Holo-transcobalamin is an indicator of vitamin B-12 absorption in healthy adults with adequate vitamin B-12 status¹⁻³


ABSTRACT

Background: It has been hypothesized that the response of holo-transcobalamin (holo-TC) to oral vitamin B-12 may be used to assess absorption. To develop a reliable clinical absorption test that uses holo-TC, it is necessary to determine the optimal timeline for vitamin B-12 administration and postdose assessment.

Objective: The objective of this study was to assess the magnitude and patterns of change in the postabsorption response of holo-TC to oral vitamin B-12.

Design: Adult (18–49 y) male and female participants (n = 21) with normal vitamin B-12 status were given three 9-μg doses of vitamin B-12 at 6-h intervals beginning early morning (baseline) on day 1. Blood was drawn at 17 timed intervals over the course of 3 d for the analysis of holo-TC and other indicators of vitamin B-12 status.

Results: Mean holo-TC increased significantly (P < 0.001) from baseline at 6 h (11%) and 24 h (50%). TC saturation increased significantly (P < 0.001) from baseline at 12.5 h (33%) and 24 h (50%). The mean cobalamin concentration changed significantly (P < 0.001) from baseline at 24 h (15%) and 48 h (14%). The ratio of holo-TC to cobalamin increased significantly (P < 0.001) at 24 h (32%).

Conclusions: The greatest increase in holo-TC was observed 24 h after ingestion of three 9-μg doses of vitamin B-12. Our results indicate that a vitamin B-12 absorption test based on measurement of holo-TC after administration of three 9-μg doses of vitamin B-12 should run for 24 h. Am J Clin Nutr 2007;85:1057–61.

KEY WORDS Transcobalamin, holo-transcobalamin, holo-TC, vitamin B-12, vitamin B-12 absorption, healthy adults

INTRODUCTION

Vitamin B-12 is an essential nutrient functioning as a coenzyme for methionine synthase and methylmalonyl CoA mutase. Circulating vitamin B-12 is bound to 1 of 2 carrier proteins, haptocorrin (HC) or transcobalamin (TC). Although the majority of vitamin B-12 (≈80%) is bound to HC (holo-HC), only TC-bound vitamin B-12 (holo-TC) can be taken up by body cells (1). TC has a half-life of ≈18 h and is sensitive to changes in vitamin B-12 intake (2). Newly ingested vitamin B-12, as holo-TC, can first be detected in the blood 3 h after intake with a maximum plasma concentration occurring at 8–12 h. Once in circulation, holo-TC is taken up into cells within minutes (2, 3).

Depletion of total body cobalamin occurs slowly and is often a result of malabsorption, which is difficult to diagnose clinically (4–7). It is important, however, to detect and treat vitamin B-12 deficiency in the early stages before significant damage occurs. Untreated deficiency may lead to neurologic damage and an increased risk of birth defect–affected pregnancies even when the deficiency is only moderate (8–11). Pernicious anemia, the specific vitamin B-12 deficiency condition caused by a lack of intrinsic factor (IF), may result from an autoimmune response to IF or gastric parietal cells, atrophy of the gastric mucosa, chronic gastritis, and, in rare cases, a congenital defect. Currently, the only available diagnostic tests for pernicious anemia are not clinically practical. The Schilling test, which involves ingestion of radioactively labeled vitamin B-12, a flushing dose of nonlabeled vitamin B-12, and collection of urine over a period of 24 h, requires meticulous adherence to protocol, making it error prone and costly (12–14). Presence of parietal cell and IF antibodies can be measured to diagnose pernicious anemia; however, parietal cell antibodies can occur in other autoimmune diseases, and both tests are only clinically meaningful in a subgroup of patients with autoimmune conditions (15, 16). It has been hypothesized that changes in holo-TC in response to a supplemental dose of vitamin B-12 may be used to assess vitamin B-12 absorption (17–19). Bor et al (18) reported a significant increase in holo-TC and TC saturation 24 and 48 h after receiving three 9-μg doses of oral vitamin B-12. Because no blood was collected before 24 h (after baseline), the magnitude and pattern of change of holo-TC during the first 24 h could not be determined (18). In developing a clinical diagnostic test, it is important to know the optimal time after the dose at which to draw blood. The objective of this study was to evaluate the postabsorption response of holo-TC to oral vitamin B-12 relative to other indicators of vitamin B-12 status.

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SUBJECTS AND METHODS

Subjects

Twenty-one healthy adult men (n = 13) and women (n = 8) aged 18–49 y from the Gainesville, FL, community were selected on the basis of the following inclusion criteria: 1) serum vitamin B-12 concentration >350 pmol/L at the time of screening, 2) no use of vitamin B-12–containing supplements or vitamin B-12 injections during the previous year, 3) no pregnancy or lactation, 4) no use of tobacco products, 5) no history of chronic disease, 6) no anemia [hemoglobin ≥11 g/dL (7.4 mmol/L), women; ≥12 g/dL (8.1 mmol/L), men], 7) normal blood chemistry profile, 8) body mass index (in kg/m²) between 18 and 29, and 9) no blood donations within 30 d of the study.

Study design

All participants signed an informed consent form approved by the University of Florida Institutional Review Board before the initiation of the study. Participants had a fasting blood sample drawn at the University of Florida Shands General Clinical Research Center (GCRC). Subjects’ heights and weights were measured, and a medical history questionnaire was completed. Blood analyses included serum vitamin B-12, blood chemistry profile, hematologic indexes, and a pregnancy test for women. The primary objective of measuring vitamin B-12 in the screening process was to ensure that no enrolled subjects had a severe vitamin B-12 deficiency.

Eligible subjects were admitted to the GCRC the evening before (day 0) the intervention. The following morning (day 1) after an overnight fast, an indwelling catheter was inserted for all blood collections during day 1. A total of 17 timed blood draws were taken from day 1 to day 3, and three 9-g doses of vitamin B-12 were administered at 6-h intervals on day 1, beginning after the baseline blood draw (Figure 1). Immediately after taking each vitamin B-12 dose, subjects consumed a piece of bread and 236 mL (8 oz) juice to improve absorption efficiency (20). In addition to the bread and juice consumed with each vitamin B-12 dose, subjects were given a midmorning snack 2 h after and lunch 3.5 h after dose 1. Dinner was fed 4 h after dose 2, and an evening snack was 3 h after dose 3. The Recommended Dietary Allowance for vitamin B-12 was provided in the diet on day 1 and on day 2. Take-home meals were provided on day 2 of the study. Water and noncaffeinated, noncaloric beverages were allowed ad libitum. Subjects remained in the GCRC overnight and were allowed to leave on pass after a fasting blood sample the morning of day 2. Subjects returned on the morning of day 3 at which time they had the final fasting blood sample drawn.

Biochemical analysis

At each blood collection, holo-TC, total TC, cobalamin, and plasma albumin were measured. The ratios of holo-TC concentration to total-TC concentration (TC saturation) and holo-TC concentration to cobalamin concentration (holo-TC:cobalamin) were determined to assess changes in these indicators in relation to one another. Baseline concentrations of methylmalonic acid, creatinine, serum folate, and homocysteine were also measured. The vitamin B-12 supplement (9 μg cyanocobalamin) was prepared by Westlab Pharmacy (Gainesville, FL). The vitamin B-12 content of the supplement was validated by an independent laboratory (Analytic Research Laboratories, Oklahoma City, OK). Blood samples were collected and stored in a freezer at −80 °C before analysis. Serum vitamin B-12 and folate were assayed on the Advia Centaur automated immunoassay system (Bayer, Tarrytown, NY) with a total imprecision <10%.

Total-TC concentration was determined by a sandwich enzyme-linked immunoabsorbent assay with a total imprecision of 4–6% (intraassay imprecision: ≈3%) (21). After removal of the apolipoprotein TC with vitamin B-12–coated beads, holo-TC was measured by the TC enzyme-linked immunoabsorbent assay. The total imprecision for measurement of holo-TC was ≈8% (22), and the intraassay imprecision was ≈4% (23). Albumin and creatinine were measured with a Cobas Integra 800 (Roche Diagnostics, Indianapolis, IN). Total imprecision was ≈2% for albumin and <3% for creatinine.

Total homocysteine was measured by the immunologic method on the IMMULITE 2000 (Diagnostic Products Corporation, Los Angeles, CA) (total imprecision: <6%) (24), and methylmalonic acid was measured by slightly modified stable-isotope dilution capillary gas chromatography–mass spectrometry (total imprecision: <8%) (25).

Statistical methods

For each dependent variable of interest (eg, cobalamin), a linear model was fitted with independent categorical variables, subject (random effects) and time (fixed effects). The overall P value for time was obtained by the F test that tests the null hypothesis that the distribution of the dependent variable was the same at all time points. Tukey’s method (26) of multiple comparisons was used for assessment of differences between time periods. A least significant difference, as defined by Tukey’s procedure, ensures that simultaneously every target population paired difference in means will be within ± least significant difference of the corresponding difference in sample means with 95% confidence.
RESULTS

Mean baseline values for all analytes were within normal ranges (Table 1) (22, 27). Some subjects had values that were somewhat outside the normal range; however, none of the subjects were severely vitamin B-12 deficient. Measurement of vitamin B-12 at screening was done with the use of a different assay from the one used at baseline of the study and likely explains the variation rather than a true change in vitamin B-12 status. Plasma albumin fluctuated throughout the intervention period, suggesting a change in hydration status throughout day 1 and among the mornings of days 1, 2, and 3 (data not shown). Holo-TC, cobalamin, and total-TC values are reported as a ratio to albumin to adjust for diurnal changes in overall body protein concentration resulting from changes in hydration status.

Unadjusted (for albumin) means and statistical differences for holo-TC, cobalamin, and TC saturation are reported in Table 2. All time points are reported relative to baseline. Of all of the analytes, only holo-TC and TC saturation changed significantly on day 1.

Mean holo-TC concentration increased steadily after baseline and fluctuated throughout day 1. Statistically significant increases were observed in mean holo-TC during the first 24 h of the intervention, although these small increases were not maintained. Mean holo-TC concentration reached a maximum value at 24 h, which was a significant increase relative to baseline and all other time points (Figure 2). The mean percentage increase from baseline was also greater at 24 h than at all other time points with a 49% increase relative to baseline and a 29% increase relative to 12 h (Figure 3). This peak at 24 h was observed for almost all subjects, with an increase of ≥22% (22–85%) for all but 1 subject. By 48 h, mean holo-TC concentration decreased significantly relative to 24 h (33%); however, it was still significantly greater than baseline (Figure 2).

Mean serum cobalamin concentration did not increase significantly relative to baseline on day 1, although there were fluctuations in concentrations throughout the day. At 24 h mean serum cobalamin concentration was significantly greater than baseline (Figure 2). Overall, the percentage change in cobalamin concentration was smaller than for holo-TC throughout the intervention period with ranges of −2% to 15% and −1% to 50%, respectively.

Mean total-TC concentration did not change significantly during the study, varying <6% from baseline at all time points (data not shown). Mean TC saturation began to increase significantly relative to baseline at 12.5 h, with the most significant increase at 24 h (Figure 2). As observed with holo-TC concentration, the mean TC saturation and percentage change at 24 h were significantly greater than at all other time points with 48% and 15% increases from baseline and 12.5 h, respectively (Figure 2). Among all subjects, the percentage change from baseline ranged from 7% to 109%; 15 of 21 subjects had a value of ≥22%. The ratio of holo-TC to cobalamin did not increase significantly until 24 h with absolute and percentage increases of 0.15% and 22%, respectively. The range for percentage change in this ratio among all subjects was −7% to 109%; 15 of 21 subjects had an increase of ≥23% at 24 h.

DISCUSSION

In this intervention study, the changes in markers of vitamin B-12 status were measured hourly during and after administration of three 9-μg doses of oral vitamin B-12. In previous studies, the changes in response to similar vitamin B-12 doses were measured after 24 h; however, no data were collected before that time point (17–19). The data from the present study indicate that a series of three 9-μg doses of oral vitamin B-12, given over 12 h, led to small fluctuations in holo-TC concentration during the first study day followed by the previously observed maximum increase in holo-TC concentration 24 h after the first vitamin B-12 dose was given. A similarity was observed in the overall pattern of change in holo-TC, cobalamin, and TC saturation, with a gradual increase over the first day with the most pronounced increase 24 h after the initial vitamin B-12 dose and 13 h after the final vitamin B-12 dose. Because no measurements were taken between 12.5 and 24 h, we cannot unequivocally conclude that...

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Reference interval</th>
</tr>
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<tbody>
<tr>
<td>Holo-TC (pmol/L)</td>
<td>85 ± 38 (41–208)</td>
<td>40–150</td>
</tr>
<tr>
<td>Cobalamin (pmol/L)</td>
<td>407 ± 118 (241–710)</td>
<td>200–600</td>
</tr>
<tr>
<td>TC saturation (%)</td>
<td>0.12 (0.05–0.27)</td>
<td>0.05–0.20</td>
</tr>
<tr>
<td>Holo-TC:cobalamin (%)</td>
<td>0.22 (0.08–0.44)</td>
<td>0.15–0.51</td>
</tr>
<tr>
<td>Hcy (μmol/L)</td>
<td>6.6 ± 1.4 (3.9–9.3)</td>
<td>4.5–11.9</td>
</tr>
<tr>
<td>MMA (μmol/L)</td>
<td>0.134 ± 0.060 (0.08–0.32)</td>
<td>0.08–0.28</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>32.7 ± 7.3 (22.2–54.4)</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>69 ± 11.7 (48–87)</td>
<td>50–100</td>
</tr>
</tbody>
</table>

1. Holo-TC, holotranscobalamin; Hcy, homocysteine; MMA, methylmalonic acid.
2. x ± SD; range in parentheses (all such values).
3. From Nexo et al (22).

### Table 2

<table>
<thead>
<tr>
<th>Time from baseline (h)</th>
<th>Holo-TC</th>
<th>Cobalamin</th>
<th>Total TC pmol/L</th>
</tr>
</thead>
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<tr>
<td>0.0</td>
<td>85 ± 38</td>
<td>407 ± 118</td>
<td>712 ± 135</td>
</tr>
<tr>
<td>0.5</td>
<td>84 ± 38</td>
<td>395 ± 113</td>
<td>688 ± 134</td>
</tr>
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<td>1.5</td>
<td>85 ± 39</td>
<td>397 ± 109</td>
<td>706 ± 135</td>
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<td>2.5</td>
<td>89 ± 43</td>
<td>409 ± 114</td>
<td>723 ± 136</td>
</tr>
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<td>3.5</td>
<td>91 ± 43</td>
<td>421 ± 113</td>
<td>743 ± 138</td>
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<td>4.5</td>
<td>96 ± 43</td>
<td>431 ± 126</td>
<td>746 ± 147</td>
</tr>
<tr>
<td>5.5</td>
<td>97 ± 41</td>
<td>414 ± 109</td>
<td>763 ± 144</td>
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<td>6.0</td>
<td>99 ± 45</td>
<td>423 ± 117</td>
<td>752 ± 141</td>
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<tr>
<td>7.0</td>
<td>97 ± 42</td>
<td>426 ± 117</td>
<td>755 ± 128</td>
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<td>8.0</td>
<td>95 ± 41</td>
<td>424 ± 102</td>
<td>773 ± 139</td>
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<td>10.0</td>
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<td>428 ± 102</td>
<td>772 ± 144</td>
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<td>11.0</td>
<td>97 ± 39</td>
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<td>24</td>
<td>124 ± 46</td>
<td>456 ± 110</td>
<td>715 ± 129</td>
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<tr>
<td>48</td>
<td>102 ± 37</td>
<td>456 ± 115</td>
<td>758 ± 145</td>
</tr>
</tbody>
</table>

1. All values are x ± SD. Values are not reported as a ratio to albumin.
2. Holo-TC, holotranscobalamin; TC, transcobalamin.
3. Significantly different from baseline, P < 0.001 (ANOVA, Tukey’s test).
4. Significantly different from all other values, P < 0.001 (ANOVA, Tukey’s test).
The true maximum increase in concentration for all markers occurred at 24 h. The timing of vitamin B-12 absorption and metabolism may explain the pattern of change observed in holo-TC concentration during the first 12 h of the intervention. After ingesting vitamin B-12, an increase in holo-TC is first measurable in the blood at 3–4 h, and holo-TC can be taken up by cells within minutes (2). It is hypothesized that until cells are saturated with holo-TC, most of it will be taken up so quickly that no large changes would be observed initially in the blood. When intake is sufficient to saturate the cells with vitamin B-12, significant changes in holo-TC can then be measured. One potential limitation of the current protocol is that a person with a low vitamin B-12 concentration because of dietary deficiency alone may require more vitamin B-12 to saturate tissues before significant changes in holo-TC can be measured. Future investigations should compare the response to this same intervention protocol in persons with vitamin B-12 deficiency but no problems with vitamin B-12 absorption with persons with adequate vitamin B-12 status.

The absolute and percentage increases in cobalamin concentration were smaller, occurred later, and were maintained longer than those for holo-TC. This finding is not surprising because total serum cobalamin primarily consists of holo-HC, and the slower rate of HC metabolism relative to TC metabolism leads to a slower overall turnover of serum cobalamin and a slower response to changes in intake (1, 28, 29). When comparing these 2 measures among the individual subjects, holo-TC had the most consistent pattern with only 1 subject not having a change of ≥20% at 24 h. In addition the mean percentage change at 24 h was 3 times that of cobalamin. Holo-TC is clearly a more sensitive indicator of change in vitamin B-12 intake and absorption than is serum cobalamin because it increased earlier after supplementation, increased relatively more, and decreased earlier after supplementation ceased.

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Total TC did not change significantly during the intervention period. TC saturation increased in a similar manner to holo-TC (Figure 4). Both holo-TC concentration and TC saturation had comparable results even when considering individual subjects. Of all subjects, 95% and 90% had increases of >22% at 24 h for holo-TC and TC saturation, respectively. In a previous study, a larger change in TC saturation (at 24 h) than for holo-TC was observed which was due to a drop in total TC at this time point (18). No such conclusion can be made from these data because no significant difference was observed in total TC at any time point. Because TC saturation is calculated rather than a direct measure, the potential error in this value is greater than that for holo-TC. Therefore, holo-TC may be the better indicator to use.

This is the first study to monitor hourly changes in holo-TC in response to oral intake of vitamin B-12. The most significant change in holo-TC occurred at 24 h, indicating that this is the optimal time after dose at which to measure holo-TC. The three 9-μg vitamin dose sequence used in this study has previously been chosen to minimize passive absorption and to maximize the amount of actively absorbed vitamin B-12 (17, 18). This aspect of the protocol would be important in a clinical vitamin B-12 absorption test, because it is the capacity to actively absorb vitamin B-12 that is being assessed. Further studies evaluating the necessity of 3 doses and the exact timing of the doses are warranted. In addition, it is possible that the peak in holo-TC concentration at 24 h could be due to a decrease in cellular uptake at this time point. The TC receptor could undergo a similar refractory period as is observed in IF, so that fewer receptors are available for a certain time after being saturated with holo-TC, resulting in an increase of holo-TC remaining in circulation. Future studies focusing on the capacity of TC receptor to take up holo-TC might help identify whether this is indeed occurring; however, note that even if the peak at 24 h was due to a reduction in cellular uptake, if significant changes in holo-TC do not occur in the malabsorbers as was reported, the current intervention protocol would still be useful in diagnosing a vitamin B-12 malabsorption condition (17, 18).

In conclusion, holo-TC increases measurably in response to administration of oral vitamin B-12 within 6 h with a maximum peak at 24 h after an overnight period. Our results indicate that a vitamin B-12 absorption test based on measurement of holo-TC after 3 doses of 9 μg oral vitamin B-12 should run for 24 h.

KMvCR designed the experiment, collected and prepared the samples, analyzed the data, and wrote the manuscript. ALM and EN designed the experiment, analyzed the sample, interpreted the data, and wrote the manuscript. CAE supervised the clinical protocol, designed the clinical diet, and edited the manuscript. DRM managed the overall laboratory operations, sample collection, and preparation. JJS performed the statistical analyses and wrote the manuscript. JFV performed the physical examination and supervised the subjects. GPAK designed the clinical diet and edited the manuscript. LBB designed the experiment, interpreted the data, and wrote and edited the manuscript. None of the authors had a conflict of interest.

REFERENCES