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Efficacy of Methylcobalamin to Normalise Elevated Homocysteine of Vitamin B12 Deficient Vegetarians: A Double Blind Placebo Control Study

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ABSTRACT

Vegetarians are known to be deficient in vitamin B12, due to a lack of dietary animal products, which can elevate plasma total homocysteine (tHcy). Elevated total tHcy can render vegetarians susceptible to cardiovascular disease (CVD). There are a limited number of published studies in relation to the efficacy of methylcobalamin to normalise plasma tHcy of vitamin B12 deficient vegetarians. The primary objective of the present study was to explore the relationship between supplementary oral methylcobalamin and levels of tHcy of vitamin B12 deficient vegetarians; to reduce the risk of developing primary CVD. A randomised double blind placebo controlled pilot study was conducted to monitor and analyse baseline and post treatment levels of plasma tHcy, 49 volunteer vegetarians were recruited to participate in this study. Statistical analysis employing SPSS software indicated that methylcobalamin reduced mean baseline plasma tHcy of 15.5 $\mu\text{mol}\cdot\text{L}^{-1}$ (n=39) to a mean plasma tHcy level of 8.4 $\mu\text{mol}\cdot\text{L}^{-1}$ ($P < 0.001$). In a second group that contained details of ten withdrawn participants, which was conducted on an 'Intention to Treat' (ITT) basis, indicated that methylcobalamin was shown to be reduced from a mean baseline plasma tHcy of 14.7 $\mu\text{mol}\cdot\text{L}^{-1}$ (n=49) to a mean plasma tHcy level of 9.1 $\mu\text{mol}\cdot\text{L}^{-1}$ ($P < 0.001$). The findings of the study have the potential to alert vegetarians of the possible risk of becoming vitamin B12 deficient, and to help avoid the risk of developing homocysteine related CVD. The quality data obtained in the study will allow an accurate sample size to be calculated for a future definite clinical study.

Key words: Hyperhomocysteinemia, vitamin B12, lactovegetarian, lactoovovegetarian, vegan, primary cardiovascular disease.

INTRODUCTION

A recent systematic review and meta-analysis¹ demonstrated that vegetarians have higher levels of plasma tHcy and lower levels of serum vitamin B12 than omnivores, brought about by a lack of dietary animal products.

Hyperhomocysteinemia {plasma tHcy level $>15 \mu\text{mol}\cdot\text{L}^{-1}$ as defined by²} has been shown to be

linked with chronic medical conditions including cardiovascular disease (CVD)^{3, 4}. Other studies have shown that coronary heart disease (CHD) is linked to homocysteine concentrations with a substantial risk occurring at $>10 \mu\text{mol}\cdot\text{L}^{-1}$ plasma tHcy^{5, 6}. Furthermore, each $5 \mu\text{mol}\cdot\text{L}^{-1}$ increase in plasma tHcy is associated with an approximate 20% increased risk of CHD events^{3, 4}, irrespective of diet. Using epidemiologic and clinical data, it has been suggested⁷ that elevated plasma tHcy is a risk factor

in 82.8% of the CVD conditions examined. From these studies 71.4% of the reported CVD conditions showed that plasma tHcy levels can be employed as a biomarker for the risk of developing CVD⁷.

Obersby *et al*⁷ have suggested that normal levels of plasma tHcy need to be maintained, as a precaution to avoid increasing the risk of developing or exacerbating CVD.

Methylcobalamin is the only form of vitamin B12 that contains a methyl group, and as a consequence is the only form of vitamin B12 that can directly reduce homocysteine⁸.

The other forms of vitamin B12 (i.e. cyanocobalamin – artificially synthesised and widely used in supplements and fortification of food and beverages, hydroxocobalamin and adenosylcobalamin) on entry to the bloodstream must be converted in the body to methylcobalamin before they can be utilised in the folate cycle for remethylation of homocysteine to methionine^{9, 10}. This conversion process is necessary to maintain non-harmful levels of homocysteine, and in the case of cyanocobalamin may take from four to nine weeks¹¹, assuming that there is no disruption by genetic factors, age related issues and metabolic problems¹.

In order to test the hypothesis that supplementary methylcobalamin can normalise elevated plasma tHcy of vitamin B12 deficient vegetarians, a clinical trial was therefore needed. However, it is highly unlikely that an accurate calculation of the sample size of a clinical trial could be made unless it was based on existing strong evidence^{12, 13}. The calculation of the sample size required to reject the null hypothesis (H0) needs to be based on evidence from previous studies of the difference likely to be found between experimental and control groups. The paucity of published data in relation to this is reflected by the fact that just one published study by Yajnik *et al*¹⁴ could be located that tested the effectiveness of methylcobalamin to lower plasma tHcy in female Indian vegetarians. However, this was a proof of principle (POP) study. The duration of the study was just six weeks which may be too short to achieve the full effect of methylcobalamin supplementation. Furthermore, the

dose size of 500µg taken every alternate day may not be sufficient to normalise the elevated plasma tHcy of vitamin B12 deficient lactovegetarians. This assertion is supported by the results shown within Table 2 of the study by Yajnik *et al* (i.e. mean baseline 18.4µmolL⁻¹ to mean post treatment 13.4µmolL⁻¹). These authors cited a study by Kwok *et al*¹⁵ which was specifically aimed at showing that vitamin B12 supplementation improves arterial functions in vegetarians with subnormal vitamin B12 status. This study was a placebo controlled crossover trial with vitamin B12 (500µg/day) taken over twelve weeks with ten weeks washout, followed by a further 24 weeks of vitamin B12 treatment. However, the type of vitamin B12 employed and how it was administered was undisclosed. The results of that study demonstrated a reduction in plasma tHcy, but vitamin B12 did not normalise the level of plasma tHcy (i.e. mean 16.7µmolL⁻¹ at baseline to a mean 11.3µmolL⁻¹ following treatment). The conflicting findings from this study could be a result of the particular route of administration of vitamin B12 or the relatively short washout period of the ten weeks¹⁶.

Both the Yajnik *et al* and Kwok *et al* studies failed to normalise homocysteine levels to < 10µmolL^{-15, 6}.

MATERIALS AND METHODS

The investigation reported in this paper is the first clinical pilot study to effectively explore the normalisation of elevated plasma tHcy of vegetarians by the direct intervention of oral methylcobalamin.

Ethical consideration

The clinical pilot study was conducted according to the guidelines laid down in the Declaration of Helsinki¹⁷ and all procedures involving human subjects were approved by the University of West London Research Scrutiny & Ethics Subcommittee. Written informed consent was obtained from all subjects.

Registration

The clinical pilot study was registered with ClinicalTrials.gov Protocol Registration System 08 June 2012 and allocated the registration number NCT01661309.

Participants

Over the period July 2012 to December 2013 a total of 49 volunteers living in the UK were recruited via the Vegetarian Society and the Vegan Society, and who had been practicing vegetarianism for >3 years were approved to participate in the clinical pilot study. These subjects comprised lactovegetarians (LV), lactoovoovegetarians (LOV) and vegans. In our study LVs and LOVs were treated as one group, since there is no nutritional detrimental difference. Whilst LOVs consume eggs that contain traces of vitamin B12, they also contain vitamin B12 cobamides that block absorption of the actual vitamin B12 and increase the body's need for this nutrient¹⁸.

Inclusion criteria

All participants of the study successfully completed a diet, supplements & medication, medical history and lifestyle confidential questionnaire and fulfilled the following criteria: Have a measured plasma tHcy of $\geq 10 \mu\text{molL}^{-1}$, aged between 18 and 65 years old, not participating in a weight reducing diet, not consuming regularly supplementary vitamin B12 > 10 $\mu\text{g/day}$, not suffering from pernicious anaemia or other conditions resulting in vitamin B12 deficiency, not previously undergone bowel surgery or suffered from gastrointestinal disease, not pregnant, lactating or trying to conceive, a non-smoker, not consuming alcohol regularly (> 2 units/day for females, > 3units/day for males); not consuming caffeine on a regular basis (> 4 cups/day of strong tea or coffee), not suffering from genetic metabolic disease, not suffering from renal failure, diabetes, thyroid disease, CVD, dementia or cancer, not using medications known to influence nutritional status and not suffering a known blood-borne infection (e.g. Hepatitis or HIV).

From the 49 participants admitted to the study, ten either withdrew or were discounted for breaching the study protocol. In order to avoid overoptimistic estimates of the efficacy of methylcobalamin, statistical analysis was performed with the noncompliance and missing outcomes being excluded (n=39) and included (n=49) in the calculations. In the case of the inclusion of the withdrawn subjects (n = 49), intention to treat (ITT) analysis was included on the basis of using the strict

rules of last observation carried forward (LOCF) technique⁽¹⁹⁾.

The reason why the ten ITT subjects withdrew or were discounted from the study was: From the methylcobalamin group, four became omnivores; one failed to have a post treatment homocysteine test taken. From the placebo group, one failed to supply any food diaries; one was due to poor health; two disliked the treatment schedule; and one started taking high daily doses of vitamin B12.

The design and structure of the clinical pilot study was a double blind randomised placebo controlled trial with two arms. The participants, principal investigator and statistician were all unaware of the identity of the methylcobalamin and the placebo groups.

The subjects were randomly assigned to each arm, according to an Fx-115ms computer generated simple randomisation list. The oral methylcobalamin arm was an 1mg lozenge supplied and certified by Jarrow Formulas Inc, Los Angeles, USA. The rationale for choosing the dose size and frequency of consumption of the methylcobalamin was to ensure that there was an effect in the duration of the study, which also took into account any inadvertent dosage changes due to possible methylcobalamin instability.

The oral placebo arm was a lozenge supplied and certified by Lonsdale Health Products Limited, Ingleton, UK. Each participant conducted a baseline plasma tHcy measurement by means of an accredited and validated blood self-sample testing kit, supplied by YORKTEST Laboratories Limited, York, UK. Each group dissolved one lozenge in the mouth, following a meal, every other day for sixteen weeks. A detailed seven day diet diary for the first and final weeks of the study was provided by each participant, which was supervised by a Nutritional Therapist. A post treatment homocysteine measurement was taken by each subject using a further self-sample testing kit supplied by YORKTEST.

The lozenge type of methylcobalamin dissolved in the mouth was preferred over a

swallowed whole tablet because it has been shown that vitamin B12 not dissolved in the mouth can be up to 88% mal-absorbed⁽²⁰⁾, due to the lack of R-binder mostly obtained from saliva, which is required to start the absorption process.

Data collection and distribution of lozenges

- 1) Each participant was allocated an Fx-115ms computer generated randomised code number.
- 2) Distribution of the lozenges by the study supervisor was in two containers each holding 28 lozenges of either type A or type B.
- 3) Participants, Principal Investigator and Statistician were unaware which arm of the study the participants belong to, and to the identity of lozenges A & B.
- 4) Compliance was checked by counting unused lozenges following the return of the containers.

The analysis of the outcome of the study took place at the University of West London, UK, from the supervised diet diaries provided by the participants, and plasma tHcy measurements certified by YORKTEST, on an 'ongoing' basis. A pictorial representation of the clinical pilot study is presented in Figure 1.

Homocysteine assay

The YORKTEST self-sample system enables immediate separation of blood plasma, so that a finger prick blood sample can be collected using a sterilised lancet. The sample is taken on arising in the morning, following an overnight fast. The device immediately separates the blood cells

and collects the plasma in a collection disc. The sample is returned by post, which would be received by YORKTEST well within the stability life of the returned sample, which is up to ten days, even when stored at 37°C⁽²¹⁾. YORKTEST have patented this system and hold accreditation to the relevant EC Directives.

Statistical analysis

Statistical analysis of the results was performed with IBM Corp SPSS software, version 21. A 'Paired Samples t Test' was performed for changes in plasma tHcy with the null hypothesis (H0) being defined as 'there will be no change in plasma tHcy following treatment'. H0 was rejected if the change in plasma tHcy was significantly different from zero, but accepted if it was not significantly different from zero.

Baseline and post treatment mean, SD, *P* and CI95% values of plasma tHcy for the methylcobalamin and placebo groups for participants who completed the study (n=39) and which contained the ITT subjects were also tabulated.

Also, following analysis of the diet diaries of the participants who completed the pilot study (n=39) the mean and SD of daily intakes of nutrients which are involved in the bio-regulation of homocysteine levels were calculated for the first and final weeks of the study and compared with RDA and daily adequate intake (AI) values. In view of the fact that the generation of homocysteine is totally dependent

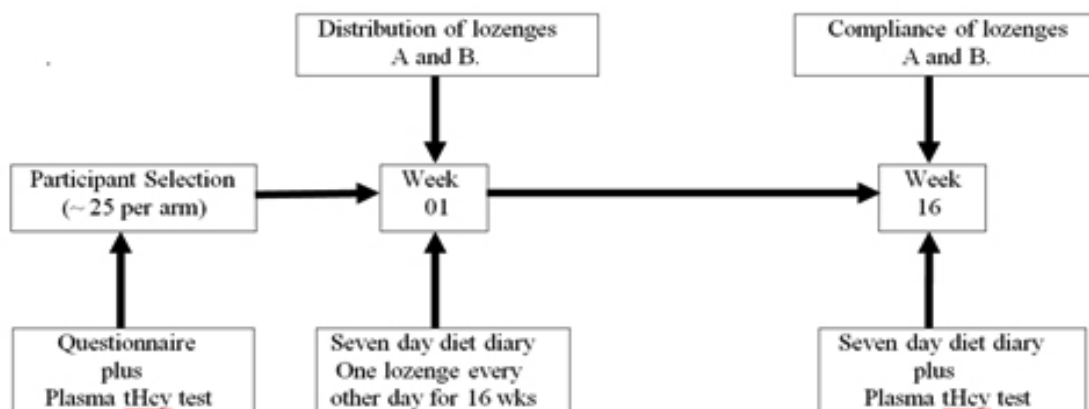


Fig. 1: Pictorial representation of randomised double blind, placebo controlled, clinical pilot study.

on the amino acid methionine⁽²²⁾, the methionine content of the participants' diet diaries was compared with the baseline plasma tHcy, to establish if a correlation exists.

RESULTS

The socio demographic characteristics of age, gender, and vegetarian classification of the participants of the pilot study are presented in Table 1. The comparison of mean values and SD's of baseline and post treatment values of plasma tHcy, for subjects who completed the pilot study and the group containing ITT subjects are presented in Table 2. There were no reported adverse events from the treatments reported by the participants who completed the study. Comparison of the nutrients which are involved in the bio-regulation

of homocysteine levels and RDA and AI values are presented in Table 4A and Table 4B.

In Table 4A and Table 4B, it was deemed unnecessary to conduct a test of significance for mean nutrient levels (except vitamin B12) that are equal to or exceeded the RDA. With regard to the mean intake of TMG, a point value for the AI was taken as 750mg. All groups with the exception of male vegans (due to small sample) were found to be all significantly less than the AI, for both the first and final seven days of the diet diaries. The choline mean intake for all groups with the exception of male vegans (due to small sample) was found to be all significantly less than the AI for both the first and final seven days of the diet diaries.

Table 1: Socio demographic characteristics of clinical pilot study participants

Socio demographic characteristics	n=25	Methylcobalamin group			Placebo group			
		Mean	SD	95% CI	n=24	Mean	SD	95% CI
Age (years)								
Male		53.4	13.2	(43.7, 63.2)		48.2	15	(36.2, 60.2)
Female		43.4	13.8	(37.1, 49.8)		44.7	14.9	(37.8, 51.6)
Gender								
Males	5 (20%)				6 (25%)			
Female	20 (80%)				18 (75%)			
Classification								
Lactovegetarian	2 (8%)				1 (4.2%)			
Lactoovovegetarian	14 (56%)				12 (50%)			
Vegan	9 (36%)				11 (45.8%)			

Table 2: Comparison of mean values of baseline and post treatment of plasma tHcy, for participants who completed the clinical pilot study (n=39) and which contained the ITT subjects (n=49)

Lozenge type	n	Baseline		Post treatment		
		Mean	SD	Mean	SD	P (2-tailed)
Completed study						
Methylcobalamin						
Homocysteine (µmolL ⁻¹)	20	15.5	3.7	8.4	3.1	< 0.001
Placebo						
Homocysteine (µmolL ⁻¹)	19	13.7	2.6	12.5	4.5	0.16
ITT						
Methylcobalamin						
Homocysteine (µmolL ⁻¹)	25	14.7	3.7	9.1	3.1	< 0.001
Placebo						
Homocysteine (µmolL ⁻¹)	24	14.1	2.8	12.9	4.5	0.54

The mean vitamin B12 for the first and final days of the diet diaries, female LV-LOVs are significantly higher than the RDA, whilst both genders of vegans are significantly less than the RDA. This also applies to male LV-LOVs for the first seven days of diet diaries.

Only female LV-LOVs for the first seven days of the diet diaries contain mean levels of vitamin B12 that is significantly higher than the RDA, whilst both genders of LV-LOVs significantly meet the RDA for the final seven days of the diet diaries. It was observed from the confidential questionnaire, completed by all applicants that 53% (n = 34) were

Table 3: Paired samples t test, change in plasma tHcy, for participants who completed the pilot study (n=39) and the group containing 'intention to treat' subjects (n=49), with in all cases test value = 0.

Lozenge type and test ($\alpha = 0.05$)	df difference	P (2-tailed) interval	Mean	95% Confidence interval
Change in plasma total homocysteine ($\mu\text{mol/L}$) Completed study (n=39)				
Methylcobalamin	19	0.00	-7.1	(-9.35, -4.85)
Placebo	18	0.16	-1.11	(-2.70, 0.48)
Intention to treat (n=49)				
Methylcobalamin	24	0.00	-5.6	(-7.78, -3.42)
Placebo	23	0.54	-1.21	(-2.6, 0.18)

Table 4A: Comparison of daily intake of nutrients and have intake of nutrients involved in the bio-regulation of homocysteine levels of participants who completed the clinical pilot study (n=39), taken from the diet diaries for the first seven days of the study. [RDAs and AIs taken from the Institute of Medicine of the Academy of Science⁽²³⁾. Dietary nutrients are in accordance with Foods Standards Agency⁽²⁴⁾ and USDA Agricultural Research Service⁽²⁵⁾]

Nutrient	Lactovegetarian /lactoovovegetarian				Vegan			Adult values	
	n	Mean	SD	P(2-tailed)	n	Mean	SD	P(2-tailed)	RDA AI
Males	11				2				
Vitamin B2 (mg)		1.7	0.71			1.3	0.14		1.30
Vitamin B6 (mg)		2.2	0.58			1.5	0.28		1.30
Vitamin B12 (μg)		2.0	0.49	0.031		1.0	0.65	0.208	2.40
Folate (μg)		533	133			402	67.9		400
Zinc (mg)		12.2	3.0			11.0	3.1		11.0
Choline (mg)		160	48.6	0.000		215	72.1	0.152	550
Trimethylglycine (mg)		142	71.6	0.000		130	17	0.012	500-1000
Females	14				12				
Vitamin B2 (mg)		1.3	0.68			1.3	0.92		1.10
Vitamin B6 (mg)		1.5	0.83			1.4	0.73		1.30
Vitamin B12 (μg)		3.4	0.41	0.000		0.7	0.33	0.59	2.40
Folate (μg)		406	201			420	210		400
Zinc (mg)		12.4	2.5			14.0	2.9		8.0
Choline (mg)		227	56.3	0.000		180	52.3	0.000	425
Trimethylglycine (mg)		268	28.1	0.000		247	42.1	0.000	500-1000

oblivious to the potential detrimental effect to health of elevated plasma tHcy, as a result of a deficiency of vitamin B12 in vegetarian diets.

that there exists a positive correlation between baseline plasma tHcy and mean dietary methionine intake of the form $Y=A.I_n(X)-C$ (see Figure 2) of vegetarian deficient vegetarians. This dispersed following methylcobalamin intervention due to the

Analysis of the diet diaries of the participants who completed the study strongly indicate ($R^2 = 0.79$)

Table 4B: Comparison of daily intake of nutrients and have intake of nutrients involved in the bio-regulation of homocysteine levels of participants who completed the clinical pilot study (n=39), taken from the diet diaries for the final seven days of the study. [RDAs and AIs taken from the Institute of Medicine of the Academy of Science ⁽²³⁾. Dietary nutrients are in accordance with Foods Standards Agency ⁽²⁴⁾ and USDA Agricultural Research Service ⁽²⁵⁾]

Nutrient	Lactovegetarian/lactoovovegetarian				Vegan			Adult values	
	n	Mean	SD	P(2-tailed)	n	Mean	SD	P(2-tailed)	RDA AI
Males	11				2				
Vitamin B2 (mg)		2.5	0.32			1.3	0.14		1.30
Vitamin B6 (mg)		1.4	0.68			2.1	0.99		1.30
Vitamin B12 (µg)		3.0	0.3	0.000		1.6	0.57	0.68	2.40
Folate (µg)		489	97			448	139		400
Zinc (mg)		11.2	2.1			14	1.4		11.0
Choline (mg)		234	51	0.000		195	12.7	0.025	550
Trimethylglycine (mg)		130	51.7	0.000		166	39.6	0.030	500-1000
Females	14				12				
Vitamin B2 (mg)		1.3	0.84			1.3	0.77		1.10
Vitamin B6 (mg)		1.3	0.97			2.2	0.74		1.30
Vitamin B12 (µg)		3.0	0.68	0.000		1.7	0.48	0.038	2.40
Folate (µg)		408	221			448	215		400
Zinc (mg)		8.4	2.3			16.6	0.99		8.0
Choline (mg)		201	46.9	0.000		186	54.4	0.000	425
Trimethylglycine (mg)		208	85.4	0.000		199	86.3	0.000	500-1000

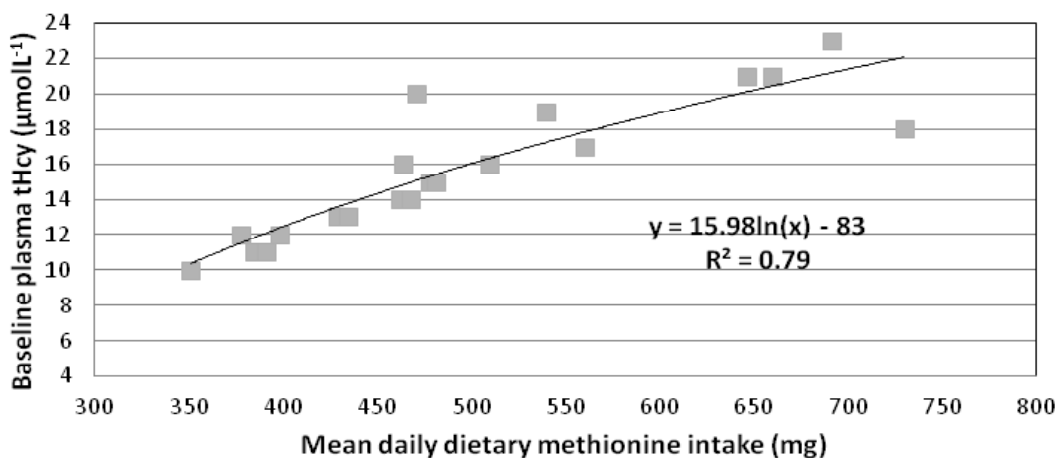


Fig. 2: Correlation between baseline plasma tHcy and mean dietary methionine intake, for vitamin B12 deficient vegetarians from the intervention group, who completed the clinical pilot study

normalisation of participants' plasma tHcy.

DISCUSSION

The statistical results presented in Table 2 demonstrate that methylcobalamin reduced mean baseline plasma tHcy level of $15.5\mu\text{molL}^{-1}$ ($n=39$) to a post treatment plasma tHcy level of $8.4\mu\text{molL}^{-1}$ ($P<0.001$). In the ITT group this also demonstrated that methylcobalamin reduced a mean baseline plasma tHcy level of $14\mu\text{molL}^{-1}$ ($n=49$) to a mean post treatment plasma tHcy level of $9.1\mu\text{molL}^{-1}$ ($P<0.001$). Both sets of results indicate that methylcobalamin normalises plasma tHcy levels, whereas, the placebo failed to achieve this. The above results are supported by the results presented in Table 3, which demonstrates that for subjects that completed the trial ($n=39$) and the ITT group ($n=49$) H_0 can be rejected for the methylcobalamin group, since $P<0.05$ and CI95% does not pass through zero. In both the placebo groups H_0 is accepted, since $P>0.05$ and CI95% passes through zero. No previously published trials were found that could be directly compared with the outcomes of the present study. The very positive effect of methylcobalamin on lowering plasma tHcy leads to the conclusion that methylcobalamin can even at this pilot study stage be recommended as a food supplement for all categories of vegetarianism, to help maintain homocysteine at non-harmful levels.

Since methylcobalamin was administered in the lozenge form, this ensured that absorption took place by mediated intrinsic factor, non-intrinsic mediated diffusion and sublingual intake. This is particularly advantageous to individuals who cannot readily absorb vitamin B12 by mediated intrinsic factor due to autoimmune problems, resulting in partial or full pernicious anaemia ⁽²⁶⁻²⁸⁾.

Table 4.9A and Table 4.9B illustrates that those subjects completing the study consumed at least the RDA and AIs range of mean values of nutrients that can influence homocysteine reduction with the exception of vitamin B12 for vegans and male LV-LOVs and both genders for LV-LOV and vegans for choline and trimethylglycine (TMG). However, the TMG reaction for the remethylation of homocysteine, can only take place in the liver and kidney of human beings as the enzyme involved,

betaine homocysteine methyltransferase, is not present in other organs of the body ⁽²⁹⁾. The influence on elevated plasma tHcy is therefore considered to be minimal in the TMG reaction.

Vitamin B12 was not measured since there are no accredited or validated assays available that have sufficient sensitivity and specificity to measure methylcobalamin that has been absorbed into cells. Some participants independently arranged for their physician to have their vitamin B12 status measured. In most cases the results indicated that their vitamin B12 status was normal i.e. $> 150\text{pmolL}^{-1}$ ⁽³⁰⁾, yet their homocysteine level was found to be elevated. This is explained by the fact that NHS vitamin B12 assays measure total serum vitamin B12 ⁽³¹⁾. The standard NHS vitamin B12 assay measures total plasma cyanocobalamin, following chemical modification of the other forms of cobalamin to cyanocobalamin by potassium cyanide. The procedure renders the assay impossible to identify each fraction of vitamin B12 being measured. As a consequence, it can be reasonably deduced that under these circumstances these participants were actually methylcobalamin deficient leading to a rise in plasma homocysteine levels.

Verification of participant methylcobalamin deficiency is strongly indicated by the fact that these participant plasma tHcy was normalised (i.e. $< 10\mu\text{molL}^{-1}$) by the intervention of methylcobalamin during their participation in the present clinical pilot study.

It is very clear from the results of the clinical pilot study and analysis of the participants' diet diaries which show that the diets are rich in the homocysteine lowering nutrients. In addition, participants exhibited subnormal levels of methylcobalamin, resulting in elevated plasma tHcy, which according to the results of the study have been normalised, probably due to: The type of intervention employed, dose size, duration of treatment, methylcobalamin supplement dissolved in the mouth and therefore mixed with saliva.

In conclusion, the primary objective was successful since the results of this study strongly suggest that supplementary methylcobalamin will normalise elevated plasma tHcy of vitamin B12

deficient vegetarians provided that they are not deficient in other bio-regulation of homocysteine nutrients and enzymes. According to Huang *et al* ⁽³²⁾ vitamin B6 and folate have little or no effect on plasma tHcy concentration when individuals have adequate dietary vitamin B6 and folate intakes. Furthermore, it will allow an accurate sample size to be calculated for a further definitive clinical trial. Analyses indicate that the RDA for vitamin B12 maybe too low. This supports previous research ⁽³³⁾, which demonstrates that doses of vitamin B12 of $\geq 6\mu\text{g/day}$ appear to be sufficient to maintain a steady-state concentration of serum vitamin B12.

supplying the homocysteine test kits, testing and validating the test results of the participant blood samples and Jarrow Formulas Inc USA and Lonsdale Health Products Limited UK for supplying the methylcobalamin and placebo lozenges employed in the study.

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It was discovered that a logarithmic relationship exists between baseline plasma tHcy of vitamin B12 deficient vegetarians and dietary methionine, which no longer exists, following intervention by methylcobalamin.

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