Sublingual vitamin B12 compared to intramuscular injection in patients with type 2 diabetes treated with metformin: a randomised trial

Amber Parry Strong, Sylvan Haeusler, Mark Weatherall, Jeremy Krebs

ABSTRACT

AIM: To compare a single 1mg intramuscular hydroxocobalamin injection with a 3-month course of 1mg/day sublingual methylcobalamin supplements on serum vitamin B12 concentrations in participants with type 2 diabetes treated with metformin.

METHOD: Participants on metformin treatment with vitamin B12 concentrations below 220pmol/L were recruited through hospital diabetes clinics and primary care practices. They were randomised to receive either the injection or sublingual treatment. The primary outcome was serum vitamin B12 level after 3 months adjusted for baseline assessed by analysis of covariance (ANCOVA). The trial was registered on the Australia New Zealand Clinical Trial registry (ACTRN12612001108808).

RESULTS: A total of 34 participants were randomised; 19 to the tablet, and 15 to the injection. The mean (SD) age, duration of diabetes, and duration of metformin use were, 64.2 (7.3) years, 13.7 (6.4) years, and 11.6 (5.0) years, respectively. After 3 months, the mean (SD) vitamin B12 was 372.1 (103.3) pmol/L in the tablet group (n=19) compared to 251.7 (106.8) pmol/L in the injection group (n=15), ANCOVA estimated difference -119.4 (95% CI -191.2 to -47.6), p=0.002. After 6 months, the mean (SD) serum B12 was 258.8 (58.7) pmol/L in the tablet group (n=17) and 241.9 (40.1) pmol/L in the injection group (n=15); ANCOVA estimated difference -15.2 (95% CI -50.3 to 19.8), p=0.38. Higher metformin dose was associated with lower serum B12 at 3 months, but not at baseline or 6 months.

CONCLUSION: Decreased serum vitamin B12 level in patients with type 2 diabetes who are treated with metformin can be corrected through treatment with either hydroxocobalamin injections or methylcobalamin sublingual supplements.

Metformin, the most common first-line treatment for type 2 diabetes mellitus (DM), reduces serum B12 concentrations in between 10 and 20% of patients using this medication.[1-4] The prevalence of low B12 concentration (<220 pmol/L) in those taking metformin in New Zealand is 18.6%.[5] Metformin impairs calcium-dependent membrane activity in the ileum, which leads to malabsorption of vitamin B12 bound to intrinsic factors.[6,7] Measurable reductions in vitamin B12 levels occur as quickly as 3 months after starting metformin, although symptomatic deficiency may take between 5 and 10 years to develop.[6]

Vitamin B12 plays a crucial role in the nervous system. It is a coenzyme for methyl malonyl-CoA mutase, the action of which is required for myelin synthesis. Impaired myelin formation can lead to neuropathy, neuropsychiatric abnormalities, myelopathy, and optic nerve atrophy. Clinical evidence of vitamin B12 deficiency-related neuropathy includes loss of vibratory sensation, diminished proprioception, and loss of cutaneous sensation in the lower limbs.[8] Cognitive impairment and

depression are neuropsychiatric syndromes associated with vitamin B12 deficiency.[9] In patients with DM, these clinical manifestations are particularly important because neuropathy is also a complication of DM, and depression is a common comorbidity associated with a chronic health condition.

Internationally, the most common method for treatment of vitamin B12 deficiency is intramuscular hydroxocobalamin injections, initially using a loading regimen, followed by a maintenance administration for long-term treatment.[1] In New Zealand, recommendations are for 1,000 mcg every 2 or 3 months for prophylaxis of macrocytic anaemia associated with vitamin B12 deficiency resulting from gastrectomy, malabsorption syndromes, and strict vegetarianism.[10] However, there are no specific recommendations for metformin induced vitamin B12 deficiency. Intramuscular injections generally require visits to a health clinic for administration. This is costly, both for the clinic and the patient. Intramuscular injections can also be painful and lead to reduced adherence.

Oral vitamin B12 supplementation is possible, but the efficacy is questionable, likely due the compromised route of absorption in the gut. The The National Health and Nutrition Examination Survey (NHANES) data reported that oral B12 supplementation reduced the rate of B12 deficiency by two-thirds in those without diabetes, but there was no association seen in those taking metformin.[11] Sublingual vitamin B12 bypasses the mechanism of interaction of metformin on absorption of vitamin B12, and is a feasible alternative to injections. Sublingual treatment is as effective as oral doses in patients who are not treated with metformin, as assessed by serum vitamin B12 and biomarkers of vitamin B12 functionality.[12-14]

The aim of the study reported here is to evaluate the effectiveness of sublingual vitamin B12 supplementation compared with intramuscular injection for patients using metformin who have low serum vitamin B12 in a randomised trial.

Methods

This was a randomised study of 3 months sublingual treatment with methylcobalamin 1mg/day, compared to a single intramuscular injection of hydroxocobalamin 1mg. Definitions of low serum B12 and B12 deficiency vary. For this study we chose that used by de Jager and colleagues, with vitamin B12 deficiency defined as serum B12<150 pmol/L, and low B12 as concentrations between 150 and 220 pmol/L.[7] Participants were patients with type 2 DM who were being treated with metformin. The particular intramuscular dose was chosen to replicate the effect of a general practitioner following the New Zealand guidelines in primary care. As there was little guidance in the literature as to what dose would be effective, it was decided the sublingual B12 dose should be 1mg/day, based on the study by Yazaki et al.[12] Both randomised groups were reviewed after 3 months and a second round of treatment started if serum vitamin B12 had not risen above 220 pmol/L. The primary outcome variable was serum vitamin B12 level after 3 months adjusted for baseline vitamin B12. The secondary outcomes were the vitamin B12 after 6 months and the Michigan Neuropathy Screening Instrument (MNSI) score after 6 months.

Recruitment and study visits

Participants were recruited through hospital and primary care clinics by invitation, and randomised based on the results of vitamin B12 screening. Inclusion criteria were a diagnosis of type 2 DM, treatment with metformin for 12 months or longer, and a screening serum vitamin B12 of < 220 pmol/L. Participants were excluded if they were already on treatment for vitamin B12 deficiency (including over-the-counter vitamin supplementation containing vitamin B12), were anaemic for another reason, had prior gastric surgery (eg, gastric bypass), pregnant or breastfeeding, reported past cobalamin allergy, or other reason in the judgement of the investigators as to why vitamin B12 could not be administered. Written informed consent was obtained at the baseline visit. Clinical variables that could affect serum vitamin B12 concentrations, including smoking and alcohol use, and medications known to effect serum B12, in particular omeprazole,

were recorded. The screening serum vitamin B12 level was considered baseline, as all participants were invited to take part in the study immediately after receipt of screening bloods.

After collection of baseline data, participants were randomised on a 1:1 allocation to receive either a single 1mg intramuscular injection of hydroxocobalamin or a 3-month course of sublingual methylcobalamin supplements of a 1,000 mcg/day dosage. The injection was hydroxocobalamin ABM, 1 mL ampoules containing 1 mg hydroxocobalamin acetate per mL equivalent to 0.96 mg of hydroxocobalamin per mL. Other ingredients were sodium chloride, sodium acetate, acetic acid and water for injections. The injections were prescribed by the study doctor, obtained through the hospital pharmacy and administered by a research nurse during the study visit. Prescription records were kept accordingly. The sublingual tablet used was 'Bronson' sublingual B12 1,000mcg—methylcobalamin (Bronson Laboratories, Utah, US). Other ingredients were microcrystalline cellulose, mannitol, fructose, sorbitol, magnesium stearate, lecithin, croscarmellose sodium and artificial cherry flavor. The sublingual tablets were purchased without a prescription online and were all manufactured in the same batch. The sublingual tablets were signed out of a log each time, and given out in 3-month batches by one investigator who undertook all baseline visits to ensure consistency.

Randomisation was carried out using an internet based randomisation tool, located at www.randomizer.org (accessed 29/1/2013), to generate a random order sequence for participant allocation to treatment. Participants were randomised in order of recruitment, blinded to the results of their B12 screening status. Randomisation was not blocked. Due to the nature of the interventions, the participants were unable to be blinded as to their treatment, but the statistician and primary investigator remained blinded throughout the study. After randomisation, participants completed a Food Frequency Questionnaire, and the Michigan Neuropathy Screening Instrument. The food frequency questionnaire is validated for micronutrient intake, and used to assess background dietary B12 intake at baseline (University of Otago Food Frequency Questionnaire, Dunedin, New Zealand). The Michigan Neuropathy Screening Instrument (MNSI) was used to assess neuropathy at baseline and after 6 months.[15] This includes a physical examination (foot inspection, assessment of vibration sensation and muscle stretch reflexes, and monofilament testing) and a questionnaire to elicit indicators of neuropathy. The MNSI was used to detect neuropathic symptoms as a score of ≥7 on the patient questionnaire and a score of ≥2 on the physical examination for neuropathy.

Participants were retested for serum B12 after 3 months. These tests were processed through routine laboratory runs at a central laboratory in order for results to be returned in time for the visit. If the B12 level was below 220 pmol/L, they received a further 3-month treatment course of the same treatment to which they were originally allocated. If the B12 level was above 220 pmol/L, the participant was not provided additional treatment and observed for another 3 months. Participants returned tablet containers for a tablet count after 3 and 6 months as applicable. A compliance percentage figure was then able to be calculated by subtracting the number of remaining tablets from the number of days since the last visit. As the injections were administered during the study visit, compliance was witnessed by the investigator conducting the visit, and checked by an investigator review of the prescription charts. After 6 months, participants returned for a follow-up serum B12 and folate, and a repeat of the MNSI questionnaire, and then were discharged to the care of their general practitioner.

Statistical analysis and ethical review

This was a randomised parallel group superiority study. The primary analysis was analysis of covariance (ANCOVA) to estimate the mean difference in serum vitamin B12 between tablet and injection, with baseline Vitamin B12 as a covariate. As an intention-to-treat analysis, all available data was included. Secondary analysis used ANCOVA

Figure 1: CONSORT diagram of recruitment and retention.

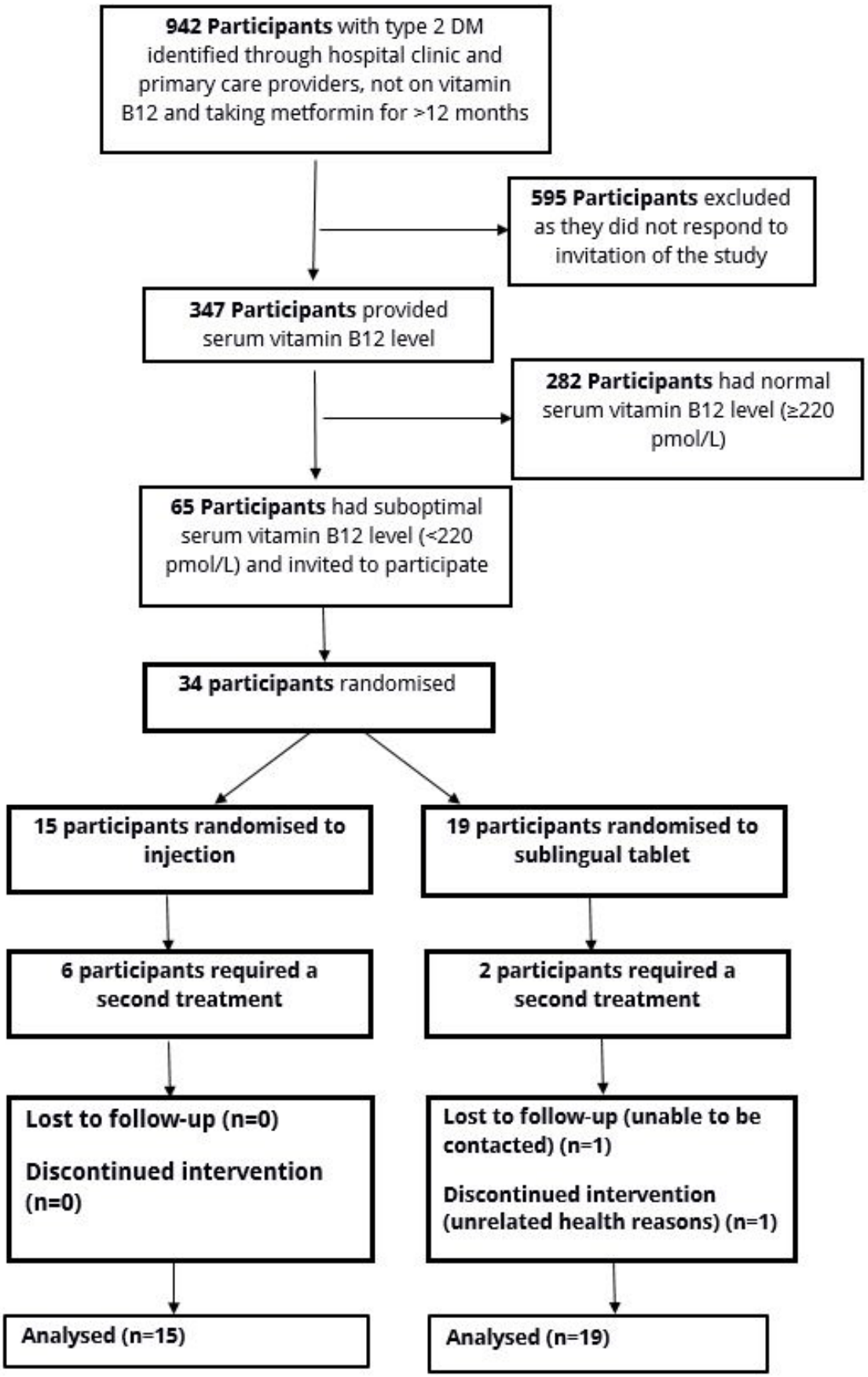

Table 1: Participant description.

Mean (SD)			
Variable	**Injection n=15**	**Sublingual n=19**	**All n=34**
Age (years)	64.9 (6.8)	63.7 (7.8)	64.2 (7.3)
Diabetes mellitus duration (years)	14.2 (4.8)	13.3 (7.4)	13.7 (6.4)
Duration of metformin use (years)	12.5 (5.0)	10.9 (4.9)	11.6 (5.0)
Metformin dose (mg)	2357 (767)	2013 (507)	2165 (657)
Alcohol units per week	2.0 (1.8)	6.2 (7.1)	4.5 (6.0)
Smoking packs per week	0.5 (0.6)	0	0.5 (0.6)
Dietary Calcium intake (mg per day)	707 (114.6)	592.1 (154.3)	640.5 (143.5)
Dietary folate intake (µg per day)	795.0 (352.8)	560.5 (244.5)	659.25 (316.9)
Serum Folate (nmol/L)	21.4 (9.9)	27.2 (6.5)	24.6 (8.4)
N per category			
Sex Male	9	11	20
Ethnicity:			
European	9	16	25
Māori	0	1	1
Indian	3	2	5
Other	3	0	3
Medication use:			
Sulphonylureas	6	4	10
Insulin	6	12	18
Omeprazole	6	6	12
Alcohol use	6	9	15
Smokers	3	0	3

with the addition of duration of metformin use as a continuous covariate. The MNSI scores were compared between randomised groups by independent t-tests and within randomised groups (change from baseline) with paired t-tests. SAS version 9.3 was used for the analysis.

Sample Size

The standard deviation of serum vitamin B12 after 2 months treatment with sublingual B12 was 75 pmol/L in a study of participants with known vitamin B12 deficiency (serum vitamin B12 <138 pmol/L).[13] We were uncertain of the clinically significant difference between two treatments for vitamin B12 in the setting of metformin use, so based the sample size calculation on detecting an effect size of one standard deviation. This required recruitment of 17 participants in each arm, for 80% power with a type I error rate of 5%. Use of ANCOVA with baseline vitamin B12 is likely to improve the statistical power to detect differences. We did not factor in drop-outs. If the standard deviation was the same as in the previous research, this study had sufficient power to detect a 75 pmol/L difference in vitamin B12.

Ethical approval for the study was obtained from the New Zealand Central Health and Disability Ethics Committee (12/CEN/65/AM02). The trial was registered on the Australia New Zealand Clinical Trial registry (ACTRN12612001108808). The study was funded by a University of Otago Research Grant.

Results

Figure 1 shows the process of recruitment and randomisation in the CONSORT Diagram. A summary of demographic information for the 34 participants is shown in Table 1. Most participants were male Europeans with a mean (SD) age of 64.2 (7.3) years. The mean (SD) duration of Type 2 DM was 13.7 (6.4) years, with mean (SD)

Table 2: Serum vitamin B12 concentrations by treatment and measurement time, comparison by ANCOVA with baseline vitamin B12 as a covariate.

	Vitamin B12 (pmol/L) Mean (SD)		Injection minus tablet (95% CI)	p=
Time	**Injection**	**Sublingual**		
Baseline	166.7 (36.5) (n=15)	170.2 (39.0) (n=19)		
3 months	251.7 (106.8) n=15	372.1 (103.3) n=18	-119.4 (-191.2 to -47.6)	0.002
6 months	241.9 (40.1) n=15	258.8 (58.7) n=17	-15.2 (-50.3 to 19.8)	0.38

SD Standard deviation, CI Confidence Interval

Table 3: Mean (Standard Deviation) MNSI scores for both treatment arms over 6 months.

	Hydroxycobalamin injection (n=15)		Methylcobalamin sublingual tablet (n=19)	
MNSI Score	Questionnaire	Physical	Questionnaire	Physical
Baseline	2.3 (2.1)	1.0 (1.5)	2.0 (1.5)	0.9 (1.4)
6 months	1.9 (2.2)	1.2 (1.7)	1.9 (2.9)	1.4 (1.9)

duration of Metformin treatment of 11.6 (5.0) years of Metformin treatment. Two participants were identified through the food frequency questionnaire as being vegetarian (no meat or fish), both randomised to the injection group. Thirty two participants completed the study; two participants withdrew from the tablet group (one due to unrelated health reasons, the other lost to follow up). Compliance in the tablet group was 89.2%.

Mean serum B12 concentration increased from baseline after 3 and 6 months in both treatment groups (Table 2). For the primary outcome variable, and when adjusted for baseline B12 concentration, serum B12 concentration was greater in the sublingual treatment than intramuscular injection after 3 months. There was no evidence of a difference between treatments after 6 months. In the secondary analysis of vitamin B12 after 3 months additionally adjusting for metformin dose, we found that metformin dose was associated with lower vitamin B12 concentrations; -75.3 units per 1,000 mg higher metformin dose (95% CI -125.0 to -25.5), p=0.004.

The treatment was repeated at 3 months for six individuals in the injection group, and two individuals in the tablet group. These eight individuals were older than the average participant, had longer duration of both diabetes and metformin use and a higher metformin dose. Seven of these were vitamin B12 deficient (150 pmol/L or less) at baseline, and two were vegetarian.

No patients had neuropathy at baseline according to the MNSI, but one participant in the tablet group registered a neuropathic score after 6 months (Table 3). There was no difference in mean score between the two treatment groups at baseline or after 6 months for the questionnaire or physical examination. There was also no difference in the injection group between baseline and 6 months for either the questionnaire or physical examination. In the tablet group, there was no difference from baseline to 6 months for the questionnaire, but there was a significant deterioration in the physical examination score.

Discussion

This study provides evidence that the treatment of patients with decreased serum vitamin B12 concentration with methylcobalamin sublingual supplementation is as effective as hydroxocobalamin injection in correcting a low vitamin B12 status over 6 months. Both treatments resulted in improvements in serum vitamin B12 concentration. After

treatment, concentrations in both groups remained in the reference range, and over-correction was not seen.

Currently, the most common treatment for vitamin B12 deficiency is intramuscular hydroxocobalamin injections. These have been shown to improve both biochemical markers for B12 deficiency and also improve physiological functions of vitamin B12 deficiency.[16-19] The administration of a single dose of 1mg hydroxocobalamin was seen to correct serum vitamin B12 status in 60% of the patients treated in this study at 3 months. There was an association between B12 at 3 months and metformin dose, with a higher metformin dose predicting a lower B12 level, but this was not apparent at baseline or 6 months. Closer inspection of the individuals receiving a second dose indicated several possible risk factors; lower initial vitamin B12 level, greater age, longer diabetes and metformin duration and higher metformin dose.

Some current treatment protocols for vitamin B12 deficiency dictate that a loading period of hydroxocobalamin should be used in order to raise baseline concentrations before a periodic maintenance regimen should be initialised. One study suggests a daily 1 mg administration of hydroxocobalamin daily for 1 week, followed by 1 mg once weekly for 4 weeks, subsequent maintenance of B12 is achieved through 1 mg administration every 2 to 3 months.[1] While we did not compare the New Zealand recommendation with a loading regimen, it is likely from our study that at least two intramuscular injections are required to correct serum B12 concentrations, with on-going monitoring. Further research is required to assess whether on going sublingual supplementation will be required and the optimal injection frequency for maintenance of B12 concentrations in patients taking long-term metformin.

The use of sublingual delivery is a growing trend in vitamin supplementation.[14] This route of administration avoids the issues of interference with absorption in the lower GI tract, and of the first pass metabolism in the liver.[13] This is particularly relevant for metformin induced vitamin B12 malabsorption, where metformin interferes with absorption of intrinsic factor bound vitamin B12.[12] While studies have shown the absorption of sublingual and oral B12 to be similar when treating B12 deficiency from pernicious anaemia, it would be very useful to see whether the same is true in metformin-induced B12 deficiency.[13] The dosage regimen for the sublingual treatment was higher than that of the single 1 mg injection, and although the bioavailability of sublingual administration is less than intramuscular injection, this probably explains the greater concentration of B12 observed with the sublingual treatment after the initial 3 months. The difference in the pattern of response to the two treatment groups may also be attributed to the different clearance and excretion properties of the two cobalamin vitamers. Almost a third of a 1 mg intramuscular dose of hydroxycobalamin is excreted in the urine in the first 72 hours.[20] The excretion rate of the sublingual dose is unknown. In addition, metformin increases preferential hepatic storage of vitamin B12.[21] This, coupled with the higher affinity of hydroxocobalamin to bind to hepatic parenchymal cells via preferential trafficking, may also explain the lower serum B12 concentration after hydroxocobalamin injection compared with methylcobalamin.[22] Total body vitamin B12 stores however may be similar, due to increased hepatic storage.

This study was not specifically designed to assess the effects of treatment on clinical consequences of B12 deficiency, which is perhaps the most important question. No significant symptoms of vitamin B12 deficiency were observed in our study using the MNSI. Only one patient met the criteria for neuropathy, and they had previously documented treatment of diabetic neuropathy.[15] The MNSI score for this participant deteriorated markedly over the 6 months despite replacement of B12, and explained the significant score increase for the sublingual treatment at 6 months. Therefore, there is no evidence from this study that using the sublingual route of administration is less effective than the intramuscular route with regard to preventing neuropathy. Clearly, much bigger and longer studies are required to fully address this question. Similarly, a longer observation period post treatment and use of MMA and tHcys

concentrations would allow more investigation into sustainability of an initial treatment and whether on-going maintenance treatment is required for either of the cobalamin forms. Although the sample size was similar to previous studies,[12,13] it would have been preferable to have a larger sample size, and factor in an arm for an oral supplement. The screening for serum B12 concentrations in this study occurred across primary health organisations and a regional hospital. Due to laboratory contracts, this resulted in two different laboratories conducting these initial screening tests. However, every effort was made to ensure inter-laboratory standardisation. A strength of this study was the randomised design. While a full double-dummy double-blinded randomised controlled trial would have provided stronger evidence, such rigour was not possible within the funding constraints of this study, and may have resulted in false positives due to a placebo effect.

In conclusion, this study has shown that decreased serum vitamin B12 level in patients with type 2 diabetes on long-term metformin treatment can be corrected through treatment with either hydroxocobalamin injections or methylcobalamin sublingual supplements. Further study is required to determine the optimal long-term dosing regimen and monitoring duration for both treatments, and clinical significance of metformin related B12 deficiency.

Competing interests:
Amber Parry-Strong reports grants from Otago University and 'other' from Victoria University during the conduct of the study.

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Author information:
Amber Parry-Strong, Research Fellow, Centre for Endocrine, Diabetes and Obesity Research, Capital and Coast District Health Board, Wellington; Sylvan Haeusler, MSc Student, Centre for Endocrine, Diabetes and Obesity Research, Capital and Coast District Health Board, Wellington; Mark Weatherall, Department of Medicine, University of Otago Wellington, Wellington; Jeremy Krebs, Associate Professor, Department of Medicine, University of Otago Wellington, Wellington, New Zealand

Corresponding author:
Amber Parry-Strong, Centre for Endocrine, Diabetes and Obesity Research, Capital and Coast Health, Private Bag 7902, Wellington, New Zealand.
amber.parry-strong@ccdhb.org.nz

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