Nonradioactive Vitamin B₁₂ Absorption Test Evaluated in Controls and in Patients with Inherited Malabsorption of Vitamin B₁₂

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Background: Current tests for evaluation of vitamin B₁₂ absorption are problematic because they involve the use of radioactively labeled vitamin B₁₂. We describe a vitamin B₁₂ absorption test that circumvents this problem.

Methods: We measured cobalamin or transcobalamin saturated with cobalamin (holo-TC) 24 h after three 9-μg doses of vitamin B₁₂ given orally at 6-h intervals. We studied 17 patients with inherited malabsorption of vitamin B₁₂ attributable to Imerslund–Grasbeck syndrome (n = 13) or intrinsic factor deficiency (n = 4), their obligate heterozygous biological parents (n = 19), and healthy controls (n = 44).

Results: In the patients, the median (range) change of holo-TC after the B₁₂ load was not significant [1 (−42 to 5) pmol/L], nor was the change of cobalamin [−3 (−32 to 22) pmol/L], consistent with a lack of measurable active or passive absorption. In controls, however, the median (range) increases of holo-TC and cobalamin were 26 (−6 to 63) pmol/L and 41 (−37 to 109) pmol/L, respectively. Similarly, the parents showed increases of 23 (−2 to 47) pmol/L and 27 (−15 to 94) pmol/L. The mean areas under the ROC curves (95% confidence intervals) were 0.97 (0.93–1.0) for holo-TC and 0.87 (0.79–0.94) for cobalamin, distinguishing patients from controls. At a cutoff of 6 pmol/L for holo-TC, the diagnostic sensitivity (95% confidence interval) was 100 (81–100)% and the diagnostic specificity was 92 (82–97)%.

Conclusion: Measurement of holo-TC after administration of vitamin B₁₂ is a promising approach for evaluating vitamin B₁₂ absorption.

Evaluation of the intestinal absorption of vitamin B₁₂ is a necessary step in determining the etiology of vitamin B₁₂ deficiencies. All available tests, including the classic Schilling test, rely on measurement of radioactively labeled vitamin B₁₂ after an oral dose of the vitamin (1). The use of a radioactively labeled compound has made the tests increasingly unacceptable (2). An alternative approach is therefore needed for estimation of the absorption of vitamin B₁₂. Measurement of vitamin B₁₂–saturated transcobalamin (holo-TC)⁴ or total cobalamin after oral ingestion of vitamin B₁₂ may represent such a test.

We recently demonstrated that a consistent and significant increase in holo-TC occurs in healthy individuals after oral intake of three 9-μg doses of vitamin B₁₂ given at 6-h intervals, indicating that this analyte reflects active vitamin B₁₂ absorption (3). In the present study, we evaluated the use of this new approach as a vitamin B₁₂ absorption test in patients with inherited malabsorption of vitamin B₁₂ attributable to Imerslund–Grasbeck syndrome (IGS) or lack of intrinsic factor (IF), their obligate heterozygous parents, and healthy controls.

Materials and Methods

The participants, all Turkish, were divided into 3 groups: patients [n = 17 (8 females and 9 males); median (range) age, 17 (8–32) years; 13 with IGS and 4 with hereditary IF deficiency]; their biological parents [n = 19; 9 females and 10 males; median age, 40 (30–57) years], and volunteers assumed to be healthy [n = 44; 22 females and 22 males; median age, 25 (9–58) years]. Patient characteristics are shown in Table 1.

All 17 patients have been described previously (4–8) and had been given vitamin B₁₂ from initial diagnosis. The treatment schedule was 1000 μg of oral vitamin B₁₂ given at 2-week intervals for the last 5 years (5). The Schilling tests indicating no ability to absorb vitamin B₁₂
had been performed in 13 of the patients around the time of diagnosis (Table 1).

IGS and hereditary IF deficiency are characterized by recessive inheritance. Thus, the biological parents (father and mother) of these patients should be heterozygous, but genetic analyses were not available for any of the parents. One of the parents of 3 patients and both parents of 8 patients participated in the study.

The controls were healthy individuals not suffering from chronic diseases or taking any form of medicine or vitamins.

Written informed consent was obtained from all participants and the parents of patients who were under the age of 18. The Research Ethics Committee of Hacettepe University Hospital approved the study protocol. The study was carried out from November 2003 to October 2004.

The nonlabeled oral vitamin B₁₂ absorption test is based on measurement of serum holo-TC before and after oral intake of 3 oral 9-μg doses of vitamin B₁₂ given at 6-h intervals (3). Blood samples were taken at 0800 on the day before the start of the study (day 0) and on day 1. After the blood sample was taken on day 0, oral 9-μg doses of vitamin B₁₂ (Natur Drogeriet A/S) were administered with a glass of water 3 times, (0800, 1400, and 2000; time points were allowed to deviate ±45 min). The participants were allowed to have a light breakfast, not including meat or any dairy products, 30–60 min before blood sampling but were otherwise allowed to eat their typical diet. For those patients taking vitamin B₁₂ as a treatment for disease, the test was performed 2 weeks after the last oral intake of vitamin B₁₂.

The blood samples were centrifuged within 1 h and stored at −20 °C until further processing.

Serum holo-TC was measured by ELISA (9) and serum cobalamin on a Centaur analyzer (Bayer Corporation).

Spearman correlation coefficients were used to describe the correlation between continuous variables. P values <5% were regarded as statistically significant. ROC curves and areas [with 95% confidence intervals (CIs)] were used to estimate diagnostic accuracy for an increase in holo-TC and cobalamin as a diagnostic marker for diagnosing vitamin B₁₂ malabsorption. Data were analyzed with Prism 4 (GraphPad) software.

### Results

We evaluated an oral nonradioactive vitamin B₁₂ absorption test in 3 groups of individuals. One group consisted of 17 patients unable to actively absorb the vitamin because of inherited lack of IF (n = 4) or defects in the IF receptors cubillin or amnionless (n = 13; Table 1). The second group was 19 parents of patients, all expected to

<table>
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<th>Mutated gene</th>
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a A genetic diagnosis was available for 12 patients, as described in Tanner et al. (7) for patients 1, 2, 5, 7, 10, 13, and 14 and in Tanner et al. (8) for patients 3, 4, and 6. The genetic diagnoses for patients 16 and 17 have been determined but not yet published. Five patients (?) are still under evaluation, but they are believed to have IGS.

b Patients who are siblings are indicated by the same letter.

c Reference interval for Schilling test (^58Co): 11%–28%.

d Reference interval >50 pmol/L.

e Reference interval 200–600 pmol/L.

f AMN, amnionless; GIF, gastric IF; NA, not available; CUBN, cubillin.

g Genetic analysis has not been completed.
be heterozygous for nonactive absorption of vitamin B₁₂. The last group consisted of 44 healthy controls.

The patient group showed no significant change in either holo-TC or cobalamin as measured in blood samples collected after the intake of the test dose of vitamin B₁₂ (Fig. 1). The median (range) changes were 1 (−42 to 5) pmol/L for holo-TC and −3 (−32 to 22) pmol/L for cobalamin. There was no difference between findings in the patients with ICS and those with hereditary IF deficiency.

The control group showed a highly significant increase in holo-TC and cobalamin (P < 0.001 for both) 1 day after an oral dose of vitamin B₁₂. There was no relationship between sex and the observed increases in cobalamin and holo-TC, whereas we found a significant negative relationship between age and the increase in holo-TC (Spearman ρ = −0.51; P = 0.0004). We also found a significant negative relationship between the starting concentrations of holo-TC (Spearman ρ = −0.4; P = 0.007) and cobalamin (Spearman ρ = −0.34; P = 0.02) and the increment in these analytes after the oral dose of vitamin B₁₂.

In the parents, concentrations of both holo-TC and cobalamin increased significantly (P < 0.001 and P < 0.01, respectively; Fig. 1) after the test dose of vitamin B₁₂. The absolute increases in holo-TC and cobalamin concentrations did not differ significantly between the parents [median (range) increase, 23 (−2 to 47) and 27 (−15 to 94) pmol/L, respectively] and the controls [26 (−6 to 63) and 41 (−37 to 109) pmol/L, respectively].

We used ROC curves to compare the diagnostic accuracy of holo-TC and cobalamin as an oral vitamin B₁₂ absorption test (Fig. 2). For this purpose, we used changes in holo-TC and cobalamin concentrations after the absorption test observed in patients (n = 17) and in controls (n = 44). The areas under the ROC curves were 0.97 (95% CI, 0.93–1.0) for holo-TC and 0.87 (0.79–0.94) for cobalamin. A cutoff limit of 6 pmol/L for holo-TC gives diagnostic sensitivity of 100% (95% confidence, 81%–100%) and diagnostic specificity of 92% (82%–97%).

**Discussion**

The results of this study indicate that measurement of holo-TC after an oral dose of vitamin B₁₂ can identify patients with hereditary disorders of absorption of vitamin B₁₂. The patient group was not able to actively absorb vitamin B₁₂; therefore, any absorption of the vitamin during the proposed test would suggest the occurrence of passive absorption. Passive absorption did not take place with the vitamin B₁₂ dose used in our study: the patient group showed no significant change in either holo-TC or cobalamin measured in blood samples after the vitamin B₁₂ load. This result is an important prerequisite for the use of the proposed test. Passive absorption of vitamin B₁₂ is believed to account for ~1% of the administered dose of the vitamin; we were therefore concerned whether the high physiologic dose used in our new test would lead to passive absorption, mimicking...
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Vitamin B₁₂ absorption has been studied by various methods, including the urinary excretion of orally administered radioactively labeled vitamin B₁₂ alone (Shilling test I) or in combination with IF (Shilling test II) (1). Performance of these tests has been increasingly difficult because of the limited availability of radioactively labeled vitamin B₁₂ and decreasing acceptance of a radioactively labeled vitamin in a diagnostic test (2). Moreover, the IF of human origin used in these tests has been removed from the market in most countries. Our new test may represent a suitable alternative to the Shilling test and have the advantage of requiring neither labeled vitamin B₁₂ nor the collection of a 24-h urine sample. In the present study, we tested only the absorption of free vitamin B₁₂. Recombinant human IF is now available commercially (14, 15), and as soon as it becomes available for human use, our design can be used to test whether IF can correct negative absorption of free vitamin B₁₂. In our patient group, this would help distinguish those with vitamin B₁₂ malabsorption attributable to a defective receptor from those with inherited lack of IF. Only in the latter group would one expect to be able to correct vitamin B₁₂ absorption by addition of IF.

In conclusion, measurement of holo-TC before and after oral intake of vitamin B₁₂ is a promising approach to evaluate vitamin B₁₂ absorption and may constitute a substitute for the Schilling test I.

We warmly acknowledge the excellent technical assistance of Anna-Lisa Christensen and Jette Fisker Pedersen. This work was supported in part by a European Union demonstration project on the diagnostic utility of holo-TC (QLK3-CT-2002-01775). Ebba Nexo is a cofounder and a member of the board of Cobento Biotech A/S. This company produces recombinant human IF in plants. The company has patents pending for the use of this protein, which has been named CobaSorb, that also cover its use in the vitamin B₁₂ absorption tests described in this report.

References

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