Impact of Chronic Use of Proton Pump Inhibitor Drugs on Vitamin B12 Status, and the Role of Fortified Foods

This project report has been prepared in accordance to the instructions to authors for *British Journal of Nutrition*

This paper reports on project data collected as part of a larger on-going study. Together with another student, I was involved in input of participant information, maintenance of accurate records of stored blood samples, maintenance of accurate compliance records and entry of diet records onto diet analysis software between October 2012 and April 2013. The data used for the purposes of this paper represents data collected over a longer period which I was permitted to analyse. I undertook all of the statistical analyses reported in this paper.

I hereby confirm that I am the major Author of this paper and work described therein and that this work has been conducted using humane and ethical procedures, in accordance with Research Ethics and Governance Policies and Procedures, and other Research Policies and Procedures including the Code of Practice for Professional Integrity in the Conduct of Research. I also confirm that this work is not plagiarised, does not infringe copyright and provides a full disclosure of relevant interests.

Abstract

Subnormal vitamin B12 status is a widespread problem and has been associated with chronic disease. Proton pump inhibitor (PPI) drugs reduce gastric acid secretion, possibly impairing absorption of food bound vitamin B12 but not free B12 found in fortified foods. The aim of this study was to compare vitamin B12 status between chronic PPI users and non PPI users, and to consider the impact of fortified foods on B12 status in PPI users. The sample in this observational study consisted of chronic (>1 year) PPI users (n = 109) and non PPI users (n = 96) who were matched for age and fortified food consumption. Differences in vitamin B12 status between these groups were assessed using serum vitamin B12 and serum holotranscobalamin (holoTC) measurements. Among PPI users differences in vitamin B12 status were assessed between those who consumed fortified foods and those who did not. Unexpectedly PPI users had a higher (P = 0.018) median serum vitamin B12 concentration of 334pmol/L compared to non PPI users (268pmol/L); however, PPI users had higher intakes of vitamin B12 than non PPI users, bordering on statistical significance (P = 0.076). Among PPI users higher concentrations of both biomarkers were observed in those who consumed fortified foods compared with those who did not (P < 0.001), correspondingly no fortified food consumers had biomarker concentrations indicative of deficiency compared with non-fortified food consumers (10%). In conclusion, these results do not provide evidence that PPIs impair vitamin B12 status. Vitamin B12 status in PPI users can be improved by the consumption of fortified foods.
Word Count: 258

Keywords: Proton Pump Inhibitors, Vitamin B12, Cobalamin, Fortified Food, Malabsorption.

Abbreviations: SCD, Subacute Combined Degeneration; PPI, Proton Pump Inhibitor; GORD, Gastrooesophageal Reflux Disease; HoloTC, Holotranscobalamin; EI, Energy Intake; FFC, Fortified Food Consumer; MMA, Methylmalonic acid; DRV, Dietary Reference Value.

Introduction

Subnormal vitamin B12 (cobalamin) status is a major public health problem and is estimated to affect up to 43% of those aged over 60 years (Wolters *et al.* 2004). Classical deficiency leads to macrocytic anaemia and irreversible subacute combined degeneration (SCD) of the spinal cord, which is fatal if left untreated. Classical deficiency, referred to as pernicious anaemia, is an autoimmune disease involving intrinsic factor deficiency and is relatively uncommon. It is most prevalent in the elderly and is estimated to affect 5-10% of those aged over 65 years (Clarke *et al.* 2004). A far more widespread and potentially more problematic issue is subclinical deficiency of vitamin B12 which is most often caused by food bound B12 malabsorption. Subclinical deficiency is an asymptomatic and more subtle depletion of cobalamin so is more likely to go undetected. It is not associated with classical deficiency symptoms mentioned above, but vitamin B12 status will be suboptimal. The major problems that can arise due to subclinical deficiency are considered to be as a result of abnormal status of vitamin B12 biomarkers as opposed to low B12 per se. Abnormal concentrations of these biomarkers have been associated with a number of chronic diseases such as vascular disease (Huang *et al.* 2012), bone disease (Herrmann *et al.* 2007) and cognitive dysfunction (Van Dam *et al.* 2009), meaning that subclinical vitamin B12 deficiency could have very serious long-term consequences at both an individual and population level.

Normal release of vitamin B12 from food proteins and subsequent absorption rely on adequate gastric acid and a low gastric pH, otherwise food bound B12 malabsorption may occur (Carmel 2003). It is thought that proton pump inhibitors (PPIs) reduce the efficacy of this crucial step in vitamin B12 absorption. PPIs have an inhibitory effect on H^+/K^+ Adenosine Triphosphatase (proton pumps) of parietal cells and thereby inhibit gastric acid secretion in affected cells (Shi *et al.* 2008). Long-term use of PPIs can lead to prolonged hypochlorhydria; giving rise to concerns that they may decrease vitamin B12 release, and thereby absorption, from food. This may lead to subclinical deficiency or even classical deficiency in those whose vitamin B12 status is already compromised (Ali *et al.* 2009; Hirschowitz *et al.* 2008). The use of PPIs is widespread and they are widely prescribed (Business Services Organisation 2012). PPIs are commonly prescribed for the treatment of conditions characterised by excess gastric acid secretion such as gastro oesophageal reflux disease (GORD) and peptic ulcers. They are also prescribed to prevent ulceration in those on long-term treatment with non-steroidal anti-inflammatory drugs (Shi *et al.* 2008).

The scientific literature regarding vitamin B12 and PPIs has proved to be conflicting and often inconclusive. Many of the results are based on measurements of serum total vitamin B12; however more sensitive measurements are now available, such as plasma homocysteine and serum holotranscobalamin (holoTC), to assess both clinical and subclinical deficiency. A number of papers suggest that long-term use of PPIs decrease serum vitamin B12 concentrations and this is particularly evident in the elderly who are already more likely to suffer from food bound B12 malabsorption due to gastric atrophy (Wolters *et al.* 2004; Schenk *et al.* 1996; Kapadia 2000). An inverse dose-response relationship between PPIs and vitamin B12 absorption has also been shown (Marcuard *et al.* 1994). Hirschowitz *et al.* (2008) found that serum vitamin B12 concentrations declined in those on long-term PPI treatment and that subclinical B12 deficiency was present. Den Elzen *et al.* (2008), on the other hand, found no association between PPI use and vitamin B12 status. Reviews that have been carried out vary greatly in their design and many focus their end-point on classical clinical deficiency of vitamin B12 with little emphasis being placed on subclinical deficiency, and as such do not recognise any major effect of PPIs on B12 biomarker status (Thomson *et al.* 2010; Cote *et al.* 2008). Furthermore many of the studies were not adequately randomised or were poorly controlled. It does appear that the elderly are at particular risk due to the age-related decline in gastrointestinal function (Ali *et al.* 2009; Sheen *et al.* 2011).

Food bound vitamin B12 malabsorption is the primary cause of subclinical deficiency (Carmel *et al.* 2003) so fortified foods, containing free B12, provide a potential means by which people with food bound B12 malabsorption can obtain and effectively absorb B12 from their diet. Free vitamin B12 is not dependent on a low gastric pH for absorption as it is unbound to food proteins. Those taking PPIs for prolonged periods are at greatest risk of poor vitamin B12 status (Sheen *et al.* 2011) but in theory any noticeable decline in B12 may be countered by fortified food consumption. This is not surprising considering that a poor vitamin B12 status in UK adults has been associated with low consumption of breakfast cereals (Scientific Advisory Committee on Nutrition 2008), which are commonly fortified with B12. It is recommended that the concentration of vitamin B12 found in these foods should be adequate to protect against subclinical deficiency in those with food bound B12 malabsorption but current vitamin B12 fortification levels are typically low in the UK (Blacher *et al.* 2007; Carmel 1997).

The aim of this study was to compare biomarkers of vitamin B12 status between chronic PPI users and non PPI users matched for age and fortified food consumption, and to consider the potential impact of fortified foods on B12 status in PPI users.

Methods

Participants and study design

This project was carried out as part of a larger on-going study regarding chronic PPI use and vitamin B12 status. The current study was an observational investigation involving the recruitment of two distinct groups: chronic PPI users and non-PPI users. Non-PPI users were selected (Appendix 1: Figure 1) from a previous study in which data on B vitamin intake and biomarker status had already been gathered (Hoey *et al.* 2007). These were healthy adults aged ≥18yrs. The initial sample was comprised of 662 people, of which 441 provided data on both B vitamin intake and status. The following exclusion criteria were implemented: pregnancy; use of B vitamin supplements in previous 6 months; self-reported history of gastrointestinal, cardiovascular, hepatic, renal or haematologic disease; use of medications that interfere with B vitamin metabolism; plasma creatinine of >130μmol/L; and a score of <25 on Folstein's Mini-Mental State Examination in those ≥60years of age, as those with a lower score may be less able to accurately recall food intake. Of the 441 available participants 96 were matched for age and fortified food consumption with the PPI user group.

The initial sample for the PPI user group was identified from a local General Practitioner practice patient list (n=1291). From this sample potential participants were excluded who were not regular users of PPIs or who had not been prescribed PPIs for >1year. Those who were receiving B12 injections or taking multivitamins were also excluded. Further exclusions were also made based on health and lifestyle factors as detailed in Appendix 1: Figure 1. The remaining group (n=288) were contacted via their GP to gain verbal consent to be contacted by the research team in relation to the study. A final sample of 109 suitable people agreed to take part in the current study. Ethical approval was obtained from the Office for Research and Ethics Committees Northern Ireland and all participants provided written informed consent (Ref: 06/NIR01/116).

Dietary analysis

Participants completed both a four-day food diary and a FFQ, a method which was developed and validated in a previous study at this centre to measure B vitamin intake against biomarker data (Hoey *et al.* 2007). Participants completed a four-day food diary to give an indication of normal dietary intake. The diary included a Saturday and Sunday for all participants. Written and verbal instructions were given to participants for carrying out the food records. The FFQ measured the intake of fortified foods such as certain food

groups or specific brands that are fortified and also acted as a means of assessing the accuracy of the information recorded in the food diary. Fortified food consumers were defined as those who consumed foods fortified with B vitamins at least once per week. Any discrepancies that arose in participants' food records were discussed with the participant and corrected within one week of completing the record.

The recorded dietary intake was analysed using the Weighed Intake Software Package (WISP, version 3; Tinuviel Software, Anglesey, United Kingdom), in particular energy and Vitamin B12 intake. The WISP database was modified to allow differentiation between synthetic B vitamin intake and natural B vitamin intake. The database was customised to include the synthetic forms of the vitamins as new nutrients; new food codes were then created to enter the nutritional information for foods that were fortified with the synthetic forms of these B vitamins. This information was acquired directly from the manufacturers where possible or otherwise from labels on the food packaging (Hoey *et al.* 2007).

Height and weight was measured for all participants and BMI was calculated. This allowed for the accuracy of the 4-day food records to be tested by estimating basal metabolic rates using the Oxford equations (Henry 2005). The ratio of energy intake (EI) to BMR could then be calculated and used to assess the accuracy of the food records and identify those likely to have underreported dietary intakes (Black 2000).

Statistical analysis

Statistical analysis was carried out using SPSS software (version 20; SPSS UK Ltd, Chersey, United Kingdom). Prior to analysis skewed data were log transformed to allow a normal distribution to be established, and $P < 0.050$ was considered significant. The participants were categorised as either PPI users or non PPI users. Differences between these groups were analysed using independent sample *t* test for numerical values and chi-square tests for categorical variables. The above groups were further subdivided into fortified food consumers and non-fortified food consumers giving rise to a total of four groups; differences between these groups were examined using one-way analysis of variance with Bonferroni's post hoc test. Among the PPI user group differences between those who consumed fortified foods and those who did not were analysed using independent samples *t* test. Correlations between variables were carried out using Pearson's correlation coefficients. Dietary data was only available for 49 of the 109 PPI users.

Results

Of the 109 PPI users identified for the study, 53 were matched for age and fortified food consumption with the non PPI users (n=96). The general characteristics of PPI users and non PPI users are shown in **Table 1**. There were no significant differences in the general characteristics between the two groups. As per matching protocol, both groups were of similar age and had a similar proportion of fortified food consumers. There were no differences between the groups in relation to gender or BMI. Among the PPI users, the median duration of PPI usage was 6 years. Dietary intakes were also similar between the groups, however, the median EI:BMR ratio was 1.0 in PPI users and 1.2 in non PPI users and this difference was significant with $P = 0.013$; probably indicative of a greater degree of under-reporting of energy intakes in the PPI user group. Intakes of both total vitamin B12 and natural B12 tended to be higher in PPI users, with these differences approaching statistical significance in both cases. The B vitamin biomarker concentrations shown in **Table 2** were all within the reference ranges. Contrary to expectations PPI users had a significantly higher serum vitamin B12 value ($P = 0.018$) probably reflecting their higher vitamin B12 intakes, though in the case of serum holoTC this did not reach significance.

The association between B vitamin dietary intakes and biomarker status was also investigated (data not shown). Among the sample comprising of both PPI users and non PPI users with available dietary data (n = 115), there was a good correlation between dietary intake of folate with both serum folate concentrations (r = 0.280, $P = 0.003$) and red blood cell folate (r = 0.308, $P = 0.001$). In the case of vitamin B12, however, no significant correlations were observed. Serum holoTC concentrations tended to be better correlated with vitamin B12 intake (r = 0.106, $P = 0.272$) than did serum vitamin B12 (r = - 0.025, $P = 0.792$), however, not significantly so in either case. Similar and also non-significant trends for vitamin B12 were also seen when analysing both PPI users and non PPI users separately. **Table 3** shows the differences in dietary intake and laboratory analysis across the four groups of PPI users and non PPI users, both of which have been further subdivided into fortified food consumers and non-fortified food consumers.

Figure 2 and **3** examine the effect of fortified food consumption among the total group of PPI users (n = 109). There were significantly higher concentrations of both serum B12 ($P < 0.001$) and serum holoTC ($P < 0.001$) in fortified food consumers compared with non-fortified food consumers. In addition, no participant was found to have a biomarker value indicative of vitamin B12 deficiency among PPI users who consumed fortified foods compared with those who did not (10%).

The impact of fortified food on vitamin B12 status in PPI users was also examined in relation to duration of PPI usage (**Table 4**). Among those using PPIs for ≤ 4 years the fortified food consumers had a higher serum vitamin B12 concentration ($P = 0.026$), yet the difference in serum holoTC concentrations was not significant ($P = 0.228$). Among those who had been using PPIs for > 4 years, significantly higher concentrations of both biomarkers were found in fortified food consumers.

Table 1. Selected characteristics of study participants according to Proton Pump Inhibitor (PPI) usage.

	PPI Users (n = 53)[1]	Non PPI Users (n = 96)	*P*[2]
General Characteristics			
Age (yrs)	65.0 (56.5, 72.0)	63.0 (56.3, 72.0)	0.910
Gender (% female)	65	53	0.160
Weight (kg)	74.5 (63.0, 83.7)	80.0 (66.7, 92.5)	0.095
BMI (kg/m^2)	27.7 (25.8, 30.5)	27.4 (24.9, 30.0)	0.865
Fortified Food Consumers (%)	75	70	0.461
Duration of PPI usage (years)	6.0 (4.5, 6.8)		
Smoker (%)	16	4	0.071
Dietary intake[3]			
Energy (MJ/d)	6.660 (6.170, 8.100)	7.39 (6.145, 8.450)	0.228
Energy intake: BMR	1.0 (0.9, 1.3)	1.2 (1.0, 1.4)	0.013
Total vitamin B12 (µg/d)[4]	3.8 (2.8, 6.1)	3.5 (2.6, 4.5)	0.076
Natural Vitamin B12	3.8 (2.6, 5.9)	3.4 (2.5, 4.5)	0.062
Free Vitamin B12	0.0 (0.0, 0.2)	0.1 (0.0, 0.2)	0.609
Total folate (µg/d)[4]	195 (180, 251)	233 (187, 272)	0.223
Natural Folate	184 (161, 236)	197 (165, 227)	0.713
Folic Acid	9 (0, 41)	25 (0, 50)	0.438

Values given as median (IQR) unless otherwise stated.

[1] Of these 53, dietary intake data were only available for 19.

[2]For continuous variables, the independent-samples *t* test was used; for categorical variables the chi-squared test was used, on log-transformed data where applicable. P<0.050 is considered significant.

[3]The RNI for Vitamin B12 is 1.5µg/d, the RNI for folate is 200µg/d for males and females >50 years.

[4] Total vitamin B12 intake comprised of B12 present naturally in food and synthetic free B12 added voluntarily to foods by manufacturers; total folate intake comprised of intake of folate present naturally in food and intake of synthetic folic acid added voluntarily to foods by the manufacturers.

Table 2. Selected characteristics and laboratory analysis of study participants according to Proton Pump Inhibitor (PPI) usage.

	PPI Users (n = 53)[1]	Non PPI Users (n = 96)	*P*[2]
General characteristics			
Age (yrs)	65.0 (56.5, 72.0)	63.0 (56.3, 72.0)	0.910
Fortified Food Consumers (%)	75	70	0.461
Dietary intake[3]			
Total vitamin B12 (µg/d)[4]	3.8 (2.8, 6.1)	3.5 (2.6, 4.5)	0.076
Natural Vitamin B12	3.8 (2.6, 5.9)	3.4 (2.5, 4.5)	0.062
Added Vitamin B12	0.0 (0.0, 0.2)	0.1 (0.0, 0.2)	0.609
Total folate (µg/d)[4]	195 (180, 251)	233 (187, 272)	0.223
Natural Folate	184 (161, 236)	197 (165, 227)	0.713
Folic Acid	9 (0, 41)	25 (0, 50)	0.438
Laboratory analysis			
Serum vitamin B12 (pmol/L)	334 (260, 392)	268 (199, 347)	0.018
Serum holoTC (pmol/L)	57.6 (44.7, 82.1)	49.7 (38.8, 66.5)	0.141
Plasma homocysteine(µmol/L)	10.8 (8.7, 13.6)	10.6 (8.5, 12.5)	0.628
Red blood cell folate (nmol/L)	862 (629, 1355)	753 (599, 1023)	0.042
Serum folate (nmol/L)	19.6 (11.4, 28.6)	17.5 (11.5, 25.4)	0.583

Values given as median (IQR) unless otherwise stated.

[1] Of these 53, dietary intake data were only available for 19.

[2]For continuous variables, the independent-samples *t* test was used; for categorical variables the chi-squared test was used, on log-transformed data where applicable. P<0.050 is considered significant.

[3]The RNI for Vitamin B12 is 1.5µg/d, the RNI for folate is 200µg/d for males and females >50 years.

[4] Total vitamin B12 intake comprised of B12 present naturally in food and synthetic free B12 added voluntarily to foods by manufacturers; total folate intake comprised of intake of folate present naturally in food and intake of synthetic folic acid added voluntarily to foods by the manufacturers.

Table 3. Selected characteristics of proton pump inhibitor (PPI) users and non PPI users according to fortified food consumption.

	PPI Users (n = 53)		Non PPI users (n = 96)		
	Non FFC	FFC	Non FFC	FFC	
	(n = 13)	(n = 40)	(n = 29)	(n = 67)	P^1
Age	65.0 (55.0, 74.0)	63.5 (56.5, 71.8)	64.0 (59.5, 71.0)	63.0 (54.0, 73.0)	0.981
Dietary intake[2]					
Energy (MJ/d)	7.390 (6.205, 8.800)	6.660 (5.960, 7.960)	7.510 (6.220, 8.795)	7.170 (6.130, 8.430)	0.423
Total vitamin B12 (µg/d)	4.3 (3.4, 7.7)	3.8 (2.8, 6.1)	3.6 (2.7, 5.0)	3.3 (2.6, 4.3)	0.246
Total folate (µg/d)	214.0 (172.8, 250)[a, b]	195.0 (179.8, 263)[a, b]	192.0 (161.5, 227.5)[a]	246.0 (205.0, 277)[b]	0.001
Laboratory analysis					
Serum vitamin B12 (pmol/L)	262 (226, 346)[a, b]	338 (280, 402)[a]	246 (217, 315)[b]	278 (196, 360)[b]	0.042
Serum HoloTC (pmol/L)	48.8 (31.9, 94.6)	61.2 (44.9, 82.1)	52.3 (38.2, 70.4)	48.7 (38.8, 66.5)	0.386
Plasma homocysteine(µmol/L)	13.4 (12.7, 15.7)[a]	10.0 (8.3, 12.1)[b]	12.2 (9.2, 14.4)[a, b]	10.5 (8.3, 11.9)[b]	0.009
Red blood cell folate (nmol/L)	724 (487, 990)[a, b]	911.6 (727, 1412)[a]	696.0 (602, 842)[b]	768.0 (595, 1039)[a, b]	0.022
Serum folate (nmol/L)	10.6 (9.0, 16.9)[a]	22.9 (15.6, 30.4)[b]	14.5 (9.9, 19.8)[a, c]	18.9 (12.3, 25.9)[b, c]	<0.001

Values given as Median (IQR)
FFC, Fortified Food Consumer; HoloTC, Holotranscobalamin
[1]ANOVA was used with Bonferroni post hoc test on log transformed data where applicable, different lettering within rows indicate significant differences. P<0.050 is considered significant.
[2]The RNI for Vitamin B12 is 1.5µg/d, the RNI for folate is 200µg/d for males and females >50 years.

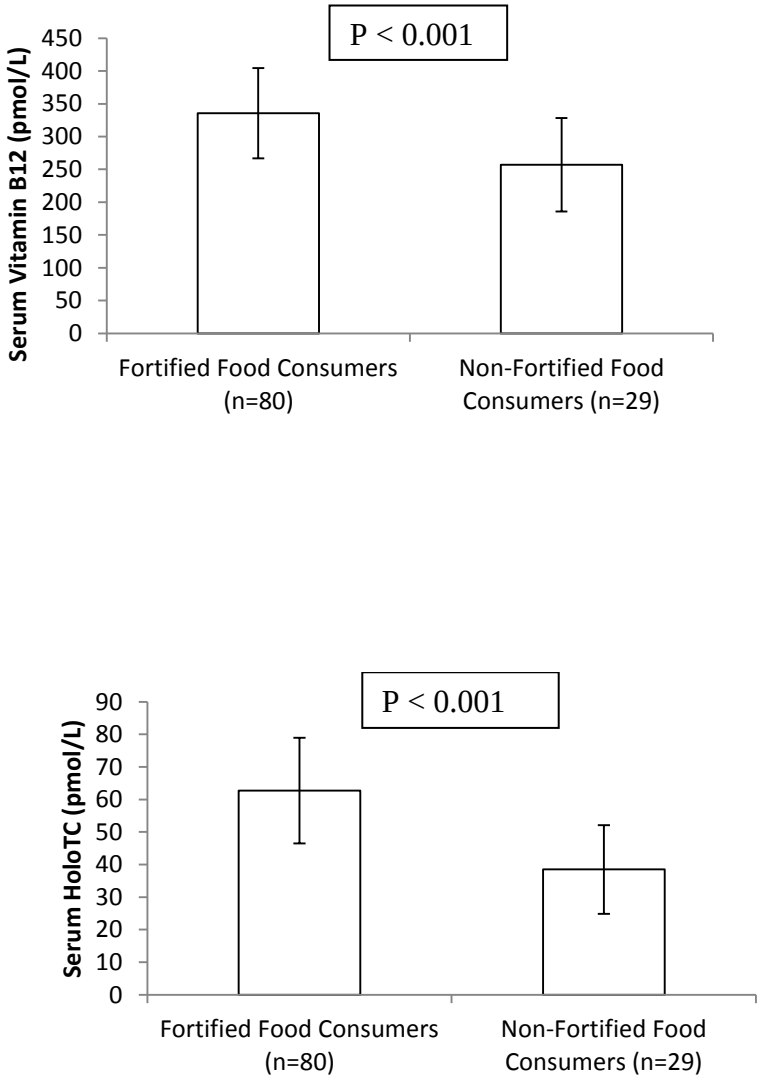

Figure 2. The impact of fortified food consumption on vitamin B12 status based on serum vitamin B12 (upper plot) concentrations and serum holotranscobalamin concentrations (lower plot). Columns represent median and error bars represent interquartile range.

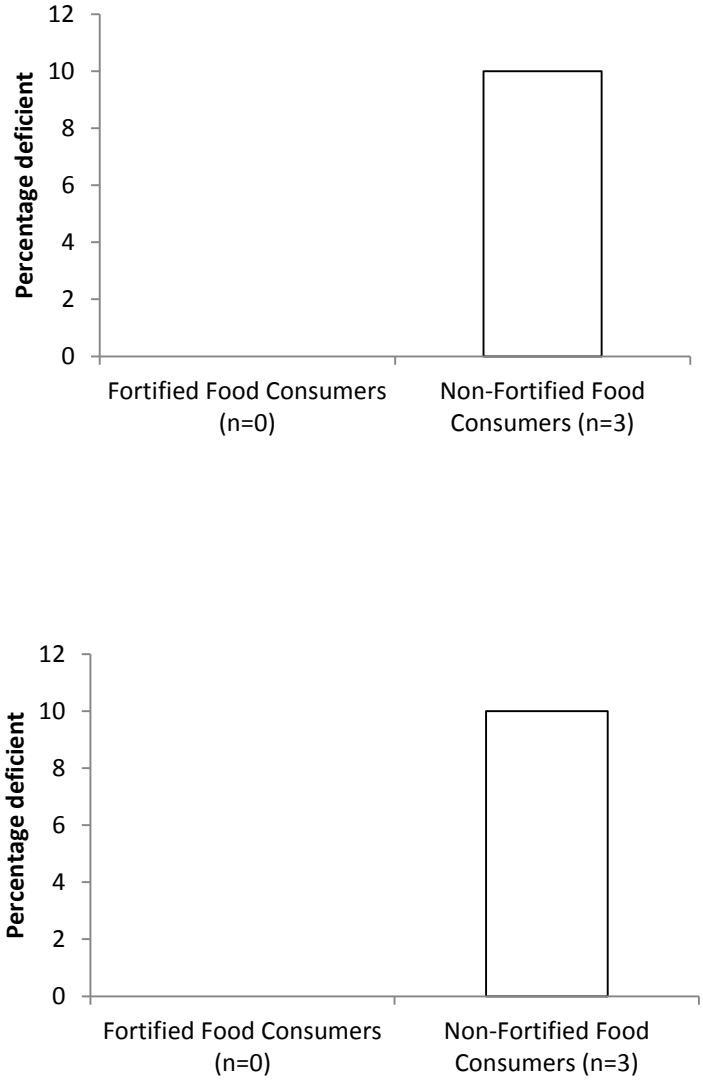

Figure 3. The impact of fortified food consumption on vitamin B12 status based on percentage of participants deficient as defined by serum vitamin B12 (upper plot) and holotranscobalamin (lower plot).

Table 4. The effect of duration of Proton Pump Inhibitor (PPI) usage based on fortified food consumption.

	Serum vitamin B12			HoloTC		
	FFC (n = 44)	Non FFC (n = 19)	*P*	FFC (n = 44)	Non FFC (n = 19)	*P*
Duration of PPI usage						
≤ 4 years (n = 17)	309 (241.4, 443.0)	194 (91, 436)	0.026	65.2 (54.6, 85.0)	38.5 (38.5, 38.5)	0.228
> 4 years (n = 46)	307 (258, 374)	260 (193, 290)	0.017	64.2 (49.0, 82.8)	39.4 (28.5, 52.0)	<0.001

Values given as median (IQR).
FFC, Fortified Food Consumer
The independent samples *t* test was used on log transformed data where applicable; P<0.050 was considered significant.
No significant differences were observed between FFC and non FFC for age (p= 0.518) or vitamin B12 intake (p=0.735).

Discussion

This observational study investigated the potential consequences of chronic PPI usage on vitamin B12 status, and the impact of fortified food consumption (providing vitamin B12 in the free form) on the vitamin B12 status of PPI users. When biomarker concentrations of vitamin B12 were compared between PPI users and matched controls (non PPI users), there was no evidence that PPI usage was associated with impaired vitamin B12 status. Fortified food consumption among PPI users was shown, however, to have a significant beneficial impact on vitamin B12 status.

Several previous studies have considered the effect of PPI drugs on vitamin B12 status, yet no definite or consistent conclusions have been reached in this area. In agreement with the results of this study, some previous studies (Valuck *et al.* 2004; Cotter *et al.* 2011) also concluded there was no significant adverse impact of chronic PPI usage on vitamin B12 status, the latter however was carried out in a hospital setting and the presence of certain diseases may have confounded the results. These conclusions are also supported by a case and control study using PPI users and their partners as controls (Den Elzen *et al.* 2008). Contrary to the findings of the current study and the aforementioned studies others concluded that PPIs negatively impact on vitamin B12 status and showed a 46% decline in vitamin B12 status among PPI users, after controlling for age (Hirschowitz *et al.* 2008). Likewise Dhamiarajan *et al.* (2008) in a cross-sectional study in the US found a highly significant association between long term use of PPIs and decline in vitamin B12 status, studying a large sample of 659 adults. It must be highlighted, however, that in the current study the higher intakes of vitamin B12 seen in PPI users (only marginally missing statistical significance) may well have contributed to the relatively high biomarker concentrations among PPI users compared with non PPI users. Nevertheless, the current study does provide some evidence that PPIs negatively impact on the absorption of food bound vitamin B12. Among PPI users there were no differences between fortified food consumers and non-fortified food consumers with regard to age or total dietary intake of vitamin B12. Despite this, those who had been using PPIs for the longest and did not consume fortified foods, therefore only consuming vitamin B12 in its food bound form, have a significantly poorer vitamin B12 status than fortified food consumers, who consumed a combination of both food bound and free vitamin B12.

At least part of the inconsistencies that exist in the literature regarding PPIs and vitamin B12 may be related to the choice of B12 biomarker used to assess status. In the current study three biomarkers, serum vitamin B12 and serum holoTC and plasma

homocysteine, were used to assess vitamin B12 status. It must be noted that questions have been raised over the reliability of serum vitamin B12 to identify low, but not necessarily deficient, vitamin B12 status and so it is limited as sole biomarker of vitamin B12 status. It is recommended that a combination of biomarkers should be used in order to confirm low vitamin B12 status (Nilsson-Ehle 1998). In the study carried out by Hirschowitz *et al.* (2008), previously referred to, subclinical vitamin B12 deficiency was observed in 31% of patients with a normal serum vitamin B12 concentration when using the more sensitive and specific biomarker methylmalonic acid (MMA) (Chatthanawaree 2011). HoloTC transports vitamin B12 in the blood and accounts for approximately 25% of vitamin B12 in circulation; it represents the metabolically active form of vitamin B12 and has been proposed as a more sensitive and specific marker of vitamin B12 status (Nexo *et al.* 2011). It is more strongly correlated with MMA than serum vitamin B12 and is thought that it will identify changes in vitamin B12 status earlier than other biomarkers (Clarke *et al.* 2007). Those findings are also supported by the results of the current study; there was a trend, although non-significant, towards better correlations between vitamin B12 intake and serum holoTC concentrations than with serum vitamin B12 concentrations. Plasma homocysteine is a sensitive functional marker of vitamin B12 (with higher concentrations indicative of lower status); however, it is not specific to vitamin B12 and will also be raised with low status of other B vitamins, in particular folate. Although differences in plasma homocysteine concentrations are reported in this current study, this is most likely to be as a result of differing folate status; vitamin B12 only becomes the main determinant of homocysteine concentrations when folate status has been optimised (Quinlivan *et al.* 2002).

In the current study both serum vitamin B12 and serum holoTC concentrations were significantly higher in PPI users who consumed fortified food than those who did not, correspondingly fortified food consumption by PPI users also appeared to provide some protection against vitamin B12 deficiency. No such findings were observed among the non PPIs. This could have implications for the food industry; although current levels of vitamin B12 added to fortified food appear to be beneficial for PPI users, they may not be sufficient to have any meaningful impact on B12 status among the general population. Although some studies have examined the role of vitamin B12 supplementation in PPI users (Blacher *et al.* 2007; Dhamiarajan *et al.* 2008; Rajan *et al.* 2002), the role of fortified foods does not appear to have been considered in any great detail in the literature. The results of the current study and potentially future results of other similar studies could

possibly have wider public health implications regarding mandatory food fortification. Presently, mandatory food fortification policy for folic acid is in place in some countries such as Canada and the United States. In the US it has been in place since 1996 and has been successful in reducing the occurrence and reoccurrence of neural tube defects in new-borns (Honein *et al.* 2001). Although this issue has been on the agenda for some time, no such policy yet exists within the UK or any other European countries over concerns that high intakes of folic acid could impair health as reviewed by Hughes *et al.* (2013). Specifically, there is concern that high intakes of folic acid could mask the symptoms of vitamin B12 deficiency (Reynolds 2006; Chanarin *et al.* 1997). However, studies carried out after the implementation of the fortification policy in the US found no evidence of this and it has been argued that the levels of fortification are not high enough to mask vitamin B12 deficiency (Mills *et al.* 2003). To overcome this potential problem and to address the issue of subnormal vitamin B12 status which is a widespread problem among the elderly (Wolters *et al.* 2004), it has been suggested that vitamin B12 should be considered in conjunction with folic acid for any fortification policy (Allen *et al.* 2010; Selhub *et al.* 2011). The results of this current study do suggest that the consumption of fortified food has a benefit on vitamin B12 status among PPI users; further studies are needed to examine this issue further. Any widespread fortification of the food chain with vitamin B12 is only likely to have a beneficial effect on those with food bound vitamin B12 malabsorption (both age related and owing to PPI usage), and not on people suffering from classical B12 deficiency. In addition to this there is a plethora of unanswered questions regarding mandatory vitamin B12 fortification relating to issues such as nutrition, epidemiology, toxicity, and efficacy as reviewed by Carmel (2011) and Green (2009), that must be addressed before any conclusive recommendations can be developed.

The impact of fortified food on vitamin B12 status in PPI users may be influenced by the duration of PPI usage. Any decline in vitamin B12 status is only likely to be noticeable, either by biochemical markers or clinical signs of deficiency, years after intake or absorption of the vitamin has decreased or stopped (Carmel 1997). Relatively large stores of vitamin B12 and its efficient enterohepatic circulation means that vitamin B12 depletion is a slow and subtle process, and the length of time before this depletion becomes evident can vary considerably between individuals depending on the initial stores available, the level of dietary intake of vitamin B12 and the condition of the gastrointestinal tract (Markle 1996). This makes it very difficult to assess any changes in vitamin B12 absorption and could, at least in part, explain the reason for the controversy

and disagreement in the literature with regard to PPI drugs and vitamin B12 status. It may be plausible that free vitamin B12 has a greater bioavailability, similar to that of folic acid, among those with malabsorption. The importance of free vitamin B12 is recognised in the US dietary reference intakes where adults >50 years are recommended to consume most of their vitamin B12 from fortified foods or a supplement containing vitamin B12, which would address the issue of food bound malabsorption (Institute of Medicine 1998). Surely a similar recommendation in the UK would clearly emphasise the value of obtaining some of the daily intake of vitamin B12 from sources of the free form? Based on the results of the current study, consuming free vitamin B12 would prove beneficial to PPI users. It may possibly also benefit the elderly population also who are more likely to suffer from food bound vitamin B12 malabsorption (Wolters *et al.* 2004), however, to achieve this it seems that the level of vitamin B12 normally added to fortified food would need to be increased. This may be of interest to producers of fortified foods.

There are a number strengths and limitations to this study which must be considered before any conclusions can be drawn. The major strength of this study the stringent selection process used to select the current sample of PPI users. This ensured that all PPI users included, were in fact chronic and regular users of PPIs, among whom other confounding factors were excluded such as vitamin B12 supplementation or certain disease states. In addition, the use of serum holoTC as a very specific and sensitive biomarker of vitamin B12 status, enhanced the results. This study was also one of the first to investigate the role of fortified foods in promoting a good vitamin B12 status among PPI users. Despite using a combination of a four day food diary and a FFQ, analysis of dietary intake, in this and dietary studies in general, is subject to bias and has limitations as discussed elsewhere in detail (Gibson 2005; Lee *et al.* 2013). The major limitation in the current study was that, even in light of lower reported energy intakes and in spite of being closely matched for age and fortified food consumption, vitamin B12 intakes were higher in PPI users compared to non PPI users albeit of borderline statistical significance. This is likely to be a key factor in the unexpected higher serum vitamin B12 concentrations observed in PPI users. This might indicate that PPI users are consuming a more nutrient dense diet, particularly rich in vitamin B12 from foods of animal origin such as dairy products and red meat. PPI users also had a significantly lower EI:BMR ratio so as a group were more likely to underreport energy intake than the non PPI users, although due to time constraints the data were not reanalysed excluding possible under-reporters. However, had likely under-reporters been excluded the greater intakes of vitamin B12 observed in PPI users may well

have reached statistical significance. In addition to this even if total vitamin B12 intakes had been closer, the daily intakes of free vitamin B12 in both of these groups were so low that any impact it may have had on absorption could have been missed. Other issues that would have impacted on the power of the analyses carried out is the fact that dietary intake data were only available for a proportion of the PPI users and that the number of PPI users who were non fortified food consumers was quite low.

Conclusions

This study shows no evidence that chronic use of PPIs impairs vitamin B12 status, but this must be interpreted with caution considering the probable higher vitamin B12 intakes in PPI users. It does appear that vitamin B12 status of PPI users can be improved by the consumption of fortified foods, and that this should be considered in relation to food fortification policy and setting of DRVs. Future work in this area is needed to further investigate these issues and should ensure close matching of vitamin B12 intake between groups with greater consideration for the presence of underreporting and a focus on recruiting a greater number of PPI users who are do not consumer fortified foods.

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Appendix 1

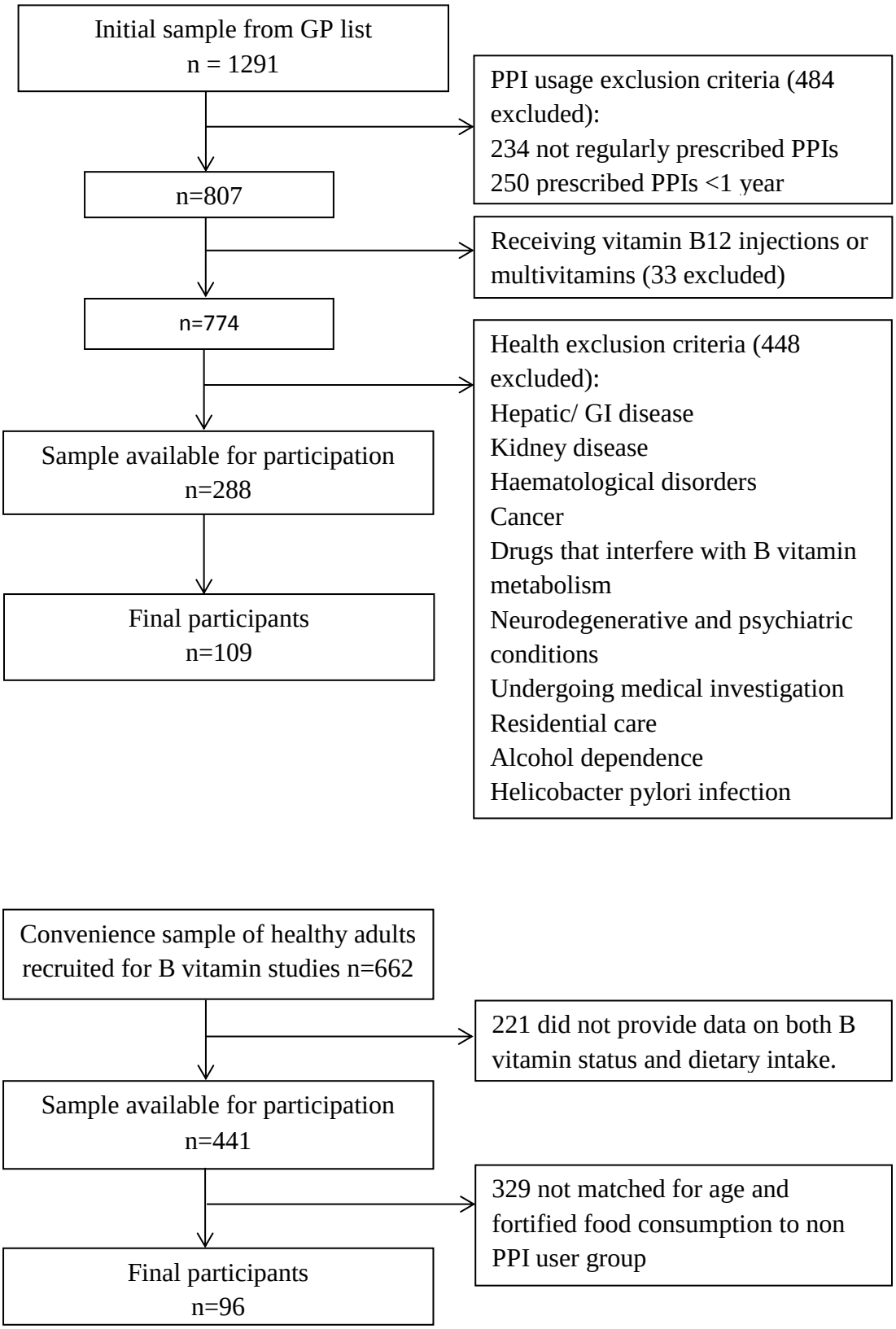

Figure 1. Identification of study participants for PPI users group (upper plot), and identification of study participants for non PPI user group (lower plot).

Appendix 2

Table 5. Raw data used to construct figure 2

	FFC (n = 80)	Non FFC (n = 29)
Serum Vitamin B12 (pmol/L)	336 (138)	257 (142)
Holotranscobalamin (pmol/L)	62.7 (32.4)	38.5 (27.2)

Values given as median (IQR)
FFC, Fortified Food Consumer

Table 6. Raw data used to construct figure 3

	FFC (n = 0)	Non FFC (n = 3)
Serum Vitamin B12 (% deficient)	0	10
Holotranscobalamin (% deficient)	0	10

FFC, Fortified Food Consumer