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Holo-Transcobalamin Is an Early Marker of Changes in Cobalamin Homeostasis. A Randomized Placebo-controlled Study

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Background: **We examined the effect of oral vitamin B_{12} treatment on fluctuations in plasma total cobalamin and its binding proteins transcobalamin (TC) and haptocorrin (HC).**

Methods: **Patients (n = 88; age range, 38–80 years) undergoing coronary angiography (part of the homocysteine-lowering Western Norway B-Vitamin Intervention Trial) were allocated to daily oral treatment with (*a*) vitamin B_{12} (0.4 mg), folic acid (0.8 mg), and vitamin B_6 (40 mg); (*b*) vitamin B_{12} and folic acid; (*c*) vitamin B_6; or (*d*) placebo. EDTA blood was obtained before treatment and 3, 14, 28, and 84 days thereafter.**

Results: **The intraindividual variation for patients not treated with B_{12} was ~10% for plasma total cobalamin, total TC, apo-TC, and apo-HC, and <20% for holo-TC and TC saturation. In B_{12}-treated patients, the maximum change in concentrations was observed already after 3 days for total TC (−16%), holo-TC (+54%), and TC saturation (+82%). At this time holo-HC (+20%) and plasma total cobalamin (+28%) showed an initial burst, but had increased further at 84 days. All changes were highly significant compared with the control group (P <0.0001).**

Conclusions: **Oral vitamin B_{12} treatment produces maximal effects on total TC, holo-TC, and TC saturation within 3 days, whereas maximal increases in holo-HC and plasma total cobalamin occur later. The results support the view that holo-TC is an early marker of changes in cobalamin homeostasis.**

In plasma, the majority of total cobalamin (vitamin B_{12}) is bound to two carrier proteins, transcobalamin (TC)[7] and haptocorrin (HC) *(1)*.

TC is essential for the transport of cobalamin from the intestine and into most cells of the body; patients lacking this protein eventually develop cobalamin deficiency *(2)*. TC carries one-third of the circulating cobalamin (holo-TC), but the major portion of this protein is present in its unsaturated form (apo-TC) *(1, 3)*. Recently, improved methods for the measurement of holo-TC have been developed *(4, 5)*. Whether measurement of holo-TC can help in the elucidation of cobalamin metabolism is a current topic of discussion *(6)*.

HC carries most of the circulating cobalamin (holo-HC), and typically, only a minor fraction of this protein is present in its unsaturated state (apo-HC) *(1, 3)*. The function of HC is still being debated *(7)*.

In the present work we studied the effect of oral vitamin B_{12} treatment on plasma total cobalamin and its binding proteins in patients not suffering from vitamin B_{12} deficiency.

Materials and Methods

PATIENTS AND BLOOD SAMPLES

The investigation was a substudy of the Western Norway B-Vitamin Intervention Trial (WENBIT), a prospective randomized double-blind study on the effects of homo-

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[7] Nonstandard abbreviations: TC, transcobalamin; HC, haptocorrin; and WENBIT, Western Norway B-Vitamin Intervention Trial.

cysteine-lowering therapy on mortality and cardiovascular events. Adult patients undergoing coronary angiography are eligible for WENBIT irrespective of subsequent therapy. Exclusion criteria are malignant disease, alcohol abuse, mental illness, patient unwillingness to participate in long-term follow-up, and patient participation in other studies. A total of 90 consecutive patients (age range, 38–80 years; 21 females and 69 males) recruited to Haukeland University Hospital from April 1999 to September 1999 participated in this substudy. Except for one patient in the control group, none of the patients was judged to have vitamin B_{12} deficiency based on measurement of plasma cobalamin (reference interval, 200–650 pmol/L) and plasma methylmalonic acid (reference interval, 0.08–0.28 μmol/L). Written informed consent was obtained from all patients, and the regional ethics committee approved the study protocol.

Recruited patients were randomized into four groups in a 2 × 2 factorial block design to daily oral treatment with vitamin B_{12} (0.4 mg), folic acid (0.8 mg), and vitamin B_6 (40 mg; group A); vitamin B_{12} (0.4 mg) and folic acid (0.8 mg; group B); vitamin B_6 (40 mg; group C); or placebo (group D). For the first 2 weeks the folic acid groups (A and B) received an additional capsules with a loading dose of folic acid (5 mg/day); the other two groups (C and D) received additional placebo capsules. Packages of study capsules were prepared and given serial numbers in random order by Alpharma ApS (Copenhagen, Denmark).

Patients were grouped according to treatment with vitamin B_{12} (A and B; treatment group) or without vitamin B_{12} (C and D; control group). Two patients in the control group were excluded because of regular treatment with B_{12} injections or multivitamins containing vitamin B_{12}.

EDTA blood was collected before and 3, 14, 28, and 84 days after the start of vitamin treatment. Eleven patients (6 treated and 5 controls) had only four, 1 control had only three, and 1 control had only two samples collected. The blood samples were immediately placed on ice and centrifuged within 30 min; plasma was stored at −80 °C until additional processing.

BIOCHEMICAL ANALYSES

Plasma total cobalamin was determined by a commercial method (Bayer Corporation) on a Centaur instrument (analytical imprecision <10%).

Plasma total TC and plasma holo-TC were measured by ELISA as described recently (analytical imprecision, 5% for total TC and 8% for holo-TC) *(4, 8)*.

Plasma apo-HC and apo-TC were measured after separation of the cobalamin-saturated proteins with silica gel (analytical imprecision <10%) *(9)*.

STATISTICAL ANALYSIS

Biochemical markers for the cobalamin-treated group (A plus B) and the control group (C and D) were compared by use of an independent-samples *t*-test. We also used independent-sample *t*-tests to compare changes in the biochemical markers between the treatment group and control group. The Levene test was used to test for equal variances. Linear regression analysis was used for analyzing the association between the biochemical markers and age. The intraindividual variation was estimated from the SD of the biochemical markers. The analyses were carried out according to the intention-to-treat principle. Data were log-transformed as appropriate to assure gaussian distribution. *P* values <5% were regarded as statistically significant. Data were analyzed using SPSS 10.0 (SPSS Inc.).

Results

Before intervention with vitamin or placebo, the concentrations of plasma total cobalamin and its binding proteins were not different among the four groups (Table 1).

After intervention, no differences were observed regarding changes in total cobalamin and cobalamin-binding proteins between the two groups not receiving vitamin B_{12} (C and D) or between the two groups receiving vitamin B_{12} (A and B; data not shown). Thus, intervention with vitamin B_6 had no influence on the markers studied.

The intraindividual variation was calculated from the serial measurement of the analytes in the control group (C and D). The intraindividual variation was ~10% for plasma total cobalamin and its binding proteins except for plasma holo-TC and TC saturation, which showed somewhat higher values (Table 1).

The changes in plasma total cobalamin and cobalamin-binding proteins were followed at timed intervals after treatment with oral vitamin B_{12} or placebo (Fig. 1). No major changes in these variables were observed for the control group, whereas the treatment group showed

Table 1. Range of values and intraindividual variation for plasma total cobalamin and its binding proteins in patients (n = 88) participating in the WENBIT study.

Biochemical marker	Range (median) at baseline, pmol/L	Intraindividual variation,[a] %	Reference interval,[b] pmol/L
Holo-TC	30–330 (97)	16	40–150
Apo-TC	360–1100 (640)	8	400–930
Total TC	580–1500 (980)	9	600–1500
TC saturation, fraction[c]	0.02–0.29 (0.10)	17	0.05–0.20
Holo-HC[d]	110–870 (240)	12	
Apo-HC	62–340 (100)	10	90–275
Total cobalamin	140–930 (340)	9	200–650

[a] Intraindividual variation was calculated from results obtained on days 0, 3, 14, 28, and 84 for 43 patients not receiving B_{12} for all markers except apo-TC and apo-HC, for which the calculations were based on values obtained on days 0 and 84 from the 38 patients participating on both occasions.

[b] Information on reference intervals was obtained from Gimsing et al. *(3)* and Nexo and coworkers *(4, 8)*.

[c] Calculated as holo-TC/total TC.

[d] Calculated as plasma total cobalamin minus holo-TC.

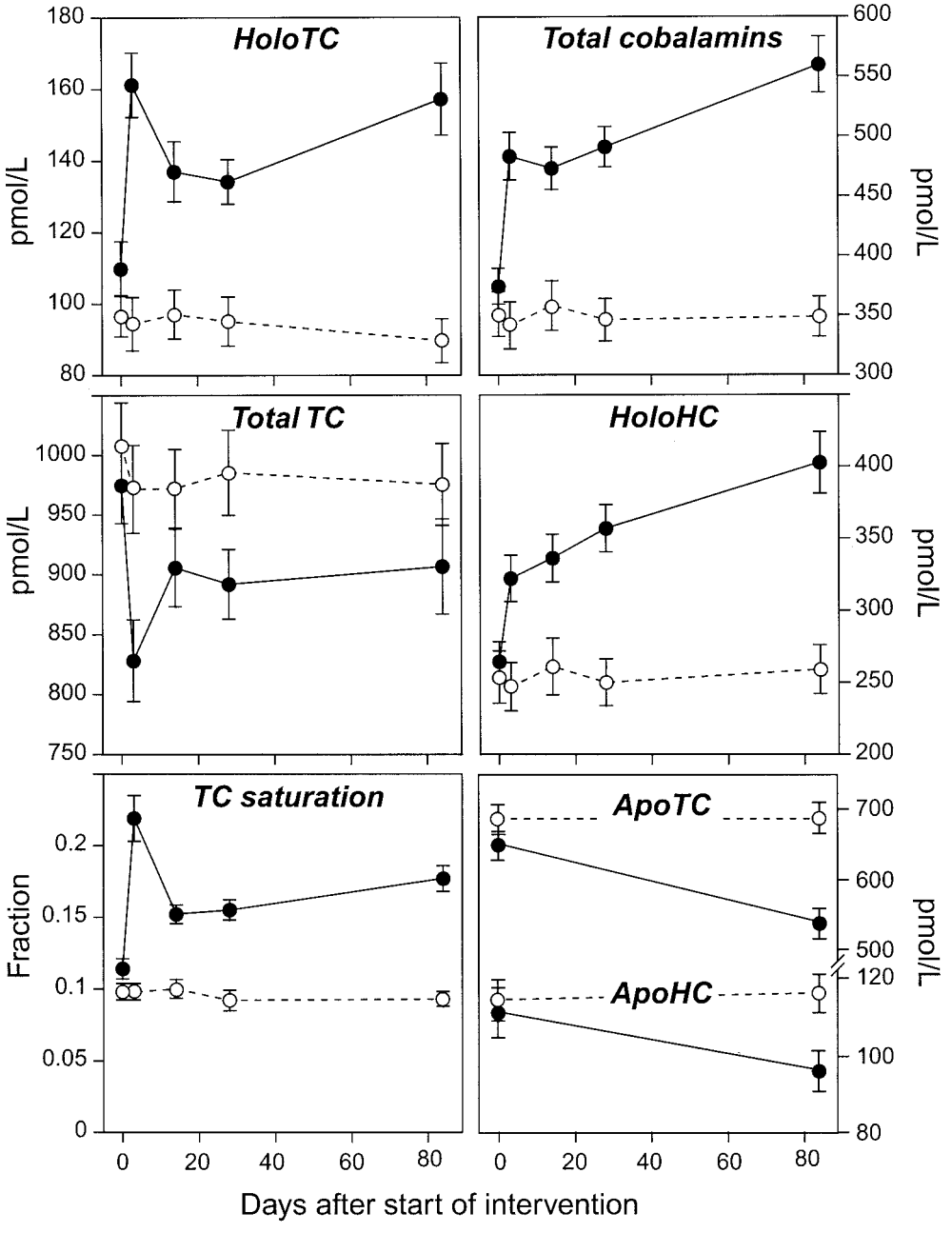

Fig. 1. Plasma total cobalamin and its binding proteins in the treatment (n = 45; ●) and control group (n = 43; ○) before intervention and 3, 14, 28, and 84 days thereafter.
Mean and SE (*error bars*) are indicated.

highly significant changes in all variables when compared with the baseline values and with values obtained for the control group ($P < 0.0001$ for all days studied). The most interesting patterns were observed for holo-TC, TC saturation, and total TC. After 3 days, both holo-TC and TC saturation reached their highest values (means, +54% and +82%, respectively), whereas total TC reached its lowest value (mean, −16%). At this point the TC saturation showed a broad range of values, reflecting a combination of opposite changes in holo-TC and total TC, but after 84 days the interindividual variation was low and only one participant had plasma TC saturation <0.1.

The concentrations of holo-HC and plasma total cobalamin increased to means of +20% and +28%, respectively, after 3 days, but continued to increase and reached mean values of +44% and +45%, respectively, after 84 days. At this point, no participant had a plasma holo-HC <175 pmol/L or a plasma total cobalamin <325 pmol/L.

Discussion

We describe the intraindividual variation for markers related to the metabolism of cobalamin in non-B_{12}-deficient patients, and we describe the changes observed after treatment with oral vitamin B_{12}. We conclude that TC-related markers are early and responsive indicators of changes in vitamin B_{12} status, whereas holo-HC and plasma total cobalamin may reflect accumulation of the vitamin.

The dose of vitamin B_{12} used in the present study is more than 100 times the daily requirement, but it is low compared with the dose recommended for oral treatment of patients suffering from vitamin B_{12} deficiency attributable to a lack of intrinsic factor *(10)*.

Absorption of an increased amount of cobalamin was evident after 3 days of treatment. The most remarkable changes were observed for the TC-associated markers. Holo-TC increased and total TC decreased, whereas their ratio (TC saturation) increased from a median of 0.14 to almost twice this value. The variations in TC saturation were considerable, but TC saturation did not exceed 0.6 in any of the patients, indicating that the carrier capacity of TC was in excess of the amount of cobalamin absorbed. This is in contrast to patients receiving parental vitamin B_{12}. In these patients [e.g., as part of the Schilling test *(11)*], TC is completely saturated with cobalamin and the excess free vitamin is excreted into the urine.

The reason for the significant decrease in total TC that we observed is unknown. One possibility is that the plasma clearance of holo-TC is faster than the clearance of apo-TC. Another possibility is down-regulation of TC synthesis by excess cobalamin.

The pattern observed for plasma total cobalamin and especially holo-HC was different from the changes in the TC-derived markers. Holo-HC showed an increase after 3 days and continued to increase moderately thereafter throughout the 84 days of the study. The corresponding reduction in apo-HC was considerably smaller than the increment observed for holo-HC, suggesting a net increase in the amount of circulating HC. We did not analyze these changes in detail because it would involve a very indirect calculation of total HC as plasma total cobalamin minus holo-TC plus apo-HC.

In summary, our study suggests that holo-TC and TC saturation reflect sudden changes in vitamin B_{12} homeostasis, whereas holo-HC and total plasma cobalamin seem to reflect the accumulation of vitamin B_{12}.

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