Methylmalonic Acid Levels in Non-Elderly Adult Chronic PPI Users

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Abstract

The use of proton pump inhibitors in the elderly population may increase the risk of vitamin B12 deficiency, as gastric acid is needed for vitamin B12 absorption. It is unclear whether the same risk is present for non-elderly adults reporting chronic use of proton pump inhibitors. The aim of this study was to determine whether chronic use of proton pump inhibitors results in increased urinary methylmalonic acid levels in non-elderly adults, indicating risk for vitamin B12 deficiency. Fifteen men and women who had been taking proton pump inhibitors daily for a minimum one year or longer were recruited. Fifteen proton pump inhibitor non-users, age- and gender-matched to subjects in the user group, were recruited. Urinary methylmalonic acid levels determined vitamin B12 status. Urinary methylmalonic acid levels were within the normal range for all study participants. There was no significant difference in urinary methylmalonic acid levels between the proton pump users Mdn=1.50 mcg uMMA /mg creatinine, and non-users Mdn=1.70 mcg uMMA/mg creatinine, p=0.29. Chronic use of proton pump inhibitors did not affect vitamin B12 status of subjects in this pilot study of non-elderly adults. Regular monitoring of vitamin B12 status does not appear to be needed in this age group; however, studies using larger groups are indicated.

INTRODUCTION

Over the last two decades the prevalence of gastroesophageal reflux disease (GERD) has increased in North America and Europe [1]. GERD is a digestive disorder in which stomach content refluxes into the lower esophagus, causing heartburn, chest pain, or regurgitation on a weekly basis [1-3]. GERD often is associated with a decrease in lower esophageal sphincter pressure, which can be caused by increases in abdominal pressure due to obesity and/or overeating, intake of certain foods and beverages, and smoking [2,4]. Left untreated, GERD can lead to erosive esophagitis and “Barrett’s esophagus,” a pre-cancerous condition in which cells in the esophagus become abnormal [1]. The primary medical treatment for GERD includes antacid medications, with proton pump inhibitors (PPIs) constituting the most effective of these because they prevent secretion of gastric acid by the parietal cells in the stomach for 24-48 hours [5]. Chronic use of PPIs has been associated with vitamin B12 deficiency, since vitamin B12 requires gastric acid in order to be absorbed. Without gastric acid, over time, the absorption and assimilation of vitamin B12 in the digestive tract may be severely impaired. Vitamin B12 participates in various biological functions in the body, including DNA synthesis and methylation. In the brain, it maintains the myelin sheath, facilitating normal brain and nervous system functions. Deficiency of vitamin B12 can cause megaloblastic anemia and neuropathy, which commonly are seen in the elderly [6]. Early detection of deficiency and subsequent supplementation with vitamin B12 are necessary steps to prevent irreversible neurological damage [7]. Normally when vitamin B12 is consumed orally, it binds to meat and/or dairy proteins. Gastric acid and pepsin in the stomach help to cleave vitamin B12 from its peptide bond, and vitamin B12 then attaches to an R binder protein called “haptocorrin” [7]. Vitamin B12 cannot be absorbed or reabsorbed as long as it is attached to haptocorrin [7,9]. When the attached vitamin B12 enters the duodenum, the pancreas secretes proteases which selectively digest haptocorrin, allowing vitamin B12 to bind to intrinsic factor (IF) (a binding protein made by the stomach with a specific affinity for vitamin B12) [7-8]. The vitamin B12-IF complex then travels to the ileum, where
it binds to the IF receptor and other proteins on enterocytes; this process then triggers vitamin B\textsubscript{12} absorption via endocytosis [7,9]. Without gastric acid to initiate the vitamin B\textsubscript{12} absorption process, however, depletion of this crucial vitamin may take place. While one study indicated no vitamin B\textsubscript{12} deficiency in elderly PPI users [10], a number of studies have shown that elderly individuals taking PPIs had lower serum vitamin B\textsubscript{12} levels [11-15] and higher homocysteine [12], and serum methylmalonic acid levels [12,13,15] but whether this is true for non-elderly PPI users is unknown. It appears that chronic use of PPIs can induce hypochlorhydria, which leads to malabsorption of vitamin B\textsubscript{12} as gastric acid is required to release vitamin B\textsubscript{12} from food. This can occur across the age spectrum, but seems to have the most impact on elderly individuals and those with low vitamin B\textsubscript{12} stores to start [14-15]. While it would seem that measurement of serum vitamin B\textsubscript{12} would be the best gauge of sufficiency of this nutrient, determination of serum methylmalonic acid (sMMA) or urinary methylmalonic acid (uMMA) levels have been found to be the most precise tests for determining acute tissue stores of vitamin B\textsubscript{12} in the body. Hirschowitz and colleagues [12] found that when only serum vitamin B\textsubscript{12} levels were measured, many vitamin B\textsubscript{12} deficiencies went undiagnosed [16-17]. When homocysteine and sMMA levels were measured, subclinical vitamin B\textsubscript{12} deficiency was detected in an additional 31% of patients whose serum vitamin B\textsubscript{12} levels had been reported as within normal limits. Both sMMA and uMMA levels have higher sensitivity and specificity than the determination of serum vitamin B\textsubscript{12} levels [12]. In the case of vitamin B\textsubscript{12} deficiency, the concentration of methylmalonic acid starts to increase in the blood or urine; therefore, elevated MMA in the blood or urine is an early indicator for Vitamin B\textsubscript{12} deficiency [12,17-19]. Urinary MMA is excreted very efficiently by the kidneys, making it a sensitive indicator of tissue repletion [7]. In addition, the uMMA measurement is less prone to giving false positive results like sMMA [16]. No studies were found that tested whether chronic use of PPIs by non-elderly adults has the same effect on MMA levels as it does on elderly users [20].

The purpose of this study was to determine whether chronic use of PPIs would alter uMMA levels in non-elderly adults.

**MATERIALS AND METHODS**

**Participants**

A quasi-experimental study design was used for this project. Participants were age- and gender-matched to control for these two variables. Thirty men and women were recruited through informational flyers posted on a midwestern university campus and at local businesses, police stations, and fire stations within a 20-mile radius of campus. Fifteen subjects who had been taking proton pump inhibitors (PPIs) daily for a minimum of one year had been recruited first. The 15 subjects in the control group were recruited through a 20-mile radius of campus. Fifteen subjects who had been taking PPIs for at least three years. The PPI users reported that they had been taking PPIs for an average of 5 years. The range for PPI use was 1-13 years, and 67% of test group participants had been on PPIs for at least 3 years. Of the PPI users 40% were taking an over-the-counter dose of a PPI and the remaining 60% were taking a prescription dose. The subjects signed a consent form prior to participating in this study. All study procedures were approved by the Institutional Review Board of Northern Illinois University. 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.

**Measurements**

**Survey:** An informational survey was designed to collect details about demographics, lifestyle behaviors, use of vitamin supplements, and use of PPIs. Participants were queried about their a) smoking habits, b) use of vitamin B\textsubscript{12}, vitamin B\textsubscript{6}, and folic acid supplements, and c) use of specific PPIs.

**Diet analyses:** Diet analyses were performed on the 3-day food logs that participants had completed. Nutrition Calc Plus (version 3.52 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 so that the most consistent results were yielded. Analyses of the following were performed: total Kcal/kg, fat, protein, and carbohydrate as percentages of total Kcal/kg; and dietary vitamin B\textsubscript{12}, vitamin B\textsubscript{6}, and folic acid.

**Anthropometrics:** Subjects reported to the university’s nutrition laboratory where anthropometric measurements were taken on subjects 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, fat mass, fat free 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. Urine samples were collected in sterile containers and were transferred into vials which contained 5 mg of thymol as a preservative, allowing samples to be mailed unrefrigerated. The vials were stored at -20°C until all data had been collected. The samples then were shipped overnight to Norman Clinical Laboratories Inc. (Cincinnati, Ohio) for analysis. Laboratory Measurements. MMA levels of the urine samples were determined by ion monitoring isotope dilution gas chromatography mass spectrometry (GC/MS). The MMA values were normalized to urine creatinine to correct for urine dilution. Five hundred ng of deuterated MMA 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) was interfaced to a mass spectrometer (Finnigan MAT 800 ion trap detector, San Jose, CA). The GC/MS was equipped with
a Finnigan MAT A200S autosampler. 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 minute hold time. Levels of uMMA are considered to be normal if they are < 3.8 mcg MMA/mg creatinine (or < 3.6 mmol/mol creatinine) [22].

Data analyses

Due to the small sample size and the nature of the assignment to groups (matched pairs), the non-parametric related-samples Wilcoxon signed rank test was chosen to test for differences in key variables between PPI users and non-users. The groups were tested for equivalency on anthropometrics, caloric intake; carbohydrate, protein, and fat as percentages of total caloric intake; and intakes of dietary vitamin B₆, vitamin B₁₂, and folate. The related-samples Wilcoxon signed rank statistic also tested the primary hypothesis of the study, that subjects using PPIs would have higher uMMA levels than their age- and gender-matched controls who were not using PPIs.

RESULTS AND DISCUSSION

Tests for equivalency of groups indicated no significant differences between the groups on any of the following: BMI, Z = -1.65, p = 0.10, body fat percent, Z = -0.17, p = 0.87, fat mass, Z = -0.51, p = 0.61, average daily caloric intake, Z = 0.06, p = 0.96, carbohydrate as percentage of caloric intake, Z = 0.57, p = 0.57, protein as percentage of caloric intake, Z = -0.40, p = 0.69, fat as percentage of caloric intake, Z = -0.74, p = 0.46, dietary vitamin B₁₂, Z = -0.28, p = 0.78, dietary vitamin B₆, Z = -0.17, p = 0.87, dietary folate, Z = -0.91, p = 0.36, supplemental B₁₂, Z = -1.22, p = 0.18 supplemental vitamin B₆, Z = -1.22, p = 0.18, and supplemental folic acid Z = -0.96, p = 0.34. PPI users and non-users were statistically different with respect to fat free mass, Z = -2.05, p = 0.04, with PPI users having higher levels of fat free mass than non-users (see Table 1). Only 5 subjects (3 PPI users and 2 non-users) reported taking supplemental oral vitamin B₁₂. Of these, one PPI user reported supplementation with 206 mcg vitamin B₁₂, while the other 4 subjects reported supplement levels less than 20 mcg from a multi-vitamin. Urinary MMA levels ranged from 0.70 - 3.60 mcg MMA/mg creatinine for study participants. Mean and median UMAA levels in mcg/mg creatinine for PPI users and non-users were M(SD)=1.62 ± .265, Mdn=1.50 and M(SD)=1.85 ± .676, Mdn=1.70, respectively. The hypothesis, that subjects taking PPIs would have higher MMA levels than their matched controls who were not taking PPIs, was not supported. There was no significant difference in mcg uMMA/mg creatinine for the PPI users (Mdn = 1.50) and matched control PPI non-users (Mdn = 1.70), Z = 1.06, p = 0.29. This was the first study to show that there were no differences in uMMA levels between non-elderly adult PPI users and their age-and gender-matched counterpart PPI non-users. This is an important finding for several reasons. GERD has significantly increased over the past two decades [1] and with that has come an increased use of PPIs. Chronic use of PPIs has been shown to deplete stores of vitamin B₁₂ in the elderly population [11-15]. Our study results indicate that non-elderly adults taking PPIs long term (minimum 1 year) had sufficient tissue stores of vitamin B₁₂ as measured by uMMA levels.

PPIs affect vitamin B₁₂ status by interfering with absorption of food-bound cobalamin by creating a gastric pH incompatible with release of food-bound vitamin B₁₂. The results of this study seem to indicate that PPI-induced hypochlorhydria may be less common in those under the age of 51 years than it is in the elderly, allowing UMAA levels to remain within the normal range [14,15,22]. Vitamin B₁₂, unlike other water-soluble vitamins, can be stored and retained in the body for several years. In addition, vitamin B₁₂ has a low turnover and only small amounts are excreted daily due to its ability to be reabsorbed and recycled via the enterohehaptic recirculation of bile [24]. Enterohepatic

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

<table>
<thead>
<tr>
<th></th>
<th>PPI Users (n = 15)</th>
<th>PPI Non-Users (n = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mdn</td>
<td>Mean±SD</td>
</tr>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8±4.7</td>
<td>27.2</td>
<td>24.6±4.8</td>
</tr>
<tr>
<td>Body fat percent (% of total body wt.)</td>
<td>24.4±10.7</td>
<td>20.1</td>
<td>23.1±10.7</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>19.9±10.2</td>
<td>17.8</td>
<td>17.9±14.6</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>60.9±13.7</td>
<td>54.6</td>
<td>55.2±11.0</td>
</tr>
<tr>
<td><strong>Dietary/Supplement Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average daily caloric intake (Kcal/kg)</td>
<td>24.8±9.2</td>
<td>22.3</td>
<td>24.5±7.5</td>
</tr>
<tr>
<td>Carbohydrates as % of caloric intake</td>
<td>49.9±7.6</td>
<td>50.6</td>
<td>51.5±9.7</td>
</tr>
<tr>
<td>Protein as % of caloric intake</td>
<td>19.0±4.9</td>
<td>17.7</td>
<td>18.4±2.7</td>
</tr>
<tr>
<td>Fat as % of caloric intake</td>
<td>31.1±7.9</td>
<td>29.9</td>
<td>28.8±5.9</td>
</tr>
<tr>
<td>Dietary vitamin B₁₂ (mcg)</td>
<td>4.5±3.5</td>
<td>3.8</td>
<td>4.1±2.5</td>
</tr>
<tr>
<td>Dietary vitamin B₆ (mg)</td>
<td>1.7±0.7</td>
<td>1.6</td>
<td>1.7±1.0</td>
</tr>
<tr>
<td>Dietary folate (mcg)</td>
<td>343.3±326.3</td>
<td>300.4</td>
<td>283.4±150.7</td>
</tr>
<tr>
<td>Vitamin B₁₂, supplement (mcg)</td>
<td>15.1±5.14</td>
<td>0.0</td>
<td>0.8±2.1</td>
</tr>
<tr>
<td>Vitamin B₆, supplement (mg)</td>
<td>2±7.0</td>
<td>0.0</td>
<td>0.3±0.7</td>
</tr>
<tr>
<td>Folic acid supplement (mcg)</td>
<td>248.4±74.7</td>
<td>0.0</td>
<td>53.3±40.7</td>
</tr>
<tr>
<td>uMMA levels (mcg uMMA/mg creatinine)</td>
<td>1.62±2.65</td>
<td>1.5</td>
<td>1.85±0.68</td>
</tr>
</tbody>
</table>

Note. P values resulted from related-samples Wilcoxon signed rank tests. Total of percentages for carbohydrates, protein, and fat may not equal 100% due to rounding.

SD = Standard deviation
recirculation promotes production of 3-8 mcg of vitamin B₁₂ daily[24]. As a result, a vitamin B₁₂ deficiency may take up to 3 to 5 years to manifest [9], and in this sample of individuals under 51 years of age who were relatively healthy, no deficiency was evident [24]. Another possibility is that the mean dietary intake of 4.5 mcg of vitamin B₁₂ from food sources provided PPI users in this study with sufficient liver stores, lowering their risk of becoming deficient in vitamin B₁₂. A number of studies conducted with elderly subjects found chronic use of PPIs altered vitamin B₁₂ levels [11-15], but those results do not concur with the findings of our study of non-elderly adults. For example, some studies found that individuals over 65 years old who had been on PPIs for more than 12 months had lower serum vitamin B₁₂ levels [11,13,15] and significantly higher sMMA levels [13,15] both of which are indicative of a vitamin B₁₂ deficiency. Elderly adults already are predisposed to impaired gastric function, such as parietal cell destruction leading to low or zero hydrochloric acid production, and this, coupled with regular use of PPIs, may accelerate the vitamin B₁₂ deficiency seen in this age group.

CONCLUSIONS

Results of the current study do not indicate that chronic uses of PPIs are problematic for non-elderly adults, however, at least with respect to depletion of body stores of vitamin B₁₂. This study of non-elderly adult PPI users had several strengths. A major strength was that one of the most precise tests for determining acute tissue stores of vitamin B₁₂, the uMMA test, was implemented. The uMMA test has been judged to be more sensitive than the serum vitamin B₁₂ test, which underestimates vitamin B₁₂ deficiency [12,17-19], or the sMMA test, which is more prone to give false positive results [20].

This study was unique in that several studies targeting sMMA levels in elderly PPI users have been conducted, [12,13,15] but no studies using uMMA in non-elderly adults were found. It is known that liver stores of vitamin B₁₂ can become deficient in 1-3 years in individuals with insufficient gastric acid secretion, [25] thus knowledge about levels of vitamin B₁₂ in long-term users of PPIs of any age is important. In the current study, all PPI users had been taking PPIs for at least 1 year, and 2/3 had been doing so for 3-13 years; the mean number of years of PPI use was 5 years. PPI users mean daily PPI dose was 33.2 mg, and the majority of the group 9/15 were taking prescription strength doses.

Another strength of the current study was that PPI users and non-users were age-, and gender-matched to control for these possible confounding variables. Since dietary habits, supplement intake, and lifestyle behaviors such as smoking cigarettes also can influence vitamin B₁₂ status, the groups were tested for equivalency on these variables, and, with the exception of fat free mass, no significant differences were found.

A limitation of this pilot study was the small sample size. Larger studies are recommended in order to conclude that there is no clinically relevant difference in uMMA levels between groups. Additionally, the study was cross-sectional in design, which also presented a possible limitation. Ideally, measurement of uMMA levels in PPI users should take place prior to the start of the PPI treatment. Since there was no difference in the groups of PPI users and non-users after the users had been on the treatment for an average of 5 years, however, it is still reasonable to conclude that PPI use did not cause a large decrease in tissue stores of vitamin B₁₂, as assessed through uMMA levels.

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REFERENCES


