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ENDOCRINOLOGY, NUTRITION & METABOLISM | RESEARCH ARTICLE

Assessment of vitamin B_{12} tissue stores in elderly proton pump inhibitor users

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Abstract: *Background*: Adults over the age of 65 years are more prone to gastro-esophageal reflux and, as such, are commonly prescribed proton pump inhibitors (PPIs). PPIs inhibit gastric acid secretion which can have adverse effects on vitamin B_{12} absorption. This quasi-experimental study was conducted to determine whether chronic use (≥1 year) of proton pump inhibitors (PPIs) results in decreased vitamin B_{12} in adults aged 68–94 years, as indicated by increased urinary methylmalonic acid (uM-MA). *Methods*: Fifteen men and women who had been using PPIs daily for a minimum of one year were recruited. Fifteen subjects not using PPIs were age (±3 years) and gender matched to the subjects taking PPIs. Tissue stores of vitamin B_{12} were determined using uM-MA. *Results*: The majority of subjects in this study resided in assisted care living facilities (55.66%) and the remaining subjects (43.33%) were free living. There were no significant differences in uM-MA levels between those using PPIs (Mdn = 1.1 μg uM-MA/mg creatinine), and those not using PPIs (Mdn = 1.1 μg uM-MA/mg creatinine) ($p = 0.75$). *Conclusion:* Chronic use of PPIs did not alter vitamin B_{12} in healthy individuals aged 68–94 years. Larger studies are indicated to validate these findings.

Subjects: Nutrition; Pharmacology; Nutrition and Dietetics

Keywords: urinary methylmalonic acid; vitamin B12 deficiency; elderly; proton pump inhibitors

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Judith M. Lukaszuk, is a professor in Nutrition in the School of Health Studies at Northern Illinois University in DeKalb, Illinois, USA. She has been teaching for 18 years and lecturing nationally on micronutrient metabolism as well as integrative nutrition. The main focus of her research includes vitamin and mineral metabolism and nutrition supplements used to enhance exercise performance. This article is the third in a series looking at the effects of PPIs usage on Vitamin B_{12} levels across the lifespan.

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PUBLIC INTEREST STATEMENT

The purpose was to determine whether chronic use of antacid medication called "proton pump inhibitors" (PPIs) resulted in a vitamin B_{12} deficiency as measured by urinary methylmalonic acid (uM-MA) levels in elderly aged 68–94 years old. In order for Vitamin B_{12} to be absorbed, gastric acid is required. The use of PPIs limits gastric acid in the stomach, and the absorption of vitamin B_{12} may be severely impaired. Fifteen men and women who had been taking PPIs daily for a minimum of one year were age (±3 years) and gender matched to subjects not taking PPIs. There were no significant differences in uM-MA levels between those taking PPIs and those not taking PPIs ($p = 0.75$). Chronic use of PPIs (≥2 years) did not alter vitamin B_{12} status in healthy individuals aged 68–94 years.

1. Introduction

In the United States, adults over 65 years of age comprise 15.2% of the population, or 48 million people (United States Census Bureau, 2017). This is a 36% increase since 2000, and more than a tripling of the size of the older adult population since 1900 (United States Census Bureau, 2017). Adults over the age of 65 years are more prone to developing gastroesophageal reflux disease (GERD) and, as such, are commonly prescribed proton pump inhibitors (PPIs) for long-term use (Achem & DeVault, 2014). PPIs act by binding the H^+/K^+ ATPase pump of the parietal cells, thereby, inhibiting gastric acid secretion from these cells in the stomach (Yang & Metz, 2010). The resulting lack of gastric acid is thought to have adverse effects on the absorption of vitamin B_{12} bound to protein in food sources. Gastric acid activates protease enzymes, such as pepsin, which break down protein and free the B_{12} bound to protein (Yang & Metz, 2010). The free vitamin B_{12} is then available for absorption. Elderly adults are prone to additional gastric problems which also may decrease their ability to absorb nutrients so those undergoing PPI treatment may be at ever greater risk for nutrient deficiencies (Masclee, Sturkenboom, & Kuipers, 2014). The adverse effects of PPIs have been reported, but studies often are inconclusive, and the long-term effects of PPI treatment remain largely unknown (Abraham, 2012; Dharmarajan, Kanagala, Murakonda, Lebelt, & Norkus, 2008; Hirschowitz, Worthington, & Mohnen, 2008; Ito & Jensen, 2010; Masclee et al., 2014; Rozgony, Fang, & Kuczmarski, 2010; Saltzman et al., 1994; Valuck & Ruscin, 2004; Yang & Metz, 2010).

When vitamin B_{12} tissue stores decrease to a level of deficiency, irreversible neurological damage, cognitive impairment, and dementia can result (Grober, Kisters, & Schmidt, 2013). In addition, vitamin B_{12} malabsorption may be further exacerbated by age-related gastric changes (i.e. hypochlorhydria**)** in the elderly adult population (Wolters, Strohle, & Hahn, 2004). Cognitive function in older adults is important and it may be markedly improved by providing a vitamin B_{12} supplement, should low levels be detected. Unlike serum vitamin B_{12} levels, which have limited specificity and sensitivity for detecting tissue deficiency, uM-MA is specific to tissue stores of vitamin B_{12} (Matchar et al., 1987). The purpose of this study was to determine the effect of PPIs on vitamin B_{12} tissue stores as measured by uM-MA in elderly adults aged 68–94 years.

2. Methods

2.1. Participants

A quasi-experimental design was used for this study. Thirty men and women were recruited from April to August 2016 using informational flyers posted in local assisted living facilities, churches, and recreational facilities. Prior to participating in the study, all subjects signed a consent form. All study procedures were approved by the Institutional Review Board at Northern Illinois University DeKalb, IL. Fifteen subjects who had been taking PPIs daily for a minimum of one year were recruited first. Fifteen subjects in the control group, not taking PPIs were age (±3 years) and gender matched to subjects in the PPI group. Subjects were excluded if they had a history of Crohn's disease, ulcerative colitis, pernicious anemia, use of Metformin, liver disease, kidney disease (due to potential malabsorption of vitamin B_{12}), adherence to a vegan diet (due to insufficient intake of vitamin B_{12}), taking intramuscular shots of vitamin B_{12}, or taking nasal Nascobal (both of which bypass digestion and may elevate vitamin B_{12} levels) (Strativa Pharmaceuticals, Spring Valley, NY).

2.2. Data collection

2.2.1. Survey questionnaire
Each subject completed a survey questionnaire designed to collect details about demographics, use of multivitamin supplements, B vitamin supplements, and use and name of PPIs.

2.3. Diet analyses
Diet analyses were performed on 3-day food logs provided by the participants. Nutrition Calc plus (version 3.5 McGraw-Hill Companies, Columbus, OH) was utilized for assessment of the 3-day food logs. All data were computed by the same investigator on the research team to minimize any

variance. Analyses of the following sample characteristics were performed: total kcal/kg, fat, protein, and carbohydrate as percentages of total kcal/kg, and dietary B_{12}, vitamin B_6, and folic acid.

2.4. Anthropometrics

Researchers collected data at the following sites: at the individual's local assisted living residences, churches, and recreational facilities. Anthropometric measurements were taken in lightweight clothing and bare feet. Height was measured using a tape measure while subjects placed their heels, buttocks, and head against a wall. Weight, fat mass, body fat percent, lean body mass, and body mass index (BMI) were assessed using a bioelectrical impedance scale (Tanita body composition Analyzer TBF-300A, Arlington Heights, IL). BMIs were calculated by the Tanita analyzer using the standard equation (kilogram per meter squared). Urine samples were collected in sterile containers and were transferred into vials containing 5 mg of thymol as a preservative, which allowed samples to be mailed unrefrigerated. The vials were stored at −20ºC until all data had been collected. The samples were then shipped overnight for analysis to Norman Clinical Laboratory, Inc. (NCL) in Lexington, KY.

2.5. Laboratory measurements

The samples were analyzed for uM-MA by NCL at their laboratory facility located in Lexington, KY. Measurement of uM-MA levels of the urine samples were determined using LC/MS/MS.13A. The method was performed by using a Shimadzu Nexera XR HPLC system coupled with a Sciex 4500MD mass spectrometer operated in the ESI mode (capillary voltage—4,500 volts). Standards were purchased from Cerilliant, MMA (1 mg/ml) P/N MK-080 and MMA-d3 (1 mg/ml), P/N M-105. Three calibrators (20, 5,000, 20,000 ng/ml) and two quality controls (500, 10,000 ng/mL) were prepared by dilution of the Cerilliant MMA standards in deionized water. The internal standard spiking solution was prepared by adding 10 uL MMA-d3 (1 mg/mL) to 40 mL of deionized water. For analysis, 50 uL of internal standard spiking solution and 200 uL of deionized water were added to 50 uL of each sample, calibrator and quality control solution in a 96-well plate. The specimens then were centrifuged at 4,000 RPM for 20 min. The 96-well plates then were placed on an autosampler rack and 2 uL of each specimen was injected into the HPLC for analysis.

The column used was a Phenomenex Synergi Hydro-RP, 2.5 um, 3.0 × 100 mm (P/N 00B-4387-YO). Mobile phase B was 0.1% formic acid in acetonitrile and mobile phase A is 0.1% formic acid in water. The column was held at 40ºC, with a stepwise gradient as follows: Start at 0% B and go to 18% B at 1.8 min; proceed to 95% B at 1.9 min, maintain at 95% until 2.5 min; return to 0% B. The total run time per sample was 4.5 min including equilibration. The scheduled multiple reaction monitoring (MRM) table (ESI positive) is shown below:

Q1 Mass	Q3 Mass	Dwell (ms)	Analyte	DP	CE	CXP
117.0	72.9	40	MMA 1	−30	−13	−10
117.0	54.9	40	MMA 2	−30	−34	−10
120.0	75.9	40	MMA-d3	−30	−13	−10

All LC/MS/MS data were processed using MultiQuant software version 3.0 (Wolters et al., 2004).

For the data analysis, levels of uM-MA were considered to be normal if they were <3.8 μg MMA/mg creatinine or <3.6 mmol/mol creatinine (Norman & Morrison, 1993).

2.6. Statistical analysis

Due to the small sample size and the matched pairs design, this study used the non-parametric related-samples Wilcoxon signed rank test procedure (Harris, Boushey, Bruemmer, & Archer, 2008) tested the hypothesis that subjects using PPIs would have higher uM-MA levels than their age matched and gender matched controls not using PPIs, indicating vitamin B12 deficiency in PPI users.

Table 1. Characteristics of the PPI group and non-PPI group						
	PPI group (*n* = 15)		Non-PPI group (*n* = 15)		Z score	*p*-value
	Mean ± SD	Median	Mean ± SD	Median		
Anthropometrics						
Age (years)	79.8 ± 8.4	77.0	80.3 ± 8.3	78.0	−0.81	0.416
BMI (kg/m^2)	30.1 ± 3.7	29.3	27.4 ± 4.4	26.6	−2.78	0.005*
Body fat percent	37.9 ± 6.6	38.8	32.1 ± 9.2	31.9	−2.44	0.015*
Body fat mass (kg)	30.6 ± 8.0	28.0	23.5 ± 10.6	19.8	−2.67	0.008*
Lean body mass (kg)	49.9 ± 10.1	49.3	48.7 ± 13.3	43.3	−0.97	0.334
Diet/supplement intake						
Average daily caloric intake (kcal/kg)	18.0 ± 3.2	17.9	19.6 ± 6.3	17.1	−0.94	0.347
Carbohydrate as % of caloric intake	49.3 ± 7.5	48.0	54.6 ± 9.1	56.0	−0.76	0.449
Protein as % of caloric intake	15.5 ± 2.0	16.0	16.5 ± 2.4	16.5	−0.71	0.477
Fat as % of caloric intake	35.2 ± 6.8	35.0	29.0 ± 8.0	30.0	−1.73	0.084
Dietary vitamin B12 (µg)	2.8 ± 2.1	2.3	3.4 ± 1.8	3.5	−1.57	0.117
Dietary vitamin B6 (mg)	1.0 ± 0.56	1.1	1.1 ± 0.46	1.1	−0.71	0.477
Dietary folate (µg)	280.0 ± 156.8	243.8	260.7 ± 150.2	209.5	−0.39	0.695
Supplemental vitamin B$_{12}$ (µg)	17.7 ± 21.9	0.0	81.3 ± 255.8	0.0	−0.287	0.774
Supplemental vitamin B$_6$ (mg)	4.7 ± 12.8	0.0	0.8 ± 1.6	0.0	−1.532	0.125
Supplemental folic acid (µg)	273.3 ± 293.9	400	120.0 ± 182.1	0.0	−1.562	0.118

Notes: Values are means ± SD. Total % carbohydrate, protein and fat may not equal 100% due to rounding. Only 13 pairs were available for analysis of dietary intake.

*p < 0.05 PPI group vs. non-PPI group.

This test also determined differences between the PPI group and the non-PPI group on anthropometrics and diet/supplement intake variables (Table 1).

The mean (M) and standard deviation (SD) were determined for each key variable between the PPI group and non-PPI group are provided as M ± SD. The median (Mdn) was also provided for each key variable. The tests for equivalency between key variables of each group were provided as a Z-value. The testing of the hypothesis was also reported as a Z-value. Statistical significance for all data analyses were accepted at the alpha level of p < 0.05. Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows (Version 23.0, 2015, SPSS, Inc, Chicago, IL).

3. Results

3.1. Participants
Participants ranged in age from 68 to 94 years, with a mean age of 80.17 years. Participants were age matched (±3 years) and gender matched. Six males and 24 females participated in the study. Most participants in both groups were Caucasian 96.6% (*n* = 29); while one PPI user was Hispanic.

3.1.1. Details regarding PPI use

The PPI group had been taking PPIs for 2–15 years, with a mean of 6.7 years M ± SD = 6.7 ± 3.6, Mdn = 6.0. From the PPI group, 12 participants reported taking over-the-counter PPIs (80%, $n = 12$) ($n = 8$ Omeprazole; $n = 4$ Pantoprazole), while 3 participants were taking prescription PPIs (20%, $n = 3$) ($n = 2$ Protonix; $n = 1$ Nexium). Fourteen PPI users reported acid reflux or GERD as the reason for the prescription of the PPI. The remaining PPI user reported use of PPIs for Barrett's esophagus.

3.2. Equivalency of groups

3.2.1. Anthropometrics

The PPI group and non-PPI group were tested for equivalency using the related-samples Wilcoxon signed rank test. The PPI group and non-PPI group were significantly different on BMI ($Z = -2.78$, $p = 0.005$), body fat percent ($Z = -2.44$, $p = 0.015$), and body fat mass ($Z = -2.67$, $p = 0.008$). That is, the PPI group had a higher BMI, body fat percent, and body fat mass. The groups were not significantly different for lean body mass ($Z = -0.97$, $p = 0.334$) (Table 1).

3.2.2. Diet intake

Diet intake data were not available for two PPI participants and one non-PPI participant; therefore, the data presented for diet intake reflects the related-samples Wilcoxon signed rank test using $n = 27$ instead of 30 as was previously conducted with the full data-set. Diet intake descriptive values were calculated with 27 participants and equivalency of groups regarding diet intake was analyzed using 13 rather than 15 pairs. Wilcoxon tests for equivalency of groups indicated that there were no statistically significant differences between average daily caloric intake (kcal/kg) ($Z = -0.94$, $p = 0.347$), carbohydrates as percentage of caloric intake ($Z = -0.76$, $p = 0.449$), protein as percentage of caloric intake ($Z = -0.71$, $p = 0.477$), fat as percentage of caloric intake ($Z = -1.73$, $p = 0.084$), dietary vitamin B_{12} (µg) ($Z = -1.57$, $p = 0.117$), dietary vitamin B_6 (mg) ($Z = -0.71$, $p = 0.477$), and dietary folate (µg) ($Z = -0.39$, $p = 0.695$) (Table 1).

3.2.3. Supplement intake

Eight PPI users reported no multivitamin use, while the remaining PPI users consumed a multivitamin with vitamin B_{12}, vitamin B_6, and folic acid with one PPI user taking a super vitamin B-complex daily. Ten non-PPI users reported using no multivitamins while the remaining five non-PPI users consumed a multivitamin daily.

Wilcoxon tests for equivalency of groups regarding supplement intake indicated that there were no statistically significant differences between supplemental vitamin B_{12} (µg) ($Z = -0.287$, $p = 0.774$), supplemental vitamin B_6 (mg) ($Z = -1.532$, $p = 0.125$), and supplemental folic acid (µg) ($Z = -1.562$, $p = 0.118$) (Table 1).

3.3. Hypothesis testing

Values for uM-MA among the PPI and non-PPI group can be found in Table 2. The hypothesis that the PPI group would have higher uM-MA levels than their gender and age matched controls, indicating vitamin B12 deficiency, was not supported ($Z = -0.42$, $p = 0.967$) (Table 3).

Table 2. Observed uM-MA for PPI group and non-PPI group					
	PPI group ($n = 15$)		Non-PPI group ($n = 15$)		Z score	p-value
	Mean ± SD	Median	Mean ± SD	Median		
uM-MA levels (µg/mg creatinine)	1.1 ± 0.29	1.1	1.3 ± 0.91	1.1	−0.42	0.967

Note: Values are means ± SD.

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Table 3. Observed uM-MA for PPI group and Non-PPI group by gender								
Variable	**PPI group**				**Non-PPI group**			
	Female (*n* = 12)		Male (*n* = 3)		Female (*n* = 12)		Male (*n* = 3)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
uM-MA (μg/mg creatinine)	1.06 ± 0.26	1.0	1.33 ± 0.35	1.3	1.31 ± 1.00	1.0	1.29 ± 0.48	1.2

Notes: Values are means ± standard deviation (M ± SD), Median (Mdn).

4. Discussion

This study showed that PPI use for an average of 6.7 years among those 68–94 years did not deleteriously affect vitamin B_{12} tissue stores as measured by uM-MA levels. These findings concur with a 2008 study of 125 community living elderly over the age of 65 years who had been using PPIs for three years in that serum vitamin B_{12} levels were not deleteriously affected (Den Elzen et al., 2008). The results of this study also are consistent with two previous studies by Lukaszuk et al., which evaluated uM-MA levels in two younger groups aged: 22–50 years and 50–70 years. Both studies showed that chronic use of PPIs did not affect uM-MA levels (Lukaszuk, Prawitz, Shokrani, Umoren, & Norman, 2013; Lukaszuk, Umoren, Warner, Shokrani, & Norman, 2015).

In direct contrast, three other studies showed that PPI use negatively affected serum vitamin B_{12} status (Dharmarajan et al., 2008; Lam, Schneider, Zhao, & Corley, 2013; Valuck & Ruscin, 2004). Lam et al. (2013) conducted a large population-based study matching 25,956 individuals diagnosed with a B_{12} deficiency to patients without a B_{12} deficiency based on geographical residence, age (±1 year), gender, and race/ethnicity. Results indicated that those at greatest risk of B_{12} deficiency were > 60 years of age; taking PPIs for two or more years and taking the highest mean daily dose (>1.5 PPI pills/day) (Lam et al., 2013). The similarities between the Lam et al. (2013) study and the current one is that everyone was > 60 years old and had been taking PPIs for more than two years and most subjects were taking > 1.5 PPI pills/day. It is difficult to do a direct comparison on PPI doses with the Lam et al. (2013) study and this study. Lam et al. (2013) quantified the PPI dose by the number of PPI pills taken per day, but did not specify the dosage per day. In the current study, 80% of the subjects were taking > 1.5 PPI pills per day. More specifically, 73% subjects were taking 40 mg/day of a PPI and 27% were taking 20 mg/day. The difference in findings between the Lam et al. (2013) study and this current study may have been due to differences in the total dose of PPIs taken per day and the difference in the sample sizes of the two studies.

Dharmarajan et al. (2008) examined serum vitamin B_{12} levels and use of PPIs in 542 older adults ages 60–102 years over a duration of six years. Their results indicated that low/marginal serum vitamin B_{12} status was observed in older adults PPI users with 19.9% in the nursing home population and 29.2% in the community living population. The authors concluded that deficiency is indeed common among the older adult population, and that more attention may be indicated to monitor vitamin B_{12} levels in older adults using PPIs.

Lastly, a case-control study, (Valuck & Ruscin, 2004) investigated the association between vitamin B_{12} deficiency and use of PPIs in adults aged 65 or older. Inclusion criteria included ≥65 years old and a documented serum vitamin B_{12} measurement. The data were obtained through a university-based, geriatric, primary care facility's medical records. Fifty-three people met the criteria for the vitamin B_{12} deficient group and a set of controls were matched for gender and ± 1 year of age (Valuck & Ruscin, 2004). Chronic use of PPIs was defined as greater than 12 months. Findings indicated that chronic use of PPIs was associated with significantly increased risk of vitamin B_{12} deficiency. Dharmarajan et al. (2008) and Valuck and Ruscin (2004), recommended screening of elderly individuals for vitamin B_{12} deficiency after chronic PPI use of 3–4 years to reduce complications due to untreated vitamin B_{12} deficiency (Dharmarajan et al., 2008; Valuck & Ruscin, 2004).

Previous studies have shown that as people get older, incidence of vitamin B_{12} deficiency increases because of a decreased ability to release protein-bound dietary vitamin B_{12} for absorption. This is attributed to the prevalence of atrophic gastritis seen with associated with advanced age (Baik & Russell, 1999; Carmel, 1997; Russell, 2001). Valuck and Ruscin (2004) has suggested that people with atrophic gastritis resulting in achlorhydia would be unlikely to be using PPIs for acid reflux, but no studies were found to support this. Achlorhydia results from destruction of the parietal cells in the stomach which release not only the hydrochloric acid (HCl) needed to free the dietary protein-bound vitamin B_{12}, it also releases the intrinsic factor (IF) needed for absorption of the free vitamin B_{12} (Rozgony et al., 2010). In addition to achlorhydia's effect on vitamin B_{12} absorption, lack of HCl affects protein digestion in the stomach by impeding pepsin activation, leading to an increase in intra-abdominal pressure. High intra-abdominal pressure opens up the cardiac sphincter resulting in gastric reflux. In the current study, participants were not assessed for atrophic gastritis.

4.1. Physical characteristics
In the current study, the PPI group was significantly different from the PPI group regarding BMI, body fat percent, and body fat mass, with the PPI group displaying a higher BMI, body fat percent, and body fat mass than the non-PPI group. The BMI of the PPI group M ± SD = 30.1 ± 3.7, Mdn = 29.3 is considered obese while the BMI of the non-PPI group M ± SD = 27.4 ± 4.4, Mdn = 26.6 is considered overweight (World Health Organization, 2017). This study as well as previous studies (Corley & Kubo, 2006; El-Serag, 2008; El-Serag, Graham, Satia, & Rabeneck, 2005; Hajar, Castell, Ghomrawi, Rackett, & Hila, 2012; Wu, Mui, Cheung, Chan, & Jao-Yiu Sung, 2007), have indicated that subjects with a higher BMI are more likely to have acid reflux and thus more likely rely on PPIs, either over-the-counter or prescribed. It is important, then that medical providers monitor PPI use and vitamin B_{12} levels in patients with higher BMIs.

4.2. Dietary and supplement intake
The PPI group and non-PPI groups in the current study were not significantly different on dietary or supplemental intake of vitamins B_{12}, B_6 and folic acid. When dietary and supplemental intakes were combined, the recommended daily allowances (RDAs) for vitamin B_{12}, vitamin B_6, and folic acid were met. The only vitamin that would affect the uM-MA level was vitamin B_{12} and all subjects were ingesting a sufficient amount of vitamin B_{12}, and all subjects based on the established RDA. This indicates that if a difference in uM-MA had been detected, it would likely have been due to the use of PPIs.

4.3. Urinary methylmalonic acid
The use of uM-MA as an accurate biomarker of vitamin B_{12} status, has been confirmed by previous studies, (Hill et al., 2013; Norman, 2004; Norman & Morrison, 1993). The use of uM-MA to measure vitamin B_{12} status in PPI users was found in only two previous studies, both conducted by Lukaszuk et al. (2013, 2015). In 2013, Lukaszuk et al., investigated the effect of PPI use on vitamin B_{12} tissue stores in 22–50 year olds, and in 2015, they repeated the study with 50–70 year olds. The current study of elderly adults, along with the two previous studies of younger age groups, found that uM-MA was not significantly different for the PPI and the non-PPI groups. These three studies, although small in size, all implemented uM-MA tests rather than serum B_{12} tests to indicate B_{12} levels. All three studies using uM-MA tests found no differences in the tissue stores of vitamin B_{12} in PPI users and non-users. Larger studies utilizing the uM-MA technique are indicated to confirm these results. A large comparison study of serum B_{12} and uM-MA tests in larger dose PPI users would be useful.

The current research was limited by the small sample size ($n = 30$). Due to the small sample size, a non-parametric test was utilized, which is not as powerful as a parametric test such as the paired samples t-test. Although newer statistical research has shown the justification for using the t-test in small sample sizes, the variable being tested is required to be normally distributed (deWinter, 2013). This was not the case with the uM-MA levels among PPI users and non-PPI users' thus eliminating the ability to use a parametric test.

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The PPI and non-PPI groups were also significantly different regarding three physical characteristics. The groups were significantly different on BMI, body fat percent, and body fat mass. Ideally, the groups would have been equivalent on all characteristics. Although the ages of participants were not significantly different between the PPI group and the non-PPI group, the groups were gender and age-matched within ± 3 years. Ideally, participants would be age-matched to the same age, but due to difficulty recruiting participants, this was not feasible and the researchers determined it was appropriate to age match as closely as possible.

Competing Interests
The authors declare no competing interest.

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