Assessment of Urinary Methylmalonic Acid Levels in Older Adults on Proton Pump Inhibitors

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Abstract

Background: Proton pump inhibitors (PPIs) affect gastric acid secretion. The use of PPIs in the elderly population (> 70 years old) may increase the risk of vitamin B_{12} deficiency because gastric acid is needed for vitamin B_{12} digestion and absorption. However, it's unclear whether adults aged 50-70 years old that use PPIs chronically are at risk of vitamin B_{12} deficiency.

Aim: To determine whether chronic use of proton pump inhibitors results in increased urinary methylmalonic acid (uMMA) levels in adults aged 50-70 years old, indicating vitamin B_{12} deficiency.

Method: Fifteen men and women who had been taking proton pump inhibitors daily for a minimum of one year were recruited. Fifteen subjects, not taking proton pump inhibitors, were age-matched (± 3 years) and gender matched, to the subjects taking proton pump inhibitors. Tissue stores of vitamin B_{12} were determined using uMMA.

Results: There were no significant differences in uMMA levels between those taking proton pump inhibitors (Mdn = 1.60 µg uMMA/mg creatinine), and those not taking proton pump inhibitors (Mdn = 1.80 µg uMMA/mg creatinine) (p = 0.12).

Conclusion: Chronic use of proton pump inhibitors did not affect vitamin B_{12} status of subjects in this pilot study of healthy adults aged 50-70 years old. Regular monitoring of vitamin B_{12} status does not appear to be needed in this age group, however, studies using larger groups are indicated to confirm these findings.

Keywords: Deficiency or depletion of vitamin B_{12}; Urinary methylmalonic acid; Proton pump inhibitors; Older adults

Abbreviations: uMMA: Urinary Methylmalonic Acid; sMMA: Serum Methylmalonic Acid; mg: Milligram; µg: Microgram; PPI: Proton Pump Inhibitor; GERD: Gastroesophageal Reflux Disease; M: Mean; Mdn: Median; SD: Standard Deviation; BMI: Body Mass Index; Kcal: Kilocalorie; min: Minute

Introduction

Aging Population

The number of Americans aged 45-64 has increased by 24% over the last decade [1]. It is projected that by 2030, older adults will comprise 20% of the U.S. population [1]. The aging population often suffers from at least one chronic condition or possibly multiple co-morbidities including gastrointestinal issues. More specifically, 6-17% of the U.S. elderly population is suffering from gastroesophageal reflux disease (GERD) [2]. Older adults seem more prone to GERD due to malabsorption and higher prevalence of chronic atrophic gastritis [3-4].

GERD and Treatment

GERD is a digestive disorder in which stomach contents reflux back into the lower esophagus, causing erosion of the esophageal lining and often times causing emesis or chest pain [5-7]. If GERD is left untreated, continuous exposure of the esophageal lining to stomach acid can cause a condition called "Barrett's esophagus" in which pre-cancerous cells develop [5].

The primary medical treatment for GERD includes ingestion of antacid medications. PPIs are currently the most effective antacids available, as they prevent secretion of gastric acid by the parietal cells in the stomach for 24-48 hours [8]. Chronic use of PPIs has been associated with vitamin B_{12} deficiency because vitamin B_{12} requires gastric acid for absorption [9]. Without gastric acid, over time, the absorption and assimilation of vitamin B_{12} in the digestive tract may be severely impaired.

Role of Vitamin B_{12}

Absorption, Metabolism and Storage of Vitamin B_{12}

Vitamin B_{12} absorption begins in the stomach where food-bound or enzyme-bound cobalamin is released from proteins by gastric hydrochloric acid (HCl-) and the gastric proteolytic enzyme, pepsin [4,10-13]. After release of cobalamin, the free vitamin B_{12} is bound to an R protein (haptocorrin or transcobalamin I) from saliva, and is transported to the duodenum [4]. This R protein is thought to provide protection to vitamin B_{12} from use by intestinal bacteria [4].

Next, the vitamin B_{12}/R protein complex is acted upon by pancreatic proteases, thereby, releasing vitamin B_{12} from the R protein. Intrinsic factor (IF), a glycoprotein produced by gastric parietal cells, will only bind to free vitamin B_{12}, in a neutral environment made so by the pancreatic secretions [13]. IF is specific for vitamin B_{12}, and also protects vitamin B_{12} from use by intestinal bacteria [4]. The vitamin B_{12}/IF complex is carried from the proximal small intestine to the terminal ileum where it is taken up by the protein receptor cubilin [4, 10]. The proteins amnion less and megalin associate with the vitamin B_{12}/IF complex and facilitate the attachment of cubilin to the ileal cell plasma membrane [4,10]. The interaction of the vitamin B_{12}/IF complex and cubilin initiates a Ca^{2+} dependent receptor-mediated endocytosis which leads to the internalization of the complex [4,10]. Within the enterocyte, vitamin B_{12} is released from IF due to the low pH within the endosomes and lysosomes and then proceeds to bind with transcobalamin II for transport into the portal blood [4]. Absorption of vitamin B_{12} occurs primarily in the distal ileum and while passive diffusion may occur at very high concentrations, it is an inefficient process [10]. Therefore, lack of gastric acid will lead to the depletion of vitamin B_{12}.

Vitamin B_{12} is stored primarily in the liver in the amount of approximately 2-4 mg as adenosylcobalamin [13]. Vitamin B_{12} is also enterohepatically recycled; thus making it available for binding with IF and absorption in order to maintain the body's need for vitamin B_{12} [13]. This enterohepatic circulation contributes to the long biological half-life of vitamin B_{12}. Thus, a deficiency of vitamin B_{12}, due to an insufficient intake, would take a long length of time to develop, approximately 3-5 years. The length of time for the development of vitamin B_{12} deficiency could change if there is an underlying condition or a disease state such as lack of acid and/or pernicious anemia in which IF is lacking [13].

Vitamin B_{12} in Elderly Individuals

The most common causes of vitamin B_{12} deficiency in humans are associated with acquired malabsorption. A number of studies have shown that elderly individuals with an average age over 80 taking PPIs had lower serum vitamin B_{12} levels [14-18] and higher serum methylmalonic acid levels [15,16], but whether this is true for 50-70 year olds who are taking PPIs is unknown. It appears that chronic use of PPIs can induce achlorhydria, which leads to malabsorption of vitamin B_{12}, as gastric acid is required to release vitamin B_{12} from food [16,17]. This can occur across the age spectrum, but seems to have the most impact on elderly individuals and those with low vitamin B_{12} stores in the body.

While it would seem that measurement of serum vitamin B_{12} would be the best indicator for the sufficiency of this nutrient, determination of sMMA or uMMA levels have been found to be the most highly sensitive functional indicators for determining tissue stores of vitamin B_{12} in the body [18,19].

Laboratory Diagnosis of Vitamin B_{12} Deficiency

Although the sMMA and uMMA respond similarly to vitamin B_{12} supplementation [20], uMMA, due to its increased specificity for vitamin B_{12}, is superior to that of sMMA. Unlike uMMA, sMMA levels may be falsely elevated due to renal insufficiency [21]. However, creatinine should be utilized, when interpreting uMMA, to account for possible kidney function impairment [10]. The test for uMMA, which is 40 times more concentrated than the levels of sMMA, is non-invasive, only requires 1 mL of urine, and is stable when frozen for months [21,22]. However, false positives have not been reported with uMMA measurements [22]. Both serum and urinary MMA biomarkers have higher sensitivity and specificity than serum vitamin B_{12} [12]. A potential drawback is the need for implementation of a quality assurance system regarding the uMMA measurements. Researchers from the Hospital of the University of Munich, Germany found a deviation of >20% from the mean concentration of uMMA samples in a large proportion of samples submitted by ten European clinical laboratories [23]. This variable can be easily controlled by having all samples evaluated by one laboratory.

In the case of vitamin B_{12} deficiency, the concentration of MMA starts to increase in the blood or urine; therefore, elevated MMA levels in the blood or urine is an early indicator of vitamin B_{12} deficiency [12]. Urinary MMA is excreted very efficiently by the kidneys, making it a sensitive indicator of tissue repletion of vitamin B_{12} [18]. In addition, the measurements of uMMA are less prone to giving false positive results as found with sMMA [20].

The purpose of this study was to determine whether chronic use of PPIs would alter uMMA levels in adults aged 50-70 years old.

Materials and Methods

Participants

A quasi-experimental design was used for this study. Thirty men and women were recruited from January to April, 2015 using informational flyers posted at the campus of Northern Illinois University (NIU), at local assisted care living facilities and other community locations. Prior to participating in this study, subjects signed a consent form. All study procedures were approved by the Institutional Review Board at NIU. On the day of their scheduled appointment, subjects brought a completed 3-day food record with them, completed a brief informational survey, had their anthropometrics assessed, and then provided a urine sample for the uMMA assay.

Fifteen subjects, who had been taking proton pump inhibitors (PPIs) daily for a minimum of one year, were recruited first. The 15 subjects in the control group were not taking PPIs and were age-matched (± 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, diabetes, liver, kidney or heart disease, were following a vegetarian diet or were taking intramuscular shots of vitamin B_{12} or nasal Nascobal (Strativa Pharmaceuticals, Spring Valley, NY).

Data Collection

Survey: Each subject completed a survey designed to collect details about their demographics, lifestyle behaviors, use of vitamin supplements, and use and name of PPIs. In addition, participants were queried about their use of vitamin B_{12}, vitamin B_6, and folic acid supplements.

Diet Analyses: Diet analyses were performed on the 3-day food logs provided by the participants. Nutrition Calc Plus (version 3.61 McGraw-Hill Companies, Columbus, OH) was utilized for assessment of the 3-day food logs. All data were computed by the same graduate-level nutrition student to minimize variance. Analyses of the following were performed: total kcal/kg; fat, protein, and carbohydrate as percentages of total kcal/kg; and dietary vitamin B_{12}, vitamin B_6, and folic acid.

Anthropometrics: Subjects reported to the university's nutrition laboratory where anthropometric measurements of subjects were taken in lightweight clothing and bare feet. Height was measured using a wall-mounted stadiometer (Ayrton S-100 Prior Lake, MN). Weight, fat mass, body fat percent, lean body mass, and body mass index (BMI) were assessed using a bioelectrical impedance scale

(InBody 520, Biospace Inc. Los Angeles, CA). BMIs were calculated by the In Body analyzer using the standard equation (kilogram per meter squared).

Urine Samples: Urine samples were collected in sterile containers and transferred into vials which contained 5 mg of thymol as a preservative, allowing samples to be mailed unrefrigerated [12,32]. The vials were stored at -20°C until all data had been collected. The samples then were shipped overnight to Norman Clinical Laboratory Inc. (Cincinnati, Ohio) for analyses.

Laboratory Measurements: The urine samples were analyzed by Norman Clinical Laboratory Inc. (Cincinnati, Ohio). Urine levels of MMA were determined using an ion monitoring isotope dilution gas chromatography mass spectrometry (GC/MS). Urinary MMA levels were normalized to urinary creatinine levels. Five hundred ng of deuterated MMA, used as an internal standard, and a gas chromatograph (Varian 3400, Varian Associates, Sugarland, TX) equipped with a capillary column (30-m, DB-5, 0.25-um film thickness, 0.5 mm inner diameter, J&W Scientific Co., Folsom, CA) were interfaced to a mass spectrometer (Finnigan MAT 800 ion trap detector, San Jose, CA). The GC/MS was equipped with a Finnigan MAT A200S auto sampler. The GC was programmed from 140°C to 225°C at 4.7°C/min and then to 280°C at 20°C/min with a 10 min hold time. For the data analyses, levels of uMMA are considered to be normal if they are < 3.8 µg MMA/mg creatinine or < 3.6 mmol/mol creatinine [12].

Data Analyses

Due to the small sample size and the matched pairs design, this study used the non-parametric related-samples Wilcoxon signed rank test to determine differences in key variables between subjects in the PPI group and subjects in the non-PPI group. This test was also used to test the hypothesis to determine if the subjects in the PPI group would have higher uMMA levels than their age- and gender-matched controls in the non-PPI group.

Simple descriptive statistics were determined for each key variable between subjects in the PPI group versus non-PPI group. The tests for equivalency between key variables of each group were provided as a Z value. Statistical significance for all data analysis was accepted at the $p < 0.05$ level of confidence. Data were analyzed using Statistical Package for Social Sciences (SPSS) for Windows (Version 21.0, 2013, SPSS, Inc, Chicago, IL)

Results and Discussion

Physical Characteristics

Thirty subjects (n = 10 males; n = 20 females) aged 50-68 years participated in this study (Table 1). Most participants (93.3%) were Caucasian, two were African American (6.7%). The PPI group reported that they had been taking PPIs for a range of 1.5-15 years (M ± SD = 7 ± 3.9 years) and 67% of the PPI group had been on PPIs for at least 5 years (n = 10).

Tests for equivalency of groups, using the Wilcoxon signed rank, test indicated the PPI group and non-PPI group were significantly different regarding BMI (Z = -2.33, p = 0.02), body fat percent (Z = -2.36, p = 0.02), and body fat mass (Z = -2.33, p = 0.02). The PPI group on average had a larger BMI, body fat percent, and body fat mass than the non-PPI group. Despite these differences, the groups did not significantly differ in age (Z = -0.11, p = 0.9) or lean body mass (Z = -0.91, p = 0.36) characteristics (Table 1).

Dietary Intake

Dietary intake data was not available for one non-PPI group participant. Therefore, the data presented for dietary intake reflects the related-samples Wilcoxon signed rank test for 14 pairs. Dietary intake descriptive values were calculated with 29 participants. Equivalence of groups in regard to dietary intake was analyzed using 14 instead of 15 pairs. Tests for equivalency of groups indicated no significant differences between average daily caloric intake (kcal/kg) (Z = 0.09, p = 0.93), carbohydrates as percentage of caloric intake (Z = 0.03, p = 0.98), protein as percentage of caloric intake (Z = -1.41, p = 0.16), fat as percentage of caloric intake (Z = 1.73, p = 0.08), dietary vitamin B_{12} (µg) (Z = -1.15, p = 0.25), dietary vitamin B_6 (mg) (Z = -1.29, p = 0.20), and dietary folate (µg) (Z = -0.66, p = 0.51) (Table 1).

Anthropometrics	PPI Group (n = 15)		Non-PPI Group (n = 15)			
	Mean ± SD	Median	Mean ± SD	Median	Z Score	P Value
Age	56.6 ± 5.5	56.0	56.5 ± 5.2	54.0	- 0.11	0.91
BMI (kg/m^2)	33.2 ± 6.6	30.9	28.3 ± 4.4	27.3	- 2.33	0.02
Body Fat Percent (%)	40.4 ± 8.2	39.8	35.1 ± 8.0	35.6	- 2.36	0.02
Body Fat Mass (kg)	38.3 ± 14.3	33.1	28.6 ± 9.6	26.3	- 2.33	0.02
Lean Body Mass (kg)	54.7 ± 10.3	53.0	52.0 ± 10.5	51.7	- 0.91	0.36
Dietary/Supplement Intake						
Average Daily Caloric Intake (kcal/kg)	19.3 ± 6.7	18.1	20.0 ± 4.7	20.4	0.09	0.93
Carbohydrate as % of Caloric Intake	40.9 ± 6.3	39.7	41.2 ± 5.5	40.8	0.03	0.98
Protein as % of Caloric Intake	20.6 ± 4.2	19.5	18.2 ± 3.3	18.0	- 1.41	0.16
Fat as % of Caloric Intake	36.7 ± 4.7	36.3	38.8 ± 4.8	38.2	1.73	0.08
Dietary Vitamin B_{12} (µg)	3.8 ± 3.1	2.9	2.7 ± 1.8	2.0	- 1.15	0.25
Dietary Vitamin B_6 (mg)	1.1 ± 0.7	0.8	1.1 ± 0.8	0.8	- 1.29	0.20
Dietary Folate (µg)	262.0 ± 195.3	185.2	178.0 ± 105.3	173.2	- 0.66	0.51
Supplemental Vitamin B_{12} (µg)	25.8 ± 35.3	15.0	64.13 ± 204.1	0.0	- 0.23	0.82
Supplemental Vitamin B_6 (mg)	3.0 ± 2.8	3.0	3.7 ± 6.5	0.0	- 0.09	0.93
Supplemental Folic Acid (µg)	246.7 ± 216.7	400.0	293.3 ± 439.91	0.0	0.00	1.00
uMMA Levels (µg uMMA/mg creatinine)	1.6 ± 0.6	1.6	2.0 ± 0.6	1.8	1.54	0.12

Table 1: *Characteristics of PPI Users and Non-PPI Users.*

Note: *P values resulted from related -samples Wilcoxon signed ranks tests. Total of percentages for carbohydrates, protein, and fat may not equal 100% due to rounding. Only 14 pairs were available for analysis of dietary intake.*
SD = Standard deviation.

Supplemental Intake

Tests for equivalency of groups regarding supplemental intake indicated no significant difference between supplemental vitamin B_{12} (µg) (Z = -0.23, p = 0.82), supplemental vitamin B_6 (mg) (Z = -0.09, p = 0.93), and supplemental folic acid (µg) (Z = 0.00, p = 1.00).

Urinary Methylmalonic Acid Levels

Urinary methylmalonic acid levels ranged from 0.90 to 2.80 µg/mg creatinine in the PPI group and 1.20 to 3.30 µg/mg creatinine in the non-PPI group. The hypothesis that the PPI group would have higher uMMA levels than the non-PPI group was not confirmed (Z = 1.54, p = 0.12).

This study shows for the first time that there were no differences in uMMA levels between those taking PPI's versus those not taking PPI's in the 50-68 year old age range. The findings of this study concur with those of Lukaszuk et al, which evaluated uMMA levels in 22-50 year old adults [24]. Two additional studies conducted in adults in their mid-fifties [15] and those who were > 65 years of age [25] also found that taking PPI's did not deleteriously affect serum vitamin B_{12} levels. In direct contrast to the findings of this study, three studies found that PPI use of at least one year to negatively affect serum vitamin B_{12} status. However, the average age of the subjects in those studies was 80-82 years old [14,16,18] which was much older than the average age of the subjects in this study, which was 57 years old.

Ruscin., *et al.* recommend monitoring of vitamin B_{12} status in individuals taking PPIs more than four years because PPIs may reduce vitamin B_{12} absorption [26]. In this current study, 10 of 15 subjects had been taking PPI's for more than five years, and 7 of 15 subjects had been taking PPI's for more than seven, yet their uMMA levels remained in a normal range. The study results seem to indicate that the PPI group, recruited for this study, had GERD, but were otherwise healthy and likely had normal vitamin B_{12} stores before being placed on PPI's. Thus; monitoring may still be recommended for those at highest risk of vitamin B_{12} deficiency.

Vitamin B_{12} is stored and endogenously recycled in the liver [13]. As stated previously, a deficiency of vitamin B_{12} would take a long length of time to develop, approximately 3-5 years [13]. The PPI users in this study have been taking PPIs for (M ± SD = 7 ± 3.9) years, yet their uMMA levels indicated that their tissue stores of vitamin B_{12} remained within a normal range.

Physical Characteristics

The PPI group and non-PPI group were significantly different 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 = 33.2 ± 6.6; Mdn = 30.9), indicated obesity, while the BMI of non-PPI group (M ± SD = 28.3 ± 4.4; Mdn = 27.3), indicated they were overweight [27]. Because there is an association between BMI and GERD, obesity commonly is seen as a risk factor that contributes to the development of GERD [28,29]. Research has indicated that obesity is associated with a 1.5 to 2 times increase in risk of GERD symptom development [30,31], and this association has been confirmed utilizing multichannel intraluminal impedance-pH monitoring [32]. The mechanism of this relationship is largely unknown; however, one study suggested that an increase in obesity is associated with a postprandial transient lower esophageal sphincter relaxation with subsequent acid reflux [29]. Subjects with a higher BMI in the aforementioned study were more likely to have acid reflux, and therefore, had a greater reliance on PPI's.

Dietary and Supplemental Intake

The PPI group and non-PPI group were not significantly different regarding dietary or supplemental intake of B vitamins. When dietary and supplemental intakes were combined, the daily reference intake for vitaminB_{12}, vitamin B_6 and folic acid was met. The only vitamin that would affect uMMA levels is vitamin B_{12}, and all subjects were ingesting a sufficient amount of vitamin B_{12} based on the established daily reference intake values. As such, there is no reason to suggest that dietary intake or supplementation had any effect on uMMA results.

Urinary Methylmalonic Acid

The only other study using uMMA to measure vitamin B_{12} status was conducted by Lukaszuk., *et al.* which investigated the effect of PPI use on vitamin B_{12} status in 22-50 year olds. That study and the current study both found that uMMA levels were not significantly different between subjects in the PPI group and subjects in the non-PPI group [24]. The use of uMMA, as a biomarker of vitamin B_{12}, has been confirmed by various studies [20-22]. No participants approached an abnormal uMMA level of > 3.8 μg MMA/mg creatinine or > 3.6 mmol/mol creatinine which would have indicated tissue store depletion of vitamin B_{12} [24].

Conclusion

The results of this study indicate that chronic intake of PPI's in 50-68 year olds recruited for this study did not deleteriously affect vitamin B_{12} status as assessed using uMMA levels. The participants in this study were relatively healthy (other than GERD) with no history of Crohn's disease, ulcerative colitis, pernicious anemia, and liver or kidney disease. Future studies should recruit a larger sample size and target higher risk populations such as those individuals with malabsorptive issues, liver disease, renal disease, vegan or gluten free individuals (who are consuming very little vitamin B_{12} from exogenous sources) or those with chronic atrophic gastritis. One may also consider recruiting individuals over the age of 70.

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