbl deficiency than a control group. Another study is needed to determine whether or not older adults with neurological dysfunction are at greater odds of a Cbl deficiency than a control group without reduced vibration sense. Furthermore, the relationship between reduced vibration sense in older and Cbl deficiency needs to be further investigated. Prophylactic use of Cbl containing supplements among older adults seems prudent. The current RDA of 2 mcg for Cbl needs to be reevaluated in terms of crystalline Cbl and protein-bound Cbl requirements in older adults.

CHAPTER ONE

I. REVIEW OF THE LITERATURE

A. Rationale for the Study

Cobalamin (Cbl) also called vitamin B_{12} has a dual function. First, Cbl is indirectly involved in cell division ie. hematopoiesis, because of its interrelationship with folate. Impaired red blood cell synthesis can lead to large malformed red blood cells or macrocytic anemia. The activity of methionine synthetase which converts methyl-folate to free folate, and homocysteine to methionine directly depends on the availability of Cbl. A Cbl deficiency, traps folate in the methyl form and consequently impairs purine and thymidine synthesis, and consequently DNA synthesis. Second, Cbl is involved in the maintenance of myelin surrounding parts of the central and the peripheral nervous system through an as of yet unidentified biochemical mechanism. Persistent Cbl deficiency can therefore cause a variety of neurological deficits. The malfunction of the two known Cbl dependent enzymes methionine synthetase and methylmalonyl CoA mutase, might be partly responsible for demyelination of the nerves (Kapedia, 1995). The Cbl dependent methylmalonyl CoA mutase converts L-methylmalonyl CoA to Succinyl CoA.

Numerous studies report that older people have a higher prevalence of low serum cobalamin (Cbl) levels than the rest of the population (Yao Y, et al. 1992; Lindenbaum, et al. 1994; Pennypacker, et al. 1992). The clinical significance of these lower serum values has not been completely sorted out since lower serum Cbl values are often unaccompanied with elevated mean cell volumes (MCV) or neurological deficits (Pennypacker, et al. 1992). Neurological dysfunction occurs commonly among older adults, raising the research question whether or not there is a relationship between the high prevalence of Cbl deficiency and neurological dysfunction among older adults. Studies which compared serum Cbl levels of patients with late onset dementia of the Alzheimer's type or other types of dementia to patients without such dementias have given conflicting results (Carmel, et al. 1995; Kristensen, et al. 1993; Basun, et al. 1994; Crystal, et al.1994). Cbl induced neuropsychiatric deficits can be complicated by the fact that older people often have multiple medical problems some of which might interact with tissue Cbl deficiency (Stabler, 1995). A study is needed to elucidate whether low "normal" serum Cbl values are part of a normal aging process or if they are more often accompanied, not only by elevated methylmalonic acid (MMA) and/or total homocysteine (tHcys), but occur more frequently among patients with detectable neurological deficiencies or symptoms. A reliable way to distinguish between low serum Cbl serum values which are accompanied with true tissue deficiency and low values which are not, is to measure serum MMA and serum tHcys levels. The latter two metabolites can become elevated with Cbl deficiency (Savage, et al. 1994, Lindenbaum, et el. 1990; Allen, et al. 1990).

Chapter One will cover the following topics. First, the absorption and transport of Cbl is discussed to prepare the ground for the different issues surrounding Cbl malabsorption. Second, the distinction between atypical and typical Cbl deficiency states is made to explain why the reported prevalence of Cbl deficiency varies so widely between older and newer studies. Third, the currently known relationships between Cbl deficiency and neurological signs, symptoms, and dementia are discussed. Fourth, Cbl deficiency induced neuropathological changes with its neurological signs and symptoms are reviewed. Fifth, the rationale for the assessment of dietary protein-bound Cbl and free Cbl among older adults is supported. Finally, the advantages of using both serum MMA and serum tHcys levels to detect Cbl deficiency are explained. The serum levels of these two metabolites may rise during Cbl deficiency.

B. Absorption, and Transport of Cobalamin

Methyl Cbl, 5'-deoxyadenosyl Cbl and hydroxyCbl are the major biologically active Cbl forms found in animal and fortified foods. In the stomach, the protein-bound Cbl, only found in animal foods, must be liberated by the action of hydrochloric acid. The freed Cbl than binds to the salivary R-binder polypeptides (transcobalamin I, Transcobalamin III, cobalophyllin, hepatocorrin) (Shevell, 1992). The R-protein Cbl complexes are transported to the duodenum, where the R-binder protein is split off by the action of pancreatic bicarbonate and trypsin. The gastric parietal cells produce intrinsic factor (IF) which also travels to the duodenum. IF binds to the freed Cbl, and the IF-Cbl complex travels to the receptors on the ileum, where it is absorbed through calcium dependent receptor mediated endocytosis into the brush border as shown in Figure 1 (Festen, 1991; Hunt, 1990).

In the enterocyte, Cbl is released from the IF, and binds to the transporter protein transcobalamin II (TC II). TCII delivers newly absorbed Cbl in the form of hydroxy or cyanoobalamin to the major tissues, including bone marrow and nerve tissue, by receptor mediated endocytosis. Lysosomal digestion releases Cbl into the cytoplasm as shown in Figure 2. Cbl in the oxidized form is either converted to reduced methyl cobalamin and used as a coenzyme for methionine synthetase, or it can enter the mitochondria and be converted to reduced 5'-deoxyadenosyl cobalamin, which is in turn used as a coenzyme for methylmalonyl CoA mutase as shown in Figure 3 (Kapadia, 1995).

C. Pathophysiology of Cobalamin Malabsorption

Protein-bound-Cbl malabsorption due to atrophic gastritis is one possible etiology for Cbl malabsorption. The prevalence of atrophic gastritis increases with age. An estimated 40% of people aged 80 or older have atrophic gastritis (Stabler, 1995). Hypochlorhydria or achlorhydria are the first symptoms of gastritis which might in turn explain the higher rate of Cbl malabsorption and lower Cbl levels among older adults. Prolonged gastritis might eventually halt the

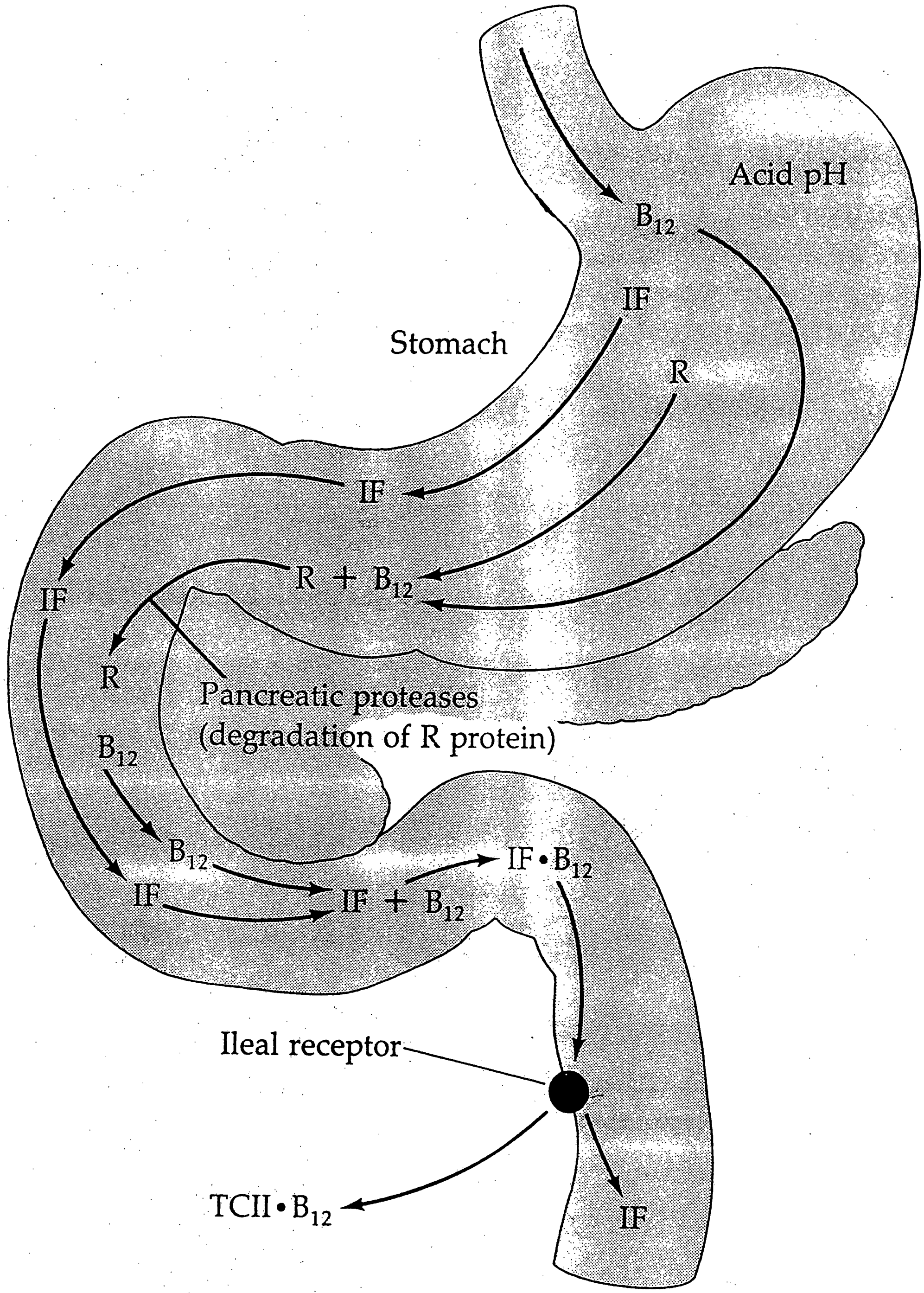

Figure 1. Vitamin B12 Absorption. (From Hunt SM and Groff JL, 1990)

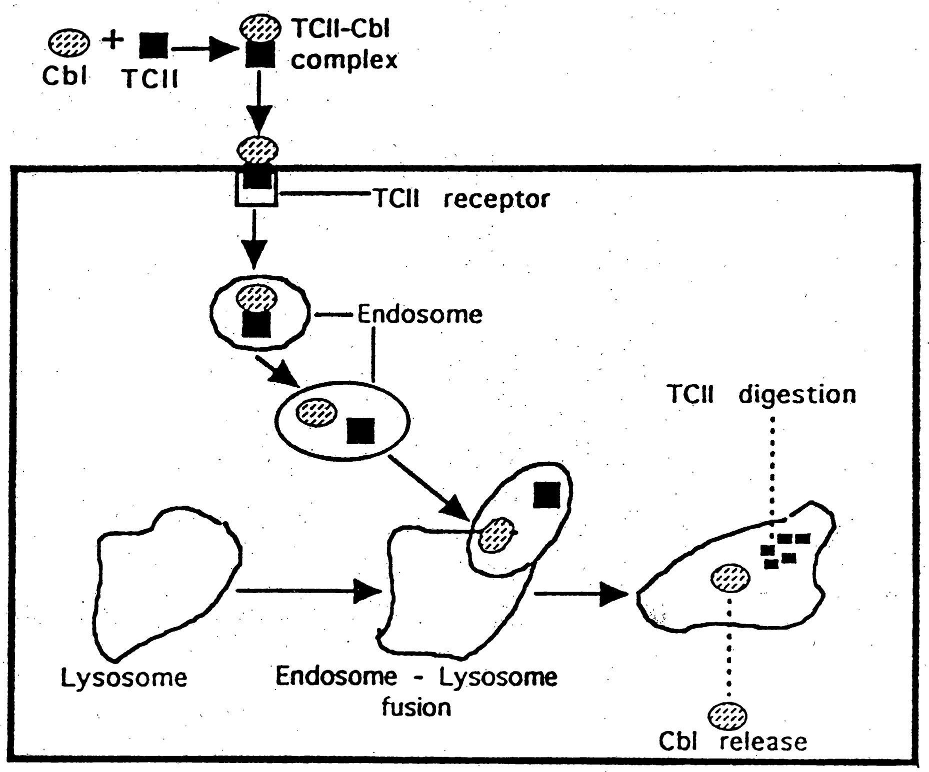

Figure 2. Receptor -mediated Endocytosis of Transcobalamin II-cobalamin Complex (TCII) into all Cell Types. (From Kapedia CR, 1995)

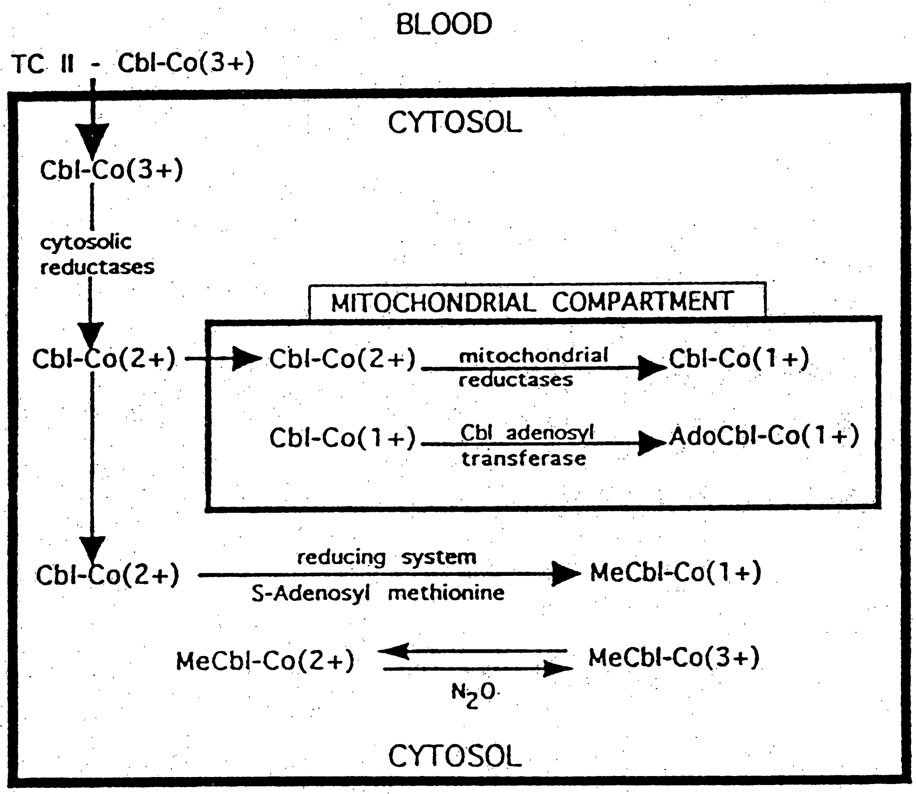

Figure 3. Generation of the Two Coenzyme Forms of Cobalamin (Cbl): Methyl cobalamin (MeCbl) in the Cytosol and 5'-deoxyadenosyl Cbl (AdoCbl) within the Mitochondria. (from Kapadia CR, 1995)

production of IF leading to malabsorption of bound as well as free Cbl (Festen, 1991). However, van Asselt, et al.(1996) reported conflicting results. Middle aged people defined as less than 65 years old compared to older people over 65 absorbed free and protein-bound Cbl equally well. Furthermore, protein-bound Cbl malabsorption was not increased in patients with mild to moderate atrophic gastritis assessed indirectly by pepsinogen A and C.

Another Cbl malabsorption problem among the elderly might be due to pancreatic insufficiency. Pancreatic enzyme production also has been shown to decrease with age (Russell, 1992). However, Schilling (1995) reported that pancreatic insufficiency rarely results in Cbl deficiency. On the other hand, diseases of the terminal ileum, such as Crohn's disease are quite often linked to Cbl deficiency. After Cbl binds to IF, a special ileal receptor is required for absorption. If the receptor is damaged, malabsorption will occur (Stabler, 1995).

In sum, malabsorption might occur because of the following. First, it might occur due to the inability to remove the Cbl from the food proteins, because of a too low hydrochloric acid production. Second, pancreatic insufficiency might lead to low bicarbonate and trypsin production, making it impossible to digest the R-protein of the R-protein Cbl complex. Third, Cbl malabsorption can by due to the halt of gastric parietal cells' IF production or IF destruction by antibodies resulting in pernicious anemia. Fourth, Cbl malabsorption might be due to the destruction of the ileum receptor absorption site after ileal disease or surgery.

The current study allowed the researcher to distinguish between patients with and without protein-bound Cbl malabsorption, and between patients who malabsorb both free and protein-bound Cbl. A food frequency questionnaire was used which specifically focused on protein-bound Cbl and free Cbl intake.

D. Atypical and Typical Cobalamin Deficiency States

The term "atypical" has arisen from the fact that many subjects with Cbl deficiency do not display the typical macrocytic or megaloblastic red blood cells. Some subjects only develop neurologic symptoms and never develop macrocytosis. Many researchers have reported that elderly subjects with neurological problems and low serum Cbl often show no abnormal hematological lab values (Healton, 1991; Pennypacker, 1992; Carmel, 1990). Previously, it was assumed that megaloblastic anemia developed prior to the neurological symptoms, but that notion has been contradicted as early as 1956 when Victor M. et al. reported several cases with subacute combined degeneration of the spinal cord who exclusively displayed neurological symptoms and signs. Atypical Cbl deficiency can be caused by protein-bound Cbl malabsorption, dietary insufficiency, nitrous oxide anesthesia, and inborn errors of metabolism (Carmel, 1990).

On the other hand, typical Cbl deficiency is caused by a lack of intrinsic factor, and usually displays the classical megaloblastic symptoms with or without neurological symptoms. However, not all subjects with pernicious anemia display megaloblastic anemia. Therefore, the term "anemia" can be misleading when anemia is absent among subjects with pernicious anemia (Carmel, 1990).

The current study specifically focused on Cbl deficiency which is atypical in its expression, namely, neurological and non-hematological, and tried to sort out how often malabsorption of protein-bound Cbl versus free Cbl occurs.

E. Laboratory Measures to Assess Cobalamin Deficiency

No true gold standard for testing tissue Cbl deficiency exists. Instead, to get an accurate picture, several laboratory measures should be taken simultaneously. The most common laboratory measures are: serum Cbl, serum methylmalonic acid (MMA), and serum total homocysteine (tHcys).

1. Serum Cobalamin as a Measure of Cobalamin Deficiency

The serum Cbl radioactive dilution assay is a laboratory test performed frequently to assess Cbl deficiency. However, serum Cbl is neither a very specific nor a very sensitive measure. In general, tissue Cbl stores have only been moderately correlated with serum Cbl levels, especially in the elderly.

Using a prospective study design, Matchar, et al. (1994) investigated whether serum Cbl measurements are useful for the diagnosis of Cbl deficiency. To identify true Cbl deficiency, subjects who had a serum Cbl of less than 180pg/ml and a control group with equal or greater 180 pg/ml of serum Cbl underwent clinical examination. Subjects were considered deficient when they excreted less than 7% of radioactive Cbl on the Schillings test, and had a hemoglobin of less than 13mg/dl which was responsive to Cbl treatment. They were also considered deficient if they either had a MCV of greater than 99 fL, a mean neutrophil lobe count greater than 3.6 per cell, or had macrocytic blood cells. Subjects were also considered deficient when they had normal Schilling tests with either a MCV of greater than 99 *fl*, a mean neutrophil lobe count greater than 3.6 per cell, or macrocytic blood cells. These diagnostic criteria were than compared against serum MMA , an objective indicator of tissue Cbl deficiency. The results were as follows. Only 16 out 72 subjects with low serum Cbl on the clinical follow up were truly deficient. The positive predictive value of serum Cbl for diagnosing true Cbl deficiency was only 22%. Eighty-eight percent of the positives were false positives.

2. Serum Methylmalonic Acid as a Measure of Cobalamin Deficiency

Cbl is the coenzyme needed for the conversion of L-methylmalonyl CoA to succinyl-CoA. This Cbl dependent metabolic pathway is needed for the breakdown of branched chain amino acids, threonine, methionine, side chains of cholesterol, thymine, and odd chain fatty acids as shown in Figure 4. However, with Cbl deficiency, D-methylmalonyl CoA undergoes hydrolysis to MMA, which then builds up in the tissues. There is one exception to this build up. A patient with chronic renal failure will also experience a high serum MMA because of impaired kidney function (Stabler, 1995).

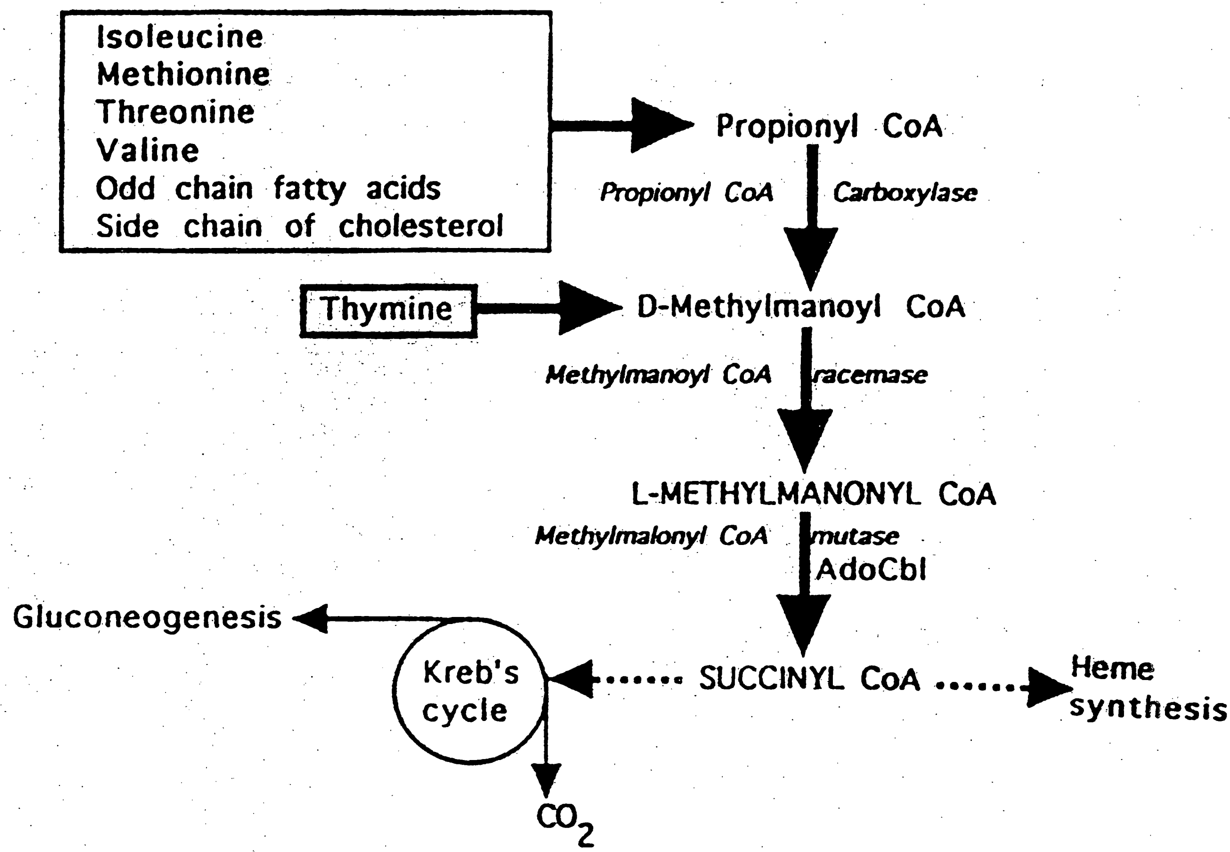

Figure 4. The Central Role of 5'-AdoCbl in Regulation of Metabolsim of Odd Chain Fatty Acids and Branched Chain Amino Acids, as well as in Gluconeogenesis and Heme Synthesis. (from Kapaedia CR, 1995)

The most specific measure to assess Cbl deficiency appears to be serum MMA. According to Savage, et al.(1994), subjects who have low serum Cbl levels (<200 pg/ml), but no serum MMA elevation, defined as a level greater than 376 nmol/L or 3 SD above the mean, can be ruled out from being in a deficient state with a 98.4% certainty.

The following two studies have used elevated serum MMA as a diagnostic tool to test for Cbl deficiency. Pennypacker, et al. (1992) prospectively screened elderly without impaired kidney function for Cbl deficiency. Of the elderly, ages 65 to 99, 38 out of 152 (25%) had a serum Cbl of less than 300 pg/ml, while of the controls (17 to 65 years), 10 out of 100 (10%) had serum Cbl levels of less than 300 pg/ml. As shown in Figure 5, serum Cbl levels between the range of 0 and 199 pg/ml were only found among the elderly subjects.

Of the 25 elderly with serum Cbl levels between 201 to 300 pg/ml, 12 (48%) had elevated serum MMA (>3 SD), while 7 out 13 (53.8%) with serum Cbl of 200 pg/ml or less had elevated serum MMA. Pennypacker, et al. (1992) concluded that the conventional "normal" serum level of 200 pg/ml is too low a reference, since the number of subjects with elevated serum MMA levels were similar among those with serum Cbl levels between 201 and 300 pg/ml and among those with 200 pg/ml or less. Figure 6 shows that only one of the 100 controls with a serum Cbl of <300 pg/ml had an elevated serum MMA, while 19 out of the 38 cases with serum Cbl of <300 pg/ml had elevated serum

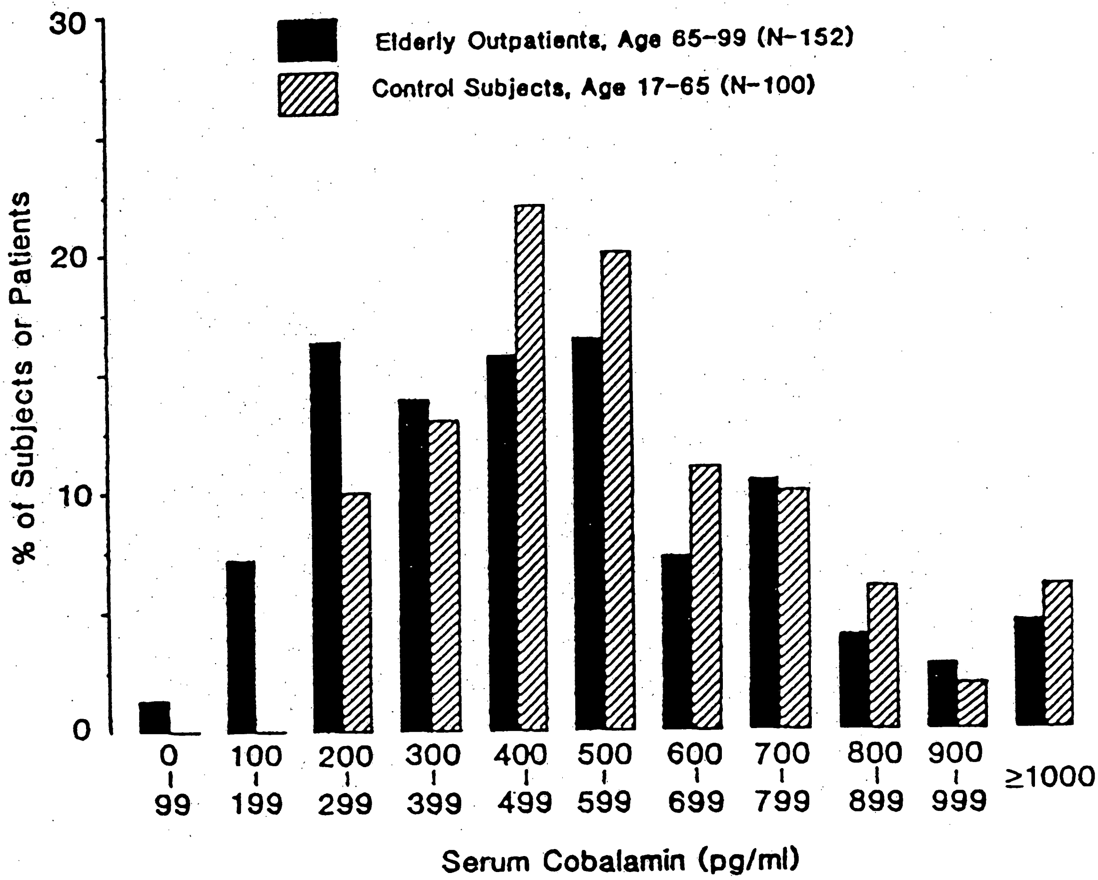

Figure 5. The Proportion of subjects with Serum Cbl levels between 0 and 100, 101 and 200 etc. is shown for Elderly Outpatients, ages 65-99, and normal controls, ages 17-65. (from Pennypacker LC, et al. 1992)

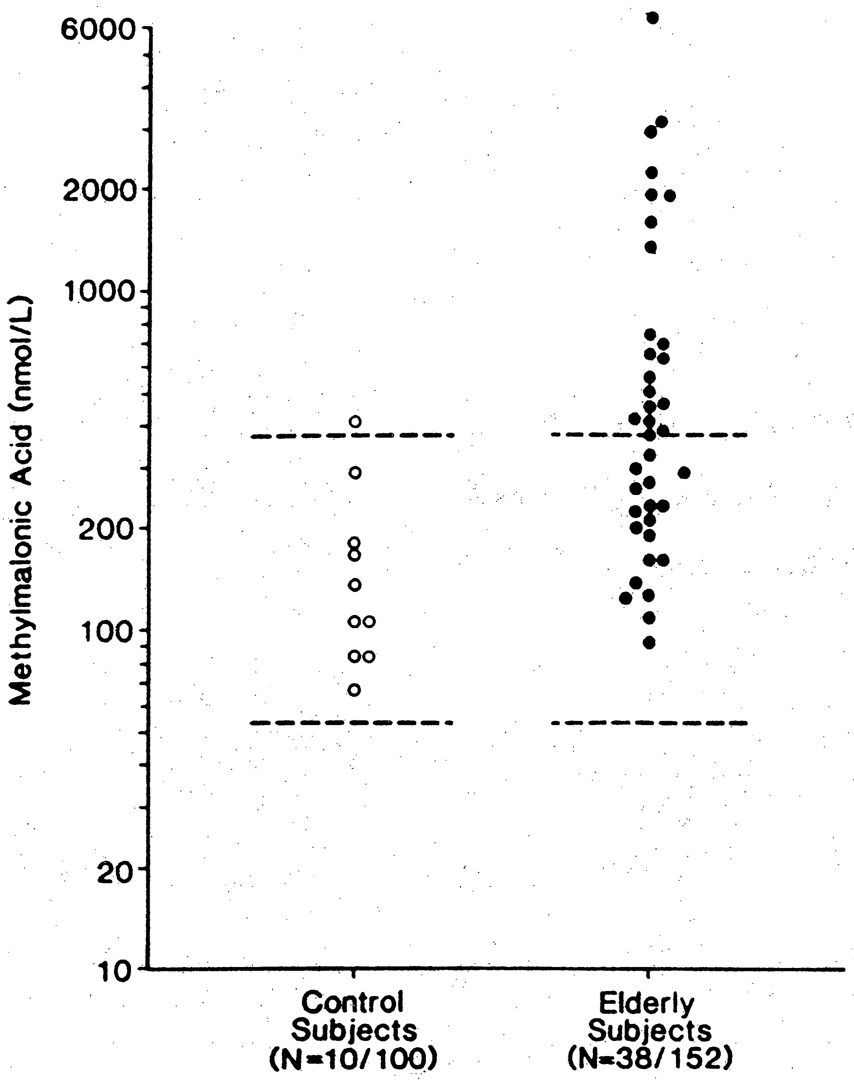

Figure 6. The Serum MMA Levels are shown for the Elderly Subjects (ages 65-99) (●) and the Controls (ages 17-65) (0), with Serum Cbl Less than or Equal to 300 pg/ml. The normal range +/- 3 SD for Serum MMA is shown by the dashed lines and is 43-3776 nmol/L. (from Pennypacker LC, et al. 1992)

MMA. Lindenbaum , et al. (1994) sought to find out whether the increased prevalence of low serum Cbl among 548 elderly subjects from the Framingham Heart study actually reflects tissue deficiency. Figure 7 shows the distribution of serum Cbl concentrations in 548 elderly subjects and 117 younger control subjects. Serum Cbl of less than 191 pg/ml was found in 40.5% of the elderly compared with 17.9% in the controls. Serum MMA were elevated in 15% of subjects with serum Cbl less than 350 pg/ml, while 10% of the subjects with serum Cbl greater than 350 pg/ml had elevated serum MMA. Of the 222 elderly who had a serum Cbl less than 191 pg/ml, 62 (27.9%) had elevated serum MMA concentrations (Lindenbaum, et al. 1994).

Often the elevation of serum MMA precedes the drop of serum Cbl in patients with established Cbl deficiency. In a previous study by Lindenbaum, et al.(1990) when Cbl deficient patients due to a gastrectomy or pernicious anemia received a less than optimal Cbl maintenance therapy for periods of 15 to 66 months, 95% experienced elevated metabolites, but only 69% of them had a serum Cbl level of less than 200 pg/ml.

Elevated serum MMA levels can vary widely from person to person. This can partly be explained by the fact that different amounts of propionic acid are produced by colonic bacteria, and by the use of antibiotics. Lindenbaum, et al. (1990) found that treatment with lincomycin, an antibiotic which changes the microbial flora of the gut, resulted in a drastic drop in serum MMA in Cbl

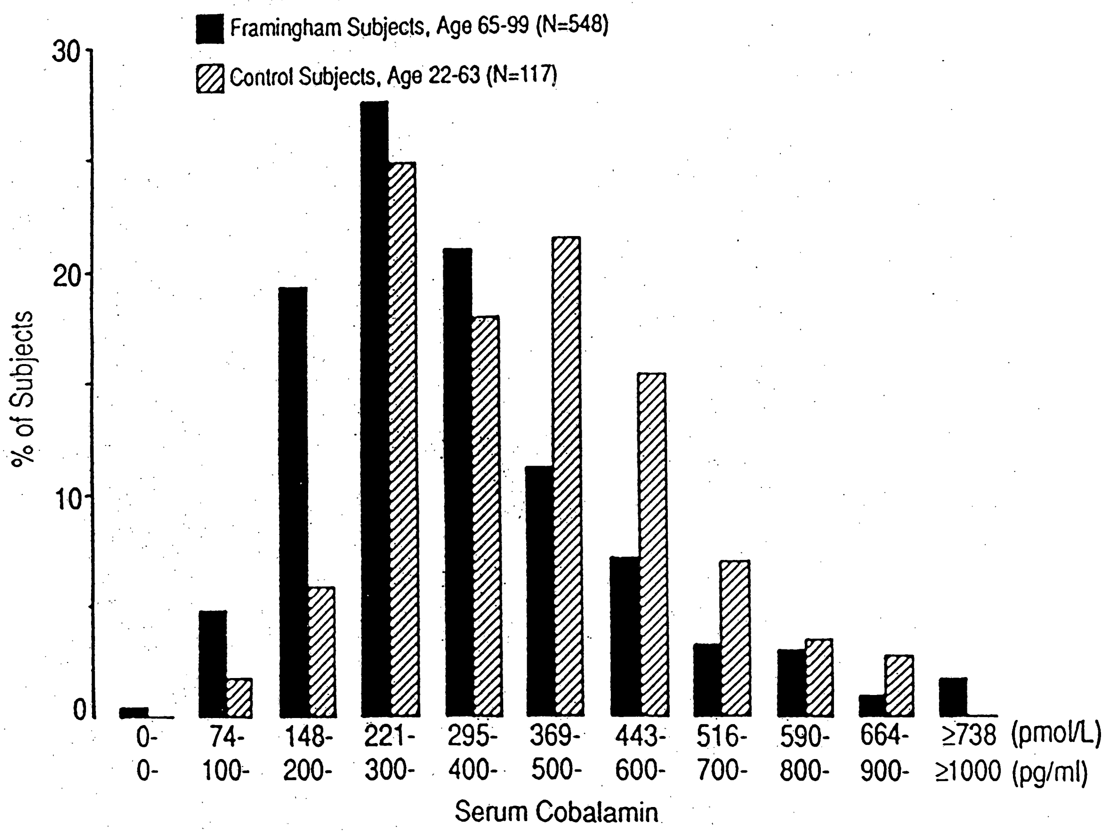

Figure 7. Distribution of Serum Cobalamin Concentrations in 548 Elderly Subjects from the Framingham Heart study and 117 Younger Control Subjects. (from Lindenbaum J, et al. 1994)

deficient subject prior to Cbl injection therapy. In conclusion, when those patients who recently have taken antibiotics, and impaired renal function are excluded, serum MMA is a very sensitive test, as indicated by the fact that serum MMA will be elevated 98.4% of the time in patients with clinically proven Cbl deficiency (Savage, et al. 1994).

3. Serum Total Homocysteine as a Measure of Cobalamin Deficiency

Serum tHcys by itself is a less specific measure than MMA, since it might either indicate a Cbl, or a folate deficiency. The enzyme methionine synthetase converts homocysteine to methionine and uses methyl Cbl as a coenzyme. In this process, the methyl group is transferred from 5'methyl tetrahydrofolate to methionine. With a folate deficiency, this methyl group is unavailable, and homocysteine builds up. On the other hand, with a Cbl deficiency, the methionine synthetase cannot convert homocysteine to methionine, and homocysteine also builds up. The metabolic interaction between Cbl and folate are shown in Figure 8.

Both Cbl deficiency and folate deficiency can result in macrocytosis and megaloblastic anemia. The red blood cells are arrested at an immature state of development as reflected by an elevated MCV. Large doses of folate supplementation can mask megaloblastic anemia caused by Cbl deficiency. However, when Cbl deficiency is wrongfully treated with folate, both serum MMA and serum tHcys, if elevated, remain elevated. The MCV often drops but

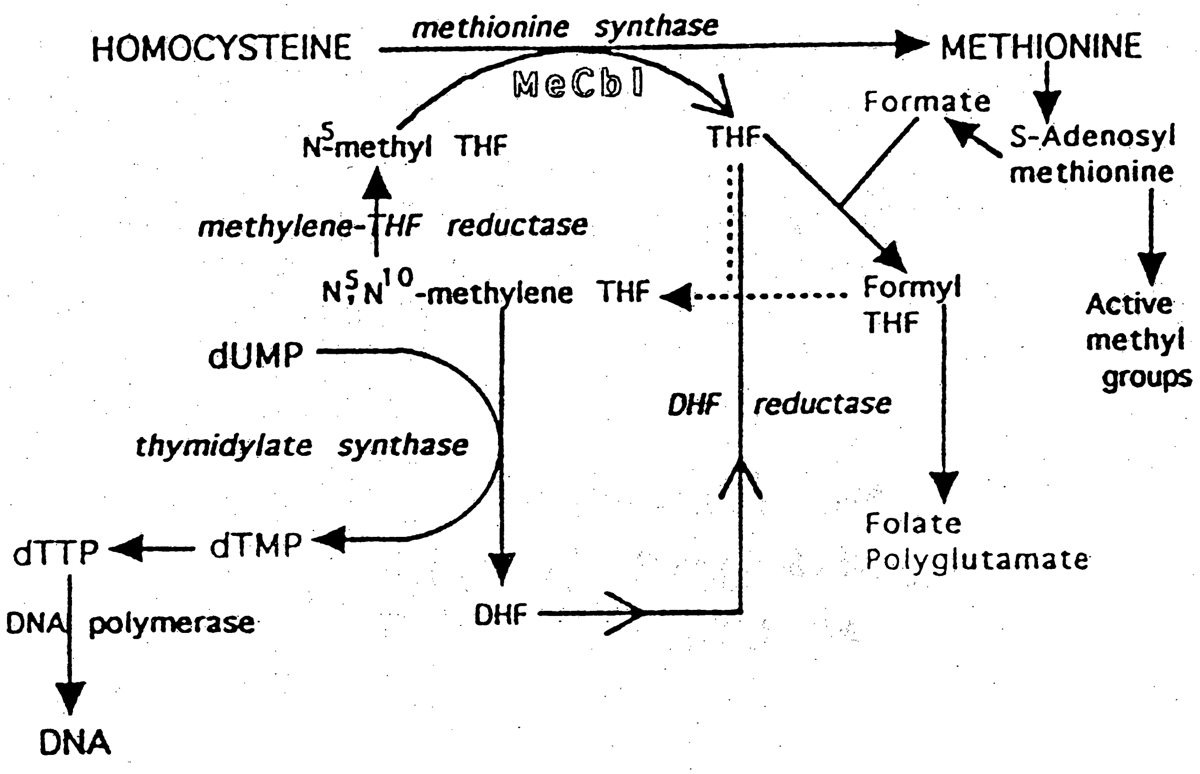

Figure 8. Interaction of the Metabolsim of Cobalamin and Folate. Methyl-Cbl via its interaction with Folate Metabolism, Indirectly Affects the Generation of DNA within the Cells. (from Kapadia CR, 1995)

neurological deficits remain if present. Not until Cbl therapy is initiated do both metabolites drop (Lindenbaum, et al. 1990).

On the other hand, when folate deficiency is wrongfully treated with Cbl, serum tHcys remains elevated, and only returns to normal after folate therapy is initiated (Lindenbaum, et al. 1990). As stated earlier, hematological values used to confirm serum tHcys accuracy in diagnosing Cbl deficiency are not reliable since the majority of elderly with low Cbl levels do not have megaloblastic anemia. Especially, patients with neuropsychiatric disorders often do not display any hematological abnormalities (Carmel, 1995; Lindenbaum, et al. 1988).

Serum tHcys level is a sensitive measure for Cbl deficiency. According to Savage, et al. (1994), subjects who have low serum Cbl levels (<200 pg/ml), but no elevated serum tHcys elevation, can be ruled out from being in a deficient state with a 95.9% certainty. Their study tested the sensitivity of serum tHcys to be able to detect Cbl deficiency in 409 Cbl deficient patients. Of the 434 episodes, 416 episodes (95.9%) were accompanied with elevated serum tHcys, defined as a level greater than 21.3 umol/L or 3 SD above the mean.

The following two studies have used elevated serum tHcys as a diagnostic tool to test for Cbl deficiency. Pennypacker, et al. (1992) found of the 152 elderly subjects, 25 with serum Cbl between 201 and 300 pg/ml, and 14 (56%) had elevated serum tHcys. Eight (62%) of the 13 subjects with serum Cbl of less 200 pg/ml had elevated serum tHcys. Figure 9 shows the serum

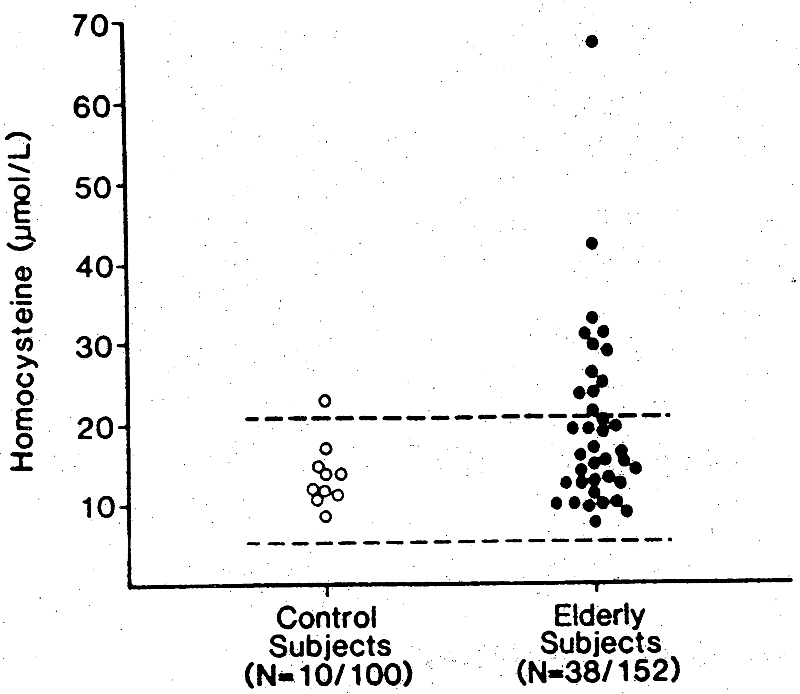

Figure 9. The serum Hcys levels for the Controls (age 17-65) (0) and Elderly subjects (age 65-99) (●) with Serum Cbl Levels Less than or Equal to 300 pg/ml. The Normal Range of +/- 3 SD for Serum Hcys is shown by the Dashed Line and is 4-21.3 *u*mol/L. (from Pennypacker LC, et al. 1992)

tHcys levels of the elderly and the controls with a serum Cbl of equal or less than 300 pg/ml. Of the controls, one out of 10 had elevated tHcys, while 13 out 38 cases had elevated tHcys.

Lindenbaum J, et al.(1994) tested 548 elderly Framingham subjects for true Cbl deficiency. Serum tHcys was elevated in 39 (7.1%) of the subjects. Serum Cbl was less than 350 pg/ml in 31 (79.5%) of the 39 subjects with tHcys elevations and was associated with an increased MMA concentrations in 21 subjects. In conclusion, serum tHcys is a very sensitive indicator of tissue deficient Cbl as indicated by its ability to rule out Cbl deficiency with 95.9% certainty (Savage DG, et al. 1994).

4. The Advantage of Using Both Serum MMA and Serum Hcys

Savage, et al.(1994) found that 98.4% of Cbl deficient patients had elevated serum MMA, and 95.0% had elevated serum tHcys. However, when looking at serum MMA and serum tHcys simultaneously, the sensitivity for detecting true Cbl deficiency increased to 99.8%.

About 1.4% to 10% of Cbl deficient patients only experience elevated serum tHcys levels (Stabler, 1995; Allen, et al. 1990). As stated earlier, this is partly due to variable gut flora between patients and recent antibiotic use. Most of subjects with folate deficiency will only experience elevated serum tHcys levels. However, Savage, et al. (1994) found that one out of 119 Cbl deficient patients experienced both elevated serum MMA and serum tHcys levels.

F. Prevalence of Cobalamin Deficiency Among Older Adults.

Between the years of 1976 and 1994, the prevalence of Cbl deficiency among older adults has been estimated to be anywhere from 3% to 32.6%. See Table 1. The reason for this great variance can be attributed to many different variables. The most obvious ones are the different biochemical methods and criteria used to determine Cbl deficiency. Samples have also been taken from different subsets of the older adult population. But, most importantly, older studies have used lower serum Cbl cut-off levels, and sometimes as the only indicator for Cbl deficiency. The more recent studies have used elevated MMA and tHcys levels, two metabolites which are more sensitive indicators of biochemical Cbl deficiency. The paragraphs that follow will give an overview of the different studies listed in Table 1. which have sought to find the prevalence of Cbl deficiency among older adults.

Elsborg , et al. (1976) was unable to differentiate between folate deficiency or Cbl deficiency in 78 of the 89 geriatric inpatients with serum Cbl levels of <200 pg/ml, because of the types of tests done. The prevalence of Cbl and or folate deficiency was estimated to be 32.6%. The following tests were performed, formiminoglutamic acid (FIGLU) excretion, MCV, Schilling test I, fecal fat excretion, D-Xylose test. These tests have many limitations in that they do not specifically test for Cbl deficiency. An impaired urinary FIGLU excretion can be either the result of a Cbl or folate deficiency. Macrocytosis can either be due

TABLE 1. PREVALENCE COBALAMIN DEFICIENCY IN OLDER ADULTS

AUTHORS	NUMBER AND TYPE OF SUBJECTS	STUDY DESIGN	PARAMETERS USED TO DETERMINE COBALAMIN DEFICIENCY	ESTIMATED PREVALENCE
Elsborg L. et al. 1976	349 geriatric inpatients	prospective pretest posttest study	Cbl of ≤ 200pg/ml microbial essay FIGLU excretion Schilling test, part I	32.6% folate or Cbl def. 47 subjects normal FIGLU 37 subjects abn. FIGLU 5 pern. anem.
Garry PJ. et al. 1984	270 healthy elderly subjects	prospective study of nutrition in the elderly	Cbl of < 220pg/ml radioessay	3% Cbl def.
Hanger HC. et al. 1991	204 randomly selected free living elderly	cross-sectional design	Cbl of 154 pg/ml	7.3% Cbl def.
Yao Y. et al. 1992	100 consecutive geriatric outpatients	cross-sectional design	if Cbl ≤ 299 pg/ml than tested serum IF, parietal cell antibodies, serum gastrin, part I Schilling, MMA/tHcys	19.7%, based on 2 SD above the mean for serum MMA and tHcys
Pennypacker LC. et al. 1992	152 consecutive geriatric outpatients	cross-sectional design	Cbl ≤300 pg/ml and > 3SD of MMA and/or tHcys	14.5% Cbl def.
Lindenbaum J. et al 1994	548 surviving members of the Framingham study	prospective observational design	Cbl of <350 pg/ml and > 3SD of MMA and/or tHcys	≥12% Cbl def.

to a Cbl or folate deficiency. The Schilling test I only tests for impaired crystalline Cbl absorption, not for food-bound Cbl malabsorption. Fat malabsorption does not necessarily indicate Cbl malabsorption although both are affected by a decreased production of different pancreatic enzymes. D-xylose test is used to distinguish diarrhea caused by pancreatic dysfunction (maldigestion) from diarrhea caused by malabsorption (Pagana, 1992). Consequently, the use of these less specific tests made it impossible to estimate the prevalence of Cbl deficiency.

Garry, et al. (1984) used a serum Cbl of less than 220 pg/ml as the cut-off for Cbl deficiency (20). A radioassay Cbl assay was used which is able to distinguish Cbl from non-Cbl analogues. Besides the MCV, plasma folate, and RBC folate, no other parameters were used. The MCV did not correlate with Cbl deficiency. None of the subjects with serum Cbl levels of less than 220 pg/ml had macrocytosis. When using a serum Cbl of 220 pg/ml as a cut-off for Cbl deficiency, only 3% of healthy people aged over 60 were deficient. Subjects with known medical illnesses were excluded. The authors concluded that Cbl status among the elderly is not a major medical problem.

Hanger, et al. (1991) used an even lower cut-off level for Cbl deficiency, namely 154 pg/ml (21). No other biochemical parameters, beside serum Cbl, were used to check for Cbl deficiency. The authors found the prevalence of Cbl deficiency among people 65 or older to be 7.3%. The exclusion criteria were more stringent than the previous study. Those subjects living in institutional care and known to take Cbl supplements, or conditions known to influence Cbl status were excluded. The use a serum Cbl of 154 pg/ml as the cut-off for Cbl deficiency might underestimate the prevalence of deficiency among older adults.

The studies to follow all use serum MMA and serum tHcys, two biochemical markers to test for true Cbl deficiency. Yao, et al. (1992) tested 100 consecutive geriatric outpatients aged 65 or above. Sixteen (16%) patients had Cbl levels of less than 200 pg/ml. Only 5 patients had their MMA and tHcys checked, since Medicare refused to pay most of the time. Of those five, four (80%) had MMA levels greater than 270 nmol/ml (>2 SD), and three (60%) had tHcys level greater than 16 nmol/ml (>2 SD). Twenty one (21%) had Cbl between 201 and 299 pg/ml. Of those 21, only nine had their MMA and tHcys levels checked. Three subject or (33%) had MMA levels greater than 270 nmol/ml, and 11% had tHcys levels greater than 16 nmol/ml. Since so few subjects had their serum MMA and serum tHcys tested, the authors could only make rough estimations of the prevalence of Cbl deficiency. Unlike the following researchers, they used a 2 SD above the mean as the cut-off for elevated MMA and Hcys. Based on this cut-off, the estimated prevalence of Cbl deficiency among older adults was 19.7% (Yao, et al. 1992).

Pennypacker, et al. (1992) screened 152 consecutive geriatric outpatients. Cbl deficiency was defined as a Cbl level of equal or less than

300 pg/ml with elevated MMA and/or tHcys levels of 3 SD above the mean. Using these criteria for deficiency, they found that the prevalence of Cbl deficiency was 14.5%. Fifty-six percent of the subjects with Cbl ranging from 201 to 300 pg/ml had elevated MMA and/or tHcys levels, compared to 62% for subjects with Cbl levels of less than 200 pg/ml.

Lindenbaum, et el. (1994) decided that a 350 pg/ml Cbl level should be the cut-off for suspecting a possible Cbl deficiency based on the fact that elevated serum MMA and serum tHcys levels occurred in 18% of the subjects with serum Cbl levels of between 300 and 349 pg/ml (14). They looked at the 548 surviving members of the Framingham Heart study, and found that 222 (40.5%) had a Cbl of less than 350 pg/ml. Of those 222, 11.3% and 5.7% had elevated MMA and tHcys level, respectively. They found the prevalence of Cbl deficiency to be 12% or greater in healthy older adults.

G. Comparing Neurological Signs in Older Adults at Different Cobalamin Levels

The Framingham study found that 40.5% of the healthy elderly subjects had serum Cbl levels below 350 pg/ml. Of those elderly subjects, 27.9% also had elevated MMA >376 nmol/L), a truthful indicator Cbl deficiency, when those with impaired renal function are excluded. This study found a true Cbl deficiency of 12% or greater among older adults, however these subjects were not systematically tested for neurological deficits (Lindenbaum , et al. 1994)

In the study of Metz, et al. (1996), the cases did not score significantly differently on the neurological test and the Mini Mental State Exam than the controls. However, that might partly be due to the fact that some of the controls were actually cases since 19% of them had elevated tHcys without concurrent folate deficiencies. Even though, the mean tHcys levels in the cases were significantly higher than in the controls, 25.0 nmol/ml versus 15.5 nmol/ml (P 0.008), 40% of the controls had elevated tHcys levels, compared to 76% of the cases. No separate neurological testing was done to compare the scores of those with true Cbl deficiency to the scores of those without Cbl deficiency.

Healton, et al. (1991) also found no correlation between the severity or presence of neurological impairment and low serum Cbl level. But, they tested only those subjects with Cbl deficiency. Consequently, these insignificant results can be due to a restriction of the Cbl range used, and the fact that serum Cbl level is an insensitive test for Cbl deficiency. They did not specifically compare serum MMA and serum tHcys levels against neurological impairment.

Pennypacker, et al. (1992) did find that those with elevated MMA and tHcys levels had a higher degree of neurological impairment than those without such elevations. A neurological score of zero indicated that the patient was neurologically completely normal. After Cbl treatment, the mean neurological impairment score dropped in the Cbl deficient outpatients, and two groups scored neurologically similarly.

Hanger, et al. 1991 looked specifically at the mental and cognitive status of older adults with low serum Cbl, defined as 154 pg/ml, and those with normal serum levels (>154 pg/ml). They found no correlation between mental status, cognitive status, and serum Cbl levels. As pointed out previously, older adults can experience Cbl deficiency at a wide serum range, and therefore serum Cbl is too insensitive a test.

From the previous studies, it can be concluded that the question of whether there is a higher prevalence of Cbl deficiency among older adults with clinically diagnosed neurological deficits than among older adults without such deficits has not yet been fully answered. The current case-control study does not stratify patients by serum Cbl levels which is very unreliable, but stratifies the patients by clinically diagnosed neurological deficits using a standard neurological exam (Healton, et al.1990; Metz, et al.1996).

H. Are Dementia Patients More Likely to be Cobalamin Deficient?

The Cobalaminergic hypothesis proposes that Cbl deficiency might be etiologically important in a sub-group of patients with Alzheimer's disease (AD) (McCaddon A, 1992). Others propose that a low serum Cbl is a marker for AD, that cognitive decline will continue in spite of normalization of serum Cbl levels after treatment. A correlation of 0.38 at $P < 0.05$ was obtained for cognitive decline as measured by the Mini Mental State Exam (MMSE) and serum Cbl values in patients with AD type dementia (Levitt, et al. 1992). On the other hand, the fourth edition of the Diagnostic Manual of Mental Disorders (DSM-IV) wants the physician to use serum Cbl as a differential diagnosis. Dementia due to other general medical conditions ie. Cbl deficiency has its own special ICD-9 code. The DSM-IV excludes the possibility of an interaction between dementia of all types and a Cbl deficiency.

Unfortunately, the current data is not so clear-cut as the DMS-IV proposes. There are many conflicting study results regarding the relationship between Cbl deficiency and AD type dementia as well as other types of dementias. Lower Cbl levels have been reported in patients diagnosed with AD type dementia compared to patients with other types of dementias or controls (Carmel, et al. 1995; Kristensen, et al. 1993). While others report that serum Cbl levels are not more often reduced in patients with AD type dementia (Basun, et al. 1994; Crystal, et. 1994). These conflicting results are partly due to different research criteria, different assays, different serum Cbl cut-offs, and whether or not serum MMA and serum tHcys were used to determine if the low Cbl levels existed concomitantly with a frank Cbl deficiency. The following paragraph will take a look at some of the currently existing conflicting research results.

Crystal, et al. (1994) performed a prospective study on elderly aged 75 to 85. After a five year follow up, they found no correlation between those who had dementia as defined by neuropsychological tests (the Blessed test of Information, Memory, and Concentration and the Fuld Object Memory Evaluation tests) and low serum Cbl values as defined by less than 150 pg/ml. However, this study used low serum Cbl as the only marker for Cbl deficiency. It lacked some vital laboratory assessments, since true Cbl deficiency cannot be determined by serum Cbl values alone. In order to distinguish between a true positive and a false positive, serum MMA and tHcys have to be measured.

Basun, et al. (1994) conducted a cross-sectional survey and compared serum Cbl levels of patients with AD type dementia, vascular dementia, other dementias, and non-demented patients. No significant difference in mean serum Cbl level was found in any of the four groups. An age effect for serum Cbl levels was only observed in the non-demented patients. Older non-demented patients had significantly lower serum Cbl values than their counterparts. Age specific prevalence rates of Cbl deficiency, defined as 200 pg/ml, for non-demented, AD type dementia, other dementias were 15.5%, 18.1%, and 14.4% respectively, and did not differ in a statistically significant way. Again, serum MMA and serum tHcys were not analyzed to assess whether the low Cbl levels corresponded with a true Cbl deficiency.

Kristensen, et al. (1993) found that older patients with AD dementia more often displayed true Cbl deficiency than older patients with other dementias, mental disorders, and the controls. Twenty seven percent of the AD patients either displayed subnormal Cbl levels and/or elevated serum MMA, while only 8% of the patients with other dementias, 4% of the patients with mental disorders, and 5% of the controls had subnormal Cbl levels and/or elevated serum MMA.

From the previous studies it can be concluded that more studies are needed to determine whether or not older dementia patients are more prone to Cbl deficiency than non-demented controls. Only Kristensen, et al (1993) assessed the serum MMA and tHcys levels. The current study attempted to assess whether or not the prevalence of Cbl deficiency is higher among demented outpatients Dementia was defined by a MMSE score of 24 or less in conjunction with a history and physical which met the DSM-IV diagnostic criteria, than a comparable non-demented control group. Cognitively impaired patients, was numerically defined by MMSE score of 25 or 26, in conjunction with a history and physical which met the DSM-IV diagnostic criteria for amnestic syndrome (Levitt, et al. 1992).

I. Cobalamin Deficiency induced Neuropathologic Changes

In 1900, the term subacute combined degeneration of the spinal cord was coined. This syndrome expresses itself neurologically, hematologically, and cellularly as epithelial atrophy of the tongue lining (Rowland, 1995). While neurological symptoms and signs are always present, the hematological and cellular signs are sometimes absent as reported by Shevell in 1956. In addition to neurological signs and symptoms, Cbl deficiency can also cause psychiatric problems such as agitation, irritability, memory loss, psychosis, hallucinations, and dementia. Others have also reported that Cbl induced neurological problems often occur in the absence of abnormal hematological findings, and that they might even be inversely related (Lindenbaum, et al. 1988; Healten, et al. 1991). Lindenbaum, et al.(1988) found that 40 of 141 (28%) patients with Cbl deficiency had no anemia or macrocytosis. As stated earlier, the type of Cbl deficiency which strictly presents itself neurologically has been called atypical Cbl deficiency, because of its atypical non-hematological presentation.

Cbl deficiency can cause a symmetric loss of myelin in the posterior-lateral columns, lateral corticospinal tracts, and spinalthalmic tracts of the spinal cord, the peripheral nerves, the cerebral cortex and cranial nerves (Victor, 1956; Allen, et al. (1995). The demyelination of the spinal cord initially affects the cervical and upper thoracic axonal tracts (Savage, 1995), then proceeds in any direction across the spinal cord (Rowland LP, 1995). When the lateral corticospinal tracts become affected, it can cause, hyper or hypo platelar reflexes or lead to absent reflexes, clonus, and babinski. Spinalthalmic tract involvement can cause decreased cutaneous sensation. Posterior column tract involvement can lead to decreased or absent vibration sense, a positive Romberg, and decreased joint position sensation (Victor, 1956). Compared to spinal cord involvement, diffuse lesions of the peripheral sensory or sensorimotor nerves can lead to a similar neurological clinical presentation. A clinical neurological examination is not able to differentiate between a the spinal cord and/or peripheral nerves involvement (Savage, et al.1995). Both, the large myelinated fibers and the small unmyelinated fibers can become damaged, reducing position and vibratory sense, pain and temperature perception, respectively (Rowland, 1995).

Currently, it is thought that the demyelination is either induced by the impaired actions of two Cbl dependent enzymes, namely, methionine synthetase or methylmalonyl CoA mutase. The first hypothesis was supported by the fact that methionine supplementation with induced Cbl deficient animals would postpone the onset of Cbl induced neuropathy (Metz, 1992). It is believed that the unavailability of methyl groups due to decreased synthesis of S-adenosyl methionine leads to impaired myelin synthesis. Myelin is predominantly made up of cholesterol, glycolipids, phospholipids, and myelin basic protein. Methyl groups are needed for the synthesis of phospholipids and myelin basic protein (Kapadia, 1995). The other hypothesis states that Cbl induced impairment of methylmalonyl CoA mutase results in the accumulation of MMA and propionyl CoA which is thought to replace malonyl CoA and acetyl CoA as substrates in fatty acid synthesis leading to the formation and incorporation of unusual fatty acids into myelin (Kapadia, 1995).

J. Neurological Symptoms and Signs of Cobalamin Deficiency

The following paragraphs give an overview of the neurological symptoms and signs in Cbl deficient patients (Healton, et al. 1991). The most common initial symptom was bilateral paresthesias, a painful burning and tingling sensation that can affect the feet or both the hands and feet. Ataxia without paresthesias can also be an initial symptom, although ataxia usually occurs at a later stage, and in combination with paresthesias.

Diminished or absent vibratory sensation in the feet or feet and legs up to the knees was the most common neurological finding. The second most common finding was diminished or absent proprioception in the toes and ankles. Sometimes the fingers and wrists were also affected. Cbl induced mental impairment such as global dementia, memory loss with a mildly reduced attention span, depression, agitation, paranoid psychosis, and personality changes was the third common finding. Diminished or absent cutaneous pain sensations was the fourth common finding. Sensations were most commonly diminished or absent in the feet or feet and legs up to the knees. However, sometimes the arms, wrist, forearms, or biceps were also involved. Weakness of the limbs as witnessed by hyper or hyporeflexia, and absent or weak tendon reflex was the fifth most common finding. A positive Romberg was the sixth most common finding. Autonomic abnormalities due to Cbl deficiency such as urinary and fecal incontinence, postural hypotension were not seen very often, as was Cbl induced visual impairments.

Healton, et al. (1992) found an inverse relationship between neurological impairment and hematocrit. The duration of neurological symptoms was positively correlated with neurological impairment. On the average, subjects without neurological impairment had lower MCVs than subjects with neurological impairment. There was no correlation found between the severity or presence of neurological impairment and the serum Cbl level. All the subjects responded to Cbl treatment, of which 47% experienced a complete recovery. The severity of neurological impairment as well as the duration of the symptoms were strong determinants of whether or not there was complete recovery.

K. Dietary Cobalamin Intake Among Older Adults

Many studies on Cbl deficiency among the older adults have been done. However, only a few selected studies have attempted to assess dietary adequacy. Most Americans eat a large variety of animal products and fortified cereals, and it is, therefore, assumed by most researchers that older adults get more than enough dietary Cbl in terms of the Recommended Daily Allowance (RDA) of 2 mcg. The following paragraphs will evaluate this assumption by evaluating some of the dietary assessments that have been done to estimate Cbl intake.

A national study of 474 participants revealed that 15.4% of the men and 17.3% of the women above the age of 74 obtained below two-thirds of the RDA for Cbl through dietary means. Between the age of 65 and 74, 8.7 of the men and 19.7 of the women obtained below two-thirds of the RDA. However, of those consuming below two-third of the RDA, 36% of the men, and 40% of the women reported taking a vitamin and/or mineral supplement. No further assessment of the supplements were performed (Ryan, et al. 1992).

Blundell. et al (1985) hypothesized that Cbl deficiency among elderly hospital inpatients is often due to a nutritional deficiency since their subjects' serum Cbl levels rose after admission to the hospital. However, this study did not systematically evaluate the patients' average dietary Cbl intake. Elsborg, et al. (1976) also found that when elderly patients were placed on a hospital diet that the serum Cbl levels rose.

Garry, et al. (1984) did do a dietary Cbl analysis, and found that the median Cbl intake was close to 3 mcg a day for the older subjects . Forty-two percent of the men had intakes below the RDA, while 10% had an intakes of less than 50% of the RDA. While, 65% of the women in this study had intakes below the RDA, and 15% had intakes of less than 50% of the RDA. Note that the RDA in 1984 was set at 3 mcg, and not at the current RDA of 2 mcg.

Carmel, et al. (1995) attempted to assess dietary histories of dementia patients, but they had too many logistical problems administering the food frequency questionnaire, and 24 hour recall. Consequently, their results on the adequacy of dietary Cbl were inconclusive.

Considering the previous studies, it is not quite clear whether or not most older adults get sufficient dietary Cbl through their diet, especially free or crystalline Cbl Free Cbl is found only in fortified foods and supplements. In light of the fact that food-bound Cbl malabsorption (Cbl in animal foods) might be quite common among older adults, no researcher seem to have systematically studied free and protein-bound Cbl intake among older adults. This current study specifically assessed free and protein-bound Cbl intake. Dietary needs of Cbl for older adults might be higher than the current RDA of 2 mcg, because of the different absorptive problems older adults can encounter with aging (Russell, 1992). Oral supplementation or food fortification with free Cbl might be needed for this older population (Russell, 1997). Furthermore, the current 1989 RDA does not distinguish between crystalline and protein-bound Cbl (National Research Counsel, 1989).

CHAPTER TWO

II. OUTLINE OF THE RESEARCH PROJECT

A. Summary of Rationale for the Research Project

The prevalence of cobalamin (Cbl) deficiency among older adults is higher than among younger adults, and is estimated to be between 14% and 23%. Persistent Cbl deficiency can cause a variety of neurological deficits. Neurological dysfunction occurs commonly among older adults, raising the research question whether or not there is a relationship between the high prevalence of Cbl deficiency and neurological dysfunction among older adults. Permanent neurological damage can occur if Cbl deficiency is mistaken for other diseases such as Alzheimer's dementia, multiple sclerosis, diabetic neuropathy, or amyotrophic lateral sclerosis (Schilling, 1995).

A study is needed to elucidate whether low "normal" serum Cbl values are part of a normal aging process or if they are more often accompanied, not only by elevated methylmalonic acid (MMA) and/or total homocysteine (tHcys), but occur more frequently among patients with detectable neurological/cognitive deficiencies or symptoms.

B. Main Study Objective and Hypotheses

1. Main Study Objective

Our primary objective is to determine whether older adults (60 years or older) with a diagnosed neurological and/or cognitive dysfunction (cases) have a higher prevalence of Cbl deficiency than a comparable group without such neurological and/or cognitive deficits (controls).

2. Hypotheses

1. The proportion (prevalence) of controls with true Cbl deficiency as defined by a serum Cbl of <400 pg/ml and a MMA of >271 nmol/L and/or a serum Hcys of >13.9 umol/L (estimated to be 18%) will be lower than for the cases (estimated to be 38%).

2. The odds of having Cobalamin (Cbl) deficiency is estimated to be 3.75 higher for subjects with neurological and/or cognitive dysfunction (cases) compared to the odds of the control subjects .

3. Older adults with neurological and/or cognitive dysfunction consume less free Cbl (from fortified foods and supplements) than the control subjects.

4. Subjects who consume a higher amount of free Cbl are less likely to be Cbl deficient.

C. The Study Design, Setting, and Sample Size Calculation

1. Study Design

A case control study which used 120 outpatients 60 years or older with and without neurological and/or cognitive deficits was conducted.

2. Setting

Subjects were recruited through the Loma Linda University Faculty Medical Offices, an outpatient clinic. Control patients were strictly recruited through the Department of General Internal Medicine and were seen by Dr. James Larson, the principal investigator, and Marion Bachra, a graduate nutrition student and author. Cases were recruited both through the Department of General Internal Medicine and the Department of Neurology and were seen by various internists, neurologists, including Dr. Daniel Giang, a co-investigator, and author.

3. Sample Size Calculation

The prevalence of Cbl deficiency in healthy free-living older adults is estimated to be around 18% (Lindenbaum, et al. 1994, Pennypacker, et al. 1990). To be able to detect an odds ratio of 3.75 or greater, at a power level of 80%, at an alpha level of 0.05, 60 cases and 60 controls were needed for this study. Epi Info Version 6.0 was used to do the power calculation. See Appendix 1 for the power calculations for the odds ratios 5 through 1.5.

D. Subjects, Exclusion Criteria and Sequence of Data Collection

1. Subjects

Thirty female and 30 male controls, 27 female and 33 male cases ages 60 through 107 were enrolled when the exclusion criteria listed below were met.

Exclusion Criteria

1. Patients with neurological demyelinating diseases, movement disorders, seizure and epilepsy syndromes, neuropathies, neuromuscular disease due to specific pathological conditions were excluded.

2. Patients with asymmetrical presentation of neurological signs or symptoms were excluded.

3. Suspected diabetics, defined as a fasting blood sugar above 140 gm/dl, or a random blood sugar above 200 mg/dl and diagnosed diabetic were excluded.

4. Patients with previously diagnosed pernicious anemia were excluded.

5. Patients with previously diagnosed Cbl deficiency ie. pernicious anemia were excluded.

6. Patients who orally took more than 25 mcg of Cbl per day, or who had previously received Cbl injection therapy were excluded.

7. Patients previously diagnosed or suspected with spinal stenosis, spinal compression, and all rediculopathy related to specific structural problems were excluded.

8. Patients receiving chemo or radiation therapy, or those who experienced peripheral neuropathy after treatment were excluded.

9. Patients with untreated or partly treated hypothyroidism were excluded.

10. Patients with multiple myeloma and those who tested positive for monoclonalgammopathy were excluded.

11. Patients with diagnosed or suspected strokes, multi-infarcts, peripheral vascular disease, cerebral hemorrhage, and brain tumors were excluded.

12. Patients with a creatinine level above 2.0 mg/dl were excluded.

2. Sequence of Data Collection Events for Cases

1. Outpatients visited either Dr. Larsen, Dr. Giang or other FMO doctors for neurological and/or cognitive complaints. Some patients were enrolled without neurological symptoms, but were tested neurologically by the MD to have a neurological deficit. To speed up the data collection process, the Clinical Lab saved serum of all patients who had their serum Cbl levels tested. The author would approach the doctor who ordered the test to see if the patient met the study criteria, and to get approval to invite the patient to enroll in the study.

2. Patient was asked to participate in the study, read and sign the informed consent form. Appendix 2 shows the two separate consent form for the cases and the controls.

3. During the first visit, the physician administered the neurological exam and the Mini Mental State Examination (MMSE) (See Appendix 3).

a. The author administered the MMSE with all the cases who strictly enrolled because of a neurological deficit. The author administered the Trail Making Test, Part B with all the cases and all the controls. Scores were filled out on the neurological score sheet and check list (Appendix 3).

b. The physician ordered a Complete Blood Count, a Chemistry Blood profile, Serological testing for syphilis (only for cognitive impairment), protein electrophoresis, a thyroid panel, and serum Cbl on a special SmithKline Beecham lab slip with a label indicating that two vials with 1.5 ml each serum should be frozen.

c. Two vials, each with 1.5 ml of serum were frozen and saved for Metabolite Laboratories, Inc. in Denver.

d. The author administered and filled out the Food frequency Questionnaire. (Appendix 4)

e. The author checked the patients' medical records and filled out a Patient Profile Sheet (Appendix 3)

4. After all the data had been collected, aliquots of those patients with a serum Cbl of equal or less than 400 pg/ml were sent to Metabolite Laboratories in Denver where serum MMA/tHcys were analyzed.

3. Sequence of Data Collection Events for Controls

1. Patients visited Dr. Larsen for a medical condition other than a neurological or cognitive complaints.

2. Patients were asked to participate in the study, read and sign the informed consent form (Appendix 2)

3. During the patients' regular visit, Dr. Larsen administered the neurological testing (Appendix 3)

a. The author administered the MMSE and Trail Making Test. All the scores were filled out on the neurological score sheet and Check list (Appendix 3).

b. Dr. Larsen ordered serum Cbl on a separate and special research SmithKline Beecham lab slip with the Grant Billing Address. CBC and Chem Profile were only ordered when medically indicated.

c. Two vials, each with 1.5 ml of serum were frozen and saved for possible transport to Metabolite Laboratories, Inc. in Denver.

d. Marion administered and filled out the Food frequency questionnaire (Appendix 4)

e. The author filled out a Patient Profile Sheet (Appendix 3)

4. After all the data had been collected, aliquots of those patients with a serum Cbl less than 400pg/ml were sent to Metabolite Laboratories in Denver where serum MMA/tHcys were analyzed.

E. Biochemical Assessment of Blood Samples

The following laboratory values were documented on the patient profile sheet. Serum Cobalamin, serum total homocysteine, serum methylmalonic acid, mean cell volume, hemoglobin, hematocrit, blood urea nitrogen, creatine, total cholesterol, albumin. (Appendix 3)

F. Neurological Impairment Measurements and Scores

The higher the neurological score the greater the neurological impairment (Healton et al, 1991; Metz et al. 1996). Maximum total points were 23 points on the neurologic testing scale (Appendix 3).

A neurological deficit was defined as a neurological score of 4 or above, excluding those who had reduced vibration sense and/or hyporeflexia in the absence of paresthesias. Absence of neurological deficit was defined as a score of 4 or less in the absence of paresthesias. Patients with peripheral neuropathy/myelopathy with and without paresthesias were enrolled. Those who experienced gait imbalance in the absence of paresthesias or peripheral neuropathy/myelopathy were also enrolled. Patients who had paresthesias in the absence of other neurological signs were also enrolled.

G. Cognitive Impairment measurements and scores

1. Mini Mental Status Examination (Tombaugh, 1992)

Maximum score is 30 points. The MMSE is divided in the following 6 categories:

1. Five points for orientation to time
2. Five points for orientation to place
3. Three points for registration of three words
4. Five points for attention & calculation
5. Three points for recall of three words
6. Nine points for language & visual construction

<u>Criterias for Cognitive impairement:</u>

27-30= no cognitive impairment

25-26= mild cognitive impairment, and based on a history & physical assessment (Levitt , et al 1992)

24-5= moderate to severe cognitive impairment (dementia of uncertain etiology, and based on a history and physical).

2. Trail Making test Part B

The Trail Making Test part B, a test of visuomotor tracking, attention, as well as cognitive impairment, was not used as a diagnostic tool since this test has not yet been standardized to account for the very pronounced age effect. (Kennedy, 1981). This test requires the subject to continuously scan the test page to identify and draw a continuous line between letters and numbers in alphabetical and numerical order. Results are reported in seconds, number of errors of omissions and commissions.

H. Dietary Assessment

A short semi-quantitative dietary questionnaire was developed by Dr. Sabaté and the author to assess protein-bound Cbl as well as free Cbl intake. This questionnaire is divided into six sections, namely, 1. vitamin supplementation, 2. eggs and dairy products, 3. meat, poultry and fish products, 4. Cbl fortified meat substitutes/meat analogues, 5. bread and Cbl fortified cereal products, and 6. Cbl fortified liquid food supplements. When the subjects reported supplement use, they were called at a later time to report the name brand, the exact amount of free Cbl per tablet(s), and were asked how often, and for how long they had taken the supplement. During their doctor's visit, the subjects were asked how often they ate a specific serving size of food, either per day, per week, per month. If they consumed a particular food less than once a month, consumption was assumed too be rarely or never, and the number zero was written in the "rarely/none" column (Appendix 4).

Nutritionist IV diet analysis, version 4.1. was used to compare dietary Cbl against the RDA of 2 mcg. For statistical analysis, the values of the cumulative Cbl intake (dietary and supplemental intake), and free Cbl intake (free Cbl from diet and supplements) were used.

I. Statistical Methods

Data were entered and analyzed using SPSS 7.0 for Windows. The following variables had strongly right skewed and/or leptokurtic distributions; age, education, MCV, MMA, Crt, BUN, dietary Cbl intake, free Cbl intake, supplementary Cbl intake, total Cbl intake, MMSE score, neurological score, and Trails B test score and were log-transformed. Parametric statistics were performed with normally distributed variables. If the log transformations did not result in a normally distributed variable, non-parametric statistics were calculated. For most of data analysis, the subjects were either stratified according to whether they were cases or controls, or whether they had a Cbl deficiency or not.

To compare the subjects, the independent *t*-tests were done for the following variables Cbl, log-MMA, tHcys, log-age, log-MCV, Hgb, Hct, log-Crt, log-BUN, and log-total Cbl intake. The Mann-Whitney was used for the MMSE score, years of education, free Cbl intake, and supplemental Cbl intake. The Mann-Whitney test was also used to test whether mean rank of free Cbl intake differed among lacto-ovovegetarians and omnivorous. The independent *t*-test was done specifically to compare controls with a neurological score of zero and controls with decreased vibration sense in terms of the mean log-serum Cbl, the mean log-serum MMA, and the mean serum tHcys.

The two-variable Chi-square test was performed to compare the frequency counts of females and males, omnivores and lacto-ovovegetarians, and among the cases and the controls, and among those subjects who were Cbl deficient or not. The two-variable Chi-square test was done to assess frequency of supplement use, dietary Cbl, free Cbl, among controls and cases by age, and among Cbl deficient and not deficient subjects.

The odds ratio was obtained to assess whether the cases were at greater odds of having a Cbl deficiency. The prevalence of Cbl deficiency was estimated to be 18% for the controls (Lindenbaum, et al. 1993, Pennypacker, et al. 1992) and 38% for the cases. The latter percentage was based on the sample size calculation. The odds ratio was also calculated for the controls with a neurological score of zero and controls with decreased vibration. Several odds ratios were calculated to assess specifically whether controls with a neurological score of zero were a greater odd of having a Cbl deficiency the cases, or those cases with specific isolated neurological deficits and/or diagnosis.

The bivariate association between variables was tested by calculating the Pearson or Spearman coefficients of correlation. To investigate the effect of the group factor (case or control) a multivariate analysis of factors predicting the following normally distributed variables; serum Cbl, log-serum MMA, serum tHcys, log-neurological score, and the log-Trials B test, the stepwise multiple linear regression model was used to select the most strongly related normally predictor variables, and to calculate a multiple unadjusted R^2.

One-way ANOVA was performed to compare serum Cbl, free Cbl intake, log-serum MMA, and the log-serum tHcys means among the different neurological diagnoses. One-way ANOVA with the Bonferroni multiple comparisons procedures was performed to compare the mean log-ages of cases and controls who had normal, reduced or absent platellar reflexes, and normal, reduced or absent vibratory sensation.

CHAPTER THREE

THE RELATIONSHIP BETWEEN COBALAMIN DEFICIENCY AND NEUROLOGICAL DYSFUNCTION IN OLDER ADULTS

Abbreviated Title:
Cobalamin Deficiency and Neurological Dysfunction

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A. ABSTRACT

Objectives: To determine whether older adults with neurological dysfunction are at greater risk of cobalamin deficiency than subjects without neurological dysfunction, and whether those with cobalamin deficiency have a lower intake of free cobalamin.

Design: Case-control study in which clinical and laboratory assessments of consecutive outpatients were surveyed.

Setting: Outpatient clinic at Loma Linda University, California.

Participants: One-hundred and twenty older outpatients aged 60 to 107.

Measurements: Neurological examination, Mini Mental State Examination, Trail Making Test - Part B, dietary Questionnaire, serum determinations of cobalamin, methylmalonic acid, total homocysteine, and folate.

Results: The prevalence of cobalamin deficiency was 16.6% for those controls with completely normal neurological exams, and 25% for the cases (OR=1.7, 95% CI .54, 5.1). Inclusion of controls with reduced vibration sense resulted in an odds ratio of 1.2 (95% CI .52, 2.8). Cbl deficiency was associated with a lack of low free cobalamin, cobalamin obtained from fortified foods and supplements. Subjects who obtained a daily average between 0 and 1 mcg of free cobalamin were most likely to be cobalamin deficient (41.5%), while those who obtained ≥2 mcg were least likely to be cobalamin deficient (13%) (P=.003).

Conclusions: Subjects with neurological dysfunction trended towards a higher odds of cobalamin deficiency when compared to controls with completely normal neurological exams ("true controls), but, because of the consequent decrease in sample size, this trend failed to reach statistical significance. Isolated reduction of vibration sense may not exclusively be a manifestation of normal aging, but it may also be an early neurological sign of cobalamin deficiency. Incorporation of free Cbl in the diet, in the form of supplements or fortified foods, may reduce the risk of cobalamin deficiency in older adults.

B. INTRODUCTION

Persistent cobalamin (Cbl) deficiency can cause a variety of neurological deficits including: myelopathy, peripheral neuropathy, and cognitive impairment (1-7). Many studies report that older adults have a higher prevalence of low serum Cbl levels as well as Cbl deficiency than the rest of the population. The estimated prevalence of Cbl deficiency among older adults is somewhere between the range of 12 to 23% depending on what cut-offs are used as serum methylmalonic acid (MMA) and serum total homocysteine (tHcys) normals (8-11). Myelopathy, peripheral neuropathy and cognitive impairment are prevalent in older adults (4), raising the possibility that Cbl deficiency may be a risk factor for these deficits. To our knowledge, no study has systematically investigated whether older people with neurological signs and/or symptoms of unknown etiology have a higher prevalence of Cbl deficiency than a comparable control group. The Cobalaminergic hypothesis proposes that Cbl deficiency might be etiologically important in a sub-group of patients with Alzheimer's disease (AD), others propose that low serum Cbl is a marker for AD (12,13). Attempts to confirm these relationships have yielded conflicting results (14-21).

Only a few selected studies have attempted to assess the dietary adequacy of Cbl intake in older adults (22-24). None of these studies assessed free Cbl intake. Free Cbl is found in both fortified foods and supplements, while protein-bound Cbl is found exclusively in animal products. Most Americans eat a large variety of animal products and fortified cereals, and it is therefore for generally assumed that older adults get more than enough dietary Cbl in terms of the recommended daily allowance (RDA) of 2 mcg (24,25). However, a national study of 474 participants revealed that 15.4% of the men and 17.3% of the women above the age of 74 obtained below two-thirds of the RDA for Cbl through dietary means, excluding supplements. Between the age of 65 and 74, 8.7% of the men and 19.7% of the women obtained below two-thirds of the RDA for Cbl, excluding supplements. Of those consuming below two-third of the RDA, 36% of the men, and 40% of the women reported taking a vitamin and/or mineral supplement (26).

Dietary needs of Cbl for older adults might be higher than the current RDA of 2 mcg, because of absorptive problems older adults may encounter with aging (27). Furthermore, the current 1989 RDA doesn't distinguish between free and protein-bound Cbl needs (28). Older adults might need oral a supplement or consume foods fortified with Cbl (29). Protein-bound Cbl malabsorption might be quite common among older adults (30,31). However, a recent study failed to confirm that older adults more often malabsorb protein-bound Cbl than middle aged adults (32). The current study addresses this contradiction, by assessing whether Cbl deficient subjects consumed less free Cbl. Specifically, free Cbl (Cbl from fortified foods and supplements) as well as dietary Cbl (protein-bound Cbl and free Cbl from fortified foods) are analyzed.

In sum, the present case-control study examined, first of all, whether subjects with neurological and/or cognitive dysfunction are at greater odds of having Cbl deficiency. Second of all, whether Cbl deficiency occurred more often among those subjects who consume less free Cbl.

C. METHOD

1. Outpatients

One-hundred and twenty patients, aged 60 or older, who visited the Faculty Medical Offices in Loma Linda California between March and October of 1997 were enrolled consecutively in a case-control study. Sixty controls were exclusively obtained from the Department of Internal medicine, while 60 cases were obtained from Internal Medicine, the Alzheimer's clinic, and the Department of Neurology. Cases were enrolled when diagnosed with a neurological and/or cognitive deficit as outlined below. This project was approved by the Institutional Review Board of Loma Linda University Medical Center.

2. Exclusion Criteria

The following types of subjects were excluded. Subjects with diagnosed demyelinating diseases, movement disorders, seizure disorders, neuropathies of known etiology, and neuromuscular diseases. Subjects with unilateral presentation of neurological signs or symptoms. Subjects with known spinal stenosis, spinal compression, and all radiculopathies related to specific structural problems. Subjects receiving chemo or radiation therapy. Subjects with partially treated hypothyroidism. Subjects with multiple myeloma and those who tested positive for monoclonal gammopathy. Subjects with suspected multi-infarcts, previous cerebral vascular accidents, and previous cerebral hemorrhage. Subjects with impaired renal function defined by a creatinine level above 2.0 mg/dl. Suspected diabetics, defined as a fasting blood sugar above 140 gm/dl or a random blood sugar above 200 mg/dl, and diagnosed diabetics. Recently diagnosed Cbl deficient subjects, and those diagnosed with pernicious anemia. Subjects who orally took more than 25 mcg of Cbl per day, or who had recently received Cbl injection therapy.

3. Diagnostic criteria for neurological deficit

Subjects were enrolled as cases when they experienced neurological symptoms or exhibited neurological signs. A neurological scoring system, as previously described by Metz et al. (1997) was used to conduct the neurological testing (33). As a general rule, subjects with a neurological score of 4 or greater were considered to have a neurological deficit, with the exception of those who displayed reduced vibration sense and hyporeflexia as their sole deficit (34). Cases were enrolled with the following diagnosed neurological symptoms and/or signs. Paresthesias in the absence of peripheral neuropathy reported by the patient as a sensation of numbness, burning or tingling. Peripheral neuropathy which was defined as one of the following minimum combinations of neurological deficits with and without paresthesias: reduced vibration and one or more errors in joint position sense; absent vibration sense and absent reflexes; and absent reflexes with or without a positive Romberg. It should be noted that a clinical examination is unable to distinguish between a peripheral neuropathy and a myelopathy, because of similar neurological signs (5). In the presence of paresthesias, the following minimum combinations of neurological deficit were also classified as peripheral neuropathy: absent vibration sense with reduced reflexes ; reduced vibration sense with absent reflexes; reduced vibration sense; and reduced cutaneous sensation. Patients with gait imbalance without any other neurological signs or symptoms were also enrolled in the study.

4. Diagnostic criteria for cognitive deficits

First time outpatients (cases) were either enrolled through the Alzheimer's clinic or through the Neurology Department. Patients received a complete work up which consisted of a history and physical examination, and neuropsychological examinations: a geriatric depression scale, activity of daily living assessment, a Mini Mental State Examination, Trail Making Test, Part B, and a neurological exam. The cases were either categorized as having mild cognitive impairment, or dementia of uncertain etiology with or without peripheral neuropathy. Suspected vascular dementia was ruled out based on the history and physical, and CT scan when available. The patient was diagnosed with dementia of uncertain etiology when the DSM-IV diagnostic dementia criteria were met, and when the MMSE score was 24 points or less (13,35). The patient was diagnosed with mild cognitive impairment when the DSM-IV diagnostic criteria for amnestic syndrome were met, and when the MMSE score was 25 or 26 (13,35).

5. Diagnostic criteria for the controls

A comparable control group was enrolled through the Internal Medicine Clinic who experienced no neurological or cognitive deficits or symptoms.

6. Nutritional assessment of Cbl intake

To assess Cbl intake, a food frequency questionnaire was developed which differentiated between protein-bound Cbl and free Cbl containing foods. Nutritionist IV diet analysis, version 4.1. was used to estimate dietary Cbl intake from Cbl containing foods. Total Cbl intake was defined as dietary and supplemental Cbl intake, free Cbl intake as Cbl from fortified foods and supplements, and supplemental Cbl as Cbl from supplements. Care givers supplied the dietary information of the cognitively impaired cases.

7. Laboratory Methods

The Bio Rad Quantaphase II B_{12} Radioassay was used to assess the patients Cbl level. In this assay the serum is combined with a solution containing dihiothreitol, labeled Cbl, and cyanide. All the various forms of Cbl bound to the binding proteins are converted to cyanocobalamin. Intrinsic factor is added, and the mixture is incubated during which the endogenous and labeled Cbl compete for the binding sites based on their respective concentrations. The supernatant with unbound Cbl is discarded while the Cbl concentration is determined by counting the pellet's reactivity and by using Bio Rad's standard curves (Bio-Rad Diagnostics group). Serum MMA and serum tHcys were measured using capillary gas chromatography-mass spectrometry as previously described in detail (36,37).

8. Definitions of Cobalamin Deficiency

Cbl deficiency was defined as a serum Cbl level of <400 pg/ml, and a serum methylmalonic acid (MMA) of >271 nmol/L (>2 SD above the reference mean) (38), or in conjunction with serum total homocystein (tHcys) of >13.9 umol/L (>2 SD above the reference mean). Cbl deficiency was also defined as a serum tHcys of >13.9 umol/L, in conjunction with a folate level >5 ng/ml (9,30). The 400 pg/ml cut-off was chosen because 97.4% of the Framingham elderly population with elevated serum MMA levels (>3 SD) had serum Cbl levels <400 pg/ml, while 2.6% with elevated serum MMA levels (>3 SD) had serum Cbl levels between 400 and 700 pg/ml (8).

Serum folate levels were checked when patients had elevated serum tHcys levels to determine whether the elevation was due to a Cbl and/or a folate deficiency. A serum tHcys level of >13.9 umol/L without elevated serum MMA levels, in conjunction with a folate level <5 ng/ml was considered to be primarily a folate deficiency with a possible Cbl deficiency.

9. Statistical Methods

Data were entered and analyzed using SPSS 7.0 for Windows. The following variables had strongly right skewed and/or leptokurtic distributions; age, education, MCV, MMA, Crt, BUN, dietary Cbl intake, free Cbl intake, supplementary Cbl intake, total Cbl intake, MMSE score, neurological score, and Trails B test score and were log-transformed. Independent *t*-tests, One Way and Two Way ANOVA, Pearson correlations, and multiple linear regressions were performed with normally distributed variables. If the log transformations did not result in a normally distributed variable, Mann-Whitney, Kruksal-Wallis, one and Two Variable Chi-square, Bionomial test, odds ratios, and Spearman correlations were performed. For most data analysis, the subjects were either stratified according to whether they were cases or controls, or whether they had a Cbl deficiency or not. Statistical significance was set at $P \leq .050$.

D. RESULTS

Outpatients

The 60 cases included 33 women and 27 men aged 60-107 with a mean (±SD) age of 77 ± 8.2. The 60 controls included 30 women and 30 men aged 65-97 with a mean (±SD) age of 77 ± 6.7. Neither their mean ages nor female to male ratio differed significantly (P=.225; P=.583). The mean years of education was 13.7 years for the controls and 13.1 years for the cases (P=.172) (Table 1).

Biochemical and Hematological Findings among the Cases and the Controls

The cases and the controls were very similar in terms of serum Cbl, Blood Urea Nitrogen (BUN), creatinine (Crt), mean cell volume (MCV), hematocrit (Hct), and hemoglobin (Hgb) levels (Table 1). Figure 1. shows the distribution of serum Cbl concentrations among the cases and the controls. One case who was also Cbl deficient, and none of the controls had a serum Cbl level below 100 pg/ml. Seven cases, three of which were Cbl deficient, and two controls who were also Cbl deficient, had serum Cbl levels between 100 and 200 pg/ml. Fourteen controls, seven of which were Cbl deficient, and ten cases, six of which were Cbl deficient, had serum Cbl levels between 201 and 300 pg/ml. Thirteen cases, four of which were Cbl deficient, and 11 controls, four of which were Cbl deficient, had serum Cbl levels between 301 and 400 pg/ml. None of the cases had serum levels above a 1000 pg/ml, while two of the controls did. The weak correlation between age and serum Cbl did not reach significance (R=.17, P=.068).

Biochemical and Hematological Findings among Subjects with and without Elevated Metabolites

Table 2. compares the biochemical and hematological findings of subjects with serum Cbl of <400 pg/ml, with and without elevated metabolites. Subjects with elevated serum tHcys levels were significantly older and included more females than subjects without such elevation (P=.002; P=.004). The mean BUN was higher for the subjects with elevated serum tHcys levels (P=.001). Subjects with elevated serum MMA levels had lower serum Cbl levels than subjects without elevated serum MMA levels (P=.021).

Frequencies of Elevated serum MMA and serum tHcys among Cases and Controls

Table 3. shows the frequencies of elevated serum MMA, serum tHcys levels, and Cbl deficiencies among the cases and controls. Thirteen controls had elevated serum MMA , and a Cbl deficiency. Five controls had elevated serum tHcys levels, four of which had a Cbl deficiency. Four controls had an elevation of both metabolites, while nine controls had only elevated serum MMA levels. One control had just an elevated serum tHcys level with serum folate of less than

5 ng/ml, and was assumed to have a folate deficiency with a possible Cbl deficiency.

Fifteen cases had elevated serum MMA , and a Cbl deficiency. Seven cases had elevated serum tHcys levels, of which four had a Cbl deficiency. Four cases had an elevation of both metabolites, while 11 cases had only elevated serum MMA. Three cases had only elevated serum tHcys levels with serum folate levels of less than 5 ng/ml, and were assumed to have a folate deficiency with a possible Cbl deficiency.

The prevalence of Cbl deficiency was 13 out of 60 (21.7%) for the controls, and 15 out of 60 (25%) for the cases. When looking at the cases by diagnosis, the prevalence of Cbl deficiency was 3 out of 13 (23%) for cases with peripheral neuropathy without paresthesias, 5 out of 17 (29.4%) for cases with peripheral neuropathy with paresthesias, 2 out of 6 (33%) for cases with paresthesias only, 1 out of 4 (25%) for cases with gait imbalance, 1 out 4 (25%) for cases with mild cognitive impairment, and 3 out of 16 (18.8%) for cases with dementia of unknown etiology (P=.666).

Cognitive and Neurological Findings

Mini Mental State Examination Scores

The mean (± SD) MMSE score was 25 ± 7 for the cases and 29 ± 1 for the controls (P=.001) (Table 1). Cases diagnosed with mild cognitive impairment and dementia of unknown the etiology, had mean MMSE scores of 25 ± 1 and

15 ± 7, respectively. The variables age and MMSE score were not significantly correlated (R=-.11; P= 0.253). The mean rank MMSE score did not differ among the different serum Cbl categories (P=.378). There was no correlation between serum Cbl and the MMSE scores (R= -.12; P=.205). MMSE scores were positively correlated with years of education for the all the subjects (R=.37, P=.001), for the cases and the controls separately (R=.34, P=.001; R=.40, P=.001).

Trails B Test Scores

The mean (± SD) Trails B test score of 153 ± 60 sec. for the cases was significantly higher than the mean Trails B test score of 128 ± 60 sec. for the controls (P=.016) (Table 1). But, the mean errors of omission and commission were no different for the cases compared to the controls (P=.496; P=.758). Cases with mild cognitive impairment (n=4) had a mean (±SD) Trails B test score of 175 ±88 sec. Only 3 of the 16 dementia patients were able to complete the Trails B test, and their mean (± SD) score was 220 ± 83 sec.

The variables age and the Trails B test scores were positively correlated for all the subjects (R=.28, P=.005), for the controls (R=.28, P=0.034), but not for the cases (R=.25, P=.098). For the cases, the Trails B test scores were a function of mental status, since the MMSE scores and the Trails B Test scores were inversely correlated (R=-.40, P=.007). Education and the Trails B Test scores were inversely correlated for all the subjects only (R=-.32, P=.001).

Neurological Scores

The mean (±SD) neurological score of 6 ± 4.2 for the cases was significantly higher than the mean score of 1 ± 1.4 of the controls (P<.001) (Table 1). But, those who were Cbl deficient did not score higher on the neurological test than those who were not Cbl deficient (P=.305) (Table 2). Twenty-eight of the 60 controls had a reduced vibration sense, and eight controls hypo platellar reflexia. Absent vibration sense, absent platellar reflexes, joint position errors, and positive Rombergs were the most commonly seen neurological signs among cases diagnosed with peripheral neuropathy without paresthesias.

Age was the only variable significantly correlated with the neurological scores. For all the subjects, the correlation was (R=.34, P<.001), for the controls it was (R=.40, P=.001) , and for the cases it was (R=.32, P=.013). Neither serum Cbl, serum tHcys, nor serum MMA were significantly correlated with the neurological scores (R=-.10, P=.294; R=.10, P=.456; R=.12, P=.383).

For the cases, impaired reflexes and impaired vibration sense were positively correlated with age (R=.35, P<.001; R=.42, P<.001). For the controls, reduced vibration sense was positively correlated with age (R=.33, P=.009). Furthermore, cases with normal vibration and with reduced vibration sense had younger mean ages of 74.6 yr and 77.9 yr, than those cases with absent vibration sense with 85.3 yr. (P=.002; P=.012). Also, cases with normal platellar reflexes had a younger mean age of 76 yr than those with absent platellar reflexes who had a mean age of 84.5 yr (P=.009). Of the controls, those with normal and reduced platellar reflexes had mean ages that did not differ significantly, 76 yr versus 79 yr. (P=.063). But, controls with normal vibration sense had a younger mean age of 74.9 yr than those with reduced vibration sense who had a mean age of 79.3 yr (P=.009).

Table 4. compares the odds ratios of Cbl deficiency for cases to the controls subjects and to "true" control subjects. A "true" control had a neurological score of zero. Even though, none of the results reached statistical significance, due to the small sample sizes, there was a definite trend of increasing odds ratios when comparing "true" controls to controls, and "true" controls to the cases. First, when the controls were compared against the cases, the odds ratio was 1.2 (.52, 2.8). Second, when only those controls with reduced vibration sense were compared to the cases, the odds ratio was 1.0 (.35, 2.8). Third, when controls with reduced vibration sense were compared against the "true" controls, the odds ratio was 1.7 (.46, 6.0). Fourth, when the "true" controls were compared to the cases, the odds ratio was 1.7 (.54, 5.1). Fifth, when the "true" controls were compared to the cases and controls with reduced vibration sense the odds ratio was also 1.7 (.59, 5.1). Because of this trend of increasing odds ratios, odds ratios were calculated for "true" controls and only those cases with either specific neurological diagnoses or deficits who

appeared to have a higher prevalence of Cbl deficiency (not statistically significant) than all the cases combined (Table 5).

Dietary findings

Two cases, one with and one without Cbl deficiency and three controls, one with and two without Cbl deficiency, had a dietary Cbl intake <2/3 of the RDA. However, only that one case with Cbl deficiency and none of the controls had a total Cbl intake (dietary and supplemental Cbl) <2/3 of the RDA. Twenty-six of the cases (43%), and 36 the controls (60%) reported using a daily supplement with Cbl (P=.068). On the other hand, 8 out of 28 (29%) subjects with a Cbl deficiency reported taking a daily supplement with Cbl, compared to 54 out of 92 (59%) subjects without Cbl deficiency (P=.005). The most frequently used Cbl dosages were either 6 mcg, 9 mcg or 25 mcg. Being older, over 74 years of age, compared to being younger, between 60 and 74 years of age, did not effect whether a case or a control consumed either less dietary Cbl, free Cbl or supplemental Cbl (P=.737; P=.975; P=.786).

Fifty of the cases and 50 of the controls were omnivores, while 10 of the controls and 10 of the cases were lacto-ovovegetarian. Of the 20 vegetarians, five had a serum Cbl levels <400 pg/ml, and three out of 20 (15%) had a Cbl deficiency. Of the 100 omnivores, 53 had a serum Cbl level <400 pg/ml, and 25 out 100 (25%) were Cbl deficient (P=.583). Omnivores had a lower mean free

Cbl intake of 5.5 ± 7.1 mcg, than lacto-ovovegetarians who had a mean intake of 9.9 ± 9.8 mcg (P=.009).

The mean (± SD) dietary Cbl intake was 4.8 ± 2.35 mcg for the cases, and was higher than the dietary Cbl intake of the controls who had an mean intake of 3.9 ± 2.0 (P=.046). However, the mean free Cbl intake was significantly higher for the controls, namely, 7.7 ± 8.1 mcg , versus 4.7 ± 7.1 mcg for the cases (P=.004), while the mean total Cbl intake did not differ, 10.7 ± 8.1 versus 9.6 ± 7.5 mcg (P=.473) (Table 1). Subjects with Cbl deficiency were more likely to consume less free Cbl (3.1 ± 6.4 mcg) than those without Cbl deficiency (5.4 ± 7.4, P=.032). Twelve subjects with elevated serum tHcys, of which all but four were also Cbl deficient, were more likely to consume less free Cbl as well as less total Cbl than subjects without elevated serum tHcys levels (2.9 ± 8.1; 4.7 ± 6.7, P=.041) (7.5 ± 10.5 ; 10.4 ± 7.5, P=.001) (Table 2.)

For the purpose of analyzing the relationship between free Cbl intake and Cbl deficiency, free Cbl intake was divided into the following three categories; 0 to 1 mcg, 1.1 to 1.9 mcg, and 2.0 mcg and up. Seventeen out of 41 (41.5%) were Cbl deficient in 0 to 1 mcg category. Two out of 10 (20%) were Cbl deficient in the 1.1 to 1.9 mcg category, and nine out of 69 (13%) were Cbl deficient in the 2.0 mcg and up category (P=.003). Of the 28 Cbl deficient subjects, 17 (61%) subjects obtained between 0 and 1.5 mcg of free Cbl , while 11 (39%) subjects obtained between 2 and 29 mcg of free Cbl (P=.571).

There was a positive correlation between serum Cbl and total Cbl intake, and serum Cbl and free Cbl intake for all the subjects (R=.23, P=.013; R=.28, P=.002), as well as for the controls (R=.38, P=.002; R=.41, P=.001). Total and free Cbl intake were also inversely correlated with serum tHcys for all the subjects (R=-.39, P=.003; R=-.42,P=.001), as well as for the controls (R=-.46, P=.015; R=-.44, P=.021), while just free Cbl intake was inversely correlated with serum tHcys for the cases (R=-.40, P=.025). Finally, free Cbl intake was inversely correlated with serum MMA for all the subjects (R=-.26, P=.047), but not for the cases and controls separately (R=-.86, P=.116; R=-.23, P=.251). Table 6. displays the Pearson or the Spearman correlation coefficients among the significantly related variables for the cases and controls separately, and combined. Of the three Cbl intake variables, only free Cbl intake is shown, since free Cbl intake correlated with more variables the other two.

Multiple-linear Regression Findings

Serum Cobalamin Predictors

Using stepwise multiple linear regression, free Cbl intake predicted 18% of the variation in the controls' serum Cbl levels (P=.002), while serum tHcys and serum MMA predicted 23% and 16% of the variation in the cases' serum Cbl levels (P=.042; P=.014). None of the other variables significantly contributed the linear regression models.

Serum Total Homocysteine Predictors

The variables age and free Cbl intake predicted 50% of the variation in the controls' serum tHcys levels (P=.001), while variables age and total Cbl intake predicted 32% of the variation in the serum tHcys levels for all the subjects (P=.001). BUN and serum Cbl predicted 37% of the variation in the cases' serum tHcys levels (P=.009). None of the other variables significantly contributed to the linear regression models.

Serum Methylmalonic Acid Predictors

The variable serum tHcys predicted 24%, 25%, and 22% of the variation in serum MMA levels for all the subjects, as well as for the cases and the controls separately (P<.001, P=.004, P=.013). None of the other variables significantly contributed to the linear regression models.

Neurological Test Score Predictors

The variables age and the Trials B test score predicted 23% of the variation in the neurological score for all the subjects (P=.008). Age predicted 16% of the variation in the neurological score for the controls (P=.001), and 10% of the variation for the cases (P=.004). None of the other variables significantly contributed to the linear regression models.

Trails B Test Score Predictors

The variable age and education predicted 21% of the variation in the Trails B test scores for all the subjects (P=.001), while the variable age predicted 7% of the variation the controls' Trails B test scores (P=.001). Sixteen percent of the cases' variation of the Trails B test scores were explained by the MMSE scores (P=.007). None of the other variables significantly contributed to the linear regression models.

E. DISCUSSION

The prevalence of Cobalamin deficiency was 16.6% for those controls with completely normal neurological exams, and 25% for the cases (OR=1.7, 95% CI .54, 5.1). Inclusion of controls with reduced vibration sense resulted in a prevalence of 21.7% (OR=1.2, 95% CI .52, 2.8). Though widely accepted as "normal" aging, inclusion of subjects with isolated reduced vibration sense may have contaminated the control group. Twenty eight of the 60 controls had reduced vibration sense. Healton, et al. (1991) reported reduced vibration sense to be the most common neurological sign among Cbl deficient patients (2). It was not clear whether this symptom ever occurred as an initial sign of Cbl deficiency. Reduced vibration sense, in the absence of other signs or symptoms, usually has been considered "normal" for older adults (34,39). In our study, controls with reduced vibration sense appeared to be at increased odds of being Cbl deficient than "true" controls (OR=1.7; 0.46, 6.0). Future studies are needed to determine, whether older adults with reduced vibration sense are at greater odds of Cbl deficiency than a control group without reduced vibration sense.

In the current study, Cbl deficient subjects did not demonstrate a higher level of neurological impairment than Cbl non-deficient subjects. Pennypacker, et al. (1992) did find that subjects with Cbl deficiency demonstrated a higher level of neurological impairment than Cbl non-deficient subjects (11), while Metz, et al. (1996) found no neurological difference among the cases and the controls

(33). However, the latter study stratified the cases and controls based on serum Cbl level, and consequently, eight of the 43 controls had definite Cbl deficiencies. No separate statistical analysis was done to compare the neurological scores of those without and with a true Cbl deficiency.

In our study, age and the Trails B Test score were the only variables which correlated with the neurological score. For older adults, age might be a better predictor of the severity of neurological dysfunction than biochemical Cbl deficiency, since in our study, a severe neurological dysfunction in the oldest subjects often occurred in the absence of a biochemical Cbl deficiency. On the other hand, biochemical Cbl deficiency quite often occurred in the absence of neurological dysfunction. The retrospective nature of this study was a major limitation, and therefore the latter observation could not be further explored, in that asymptomatic Cbl deficient subjects at the time of the study could have developed neurological signs or symptoms in the future.

The correlation between the Trails B Test and the neurological score might indicate that some subjects with neurological dysfunction have slowed visuomotor tracking abilities (40). However, for the cases, the Trails B test score was a function of mental status, not the Trails B score. MMSE scores and the Trails B Test scores were inversely correlated, while age was not significantly correlated with the Trails B Test scores. Like previous studies, education and the Trails B Test scores were inversely correlated for all the subjects (41), while age and the Trails B Test scores were positively correlated (41,42). The mean Trails B test score of the controls was higher than the mean score previously reported of a similar age group (43), while the mean score was comparable to a study in which no differentiation was made between normal cognition and of impaired cognition (41).

Unlike, previous studies, the correlation between serum Cbl and age did not reach significance (8,32), nor with the MMSE scores (13). But, like previous studies, serum Cbl did correlate with supplemental Cbl intake (8,22), but even more strongly with free Cbl intake (free Cbl form fortified foods and Cbl supplements).

This study also examined the relationship between Cbl deficiency, and free Cbl intake among older adults. Previous researchers have usually assumed that dietary Cbl intake gives little insight to the subjects' Cbl status (25), which our study confirmed. However, in terms of free Cbl intake, subjects who obtained between 0 and 1 mcg of daily free Cbl were more likely to be Cbl deficient than those subjects who obtained ≥2 mcg, (41.5% versus 13%). When looking at Cbl supplementation, 20 of 28 (71%) Cbl deficient subjects reported to not take Cbl supplements, compared to 41% of the non-deficient subjects. A previous study reported that 86% of Cbl deficient subjects, and 69% of the non-deficient subjects reported to not take Cbl supplements (8). In our study, Cbl deficiency in older adults is more associated with a lack of free Cbl, than with dietary Cbl intake. Only one of the 120 subjects obtained <2/3 of RDA for total Cbl. Incorporation of free Cbl in the diet, the form of supplements of fortified foods, may reduce cobalamin deficiency in older adults.

Savage et al. 1995 and Healton et al. 1994 reported to have never observed Cbl deficiency induced cognitive impairment in the absence of other neurological deficits (2,5). However, only two of the four cognitively impaired and Cbl deficient subjects had neurological impairment, while one of the cognitively impaired with neurological impairment did not have a Cbl deficiency. The prevalence of elevated metabolites appeared to be lower among the cognitively impaired than among cases with other neurological diagnoses as well as the controls. Joosten, et al. (1997) did observe that subjects with Alzheimer type dementia have higher elevated metabolite levels than a control group (21).

In conclusion, more studies are needed to confirm the trends found in this study that older adults with isolated reduced vibration sense are at greater odds of a Cbl deficiency than a comparable control group with completely normal neurological exam. A future study should also determine whether older adults with specific neurological deficits/diagnosis are at greater odds of Cbl deficiency than a control group with a normal neurological exam. Cbl deficiency in older adults is more associated with a lack of free Cbl, than with dietary Cbl intake. Incorporation of free Cbl in the diet, the form of supplements of fortified foods, may reduce cobalamin deficiency in older adults.

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H. TABLES AND FIGURES

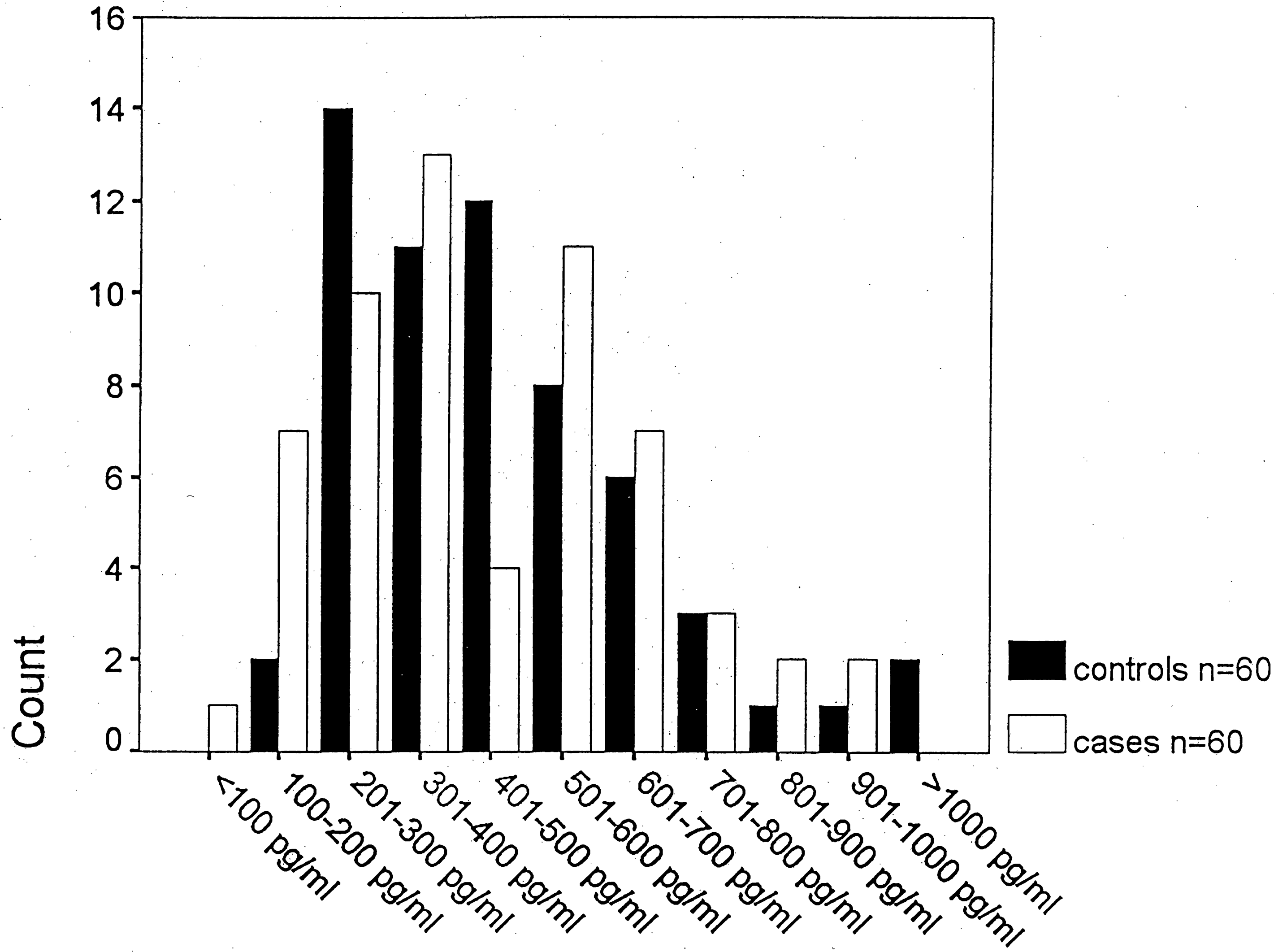

Figure 1. Distribution of Serum Cobalamin Levels among 60 cases with neurological Dysfunction and 60 Controls

Table 1. Characteristics of the Cases and the Controls

Variables	Cases n=60 Mean (SD)[¶]	Range	Controls n=60 Mean (SD)[¶]	Range	P-values (2 tailed)
Sex F/M	33/27	NA**	30/30	NA**	.583[§]
Age	79 (8.24)	60-107	79 (6.7)	65-97	.225
Education (yrs)	13.1 (2.4)	8-20	13.7 (2.4)	7-20	.175[‖]
Cbl* (pg/ml)[†]	440 (221)	74-928	462 (232)	110-1416	.781
BUN* (mg/dl)	19.8 (6.7)	7-35	18.6 (6.0)	7-39	.997
Crt* (mg/dl)	1.0 (0.25)	0.7-1.9	1.0 (0.26)	0.5-1.8	.939
MCV* (*fl*)	88.6 (13.3)	83.5-99.7	91.4 (3.9)	83.1-98.1	.291
Hct *(%)	41.8 (4.3)	31.3-49.4	40.3 (3.9)	30.3-48.3	.051
Hgb* (g/dl)	14.3 (1.5)	10.4-16.8	13.8 (1.4)	10.2-16.8	.066
Diet* Cbl (mcg)	4.8 (2.4)	0.9--10.1	3.9 (2.0)	0.8-9.7	.046
Free* Cbl (mcg)	4.7 (7.1)	0-29.5	7.7 (8.1)	0-27.5	.004[‖]
Suppl* Cbl (mcg)	4.8 (7.1)	0-25	6.7 (8.1)	0-25	.068[‖]
Total* Cbl (mcg)	9.6 (7.5)	1.3-29.7	10.7 (8.1)	1.8-34.7	.473
MMSE* score	25 (7)	5-30	29 (1)	27-30	.001[‖]
Trails B score (sec)	153 (60)	53-300	128 (60)	56-360	.016
Neuro Score	6 (4.2)	0-19	1 (1.4)	0-4	<.00

Footnotes for Table 1.

*Cbl = cobalamin, BUN = blood urea nitrogen, Crt = creatinine, MCV = mean cell volume, Hct = hematocrit, Hgb = hemoglobin, Diet Cbl = dietary Cbl intake only, Free Cbl = free Cbl obtained from fortified food and supplements, Suppl Cbl = free Cbl obtained from supplements only, Total Cbl = free and protein-bound Cbl obtained from diet and supplements, MSSE = Mini Mental State Examination

†To convert Cbl values to pmol/L, divide by 1.355

§ Chi-square test

‖ Mann-Whitney test

¶ SD = standard deviation

** NA = not applicable

Table 2. Characteristics for Cases and Controls Combined with & without Elevated Metabolites

	Subjects with Cbl <400 pg/ml N=58			Subjects with Cbl <400 pg/ml N=58		
	> 2 SD MMA Mean (SD)‡	≤2 SD MMA Mean (SD)‡	P-value (2-tailed)	> 2 SD tHcys Mean (SD)‡	≤2 SD tHcys Mean (SD)‡	P-value (2-tailed)
Sex F/M	15/13	18/12	.300	10/2	17/29	.004§
Age (yrs)	79 (7)	75 (7)	.083	82 (4)	75 (7)	.002
Cbl* (pg/ml)*	252 (89)	302 (70)	.021	257 (82)	283 (83)	.335
MMA* (nmol/L)	402 (153)	193 (47)	NA	421 (227)	261 (107)	NA
tHcys* (umol/L)	11.9 (2.8)	9.9 (3.1)	NA	15.6 (1.7)	9.7 (2.0)	NA
BUN* (mg/dl)	19 (6)	17 (5)	.265	23 (7)	17 (4)	.001
Crt* (mg/dl)	1.1 (.3)	1.0 (.2)	.076	1.2 (.4)	1.0 (.2)	.081
MCV* (*fl*)	90.7 (3.9)	91.1 (4.5)	.753	92.6 (3.9)	90.5 (4.2)	.163
Hct* (%)	48.7 (3.4)	42.7 (4.1)	.545	41.8 (2.7)	42.6 (4.0)	.566
Hgb* (g/dl)	14.4 (1.3)	14.6 (1.5)	.590	14.4 (1.0)	14.5 (1.4)	.713
Diet* Cbl (mcg)	4.9 (2.5)	3.6 (1.8)	.057	3.3 (1.3)	4.5 (2.4)	.187
Free* Cbl (mcg)	3.1 (6.4)	5.4 (7.4)	.032‖	2.9 (8.1)	4.7 (6.7)	.041‖
Suppl* Cbl (mcg)	3.6 (7.3)	4.8 (7.5)	.200‖	4.4 (9.7)	4.2 (6.7)	.204‖
Total* Cbl (mcg)	8.4 (7.7)	10.7 (7.8)	.058	7.5 (10.5)	10.4 (7.5)	.001
MMSE* score	28 (5)	28 (5)	.843‖	27 (7)	28 (5)	.410‖

Table 2. (Continued)

Trails B (sec)	123 (47)	148 (71)	.170	144 (84)	135 (57)	.847
Neuro score	4 (5)	3 (3)	.305	4 (3)	3 (4)	.325

*Cbl = cobalamin, MMA = methylmalonic acid, tHcys = total homocysteine, BUN = blood urea nitrogen, Crt = creatinine, MCV = mean cell volume, Hct = hematocrit, Hgb = hemoglobin Diet Cbl = dietary Cbl intake only, Free Cbl = free Cbl obtained from fortified food and supplements, Suppl Cbl = free Cbl obtained from supplements only, Total Cbl = free and protein-bound Cbl obtained from food and supplements, MSSE = Mini Mental State Examination.

†To convert Cbl values to pmol/L, divide by 1.355.

‡ SD = standard deviation

§ Chi-square test

‖ Mann-Whitney test

Table 3. Frequencies of Elevated Serum Methylmalonic acid and Serum Total Homocysteine among Cases and Controls

> 2 SD MMA* (>271 nmol/L) No. (%)	> 2 SD† tHcys (>13.9 umol/L) No. (%)	Both MMA & tHcys elevated No. (%)	> 2 SD MMA only No. (%)	> 2SD tHcys only No. (%)	Neither metabolites Elevated No. (%)	Definite Cbl‡ Deficiency No. (%)	Possible Cbl§ Deficiency No. (%)
Controls n=60							
13 (21.7%)	5 (8.3)	4 (6.7)	9 (15)	1 (1.7)	47 (78.3)	13 (21.7)‖	1 (1.7)
Cases n=60							
15 (25%)	7 (11.7)	4 (6.7)	11 (18.3)	3 (5)	45 (75)	15 (25)‖	3 (5)

* Normal range for serum methylmalonic acid (MMA) is ±2 SD or 73-271 nmol/L
† Normal range for serum total homocysteine (tHcys) is ±2 SD or 5.1-13.9 umol/L
‡ Definite cobalamin (Cbl) deficiency is defined as a MMA level >271 nmol/L or a MMA level >271 nmol/L & a tHcys level > 13.9 umol/L or a tHcys level >13.9 umol/L and a serum folate ≥ 5 ng/ml.
§ Possible Cbl deficiency is defined as a tHcys level >13.9 umol/L with a serum MMA level < 272 nmol/L and a serum folate level of < 5ng/L
‖ Odds Ratio = 1.21 (95% confidence interval = .52, 2.8)

Table 4. Odds Ratios of Cobalamin Deficiency for Cases to Control Subjects or "True" *Control Subjects

CONTROLS	n	CASES	n	Odds Ratio	95% Confidence Interval
Controls	60	Cases	60	1.2	(.52, 2.8)
Controls with Reduced Vibration	28	Cases	60	1.0	(.35, 2.8)
"True"* Controls	30	Controls with reduced Vibration sense	28	1.7	(.46, 6.0)
"True"* Controls	30	Cases	60	1.7	(.54, 5.1)
"True"* Controls	30	Cases & controls with reduced vibration	88	1.7	(.59, 5.1)

* "True control " are defined as controls with a neurological score of zero

Table 5. Odds Ratios of Cobalamin Deficiency for Subjects with Specific Neurological Diagnoses/Deficits to True* Controls

TRUE* CONTROLS	n	CASES WITH SPECIFIC NEUROLOGICAL DIAGNOSES	n	Odds Ratio	95% Confidence Interval
Controls	30	Cases without Cognitive Impairment & Dementia	40	1.9	(.58, 6.0)
Controls	30	Peripheral neuropathy with paresthesias	17	2.1	(.51, 8.6)
Controls	30	Peripheral neuropathy with Paresthesia & paresthesia only	23	2.2	(.59, 8.1)
TRUE * CONTROLS	**n**	**CASES WITH SPECIFIC NEUROLOGICAL DEFICITS**	**n**	**Odds Ratio**	**95% Confidence Interval**
Controls	30	Reduced vibration sense‡	62	1.7	(.57, 5.3)
Controls	30	Absent vibration sense	13	2.2	(.49, 10.2)
Controls	30	Absent reflexes	13	2.2	(.49, 10.2)
Controls	30	Reduced cutaneous sensation†	22	2.3	(.63, 8.7)
Controls	30	Paresthesias only	6	2.5	(.36, 17.6)
Controls	30	Positive Romberg	10	3.3	(.68, 16.3)

* True Controls are defined as controls with a neurological score of zero
† For this odds ratio calculation, one control with reduced cutaneous sensation is also considered a case
‡ For this odds ratio calculation, 28 controls with reduced vibration sense are also considered cases

Table 6. Pearson or Spearman Correlation Coefficients among significantly Related Variables

CASES									
Variables‡	S-MMA	S-tHcys	Neuro	Age	Reflexia§	Vibration§	Romberg§	MMSE§	Trails B
S-Cbl	-.40*	-.37*							
S-tHcys	.50†								
F-Cbl		-.40*							
Neuroscore				.32*					
Age	.39*	.43*	.32*		.35*	.42*	.28*		
BUN		.39*		.29*					
Education§								.34†	
MMSE									-.40†

CONTROLS									
Variables‡	S-MMA	S-tHcys	Neuro	F-Cbl		Vibration§		MMSE§	Trails B
S-Cbl				.44†					
t-Hcys	-.47*			-.46*					
Age		.45*	.40†			.33*			.28†
Education								.40†	
BUN	.42*								
Crt	.41*								

Tables 6. (Continued)

CASES AND CONTROLS Variables‡	S-MMA	F-Cbl	Age	BUN	Crt	Neuro	Trails B	Education§
S-Cbl		.28*						
tHcys	.49†	-.42†	.43†	.37†	.33*			
s-MMA	.49†	-.26*	.31*	.28*	.34*			
Neuro			.34†				.32†	
Vibration§			.37†					
Reflexia§			.33†					
Trails B			.28†			.32†		-.32*
MMSE§							-.30†	.37

* Correlation is significant at .05 level (2-tailed)
† Correlation is significant at .01 level (2-tailed)
‡ S-Cbl = serum cobalamin, S-MMA = serum methylmalonic acid, S-tHcys = serum total homocysteine, Neuro = neurological score, MMSE = Mini Mental State Examination, Trails B= trails B test score, BUN = blood urea nitrogen, Crt = creatinine, Hct = hematocrit , Hgb = hemoglobin, F-Cbl = free Cbl from fortified foods and supplements.
§ Spearman correlation coefficients were calculated.

CHAPTER FOUR

APPENDICES AND REFERENCES

APPENDIX 1:

SAMPLE SIZE CALCULATIONS

Unmatched Case-Control study
Comparison of Ill and not Ill
Sample sizes for 18% Exposure in not ill group

Conf.	Power	Not ill :ill	Exposure in ill	Odds Ratio	Sample Size not ill	ill	Total
95%	80%	1:1	44.87%	5.0	39	39	79
95%	80%	1:1	42.28%	4.5	45	45	90
95%	80%	1.1	39.44%	4.0	54	54	108
95%	80%	1.1	36.30%	3.5	67	67	134
95%	80%	1.1	32.81%	3.0	89	89	178
95%	80%	1.1	28.93%	2.5	131	131	262
95%	80%	1.1	24.56%	2.0	236	236	472
95%	80%	1.1	19.63%	1.5	728	728	1456

APPENDIX 2:

CONSENT FORMS FOR CONTROLS AND CASES

Loma Linda University

School of Public Health

Loma Linda, California 92350
(909) 824-4546
FAX: (909) 824-4087

CONSENT FORM FOR CONTROLS

VITAMIN B12 DEFICIENCY AND NEUROLOGIC DEFICIT IN OLDER ADULTS

Dr. James Larson, Dr. Daniel Giang, and Marion Bachra, a graduate student from Loma Linda University are conducting a study in which they are comparing the prevalence of vitamin B12 deficiency in older adults with and without neurological deficits. We invite you to join our study. When you agree to join the study, you will be part of the control group, that is, you and others will serve as a reference group who experience no neurological complaints or symptoms. The scientific literature suggest that vitamin B12 deficiency can lead to neurological problems. Some studies have shown that older adults' food intake, as well as the ability to absorb vitamin B12 decrease with age. This study is attempting to quantify the current intake levels of vitamin B12.

As a participant in this study, you are being asked to answer a food frequency questionnaire. Some blood will be drawn to assess your vitamin B12 status. You may receive the lab results. To test your neurological status, the doctor will test your reflexes.

The only risks of the study are those associated with the usual drawing of blood. These risks are the following; bruising due to needle puncture, and slight discomfort after the blood is drawn. There is no cost to you or any third party payor for any part of the study. This study may or may not benefit you, but it is hoped that the obtained information will benefit humanity by increasing the knowledge base for future research of vitamin B12 deficiency and its complication. Further, this study might indirectly lead to potential changes in standard medical practice. Under current medical practice, testing your vitamin B12 level is not medically necessary, since you have no neurological complaints.

Your participation in this study is completely voluntary. You have the freedom to withdraw at any time without compromise of your medical care.

The information you provide will be held in complete confidence. Your identity will not be disclosed in any published document.

"I________________________ have read the contents of the consent form and have listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors responsibilities. I may call Georgia Hodgkin, Ed.D., R.D. a faculty member at the LLU Department of Nutrition and Dietetics at (909)-824-4593 during routine office hours if I have additional questions or concerns. I have been given a copy of this consent form."

"I ________________________ have been told that if I wish to contact an impartial third party not associated with this study regarding any complaint I may have about the study, I may contact Jean Fahnkhanel, Patient Representative, Loma Linda University Medical Center, Loma Linda, CA 92354, Phone (909)-824-4647 for information and assistance."

I have received a copy of California Experimental Subject's Bill of Rights and have these rights explained to me.

a.________________________ ______________

Signature of Subject Date

____

Witness

b. Subject is unable to sign because________________________________

________________________ ______________ ____________

Authorized signature Relationship Date

c. "I have reviewed the contents of the consent form with the person signing above. I have explained the potential risks and benefits of the study."

__________________________ ________________ ______

Signature of Investigator Phone Date

LOMA · LINDA · UNIVERSITY
TO MAKE MAN WHOLE
1905

LOMA LINDA UNIVERSITY

School of Public Health

Loma Linda, California 92350
(909) 824-4546
FAX: (909) 824-4087

CONSENT FORM FOR CASES

VITAMIN B12 DEFICIENCY AND NEUROLOGIC DEFICIT IN OLDER ADULTS

Dr. James Larsen, Dr. Daniel Giang, and Marion Bachra, a Loma Linda University graduate student, with the help of other Faculty Medical Offices' doctors, are conducting a study. In this study they are comparing the prevalence of vitamin B12 deficiency in older adults with and without neurological deficits. Your doctor has ordered a vitamin B12 test for you, because you have a neurological deficit. We therefore, invite you to join our study. The scientific literature suggest that vitamin B12 deficiency can lead to neurological problems. Some studies have shown that older adults' food intake, as well as the ability to absorb vitamin B12 decrease with age. This study is also attempting to quantify your current dietary vitamin B12 levels.

As a participant in this study, you are being asked to answer a dietary questionnaire. As part of regular medical procedures, your blood will be drawn to assess your vitamin B12 status. You will receive the lab results. Your doctor has neurologically assessed you by performing some simple tests.

No discomfort or risks are anticipated. This study may or may not benefit you, but it is hoped that the obtained information will benefit humanity by increasing the knowledge base for future nutritional counseling and for research of vitamin B12 deficiency and its implications. This study might indirectly lead to potential changes in standard medical practice.

Your participation in this study is completely voluntary. You have the freedom to withdraw at any time without compromise of your medical care.

The information you provide will be held in complete confidence. Your identity will not be disclosed in any published document.

"I ______________________ have read the contents of the consent form and have listened to the verbal explanation given by the investigator. My questions concerning this study have been answered to my satisfaction. I hereby give voluntary consent to participate in this study. Signing this consent document does not waive my rights nor does it release the investigators, institution or sponsors responsibilities. I may call Georgia Hodgkin, Ed.D., R.D. a faculty member at the LLU Department of Nutrition and Dietetics at (909)-824-4593 during routine office hours if I have additional questions or concerns. I have been given a copy of this consent form."

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b. Subject is unable to sign because______________________________

______________________ ______________________ ________

Authorized signature Relationship Date

c. "I have reviewed the contents of the consent form with the person signing above. I have explained the potential risks and benefits of the study."

______________________ ______________________ ________

Signature of Investigator Phone Date

APPENDIX 3:

DATA COLLECTION FORMS

NEUROLOGICAL SCORING SHEET

PATIENT NAME & MEDICAL RECORD #__________________________________

PARESTHESIA Yes _____ NO_____

NEUROLOGIC TESTS	SCORES	SCORES	SCORES	SCORES	SCORES
VIBRATION SENSE	same=0	reduced=2	absent=4		
JOINT POSITION SENSE	0error=0	1error=1	2error=2	3error=3	4error=4 or 5error=5
CUTANEOUS SENSATION	same=0	reduced=2	absent=4		
PLANTAR REFLEXIA	normal reflex=0	detect. hypo-reflexia=2	detect. hyper-reflexia =2	absent reflex=4	hyper-reflexia with clonus=4
PLANTAR RESPONSE	up=2	sideways =0	down=0		
ROMBERGISM	absent=0	present=4			
MAX PTS 23 TOTAL POINTS					

MINI MENTAL STATUS EXAM

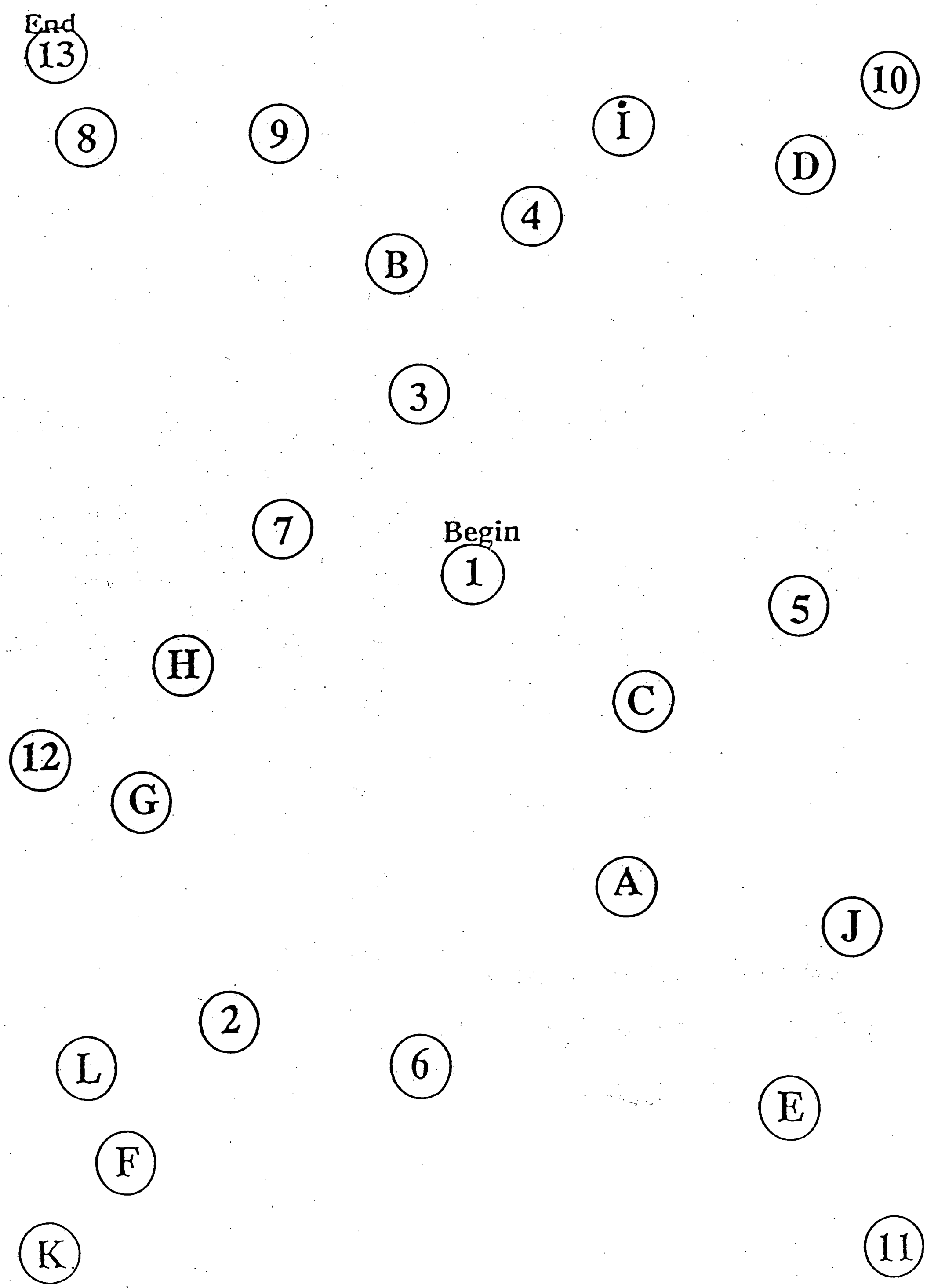
ORIENTATION TO TIME 5PTS MAX	
ORIENTATION TO PLACE 5PTS MAX	
REGISTRATION 3 WORDS 3PTS MAX	
ATTENTION & CALC. 5 PTS MAX	
RECALL OF 3 WORDS 3 PTS MAX	
LANGUAGE 8 PTS MAX	
VISUAL CONSTRUCTION 1 PT MAX	
(MAX. PTS 30) TOTAL POINTS	

The Mini Mental State Exam (MMSE)

Maximum Score	Score	
		ORIENTATION
5	( )	1. What is the (year) (season) (date) (day) (month)?
5	( )	2. Where are we: (state) (county) (hospital) (floor)?
3	( )	3. Name 3 objects: 1 second to say each. Then ask the patient all 3 after you have said them. Give 1 point for each correct. Then repeat them until s/he learns all 3. Count trial and record ____ Trials
		ATTENTION AND CALCULATION
5	( )	4. Serial 7s. 1 point for each correct. Stop after 5 answers. Alternatively, spell "world" backwards, if s/he cannot subtract.
		RECALL
3	( )	5. Ask for 3 objects repeated above. Give 1 point for each correct.
		LANGUAGE
9	( )	6. Name a pencil and watch (2 points) 7. Repeat the following "No ifs, ands or buts" (1 point) 8. Follow a 3-stage command: "Take a paper in your right hand, fold it in half and put it on the floor." (3 points) 9. Read and obey the following: "Close your eyes" (1 point) 10. Write a sentence. (1 point) 11. Copy design. (1 point)

Total (   )

Trail Making Test - Part B



PATIENT PROFILE SHEET
Name______________________LL#____________Religion_________
Sex________ Age_____Education______Ht______ Wt______ BMI______
Marital Status_______________ Living situation____________
LAB VALUES
Serum B12______ Serum MMA______ Serum HCYS______
MCV_____ Hct ______ Hgb_____ Crt_____ BUN_____
Serum Gastrin____ Alb _____ Chol_____ Other________________
DIETARY HISTORY
FFQ B12___________ B12 Supplement___________ Free B12_____
Vegetarian No___ Yes____ Type_________________
MEDICAL HISTORY
Diagnosis/ICD-9 codes______________________________________
Past Medical History_______________________________________
Alcohol use >280 gm/week yes___ no__
Gastrectomy partial ___ total ____ none____ Ileal resection yes___ no___ date______
Atrophic Gastritis (AB against parietal cell, IF, elevated yes____ no_____ gastrin, abnormal Schilling test)
Hematological malignancy no___ yes ____ type_____________
Celiacs disease (Tropical Sprue) yes____ no____
Cerebral vascular accident yes___ when_________ no__
Multiple myeloma (B12) yes___ when ___ no____
Other conditions____
MEDICATIONS
Antibiotics use: currently______ date last used _________
Type__________________
Antisecretory, Antiulcer
Histamine H2 Antagonists Yes____ No_____
Omeprazole (Prisosec)_____ Cimetidine (Tagamet) _____Other______________
Antacids Yes___ No_____
Type__________________
Corticosteroids (folate) Yes_____ No_____Type__________________
Laxatives Yes____ No_____Type _________________
Cytotoxic drugs Yes____ No______Type______________________
Sedative, Sleep Aid
Chloral Hydrate_____ Yes ____ No_____
Antihypertensive,Diuretic, K sparing
Spironolactone (Aldactone) Side effect: Ataxia, Confusion Yes____ No____
Bile acid Sequestrant (folate)
Colestyramine Yes___ No____
Dilatin (B12) yes___ no___ Diphenylhydantoin (B12) yes___ no___
CURRENT MEDS____

CHECK LIST FOR VITAMIN B12 STUDY

PATIENT NAME____

LL MEDICAL RECORDS #___________________________________

FIRST VISIT

PATIENT AND PHYSICIAN BOTH SIGNED CONSENT FORM YES______

MINI MENTAL STATUS EXAMINATION SCORE______

TRAILS B COGNITIVE EXAM TIME ______
ERRORS OF OMISSION_______ERRORS OF COMMISSION________

PARESTHESIA YES____ NO____

NEUROLOGICAL TEST SCORE______

PATIENT FILLED OUT FOOD QUESTIONNAIRE YES_____ NO______

ORDERED SERUM B12 ON SPECIAL LAB SLIP YES____

SEND PATIENT WITH SPECIAL LAB SLIP TO THE FM0 YES______

SEND CONSENT FORM, NEUROLOGICAL TEST, FOOD QUESTIONNAIRE TO
MARION BACHRA
LLU/SPH BOX 260 YES____

SECOND VISIT

SERUM B12 RESULT (PG/ML)______

ORDERED SERUM MMA/HCYS ON A REGULAR LAB SLIP
IF SERUM B12 IS <350PG/ML (CASES ONLY) YES______

SERUM MMA RESULT (NMOL/L)______

SERUM HCYS RESULT (UMOL/L_______

SEND COPY OF CHECK LIST TO MARION BACHRA

APPENDIX 4:

DIETARY QUESTIONNAIRE

LOMA LINDA UNIVERSITY
DEPARTMENT OF NUTRITION

DIETARY QUESTIONNAIRE
(Vitamin B_{12} Study)

Chart #______________________ **Date**___________

Name _______________________ **Received by**__________________

Dear Participant,

Thank you very much for your willingness to participate in this study. In order to get an idea of your dietary habits, you will be filling out this questionnaire. First, we will ask you about your vitamin supplement usage, then we will ask you about your food consumption.

If you take any vitamin supplements, orally or by injection, please indicate in the table below, the brand name (oral supplements only), how often you take them, and the amount of vitamin B_{12} in micrograms.

TYPES OF SUPPLEMENTS	BRAND NAME	HOW OFTEN	AMOUNT OF VITAMIN B_{12}
MULTIVITAMIN			Micrograms
VITAMIN B COMPLEX OR VITAMIN B_{12} PILLS			Micrograms
SUBLINGUAL B_{12} CAPSULES			Micrograms
VITAMIN B_{12} INJECTIONS			Micrograms

If you do not know the amount of vitamin B_{12} you take, could you please check at home the label on your vitamin bottle. Vitamin B_{12} may be listed as cyanocobalamin on the label. Please write down your phone number. Area code _____ telephone number____________
When is the best time to call you?

Please check off (✓), mornings ☐ afternoons ☐ evenings☐

Please turn to the back of this page

EXAMPLE OF HOW TO FILL OUT THE QUESTIONNAIRE

FOR EXAMPLE, if you drink two cups of milk once a day, never eat ice cream or frozen yogurt, eat one cup of flavored yogurt about once a week, and eat about three cups of custard three times a month, you would write that down as shown in the example below.

THIS IS AN EXAMPLE ONLY!

FOOD/NAME/ DESCRIPTION	SERVING SIZE	RARELY/ NONE	DAY	WEEK	MONTH
Milk of any type	1 cup				
Ice cream, frozen yogurt	1 cup				
Plain or flavored yogurt	1 cup				
Pudding or custard	1 cup				

EXAMPLE

PLEASE FILL OUT THE REST OF THIS QUESTIONNAIRE

For each food listed in the questionnaire, indicate the number of times, on average, you have consumed these food during the past year. Please fill it out as accurately as you can.

1. How many times do you eat the following EGGS AND DAIRY PRODUCTS, plain or as part of an entree. Please write down the number of times, if any, you eat these items on either a daily, weekly, or monthly basis.

FOOD/NAME/ DESCRIPTION	SERVING SIZE	RARELY/ NONE	DAY	WEEK	MONTH
Milk of any type	1 cup				
Ice cream, frozen yogurt	1 cup				
Plain or flavored yogurt	1 cup				
Pudding or custard	1 cup				
Cottage cheese	½ cup				
Hard cheese	1 ounce				
Cream cheese	1 ounce				
Sour cream	2 tablespoons				
Whole egg	1 each				
Egg substitute	1/4 cup				

2. How many times do you eat the following MEAT, POULTRY, AND FISH products, plain or as part of an entree.

FOOD/ NAME/ DESCRIPTION	SERVING SIZE	RARELY/ NONE	DAY	WEEK	MONTH
Beef, lamb, or pork	3 ounces				
Chicken, turkey, or duck	3 ounces				
Sausages or hotdogs	1 each				
Luncheon meat	1 slice				
Bacon	2 strips				
Organ meat	3 ounces				
Shellfish	3 ounces				
Fish	3 ounces				

3. You might eat MEAT SUBSTITUTES or MEAT ANALOGUES, if you don't, please check off (✓) this box ☐. If you do, please write down, as shown in the examples below, the brand names and types of the meat substitutes you use most of the time.

FOOD/ NAME/ DESCRIPTION	SERVING SIZE	DAY	WEEK	MONTH
For example: Worthington, Prosage sausage	1 each		3	
Worthington, Smoked turkey	1 slice	1		

Please turn to the back of this page

4. How many times do you eat the following CEREAL OR BREAD PRODUCTS.

FOOD/ NAME/ DESCRIPTION	SERVING SIZE	RARELY/ NONE	DAY	WEEK	MONTH
Pancakes or waffles	1 each				
Muffins	1 each				
French toast	1 each				
Please write below the brand names and types of hot or cold cereal(s) or granolas you use					
For example: Kellogs, Raisinbran	1 cup			3	
Post, Golden Crisp	1 cup			2	

5. You might drink LIQUID FOOD SUPPLEMENTS, like Sustacal or Ensure.
If you don't, please check off (✓) this box ☐. I you do, please write down as shown in the example below the brand names of the supplements you use.

FOOD/ NAME/ DESCRIPTION	SERVING SIZE	DAY	WEEK	MONTH
For example: Ensure Light	1 can	1		

Thank you very much for taking the time to fill out this questionnaire!

LLU Departement of Nutrition, February 1997. Developed by Marion Bachra and Joan Sabaté

VII. APPENDIX 5:

ADDITIONAL STUDY RESULTS

APPENDIX 1. Characteristics of Controls and Cases Categorized by Clinical Diagnosis

Diagnosis Variable	Control n=60 Mean (SD)‡	Peripheral neuropathy without paresthesia n=13 Mean (SD)‡	Peripheral neuropathy with paresthesia n=17 Mean (SD)‡	Paresthesia Only n=6 Mean (SD)‡	Gait Imbalance n=4 Mean (SD)‡	Mild Cognitive Impairment n=4 Mean (SD)‡	Dementia w/ and w/o peripheral neuropathy n=16 Mean (SD)‡
Age (yr)	79 (6)	82 (12)	76 (8)	77 (5)	82 (9.3)	81 (4)	79 (5)
Sex F/M	30/30	7/6	5/12	4/2	3/1	3/1	11/5
Educ. (yrs)	13.7 (2.4)	13.9 (2.6)	13.4 (2.1)	15 (2.8)	12.3 (2.6)	12 (3.3)	12 (2.0)
Cbl*(pg/ml)†	462 (232)	422 (214)	417 (221)	312 (139)	444 (269)	415 (221)	533 (196)
BUN* (mg/dl)	18.6 (6.0)	21.5 (9.6)	17.9 (4.5)	19.3 (4.8)	17.3 (2.5)	18.1 (8.9)	18.0 (6.4)
Crt* (mg/dl)	1.0 (0.3)	1.2 (.4)	1.0 (.2)	.98 (.19)	.97 (.09)	1.0 (.16)	1.0 (.20)
MCV* (*fl*)	91.4 (3.9)	90.8 (4.0)	90.8 (4.5)	92.1 (4.7)	92.6 (6.2)	87.6 (2.7)	88 (3.4)
Hct* (%)	40.3 (3.9)	42.8 (4.1)	43.5 (3.7)	43.5 (3.4)	43.4 (3.5)	36.5 (4.8)	39.6 (4.1)
Hgb* (g/dl)	13.8 (1.4)	14.6 (1.4)	14.9 (1.1)	15.0 (1.2)	15.2 (1.4)	12.3 (1.7)	13.4 (1.4)
Diet* Cbl (mcg)	3.9 (2.0)	5.4 (2.1)	5.2 (2.9)	3.3 (2.3)	4.2 (1.1)	4.3 (2.5)	4.7 (2.2)

APPENDIX 1 (continued)

Diagnosis Variable	Control n=60 Mean (SD)‡	Peripheral neuropathy without paresthesia n=13 Mean (SD)‡	Peripheral neuropathy with paresthesia n=17 Mean (SD)‡	Paresthesia n=6 Mean (SD)‡	Gait Imbalance n=4 Mean (SD)‡	Mild Cognitive Impairment n=4 Mean (SD)‡	Dementia w/ and w/o peripheral neuropathy n=16 Mean (SD)‡
Free* Cbl (mcg)	7.7 (8.1)	5.9 (8.1)	5.5 (8..8)	5.6 (10.4)	1.9 (2.9)	2.6 (2.7)	3.7 (4.4)
Suppl* Cbl (mcg)	6.7 (8.1)	4.5 (7.7)	5.3 (8.0)	9.3 (12.4)	1.5 (3.0)	3.0 (3.5)	4.2 (7.1)
MMSE* score	29 (1)	29 (1)	29 (1)	29 (1)	29 (1)	25 (1)	14 (7)
Trails B score (sec.)	128 (60)	150 (49)	141 (52)	115 (46)	196 (43)	175 (88)	220 (83)
Neuro Score	1 (1.4)	9 (4.7)	7 (3.9)	2 (.82)	5 (1.0)	4 (1.6)	3 (3)

*Cbl = cobalamin, BUN = blood urea nitrogen, Crt = creatinine, MCV = mean cell volume, Hct = hematocrit, Hgb = hemoglobin
Diet Cbl = dietary Cbl intake only, Free Cbl = free Cbl obtained from fortified food and supplements, Suppl Cbl = free Cbl obtained from supplements only, MSSE = Mini Mental State Examination.

†To convert Cbl values to pmol/L, divide by 1.355.

‡SD = standard deviation

APPENDIX 2. Frequencies of Elevated Serum Methylmalonic acid and Serum Total Homocysteine among Controls and Cases categorized by Clinical Diagnosis

Diagnostic Criteria	Controls n=60 No. (%)	Peripheral neuropathy without paresthesia n=13 No. (%)	Peripheral neuropathy with paresthesia n=17 No. (%)	Paresthesia Only n=6 No. (%)	Gait Imbalance n=4 No. (%)	Mild Cognitive Impairment n=4 No. (%)	Dementia w/ and w/o peripheral neuropathy n=16 No. (%)
> 2 SD MMA*	13 (21.7)	3 (23)	5 (29.4)	2 (33)	1 (25)	1 (25)	3 (18.8)
> 2 SD tHcys†	5 (8.3)	2 (15.4)	2 (11.8)	2 (33)	0 (0)	0 (0)	1 (6.3)
Both MMA & tHcys elevated	4 (6.7)	2 (5.4)	1 (5.9)	1 (16.7)	0 (0)	0 (0)	0 (0)
MMA only	9 (15)	1 (7.7)	4 (23.5)	1 (16.7)	1 (25)	1 (25)	3 (18.8)
tHcys only	1 (1.7)	0 (0)	1 (5.9)	1 (16.7)	0 (0)	0 (0)	1 (6.3)
None elevated	47 (78.3)	10 (77)	11 (64.7)	3 (50)	3 (75)	3 (75)	12 (75)
Cbl deficiency‡	13 (21.7)	3 (23)	5 (29.4)	2 (33)	1 (25)	1 (25)	3 (18.8)
Possible Cbl Deficiency§	1 (1.7)	0 (0)	1 (5.9)	1 (16.7)	0 (0)	0 (0)	1 (6.3)

* Normal range for serum methylmalonic acid (MMA) is ± 2 SD or 73-271 nmol/L

† Normal range for serum total homocysteine (tHcys) is ± 2 SD or 5.1-13.9 umol/L

‡ Cobalamin (Cbl) deficiency is defined as a MMA level >271 nmol/L or a MMA level >271 nmol/L & a tHcys level > 13.9 umol/L or a tHcys level >13.9 umol/L and a serum folate ≥ 5 ng/ml.

§ Possible Cbl deficiency is defined as a tHcys level >13.9 umol/L with a MMA level <272 nmol/L and a serum folate level of < 5ng/L

APPENDIX 3. Frequencies of Neurological Signs and Symptoms among Controls and Cases Categorized by Clinical Diagnosis

Diagnosis Neurological Signs	Controls n=60 No. (%)	Peripheral neuropathy w/o paresthesia n=13 No. (%)	Peripheral neuropathy with paresthesia n=17 No. (%)	Paresthesia Only n=6 No. (%)	Gait Imbalance n=4 No. (%)	Mild Cognitive Impairment n=4 No. (%)	Dementia w/ and w/o peripheral neuropathy N=16 No. (%)
Paresthesias							
Absent	60 (100)	13 (100)	0 (0)	0 (0)	3 (75)	4 (100)	15 (93.7)
Present	0 (0)	0 (0)	17 (100)	6 (100)	1 (25)	0 (0)	1 (6.3)
Vibration Sense							
Normal	32 (53)	1 (7.7)	3 (17.7)	3 (50)	1 (25)	0 (0)	5 (31.2)
Reduced	28(46.7)	4 (30.8)	11 (64.7)	3 (50)	2 (50)	4 (10)	10 (62.5)
Absent	0 (0)	8 (61.5)	3 (17.6)	0 (0)	1 (25)	0 (0)	1 (6.3)
Joint position							
0 errors	60 (100)	8 (61.5)	13 (76.4)	6 (100)	4 (100)	4 (100)	13 (81.1)
1 or more errors	0 (0)	5 (38.5)	4 (23.6)	0 (0)	0 (100)	0 (0)	2 (12.6)

APPENDIX 3 (continued)

Diagnosis **Neurological Signs**	**Controls** **n=60** **No. (%)**	**Peripheral neuropathy without paresthesia** **n=13** **No. (%)**	**Peripheral neuropathy with paresthesia** **n=17** **No. (%)**	**Paresthesia Only** **n=6** **No. (%)**	**Gait imbalance** **n=4** **No. (%)**	**Mild Cognitive Impairment** **n=4** **No. (%)**	**Dementia w/ and w/o peripheral neuropathy** **n=16** **No. (%)**
Cutaneous Sense							
Normal	59(98.3)	10 (76.9)	6 (35.3)	4 (66.7)	3 (75)	3 (75)	13 (81.3)
Reduced	1 (1.7)	3 (23.1)	11 (64.7)	2 (33.3)	1 (25)	1 (25)	3 (18.7)
Absent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Platellar Reflex							
Normal	52(86.7)	1 (7.)	2 (11.8)	4 (66.6)	2 (50)	1 (25)	10 (62.5)
Hypo	8 (13.3)	5 (38.5)	10 (58.8)	1 (16.7)	2 (50)	3 (0)	5 (31.3)
Hyper	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)	0 (0)	0 (0)
Absent	0 (0)	7(53.8)	5 (29.4)	0 (0)	0 (0)	0 (0)	1 (6.2)
Rhomberg							
Absent	60 (100)	6 (46.2)	15 (88.2)	6 (100)	3 (75)	4 (100)	16 (100)
Present	0 (0)	7 (53.8)	2 (11.8)	0 (0)	1 (25)	0 (0)	0 (0)

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