

# A Vitamin B-12 Supplement of 500 $\mu\text{g}/\text{d}$ for Eight Weeks Does Not Normalize Urinary Methylmalonic Acid or Other Biomarkers of Vitamin B-12 Status in Elderly People with Moderately Poor Vitamin B-12 Status<sup>1-4</sup>

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## Abstract

Plasma vitamin B-12 is the most commonly used biomarker of vitamin B-12 status, but the predictive value for low vitamin B-12 status is poor. The urinary methylmalonic acid (uMMA) concentration has potential as a functional biomarker of vitamin B-12 status, but the response to supplemental vitamin B-12 is uncertain. A study was conducted to investigate the responsiveness of uMMA to supplemental vitamin B-12 in comparison with other biomarkers of vitamin B-12 status [plasma vitamin B-12, serum holotranscobalamin (holoTC), plasma MMA] in elderly people with moderately poor vitamin B-12 status. A double-blind, placebo-controlled, randomized 8-wk intervention study was carried out using vitamin B-12 supplements (500  $\mu\text{g}/\text{d}$ , 100  $\mu\text{g}/\text{d}$ , and 10  $\mu\text{g}/\text{d}$  cyanocobalamin) in 100 elderly people with a combined plasma vitamin B-12 <250 pmol/L and uMMA ratio ( $\mu\text{mol MMA}/\text{mmol creatinine}$ ) >1.5. All biomarkers had a dose response to supplemental vitamin B-12. Improvements in plasma vitamin B-12 and serum holoTC were achieved at cobalamin supplements of 10  $\mu\text{g}/\text{d}$ , but even 500  $\mu\text{g}/\text{d}$  for 8 wk did not normalize plasma vitamin B-12 in 8% and serum holoTC in 12% of people. The response in uMMA was comparable with plasma MMA; 15–25% of people still showed evidence of metabolic deficiency after 500  $\mu\text{g}/\text{d}$  cobalamin for 8 wk. There was a differential response in urinary and plasma MMA according to smoking behavior; the response was enhanced in ex-smokers compared with never-smokers. uMMA offers an alternative marker of metabolic vitamin-B12 status, obviating the need for blood sampling. *J. Nutr.* 143: 142–147, 2013.

## Introduction

Cross-sectional studies from several countries point to an age-related deterioration in vitamin B-12 status. For example, the percentage of men and women in the UK with a plasma vitamin B-12 concentration <200 pmol/L increases from ~23% in the adult population (aged 19–64 y) (1) to 62% in men and women aged 65 y and older (2). Poor vitamin B-12 status may cause anemia, peripheral neuropathy, and cognitive impairment (3), which can substantially contribute to morbidity

among the elderly. Current concern relates to the potential masking of symptoms of vitamin B-12 deficiency in response to national programs of folic acid fortification of flour, which might improve anemia-related symptoms of vitamin B-12 deficiency but allow the progression of neurological damage (4). Furthermore, there is evidence that folic acid fortification might even exacerbate the anemia of vitamin B-12 deficiency (5).

Many studies use plasma vitamin B-12 as the sole measure of vitamin B-12 status. The measurement of serum or plasma vitamin B-12 is easy and reproducible and the commercial availability of high throughput protein-binding immunoassays makes the method suitable for epidemiological studies. However, low plasma concentrations are not always reflective of poor vitamin B-12 status and patients with clinical evidence of vitamin B-12 deficiency do not always present with low plasma vitamin B-12 concentrations (3,6,7). The predictive value of serum vitamin B-12 for vitamin B-12 deficiency is reported to be as low as 22% (8).

<sup>1</sup> Supported by the Department of Health (study code N05R0014).

<sup>2</sup> Author disclosures: M. H. Hill, J. E. Flatley, M. E. Barker, C. M. Garner, N. J. Manning, S. E. Olpin, S. J. Moat, J. Russell, and H. J. Powers, no conflicts of interest.

<sup>3</sup> This trial was registered at Controlled Trials Database as IRCTN92541398.

<sup>4</sup> Supplemental Figure 1 and Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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There is current interest in the potential value of serum holotranscobalamin (holoTC)<sup>9</sup> as a marker of vitamin B-12 status. The serum holoTC concentration represents the fraction of circulating vitamin B-12 that is available for uptake into cells and should therefore be a better reflection of bioavailable vitamin B-12 than total plasma vitamin B-12 (9,10). A possible drawback is that serum holoTC concentrations are reportedly elevated in people with renal impairment (11), thereby potentially limiting its application in the elderly population. However, we did not find such confounding evidence in a recent cross-sectional study of 600 healthy elderly people in the UK (12).

The plasma MMA concentration constitutes a functional marker of vitamin B-12 status and an elevation is considered to reflect metabolic insufficiency of cobalamin. Adenosylcobalamin is a cofactor for methylmalonyl CoA mutase. Impaired activity of this enzyme leads to an accumulation of methylmalonic acid (MMA) and an elevated concentration in the plasma and in the urine (13,14). Whereas plasma MMA has received a certain amount of attention over the last 2 decades, urinary MMA (uMMA) has been relatively neglected. The uMMA concentration may have advantages over plasma MMA as a functional biomarker of vitamin B-12 status. uMMA is excreted very efficiently by the kidney, which concentrates the metabolite in the urine, making it a potentially sensitive indicator of tissue depletion (15) and a potentially useful early indicator of cellular vitamin B-12 depletion. Several factors are reported to associate with elevated plasma MMA, including impaired renal function and thyroid abnormalities (16,17), limiting its specificity for evaluating vitamin B-12 status. uMMA does not appear to suffer from the problem of falsely elevated values associated with impaired renal function; indeed, impaired renal function has been associated with a lower concentration of uMMA (12), meaning that whereas plasma MMA is known to overdiagnose vitamin B-12 deficiency in a population in which renal dysfunction is prevalent, uMMA may underdiagnose deficiency.

There is potential value in using urinary biomarkers of nutrient status for large-scale nutrition surveys, where appropriate, given the often poor response rate for blood sampling (18).

However, relatively little is known about the responsiveness of uMMA to vitamin B-12 intake. The main aim of this study, therefore, is to investigate the responsiveness of uMMA to supplemental vitamin B-12, determine the time course of this response, and compare the response with other biomarkers of vitamin B-12 status.

## Subjects and Methods

### Subjects

Men and women who participated in a cross-sectional study of biomarkers for vitamin B-12 status were eligible for consideration for recruitment to the randomized controlled trial (12). To recruit 100 men and women with evidence of poor vitamin B-12 status as demarked by a combination of circulating cobalamin and uMMA, the following cutoffs were chosen: plasma vitamin B-12 <250 pmol/L and a uMMA ratio >1.5  $\mu\text{mol MMA}/\text{mmol creatinine}$ .

**Power calculation.** There is no published work to our knowledge describing the response of uMMA to increasing intakes of vitamin B-12. The sample size was estimated on the basis of a percentage reduction in

the uMMA ratio comparable with that reported for plasma MMA in response to vitamin B-12 supplements (19,20). On the basis of a clinically relevant reduction in the uMMA ratio of 35% in our population, with a mean  $\pm$  SD for the uMMA ratio of  $2.58 \pm 1.12 \mu\text{mol MMA}/\text{mmol creatinine}$  (12), a sample of 25/group was judged sufficient for a study with 80% power and significance set at  $P < 0.05$ .

**Randomization.** Supplements (500, 100, and 10  $\mu\text{g}$  cyanocobalamin and placebo) were obtained from Research Products Limited and the identity of the capsules was unknown to the researchers and volunteers. Volunteers were randomized to receive the supplements by an independent third party. As we previously showed plasma pepsinogen to correlate inversely with uMMA, albeit modestly (12), volunteers were stratified for plasma pepsinogen at the following concentrations: <72, 72–118, and >118  $\mu\text{g}/\text{L}$ , which approximated the tertiles observed in the population screened for recruitment to this study (12).

The study received ethics approval from the South Sheffield Research Ethics Committee [National Health Service (Research Ethics Committee reference no. 08/H1309/4)] and Research Governance approval was obtained from the Sheffield Health and Social Research Consortium (National Health Service).

### Study design

**Study protocol.** Volunteers attended the Clinical Research Facility at the Royal Hallamshire Hospital, Sheffield on 2 occasions (baseline and after 8 wk) for blood sampling (Supplemental Fig. 1). Meal consumption is reported to lead to an increase in uMMA excretion (21) and therefore first-voided morning urine samples were collected from fasting participants. Urine samples were collected from each volunteer's home on d 14, 28, and 42; participants brought urine samples to clinic on d 0 and 56.

Blood samples were collected from fasting participants into a serum separating tube and heparinized vacutainers. Aliquots of plasma were stored for the measurement of plasma vitamin B-12, pepsinogen 1, total folate, homocysteine, MMA, and creatinine. An aliquot of serum was stored for the measurement of holoTC. Plasma and serum samples were stored at  $-20^{\circ}\text{C}$  or below. Approximately 10 mL of the fasted urine samples was stored untreated at  $-20^{\circ}\text{C}$  for the measurement of MMA and creatinine.

### Lifestyle questionnaire and physical measurements

At the first visit, lifestyle data, including smoking habits and alcohol consumption, were collected via an interviewer-aided questionnaire. Volunteers' heights and weights were measured and BMI calculated.

### Biochemical measurements

**uMMA and creatinine.** MMA was measured by GC-MS (QP2010 GCMS; Shimadzu Scientific Instruments) as previously described (12). The mean intra-batch CV for lyphocheck quality control (mean 56.9  $\mu\text{mol}/\text{L}$ ) was 3.6%. Creatinine was measured using the kinetic Jaffé reaction on a Konelab 20 analyzer (Thermo Scientific) with a commercial kit (Konelab Creatinine 981811, Thermo Scientific). The mean intra-batch CV for control 1 (mean 7.2 mmol/L) was 9.9% and for control 2 (mean 21.5 mmol/L) was 2.8%. uMMA was expressed relative to urinary creatinine ( $\mu\text{mol MMA}/\text{mmol creatinine}$ , uMMA ratio) to correct for urine dilution.

**Plasma vitamin B-12 and total folate.** Vitamin B-12 and total folate were measured using the Bayer Advia Centaur chemiluminescent protein binding immunoassay on an automated analyzer (Bayer Centaur Immunoassay Analyzer) (12). For vitamin B-12, the mean intra-batch CV was 3.5% and the inter-batch CV was 4.8%; for total folate, the intra-batch CV was 6.1% and the inter-batch CV was 6.2%.

**Serum holoTC.** Serum holoTC was measured using the AxSYM Active B-12 immunoassay (Axis-Shield Diagnostics) (12). The mean intra-batch CV was 6.5% and the inter-batch CV was 10.5%.

**Plasma MMA.** Plasma MMA was measured by University Hospital Wales, Cardiff using a GC-MS assay (22). All samples were run in a single batch; the mean intra-batch CV was 8.0%.

<sup>9</sup> Abbreviations used: holoTC, holotranscobalamin; MMA, methylmalonic acid; tHcy, total homocysteine; uMMA, urinary methylmalonic acid.

**Plasma creatinine and total homocysteine.** Plasma creatinine and homocysteine were measured using commercially available assays for the Abbott Diagnostics ARCHITECT system by University Hospital Wales, Cardiff. All samples were run as a single batch; the mean intra-batch CV for homocysteine was <3% and for creatinine was <2%.

**Plasma pepsinogen I.** The pepsinogen I concentration was measured using a commercial immunoassay (Biohit) according to the manufacturer's instructions. The mean intra-batch CV was 5.0%, the inter-batch CV was 4.9%.

### Statistical analysis

Data were analyzed using SPSS version 20. Associations between biomarkers of vitamin B-12 status were examined using Pearson's correlation. For the uMMA ratio, which was measured at baseline and after 14, 28, 42, and 56 d of vitamin B-12 supplementation, a repeated-measures ANOVA was used with age, gender, baseline values, baseline plasma creatinine, and smoking status as covariates. Sidak correction was applied to correct for multiple comparisons. Any interaction between dose and age, smoking status, and gender was examined. Only significant interactions were included in the final model. For plasma MMA, plasma vitamin B-12, and serum holoTC, a comparison between supplementation levels after 8 wk of intervention was made using ANOVA with age, gender, baseline values, baseline plasma creatinine, and smoking status as covariates. Values are presented as means  $\pm$  SEM corrected for baseline value and baseline plasma creatinine. For the uMMA ratio and plasma MMA, ANOVA was used to compare values at baseline between the ex-smokers and never-smokers. ANOVA was also used to compare the uMMA ratio after 14 d of vitamin B-12 supplementation with baseline values. Significance was accepted at  $P < 0.05$ .

## Results

One hundred men and women were recruited to the trial on the basis of a uMMA ratio  $>1.50$  and plasma vitamin B-12  $<250$  pmol/L at the time of the cross-sectional study (12) (Table 1). One participant withdrew after giving a baseline blood sample (but no urine sample) and a second participant did not provide a postintervention blood sample. Compliance (mean  $\pm$  SD), assessed by number of capsules returned, was  $98\% \pm 4.3$ .

### Baseline biochemistry

Biochemical variables at baseline, according to supplementation group, are shown in Supplemental Table 1.

The uMMA ratio was negatively correlated with plasma vitamin B-12 ( $r = -0.34$ ;  $P = 0.001$ ) and serum holoTC ( $r = -0.39$ ;  $P = 0.001$ ) and positively correlated with plasma MMA ( $r = 0.59$ ;  $P < 0.0001$ ) and plasma total homocysteine (tHcy) ( $r = 0.34$ ;  $P = 0.001$ ).

### Effects of the intervention

When data from all participants were analyzed, the change in the uMMA ratio after 56 d of study correlated with the change in all other biomarkers of vitamin B-12 status: plasma MMA ( $r = 0.57$ ;  $P < 0.0001$ ), serum holoTC ( $r = -0.24$ ;  $P = 0.019$ ), plasma vitamin B-12 ( $r = -0.37$ ;  $P < 0.0001$ ), and plasma tHcy ( $r = 0.42$ ;  $P < 0.0001$ ) (data not shown).

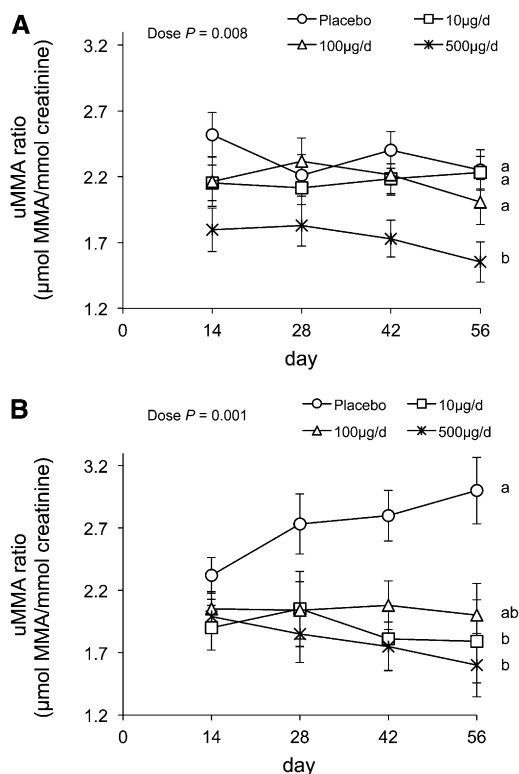
**uMMA:creatinine ratio.** Participants were classified as never-smokers ( $n = 52$ ), current smokers ( $n = 3$ ), or ex-smokers ( $n = 45$ ). There was an effect of dose ( $P < 0.0001$ ) and an interaction among dose, time, and smoking status ( $P = 0.04$ ) on the uMMA ratio. The response to all levels of supplementation occurred early in the intervention, such that values at 14 d were lower than baseline values for all levels of supplementation ( $P < 0.05$ ); the value in the placebo group did not change. When the data were examined according to smoking status, the response in the uMMA ratio was different between never-smokers and ex-smokers and for this reason it was decided to stratify the analysis by smoking status. The 3 current smokers were excluded from the stratified analysis.

There was an effect of dose on the uMMA ratio in both the never-smokers ( $P = 0.008$ ) and the ex-smokers ( $P = 0.001$ ) (Fig. 1). For both the never-smokers and the ex-smokers, the uMMA ratio was lower after 14 d of 500  $\mu\text{g/d}$  than at baseline ( $P < 0.05$ ). Among the never-smokers, the reduction in the uMMA ratio after the 56 d of supplementation was greater for the 500- $\mu\text{g/d}$  dose than for the placebo and the 10- and 100- $\mu\text{g/d}$  doses ( $P < 0.01$ ). The effects of 10 and 100  $\mu\text{g/d}$  on the uMMA ratio did not differ from placebo, nor were they different from each other. Among the ex-smokers, both the 10- $\mu\text{g/d}$  dose ( $P < 0.05$ ) and the 500- $\mu\text{g/d}$  dose ( $P < 0.001$ ) elicited a significant response during the 56 d compared with the placebo group. The reduction in response to 100  $\mu\text{g/d}$  was of borderline significance compared with placebo ( $P = 0.056$ ) and did not differ from the response to 10 or 500  $\mu\text{g/d}$ .

**TABLE 1** Characteristics of participants according to randomization group<sup>1</sup>

	All	Placebo	10 $\mu\text{g/d}$ Vitamin B-12	100 $\mu\text{g/d}$ Vitamin B-12	500 $\mu\text{g/d}$ Vitamin B-12
<i>n</i>	100	25	25	25	25
Age, <i>y</i>	71.0 (65–86)	72.5 (65–84)	69.0 (65–80)	70.0 (65–81)	71.5 (66–86)
BMI	27.1 (17.4–52.7)	27.9 (18.0–34.0)	26.96 (21.3–52.7)	25.9 (17.4–38.0)	27.5 (18.4–37.3)
Smoking status, <i>n</i>					
Never-smoker	52	12	15	10	12
Ex-smoker	45	11	10	14	13
Current smoker	3	2	0	1	0
Gender, <i>n</i>					
Male	47	11	15	11	10
Female	53	14	10	14	15
Vitamin B-12 status at screening					
Plasma vitamin B-12, <i>pmol/L</i>	201 (107–249)	188 (122–249)	202 (115–239)	216 (127–249)	183 (107–245)
uMMA ratio, $\mu\text{mol/mmol creatinine}$	2.08 (1.55–10.1)	2.15 (1.65–5.14)	2.22 (1.62–4.11)	2.05 (1.57–7.64)	2.06 (1.55–10.1)

<sup>1</sup> Values are medians (minimum–maximum). uMMA, urinary methylmalonic acid.



**FIGURE 1** The uMMA ratio after 14, 28, 42, and 56 d of intervention, according to daily supplement of vitamin B-12, in never-smokers (A) and ex-smokers (B). Values are means  $\pm$  SEM, corrected for baseline uMMA ratio and plasma creatinine,  $n = 10$ – $15$  (A) or  $10$ – $14$  (B). On d 56, means without a common letter differ,  $P < 0.05$ . uMMA, urinary methylmalonic acid.

**Plasma MMA.** As for the uMMA ratio, there was an interaction between dose and smoking status ( $P = 0.04$ ) and therefore the sample was stratified by smoking status for further analysis. Plasma MMA was higher in the placebo group in ex-smokers at baseline than in the never-smokers ( $P < 0.05$ ). Values did not differ between smoking groups at baseline for any level of supplement. In the never-smokers, there was an effect of dose on plasma MMA ( $P = 0.043$ ) (Fig. 2). Plasma MMA was lower after 56 d of supplementation with 500  $\mu\text{g}/\text{d}$  compared with placebo, 10  $\mu\text{g}/\text{d}$ , and 100  $\mu\text{g}/\text{d}$  ( $P < 0.05$ ). A significant effect of dose on plasma MMA ( $P < 0.0001$ ) was also seen in the ex-smokers; pairwise comparisons showed a reduction in plasma MMA in response to all doses compared with placebo ( $P < 0.02$ ). There were no significant differences between doses.

**Plasma vitamin B-12 and serum holoTC.** There was no effect of smoking status on response in either plasma vitamin B-12 or serum holoTC and therefore data were not stratified for either of these variables. There was an increase in plasma vitamin B-12 in response to vitamin B-12 supplements ( $P = 0.007$ ) (Fig. 3). The response in plasma vitamin B-12 was significant at all doses compared with placebo ( $P < 0.0001$ ) and the 500- $\mu\text{g}/\text{d}$  dose elicited a greater response than 10 or 100  $\mu\text{g}/\text{d}$  ( $P < 0.0001$ ). There was also a dose effect on serum holoTC ( $P = 0.018$ ); the increase was significant at all doses compared with placebo ( $P < 0.01$ ). The response to 500  $\mu\text{g}/\text{d}$  was also greater than for 10 or 100  $\mu\text{g}/\text{d}$  ( $P < 0.0001$ ).

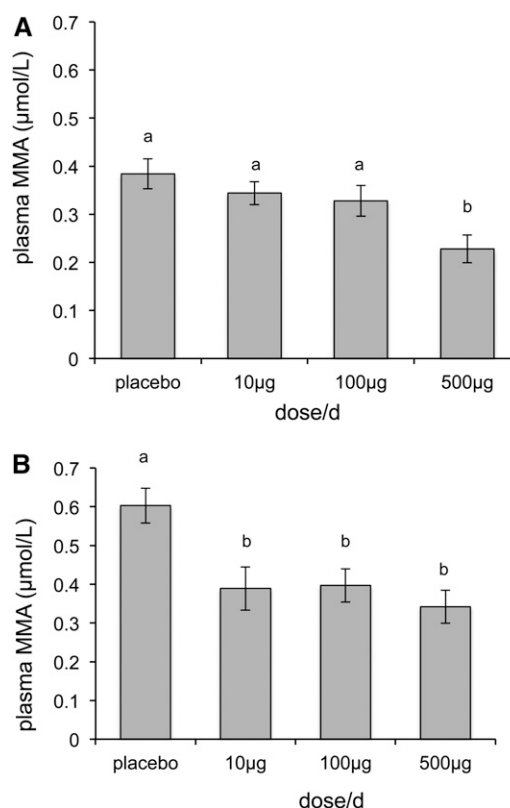
The lowest dose of vitamin B-12 was sufficient to bring values for plasma vitamin B-12 above 200 pmol/L [defined by Clarke et al. (23) as the threshold below which there is “metabolically significant

vitamin B-12 deficiency”] and serum holoTC above 37 pmol/L (24) for all but 15% of participants, whereas  $>40\%$  of participants still had a plasma MMA  $>0.35 \mu\text{mol}/\text{L}$  (3) and a uMMA ratio  $>2.0$  (15). Even after 56 d of receiving a supplement of 500  $\mu\text{g}/\text{d}$  vitamin B-12,  $\sim 10\%$  of the participants still had plasma vitamin B-12 and serum holoTC below the stated thresholds, while 23 and 15% of the participants still had elevated MMA in plasma and urine, respectively.

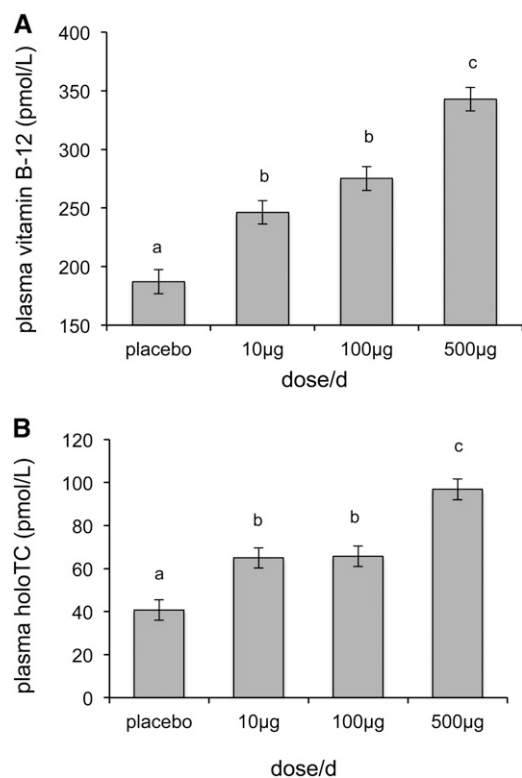
**Plasma folate and homocysteine.** There was no effect of the vitamin B-12 supplement on plasma total folate concentration. Plasma tHcy did change in response to the supplement ( $P = 0.001$ ). After 8 wk of supplementation, the concentration of plasma tHcy was lower ( $P < 0.01$ ) in the groups receiving 100  $\mu\text{g}/\text{d}$  ( $15.8 \pm 0.31 \mu\text{mol}/\text{L}$ ) or 500  $\mu\text{g}/\text{d}$  ( $15.1 \pm 0.30 \mu\text{mol}/\text{L}$ ) compared with placebo ( $14.6 \pm 0.31 \mu\text{mol}/\text{L}$ ) or 10  $\mu\text{g}/\text{d}$  ( $14.0 \pm 0.30 \mu\text{mol}/\text{L}$ ) but did not differ from one another.

## Discussion

In this study, biomarker response to cobalamin supplements was dose dependent, reflecting incremental increases in the diffusion component of cobalamin absorption against a saturation of the intrinsic factor-mediated absorption at  $\sim 1.5$ – $2.0 \mu\text{g}$  cobalamin (25). Both urinary and plasma MMA response were influenced by smoking status, with the response to supplement enhanced in ex-smokers. Few studies have examined the response of plasma MMA to vitamin B-12 supplements (19,20,26,27) and none to our knowledge reported recording smoking status of the



**FIGURE 2** Plasma MMA after 56 d of intervention, according to daily supplement of vitamin B-12, in never-smokers (A) and ex-smokers (B). Values are means  $\pm$  SEM, corrected for baseline plasma MMA and creatinine,  $n = 10$ – $15$  (A) or  $10$ – $14$  (B). Means without a common letter differ,  $P < 0.05$ . MMA, methylmalonic acid.



**FIGURE 3** Plasma vitamin B-12 (A) and serum holoTC (B) after 56 d of intervention according to daily supplement of vitamin B-12. Values are means  $\pm$  SEM, corrected for baseline value and plasma creatinine,  $n = 24$  or  $25$ . Means without a common letter differ,  $P < 0.01$ . holoTC, holotranscobalamin.

participants. We have no explanation for this effect, but the fact that the uMMA ratio differed according to smoking status in the cross-sectional study (12) and the congruence of effect for both uMMA ratio and plasma MMA suggest that the observation is real. We cannot exclude the possibility that smoking status might be a surrogate for some other, as yet unknown factor.

The decrease in the uMMA ratio occurred rapidly in the first 2 wk of supplement, with no further decrease thereafter, suggesting that the limiting effects of poor vitamin B-12 status on cellular metabolism can be rapidly mitigated by vitamin B-12 supplementation, especially at a high dose. There are no other trials to our knowledge using uMMA ratio as an outcome, for comparison, and trials utilizing serum or plasma MMA as outcome variable have made measurements only after between 6 and 16 wk of intervention (19,20,26).

Both plasma vitamin B-12 and serum holoTC had a dose response to cobalamin supplements and the response was not influenced by smoking status. Other studies have used supplements between 2.5 and 1000  $\mu\text{g}/\text{d}$  and intervention periods between 1 and 6 mo. There is reasonable consistency in the selection of participants for intervention of those with plasma vitamin B-12 in the range of 100–300 pmol/L and/or plasma MMA  $>0.26 \mu\text{mol}/\text{L}$ . Overall, results indicate that a vitamin B-12 supplement as low as 2.5  $\mu\text{g}/\text{d}$  can elicit an improvement in plasma vitamin B-12 or MMA in some people and that response rate improves as the dose increases (19,20). Eussen et al. (20) calculated that a dose of 449  $\mu\text{g}/\text{d}$  would achieve maximum increase in plasma holoTC in elderly men and women with mild vitamin B-12 deficiency, while a dose of 830  $\mu\text{g}/\text{d}$  would be required to achieve maximum reduction in plasma MMA. Rajan et al. (17) found that while 25  $\mu\text{g}/\text{d}$  for 6 wk elicited a reduction

in mean plasma MMA, a dose of 1000  $\mu\text{g}/\text{d}$  was required to lower MMA to  $<0.27 \mu\text{mol}/\text{L}$  in all but 1 of 23 participants. Our findings were similar to both these trials in some important aspects. Plasma vitamin B-12 and serum holoTC showed greater proportional increases in response to low-dose vitamin B-12 than urinary or plasma MMA and a greater percentage of participants had values in the normal range following the high-dose supplement than for uMMA ratio or plasma MMA. However, even after receiving 500  $\mu\text{g}/\text{d}$  for 8 wk,  $\sim 10\%$  of people had plasma vitamin B-12 and serum holoTC concentrations outside the normal range. Results suggest that dietary reference values for vitamin B-12 in older people may be too low; the current reference nutrient intake for adult men and women in the UK and US is 1.5 and 2.4  $\mu\text{g}/\text{d}$ , respectively. Although others have examined response in plasma MMA to vitamin B-12 supplements (19,20,28), uMMA has been relatively neglected in this regard. The uMMA ratio and plasma MMA, as indicators of metabolic impairment, had a similar pattern of response to supplementation. The use of a plasma MMA threshold of  $>0.35 \mu\text{mol}/\text{L}$ , defined as “elevated MMA” by Clarke et al. (3), rather than the 0.27  $\mu\text{mol}/\text{L}$  often used by others (19,20) probably explains the apparently more modest response in the uMMA ratio compared with plasma MMA in our study. The lack of normalization of biomarkers after 8 wk of 500  $\mu\text{g}/\text{d}$  cobalamin might reflect an underlying malabsorption (28) or, for MMA biomarkers, influences of gastrointestinal production of propionate (29), or in dietary intakes of nonstarch polysaccharides, a fermentable source of propionate (30,31). There was little evidence that renal impairment might have explained why the plasma MMA concentrations did not fall further. Only 2 men and 2 women had plasma creatinine concentrations greater than the accepted gender-specific thresholds (32). Results might, however, suggest inappropriate thresholds for vitamin B-12 biomarkers.

In their systematic review of biomarker response to intervention with vitamin B-12, Hoey et al. (33) concluded that plasma/serum vitamin B-12 and MMA are both responsive to changes in vitamin B-12 intake but with considerable heterogeneity for plasma vitamin B-12. They identified only one small trial for which plasma holoTC was an outcome and this reported a significant increase in response to 24 wk of supplementation with 1000  $\mu\text{g}/\text{d}$  cyanocobalamin. They did not identify any study using the uMMA ratio as biomarker. Our study makes an important contribution to the understanding of biomarker response to vitamin B-12 supplements in that it uses a range of supplemental levels from physiological to pharmacological and examines 4 markers of vitamin B-12 status, including 2 functional biomarkers. Furthermore, it reports the temporal response in the uMMA ratio, which is the particular biomarker of interest in this study.

In conclusion, healthy elderly people in the UK with biomarker evidence of poor vitamin B-12 status respond to daily oral supplements of vitamin B-12 in a dose-dependent manner. Although improvements in biomarkers of vitamin B-12 status can be achieved in some people at doses as low as 10  $\mu\text{g}/\text{d}$ , a dose of 500  $\mu\text{g}/\text{d}$  was required to normalize plasma vitamin B-12 and serum holoTC in  $\sim 90\%$  of people, but this was sufficient to correct metabolic deficiency in only 75–85% of people. The current Reference Nutrient Intake for dietary vitamin B-12 in elderly people may be too low to achieve vitamin B-12 status compatible with functional adequacy. The uMMA ratio responds in a comparable manner to plasma MMA and should be considered as a functionally relevant biomarker of vitamin B-12 status applicable for large surveys, especially where blood

sampling limits compliance. However, the effect of smoking behavior on response in MMA to vitamin B-12 supplements needs further consideration.

### Acknowledgments

Professor Anne Molloy of Trinity College Dublin was responsible for the measurement of serum holoTC. H.J.P. and M.E.B. conceived the study; J.R. carried out the statistical analyses; M.H.H., J.E.F., and C.M.G. carried out the intervention; S.E.O. and N.J.M. conducted the uMMA and creatinine analyses; S.J.M. carried out the measurements of plasma MMA; and H.J.P. drafted the manuscript. All authors read and approved the final manuscript.

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