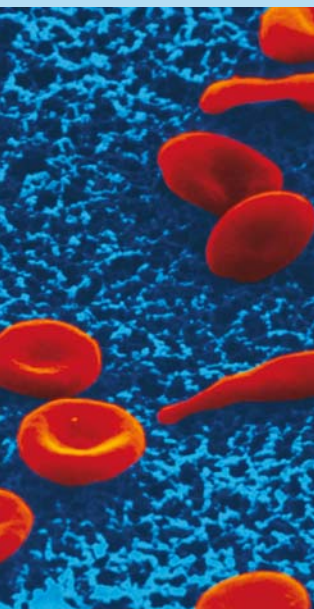


Active-B12

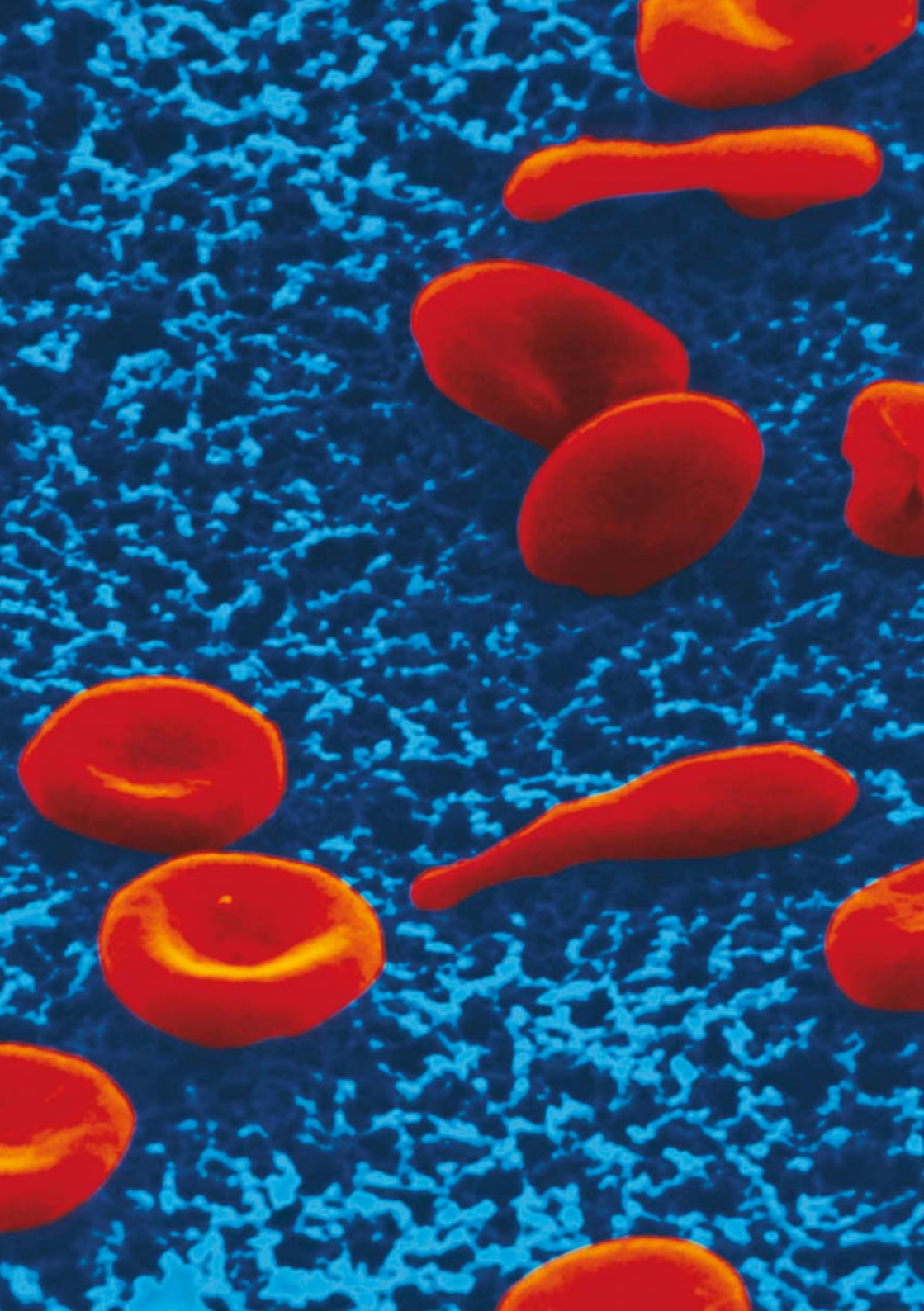
(Holo transcobalamin)

Publication Abstracts 2007



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Diagnosis and treatment of vitamin B12 deficiency – an update

Hvas AM, Nexø E

Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, Denmark.
am.hvas@dadlnet.dk

We represent an update on diagnosing and treatment of vitamin B12 deficiency. Vitamin B12 deficiency should be suspected in all patients with unexplained anaemia and/or neurological symptoms, as well as in patients at risk of developing vitamin B12 deficiency such as the elderly and patients with intestinal diseases. Measurement of plasma cobalamins is suggested as the primary analysis followed by measurement of plasma methylmalonic acid in unsettled cases. Accumulating evidence indicates that the biologically active cobalamin, plasma holotranscobalamin (holoTC), may be superior to plasma cobalamins, and measurement of holoTC is currently introduced in the clinical setting. No consensus exists concerning evaluation of the cause for vitamin B12 deficiency, and pros and cons on the different tests mainly aiming at evaluation of the function of the gastric mucosa are presented. Once the diagnosis of vitamin B12 deficiency has been confirmed efficient treatment can be ensured either by injections every 2 – 3 month or by a daily dose of 1 mg vitamin B12.

Diagnosis of megaloblastic anaemias

Wickramasinghe SN

Department of Haematology, Faculty of Medicine, Imperial College, St Mary's Campus, Praed Street, London W2 1PG, UK

There are a large number of causes of megaloblastic anaemia. The most frequent are disorders resulting in vitamin B12 or folate deficiency. The diagnostic process often consists first of establishing the presence of B12 or folate deficiency and then of determining the cause of deficiency. The blood count, blood film, serum B12 assay, and red cell and serum folate assays are the primary investigations. Other useful investigations include serum/plasma methylmalonic acid (MMA), plasma total homocysteine (tHCYS) and serum holotranscobalamin II assays. All currently used tests have limitations regarding specificity or sensitivity or both and the metabolite assays are not widely available. An understanding of these limitations is essential in formulating any diagnostic strategy. The wide use of serum B12 and metabolite assays has resulted in the increasingly early diagnosis of B12 deficiency, often in patients without B12-related symptoms (subclinical deficiency). Food cobalamin malabsorption is the most frequent cause of a low serum B12. At least 25% of low serum B12 levels are not associated with elevated metabolite levels and may not indicate B12 deficiency. Some of these are caused by partial deficiency of transcobalamin I.

Nonradioactive vitamin B12 absorption test evaluated in controls and in patients with inherited malabsorption of vitamin B12

Bor MV, Cetin M, Aytac S, Altay C, Nexø E

Department of Clinical Biochemistry, NBG, AS, Aalborg Hospital, Aarhus University Hospital, Denmark. vakurbor@hotmail.com

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 (HoloTC) 24 h after three 9-microg 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 HoloTC 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 HoloTC 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 HoloTC and 0.87 (0.79 – 0.94) for cobalamin, distinguishing patients from controls. At a cut-off of 6 pmol/L for HoloTC, the diagnostic sensitivity (95% confidence interval) was 100 (81 – 100)%, and the diagnostic specificity was 92 (82 – 97)%. **Conclusion:** Measurement of HoloTC after administration of vitamin B12 is a promising approach for evaluating vitamin B12 absorption.

A longitudinal study of serum cobalamins and its binding proteins in lactating women

Morkbak AL, Ramlau-Hansen CH, Moller UK, Henriksen TB, Moller J, Nexø E

Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus Sygehus, Aarhus, Denmark

Objective: To examine longitudinal changes in serum cobalamins, transcobalamin (TC) and haptocorrin (HC) during lactation and to investigate the influence of vitamin B12 supplementation on these parameters. **Design:** A 9-month follow-up study. **Subjects and methods:** Lactating mothers (N = 89) including 23 supplemented with vitamin B12 (1 – 18 µg/daily), 41 partly supplemented and 25 not supplemented. Blood samples collected 3 weeks (baseline) and 4 and 9 months post-partum were analysed for cobalamins, TC and HC. Both the total concentration and the cobalamin-saturated form (holo) of TC and HC were analysed. **Results:** No significant differences were observed in serum cobalamins or its binding proteins related to supplementation with vitamin B12 or the duration of lactation. Serum cobalamins remained unchanged from 3 weeks to 9 months post-partum. Total TC (HoloTC) (median ± s.e. pmol/l) decreased between 3 weeks (710 ± 23 [85 ± 12]) and 9 months (602 ± 21 [76 ± 11]) ($P < 0.0001$ [$P = 0.0002$]), whereas total HC (HoloHC) increased from (422 ± 11 [300 ± 9]) at 4 months to (455 ± 13 [317 ± 10]) to 9 months post-partum ($P < 0.0001$ [$P < 0.0001$]). **Conclusion:** We report a decrease in TC and an increase in HC during a 9-month period post-partum. No differences were observed between the vitamin B12-supplemented and the unsupplemented groups. Thus, supplementation with vitamin B12 has no impact on the circulating level of serum cobalamins or its binding proteins in a Danish population of lactating mothers.

Clinical relevance of low serum vitamin B12 concentrations in older people: the Banbury B12 study

Hin H, Clarke R, Sherliker P, Atoyebi W, Emmens K, Birks J, Schneede J, Ueland PM, Nexo E, Scott J, Molloy A, Donaghy M, Frost C, Evans JG
Hightown Surgery, Hightown Gardens, Banbury, UK

Background: Low vitamin B12 concentrations are common in older people, but the clinical relevance of biochemical evidence of vitamin B12 deficiency in the absence of anaemia is uncertain. **Objective:** To examine associations of cognitive impairment, depression and neuropathy with blood measurements of vitamin B12 and folate status in older people. **Design:** Cross-sectional study in general practice in Banbury, England. **Participants:** A total of 1,000 individuals aged 75 years or older living in the community. **Results:** Low vitamin B12 concentrations were identified in 13% of older people and were associated with memory impairment and depression. After adjustment for age, sex and smoking, individuals with vitamin B12 or holotranscobalamin (HoloTC) in the bottom compared with top quartiles had a 2-fold risk (OR = 2.17; 95% CI 1.11–4.27) and a 3-fold risk (OR = 3.02; 95% CI 1.31–6.98) of cognitive impairment, respectively. Low vitamin B12 status was also associated with missing ankle tendon jerks but not with depression. Treatment with vitamin B12 for 3 months corrected the biochemical abnormalities but had no effect on any of the clinical measurements. **Conclusions:** Low vitamin B12 concentrations are associated with cognitive impairment and missing ankle tendon jerks in older people in the absence of anaemia. Large-scale trials of vitamin B12 supplementation are required to assess the clinical significance of these associations.

The effect of recombinant human intrinsic factor on the uptake of vitamin B12 in patients with evident vitamin B12 deficiency

Hvas AM, Buhl H, Laursen NB, Hesse B, Berglund L, Nexø E

Department of Clinical Biochemistry, Skejby Hospital and Aarhus Hospital, Aarhus University Hospital, Aarhus, Denmark. am.hvas@dadlnet.dk

We report on the use of recombinant human intrinsic factor (rhIF) in a new vitamin B12 absorption test. Holotranscobalamin (HoloTC) was measured before and 24 hours after intake of three 9-mg doses of vitamin B12 (B12) and again 24 hours after intake of the same dose of B12 together with rhIF (rhIF-B12). Nine patients with evident vitamin B12 deficiency had a significantly higher increase in HoloTC after intake of rhIF-B12 than after intake of B12. Twenty-eight patients with suspected vitamin B12 deficiency showed no additional increase in HoloTC after intake of rhIF-B12. We conclude that rhIF promotes B12 absorption among patients with evident vitamin B12 deficiency.

The limited value of methylmalonic acid, homocysteine and holotranscobalamin in the diagnosis of early B12 deficiency

Goringe A, Ellis R, McDowell I, Vidal-Alaball J, Jenkins C, Butler C, Worwood M

Department of Haematology, University Hospital of Wales, Cardiff CF14 4XN, UK

Treatment of B12 deficiency is important to prevent progressive neurological and/or hematologic disease but requires a secure diagnosis. The aim of this study was to evaluate second line tests of B12 status as prognostic indicators of a hematologic response to vitamin B12 therapy. Forty-nine patients referred with low, serum vitamin B12 concentrations were treated with intramuscular B12 and re-assessed after 3 months. Methylmalonic acid, homocysteine, holotranscobalamin and neutrophil hypersegmentation index were measured before and after treatment. Before treatment 27/49 patients were anemic or macrocytic of whom 15 had a clear hematologic response. All the tests had a similar prognostic accuracy. Symptomatic improvement did not correlate with hematologic response. Supplementary tests of vitamin B12 status were not significantly better than total serum B12 concentration as predictors of a hematologic response to vitamin B12 therapy.

Measurement of total vitamin B12 and holo-transcobalamin, singly and in combination, in screening for metabolic vitamin B12 deficiency

Miller JW, Garrod MG, Rockwood AL, Kushnir MM, Allen LH, Haan MN, Green R

Department of Pathology and Laboratory Medicine, University of California, Davis, Sacramento, CA, USA

Background: The standard screening test for vitamin B12 deficiency, measurement of total plasma vitamin B12, has limitations of sensitivity and specificity. Plasma vitamin B12 bound to transcobalamin (HoloTC) is the fraction of total vitamin B12 available for tissue uptake and therefore has been proposed as a potentially useful alternative indicator of vitamin B12 status. **Methods:** We compared the diagnostic accuracy of total vitamin B12, HoloTC, and a combination of both measures to screen for metabolic vitamin B12 deficiency in an elderly cohort (age ≥ 60 years). Plasma methylmalonic acid and homocysteine were used as indicators of vitamin B12 deficiency. **Results:** Low total vitamin B12 (< 148 pmol/L) and low HoloTC (< 35 pmol/L) were observed in 6.5% and 8.0%, and increased methylmalonic acid (> 350 nmol/L) and homocysteine (> 13 μ mol/L) were observed in 12.1% and 17.0% of the study participants. In multiple regression models, HoloTC explained 5%–6% more of the observed variance in methylmalonic acid and homocysteine than did total vitamin B12 ($P \leq 0.004$). ROC curve analysis indicated that total vitamin B12 and HoloTC were essentially equivalent in their ability to discriminate persons with and without vitamin B12 deficiency. Individuals with low concentrations of both total vitamin B12 and HoloTC had significantly higher concentrations of methylmalonic acid and homocysteine than did individuals with total vitamin B12 and/or HoloTC within the reference intervals ($P < 0.001$). **Conclusions:** HoloTC and total vitamin B12 have equal diagnostic accuracy in screening for metabolic vitamin B12 deficiency. Measurement of both HoloTC and total vitamin B12 provides a better screen for vitamin B12 deficiency than either assay alone.

The cobalamin-binding proteins transcobalamin and haptocorrin in maternal and cord blood sera at birth

Obeid R, Morkbak AL, Munz W, Nexo E, Herrmann W

Department of Clinical Chemistry, Faculty of Medicine, Department of Obstetrics and Gynaecology, Faculty of Medicine, University Hospital of the Saarland, Homburg/Saar, Germany

Background: Two proteins carry vitamin B12 in plasma. Transcobalamin (TC) carries approximately 25% of total plasma vitamin B12 and is 6% to 20% saturated with cobalamin. Haptocorrin (HC) binds approximately 80% of total cobalamin and is largely saturated with cobalamin. **Methods:** We investigated the distribution and the relationship between concentrations of cobalamin, total and forms of TC, and HC in blood samples from pregnant women just before delivery ($n = 92$) and in cord blood samples from their newborn babies. We also investigated the relationship between these proteins and concentrations of methylmalonic acid (MMA), the functional marker of vitamin B12 status. **Results:** Concentrations of total serum cobalamin, total HC, holoHC, and percentage of HC saturation were higher in cord blood than in the maternal blood (mean cobalamin, 268 vs 188 pmol/L; total HC, 648 vs 538 pmol/L; holoHC, 441 vs 237 pmol/L; HC saturation, 70% vs 47%). Moreover, total TC was low in cord blood, whereas both HoloTC and TC saturation were higher in cord blood than in the maternal blood (mean total TC, 654 vs 1002 pmol/L; HoloTC, 118 vs 53 pmol/L; TC saturation, 19.8% vs 5.4%). Higher maternal serum cobalamin was associated with higher cord blood HoloTC and TC saturation ($P < 0.05$). Gestational age was also a significant determinant of baby total TC, TC saturation, total HC, and holoHC. **Conclusion:** The close correlation between the amounts of HoloTC present in cord blood and in maternal serum supports the importance of maternal cobalamin status for ensuring a sufficient supply to the baby.

Characterization of a monoclonal antibody with specificity for holotranscobalamin

Orning L, Rian A, Campbell A, Brady J, Fedosov SN, Bramlage B, Thompson K, Quadros EV

Axis-Shield AS, POB 206 Okern, N-0510 Oslo, Norway. lars.orning@no.axis-shield.com.

Background: Holotranscobalamin, cobalamin-saturated transcobalamin, is the minor fraction of circulating cobalamin (vitamin B12), which is available for cellular uptake and hence is physiologically relevant. Currently, no method allows simple, direct quantification of holotranscobalamin. We now report on the identification and characterization of a monoclonal antibody with a unique specificity for holotranscobalamin. **Methods:** The specificity and affinity of the monoclonal antibodies were determined using surface plasmon resonance and recombinant transcobalamin as well as by immobilizing the antibodies on magnetic microspheres and using native transcobalamin in serum. The epitope of the holotranscobalamin specific antibody was identified using phage display and comparison to a de novo generated three-dimensional model of transcobalamin using the program Rosetta. A direct assay for holotranscobalamin in the ELISA format was developed using the specific antibody and compared to the commercial assay HoloTC RIA. **Results:** An antibody exhibiting >100-fold specificity for holotranscobalamin over apotranscobalamin was identified. The affinity but not the specificity varied inversely with ionic strength and pH, indicating importance of electrostatic interactions. The epitope was discontinuous and epitope mapping of the antibody by phage display identified two similar motifs with no direct sequence similarity to transcobalamin. A comparison of the motifs with a de novo generated three-dimensional model of transcobalamin identified two structures in the N-terminal part of transcobalamin that resembled the motif. Using this antibody an ELISA based prototype assay was developed and compared to the only available commercial assay for measuring holotranscobalamin, HoloTC RIA. **Conclusion:** The identified antibody possesses a unique specificity for holotranscobalamin and can be used to develop a direct assay for the quantification of holotranscobalamin.

Novel and established markers of cobalamin deficiency: complementary or exclusive diagnostic strategies

Schneede J, Ueland PM

Department of Clinical Chemistry, Umeå University, Umeå, Sweden

New developments in diagnostic markers and a better understanding of the limitations of traditional diagnostic strategies have allowed diagnosis of earlier stages and atypical forms of cobalamin deficiency. Still, there are no generally accepted guidelines for the definition, diagnosis, treatment, and follow-up of cobalamin deficiency. The new trend toward defining cobalamin deficiency purely on the basis of biochemical test outcomes in the absence of overt clinical signs and symptoms could, however, be problematic and may result in overdiagnosis and overtreatment. Use of metabolic markers for the assessment of cobalamin deficiency allows the demonstration of tissue deficiency, but the establishment of the cause of deficiency should also be part of the diagnostic approach. Four groups of diagnostic tests are currently available and these include total cobalamin and cobalamin fractions (such as holotranscobalamin), tests of gastrointestinal dysfunction, tests of metabolic function, and different gene tests. Among the available tests, only homocysteine, methylmalonic acid, holotranscobalamin, and possibly methylcitric acid are considered to be useful in clinical practice to add to cobalamin. Gastrointestinal function tests may identify the cause of cobalamin deficiency, whereas the diagnostic usefulness of genetic testing needs to be evaluated. This article provides an overview of recent developments and a reappraisal of novel and established diagnostic markers for cobalamin deficiency.

Circadian variation of holotranscobalamin (HoloTC) and related markers

Hvas AM, Gravholt CH, Nexø E

Department of Clinical Biochemistry, SKS, Aarhus University Hospital, Aarhus, Denmark.
am.hvas@dadlnet.dk

We examined the circadian variation of holotranscobalamin (HoloTC), total transcobalamin (total TC) and plasma cobalamins, and the association between food intake and fluctuations in the biochemical markers. A total of 17 healthy women (mean age 33 years; range 24–40) participated. The subjects were admitted at 08:00 h after an overnight fast, and blood samples were obtained every 20 min for 24 h from 12:00 h. HoloTC and total TC were measured by an ELISA, and plasma cobalamins were measured by a routine method (Centaur*, Bayer). All subjects, except one, had 12:00 h levels within the reference interval for all variables studied, and all subjects had normal renal function as judged from plasma creatinine. We found a small intra-individual variation (12%, 10%, 10%) and a considerable inter-individual variation (56%, 26%, 43%) in the level of HoloTC, total TC and plasma cobalamins, respectively. During the night, the absolute values of all components decreased, as did plasma albumin. There was no systematic association between intake of food and fluctuation of the biochemical markers. In conclusion, we found no significant circadian variation in serum HoloTC in healthy vitamin-replete subjects on a standard diet. This supports the view that HoloTC is a marker of long-term vitamin B12 status, and that samples may also be obtained from non-fasting subjects.

* Centaur is a property of its owner Bayer

Holo transcobalamin and Total Transcobalamin in Human Plasma: Determination, Determinants, and Reference Values in Healthy Adults

Refsum H, Johnston C, Guttormsen AB, Nexø E

Department of Pharmacology, University of Oxford, Oxford, UK, and Institute of Medicine, Section of Pharmacology, University of Bergen, Institute of Basic Medical Sciences, Department of Nutrition, University of Oslo, Oslo, Norway

Background: We developed microbiological assays (MBAs) to identify determinants and to establish reference values for cobalamin bound to transcobalamin [holo transcobalamin (HoloTC)] and total TC in plasma.

Methods: We captured HoloTC with magnetic beads with TC antibodies and used a conventional MBA for cobalamin measurements. Total TC was determined as HoloTC after TC was saturated with cyanocobalamin. The new assays were compared with published methods. Determinants and reference values were determined in 500 blood donors, ages 18–69 years.

Results: Determination of cobalamin, HoloTC, and TC by MBA required < 150 μL . HoloTC and TC by MBA correlated with HoloTC by RIA ($r = 0.95$) and TC by ELISA ($r = 0.79$), respectively. Between-day CVs for HoloTC and total TC were 4%–9%. Women had approximately 20% lower HoloTC than men, but only at age < 45 years. In multivariate regression analyses, HoloTC was positively associated with age (in women only), creatinine (in men only), and plasma concentrations of total TC, folate, and cysteine, but inversely correlated with homocysteine and methylmalonic acid. For all study participants, total TC was associated with HoloTC and number of TCN2 766C alleles; in female participants only, total TC was also associated with age, homocysteine, and cysteine. Reference values were 610–1350 pmol/L for TC and 42–175 pmol/L for HoloTC, but they differed according to age and sex. **Conclusions:** Our MBAs for TC and HoloTC required low plasma volume and performed acceptably compared with other methods. Determinants of HoloTC and TC differed between men and women and according to age. Separate reference intervals for HoloTC should be considered in younger women.

Evaluation of the technical performance of novel holotranscobalamin (HoloTC) assays in a multicenter European demonstration project

Morkbak AL, Heimdal RM, Emmens K, Molloy A, Hvas AM, Schneede J, Clarke R, Scott JM, Ueland PM, Nexø E

Department of Clinical Biochemistry, AS, Aarhus University Hospital, Aarhus, Denmark, LOCUS for homocysteine and related vitamins, University of Bergen, Bergen, Norway, Clinical Trial Service Unit, University of Oxford, Oxford, UK, Department of Biochemistry, Trinity College, Dublin, Ireland, Department of Clinical Chemistry, Umeå University Hospital, Umeå, Sweden

A commercially available holotranscobalamin (HoloTC) radioimmunoassay (RIA) (Axis-Shield, Dundee, Scotland) was evaluated in four laboratories and compared with a HoloTC ELISA run in one laboratory. The performance of the HoloTC RIA assay was comparable in three of the four participating laboratories. The results from these three laboratories, involving at least 20 initial runs of “low”, “medium” and “high” serum-based controls (mean HoloTC concentrations 34, 60 and 110 pmol/L, respectively) yielded an intra-laboratory imprecision of 6–10%. No systematic inter-laboratory deviations were observed on runs involving 72 patient samples (HoloTC concentration range 10–160 pmol/L). A fourth laboratory demonstrated higher assay imprecision for control samples and systematic deviation of results for the patient samples. Measurement of HoloTC by ELISA showed an imprecision of 4–5%, and slightly higher mean values for the controls (mean HoloTC concentrations 40, 70 and 114 pmol/L, respectively). Comparable results were obtained for the patient samples. The long-term intra-laboratory imprecision was 12% for the HoloTC RIA and 6% for the ELISA. In conclusion, it would be prudent to check the calibration and precision prior to starting to use these HoloTC assays in research or clinical practice. The results obtained using the HoloTC RIA were similar to those obtained using the HoloTC ELISA assay.

The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings

Herrmann W, Obeid R, Schorr H, Geisel J

Department of Clinical Chemistry, University Hospital of the Saarland, 66421 Homburg/Saar, Germany. kchwher@uniklinik-saarland.de

Serum concentrations of homocysteine (Hcy) and methylmalonic acid (MMA) become increased in B12-deficient subjects and are therefore considered specific markers of B12 deficiency. Serum level of holotranscobalamin (HoloTC) becomes decreased before the development of the metabolic dysfunction. We investigated the usefulness of HoloTC in diagnosing B12 deficiency in some clinical settings. We measured serum concentrations of HoloTC, MMA, Hcy and total B12 in omnivores, vegetarians, elderly people and haemodialysis patients. Our results indicated that the incidence of HoloTC <35 pmol/L was highest in the vegans (76%). Low HoloTC and elevated MMA were detected in 64% of the vegans and 43% of the lacto- and lacto-ovovegetarians. An elevated MMA and a low HoloTC were found in subjects with total serum B12 as high as 300 pmol/L. The distribution of HoloTC in elderly people was similar to that in younger adults (median HoloTC 55 pmol/L in both groups). A low HoloTC and an elevated MMA were found in 16% of the elderly group. An elevated MMA and a normal HoloTC were found in 20% of the elderly group who had a relatively high median serum concentration of creatinine (106.1 $\mu\text{mol/L}$). Serum concentrations of HoloTC in dialysis patients were considerably higher than all other groups (median 100 pmol/L). This was also associated with severely increased serum levels of MMA (median 987 nmol/L). From these results it can be concluded that serum concentration of HoloTC is a much better predictor of B12 status than total B12. This was particularly evident in case of dietary B12 deficiency. Serum concentrations of HoloTC as well as MMA can be affected by renal dysfunction. Elevated MMA and normal HoloTC in patients with renal insufficiency may not exclude vitamin B12 deficiency. HoloTC seems not to be a promising marker in predicting B12 status in renal patients.

Holo transcobalamin – a first choice assay for diagnosing early vitamin B deficiency?

Hvas AM, Nexø E

Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus C, Denmark.
am.hvas@dadlnet.dk

Objectives: The performance of holo transcobalamin (HoloTC) was compared with the other markers of vitamin B₁₂ deficiency, and the influence of age, renal function, and thyroid status was examined. **Design and Interventions:** We examined 937 individuals not treated with vitamin B₁₂ but in whom vitamin B₁₂ deficiency was suspected because of a plasma methylmalonic acid (MMA) above 0.28 $\mu\text{mol L}^{-1}$ within the past 4 years. Besides laboratory tests, a structured interview and a neurological examination were performed amongst 534 individuals. Amongst these, 140 individuals qualified for a randomized trial (MMA 0.40–2.00 $\mu\text{mol L}^{-1}$). They were randomized to injections with vitamin B₁₂ or placebo and re-examined after 3 months. **Setting:** One university hospital in Aarhus, Denmark. **Results:** The ROC curves indicate that HoloTC (AUC: 0.90) compared favourable with plasma vitamin B₁₂ (AUC: 0.85) for identifying individuals likely to have vitamin B₁₂ deficiency (MMA \geq 0.75 $\mu\text{mol L}^{-1}$) and plasma total homocysteine (tHcy) \geq 15 $\mu\text{mol L}^{-1}$), and further that HoloTC (AUC: 0.91) might replace combined testing with plasma vitamin B₁₂ and the metabolites. No association was observed between the biochemical markers and symptoms and signs possibly related to vitamin B₁₂ deficiency. HoloTC, TC saturation, plasma vitamin B₁₂, MMA, and tHcy were significantly associated with plasma creatinine (all with $P < 0.001$). Only tHcy was significantly associated with thyroid stimulating hormone ($P = 0.02$). **Conclusions:** HoloTC shows promise as first-line tests for diagnosing early vitamin B₁₂ deficiency.

Transcobalamin 776C → G polymorphism negatively affects vitamin B12 metabolism

von Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, Vaughn JD, Griffin ER, Maneval DR, Theriaque DW, Bailey LB

Food Science and Human Nutrition Department and the General Clinical Research Center, University of Florida, Gainesville, FL 32611, USA

Background: A common genetic polymorphism [transcobalamin (TC) 776C → G] may affect the function of transcobalamin, the protein required for vitamin B12 cellular uptake and metabolism. Remethylation of homocysteine is dependent on the production of 5-methyltetrahydrofolate and adequate vitamin B12 for the methionine synthase reaction. **Objectives:** The objectives were to assess the influence of the TC 776C → G polymorphism on concentrations of the transcobalamin-vitamin B12 complex (HoloTC) and to determine the combined effects of the TC 776C → G and methylenetetrahydrofolate reductase (MTHFR) 677C → T polymorphisms and vitamin B12 status on homocysteine concentrations. **Design:** Healthy, nonpregnant women (n = 359; aged 20–30 y) were screened to determine plasma vitamin B12, serum HoloTC, and plasma homocysteine concentrations and TC 776C → G and MTHFR 677C → T genotypes. **Results:** The serum HoloTC concentration for women with the variant TC 776 GG genotype was significantly different (P = 0.0213) from that for subjects with the CC genotype (74 ± 37 and 87 ± 33 pmol/L, respectively). An inverse relation was observed between plasma homocysteine concentrations and both serum HoloTC (P ≤ 0.0001) and plasma vitamin B12 (P ≤ 0.0001) concentrations, regardless of genotype. **Conclusions:** These data suggest that the TC 776C → G polymorphism negatively affects the serum HoloTC concentration and provide additional evidence that vitamin B12 status modulates the homocysteine concentration in this population.

Holo-transcobalamin concentration and transcobalamin saturation reflect recent vitamin B12 absorption better than does serum vitamin B12

Bor MV, Nexø E, Hvas AM

Department of Clinical Biochemistry, AKH, Aarhus University Hospital, Norrebrogade 44, DK-8000 Aarhus C, Denmark. vakurbor@hotmail.com

Background: We evaluated whether measurement of vitamin B12-saturated transcobalamin (HoloTC) concentrations or TC saturation (HoloTC: total TC) reflects active vitamin B12 absorption in healthy individuals and patients after vitamin B12 intake. **Methods:** We obtained blood samples from 31 healthy individuals (age range, 25–57 years) before (days –1 and 0) and after (days 1, 2, and 6) oral administration of three 9- μ g doses of vitamin B12. The blood samples from seven patients (age range, 22–39 years) suspected to have decreased vitamin B12 absorption were obtained before and 1 day after the vitamin B12 intake. The blood samples were analyzed for vitamin B12, total TC, and HoloTC. The TC saturation was calculated. **Results:** Intraindividual variation was <13% for all measured values, as calculated from samples removed on day –1 and 0. In healthy individuals ($n = 31$) after intake of vitamin B12, the maximum median (range) increase (as percentages and absolute values) was in TC saturation [52 (–2% to 128)% and 0.04 (0–0.23) as a fraction], closely followed by HoloTC concentrations [39 (0–108)% and 34 (0–149) pmol/L]. All but one healthy individual had an increase of $\geq 15\%$ in these markers. Serum vitamin B12 showed a smaller increase [14 (–8 to 51)% and 36 (–27 to 290) pmol/L]. After vitamin B12 intake, three patients with Crohn disease had the lowest increases in HoloTC concentration (3, 7, and 14 pmol/L) and in TC saturation (0.004, 0.01, and 0.01) among patients and 30 healthy individuals. **Conclusion:** HoloTC concentrations and TC saturation reflect normal vitamin B12 absorption better than does serum vitamin B12.

Vitamin B12 Status of Patients Treated With Metformin: A Cross-Sectional Cohort Study

Leif Sparre Hermann; Bo Nilsson; Staffan Wettre

Diabetes Unit, Medical Department, Uddevalla Hospital, Uddevalla, Sweden,
Department of Clinical Chemistry, Uddevalla Hospital, Uddevalla, Sweden

Aim: To assess the vitamin B12 status of patients with type 2 diabetes who had been receiving metformin treatment for at least one year. **Methods:** Patients with type 2 diabetes attending a diabetes clinic were included in a cross-sectional cohort study. Patients exposed to metformin for more than one year ($n = 53$) were compared with a non-exposed control group ($n = 31$). Serum cobalamin and other variables reflecting vitamin B12 status were measured. **Results:** Patients on metformin had lower cobalamin (289 ± 137 vs. 395 ± 162 pmol/L; $p < 0.01$) and holotranscobalamin (76 ± 49 vs. 97 ± 41 pmol/L; $p < 0.05$), and higher HCy (11.3 ± 3.3 vs. 10.3 ± 2.3 $\mu\text{mol/L}$; $p < 0.05$); these changes were correlated. Eight metformin patients, but no controls, had holotranscobalamin below the normal range ($p < 0.05$). Methylmalonic acid and folate were similar in both groups. **Conclusion:** Patients exposed to long-term metformin therapy had 26.7% lower cobalamin, 21.6% lower holotranscobalamin and 9.7% higher HCy serum concentrations than control subjects. Such changes indicate a potential risk for development of vitamin B12 deficiency. Our results highlight the necessity of checking B12 status during metformin therapy.

Transcobalamin polymorphism and serum holotranscobalamin in relation to Alzheimer's disease

McCaddon A, Blennow K, Hudson P, Hughes A, Barber J, Gray R, Davies G, Williams JH, Duguid J, Lloyd A, Tandy S, Everall M, Cattell H, McCaddon A, Ellis D, Palmer M, Bogdanovic N, Gottfries CG, Zetterberg H, Rymo L, Regland B

University of Wales College of Medicine, Division of General Practice, Wrexham, UK.

andrew@mccaddon.demon.co.uk

Isoforms of the vitamin B12 carrier protein transcobalamin (TC) might influence its cellular availability and contribute to the association between disrupted single-carbon metabolism and Alzheimer's disease (AD).

We therefore investigated the relationships between the TC 776C > G (Pro259Arg) genetic polymorphism, total serum cobalamin and HoloTC levels, and disease onset in 70 patients with clinically diagnosed AD and 74 healthy elderly controls. TC 776C > G polymorphism was also determined for 94 histopathologically confirmed AD patients and 107 controls. Serum HoloTC levels were significantly higher in TC 776C homozygotes ($p = 0.04$). Kaplan-Meier survival functions differed between homozygous genotypes (Cox's F-Test $F(42, 46) = 2.1$; $p = 0.008$) and between 776C homozygotes and heterozygotes (Cox's F test $F(46, 108) = 1.7$; $p = 0.02$). Proportionately fewer TC 776C homozygotes appear to develop AD at any given age, but this will require confirmation in a longitudinal study.

Clinical utility of serum holotranscobalamin as a marker of cobalamin status in elderly patients with neuropsychiatric symptoms

Nilsson K, Isaksson A, Gustafson L, Hultberg B.

Department of Psychogeriatrics, Division of Clinical Chemistry, University Hospital, Lund, Sweden

Early diagnosis of cobalamin deficiency is crucial, owing to the latent nature of this disorder and the resulting possible irreversible neurological damage. A normal serum cobalamin concentration does not reliably rule out a functional cobalamin deficiency and there does not at present seem to be any single diagnostic approach to achieve this diagnosis. A new marker for cobalamin status is the serum concentration of cobalamin bound to transcobalamin II (HoloTC). Because methods suitable for routine use have been unavailable until recently, the clinical value of low HoloTC is still uncertain. Furthermore, there is at the moment no gold standard or true reference method to diagnose subtle cobalamin deficiency, which makes evaluation of the clinical usefulness of HoloTC and the estimation of sensitivity and specificity problematic. In this study, we aimed to assess whether low HoloTC concentrations are congruent with other biochemical signs of cobalamin deficiency in a group of psychogeriatric patients. The findings in the present study show that HoloTC is strongly related to serum cobalamin (0.68; $p < 0.001$ in both patients and controls). Distribution of the different markers for cobalamin/folate status in the 33 patients with low levels of serum HoloTC (below 40 pmol/l) showed that 17 patients had normal levels of the other markers for cobalamin status. This may indicate poor specificity of low HoloTC for cobalamin deficiency. In 23 out of 176 patients with normal levels of HoloTC we observed pathological levels of other markers for cobalamin deficiency. The use of HoloTC in the present study group did not give significant additional information other than that given by serum cobalamin and therefore cannot be recommended in this clinical setting.

Analysis of the transcobalamin II 776C > G (259P > R) single nucleotide polymorphism by denaturing HPLC in healthy elderly: Associations with cobalamin, homocysteine and holotranscobalamin II

Wans S, Schuttler K, Jakubiczka S, Muller A, Luley C, Dierkes J

Institute of Clinical Chemistry, University Hospital Magdeburg, Magdeburg, Germany

A relatively new method for the detection of single nucleotide polymorphisms is the use of denaturing high-performance liquid chromatography (DHPLC). DHPLC was used to analyse the transcobalamin II 776C > G polymorphism in DNA from 159 healthy elderly. Furthermore, cobalamin, folate, homocysteine and holotranscobalamin II (HoloTC II) were measured. The allele frequency of the G-allele was 17% with n = 55 harbouring the CC genotype, n = 77 being heterozygous and n = 27 showing the GG genotype. HoloTC II concentrations were significantly decreased in patients harbouring the GG genotype. There was no effect on cobalamin, methylmalonyl-CoA, folate or homocysteine concentrations. A new G > A variant at nucleotide position 810 in the TC II gene was detected by an altered peak pattern in the DHPLC and further elucidated by direct sequencing. The TC II G810A variant is a silent mutation without replacement of the corresponding amino acid (alanine) at position 270 in the TC II protein and was only found as a heterozygous genotype in a single patient. The new variant would have been undetected by other methods used for single nucleotide polymorphism detection, e.g., restriction fragment length polymorphism analysis. The results suggest that the common TC II 776C > G polymorphism has no major influence on vitamin B12 metabolism.

Holo transcobalamin as a predictor of vitamin B12 status

Hvas AM, Nexø E

Department of Clinical Biochemistry, Aarhus University Hospital, AKH, Aarhus, Denmark

We report on the performance of a new test, holo transcobalamin, as compared to well established markers of vitamin B12 deficiency (plasma cobalamins, methylmalonic acid, and homocysteine). Holo transcobalamin was analyzed in 143 samples by a competitive radiobinding assay (Axis-Shield). Employing a cut-off value of 50 pmol/l, holo transcobalamin showed a sensitivity of 1.00 and a specificity of 0.89 as regards discriminating between individuals with test results indicating vitamin B12 deficiency (methylmalonic acid > 0.70 $\mu\text{mol/l}$ and plasma cobalamins < 200 pmol/l, $n = 35$) and individuals with test results inside the reference intervals (methylmalonic acid < 0.29 $\mu\text{mol/l}$ and plasma cobalamins ≥ 200 pmol/l, $n = 35$). In a group ($n = 37$) with low plasma cobalamins (< 200 pmol/l) and normal methylmalonic acid (< 0.29 $\mu\text{mol/l}$), 27 individuals had low holo transcobalamin, and in nine of these individuals plasma homocysteine supported the deficiency state (homocysteine > 15 $\mu\text{mol/l}$). Holo transcobalamin was low in 12 individuals with increased methylmalonic acid (> 0.40 $\mu\text{mol/l}$) and normal plasma cobalamins (≥ 200 pmol/l) ($n = 36$), and plasma homocysteine supported the deficiency state in four of these individuals. We conclude that holo transcobalamin is likely to be a sensitive marker of vitamin B12 deficiency that also has a reasonable specificity. Large-scale clinical studies are warranted in order to clarify the usefulness of holo transcobalamin in the clinical setting.

Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk

Herrmann W, Obeid R, Schorr H, Geisel J

Department of Clinical Chemistry, School of Medicine, Saarland University, Homburg/Saar, Germany

Background: The prevalence of a sub-clinical functional vitamin B12 deficiency in the general population is higher than previously expected. Total serum vitamin B12 may not reliably indicate vitamin B12 status. To get more specificity and sensitivity in diagnosing vitamin B12 deficiency, the concept of measuring holotranscobalamin II (HoloTC), a sub-fraction of vitamin B12, has aroused great interest. HoloTC as a biologically active vitamin B12 fraction promotes a specific uptake of its vitamin B12 by all cells. In this study we investigated the diagnostic value of storage (HoloTC) of vitamin B12 and functional markers (methylmalonic acid (MMA)) of vitamin B12 metabolism in populations who are at risk of vitamin B12 deficiency.

Subjects and Methods: Our study included 93 omnivorous German controls, 111 German and Dutch vegetarian subjects, 122 Syrian apparently healthy subjects, 127 elderly Germans and finally 92 German pre-dialysis renal patients. Serum concentrations of homocysteine (Hcy) and MMA were measured by gas chromatography-mass spectrometry, folate and vitamin B12 by chemiluminescence immunoassay, and HoloTC by utilizing a RIA test. **Results:** High Hcy ($> 12 \mu\text{mol/l}$), high MMA ($> 271 \text{ nmol/l}$) resp. low HoloTC (vitamin B12) in serum were detected in 15%, 8% resp. 13% (1%) of German controls, 36%, 60%, resp. 72% (30%) of vegetarians, 42%, 48% resp. 50% (6%) of Syrians, 75%, 42%, resp. 21% (7%) of elderly subjects and 75%, 67% resp. 4% (2%) of renal patients. The lowest

median levels of HoloTC were observed in vegetarians, followed by the Syrian subjects (23 and 35 pmol/l, respectively). Renal patients had significantly higher levels of HoloTC compared to the German controls (74 vs. 54 pmol/l). In the vitamin B12 range between 156 pmol/l (conventional cut-off level) and 241 pmol/l, both mean concentrations of HoloTC and MMA were in the pathological range. HoloTC was the earliest marker for vitamin B12 deficiency followed by MMA. Vitamin B12 deficiency causes folate trapping. A higher folate level is required to keep Hcy normal. The relationship between MMA and HoloTC seemed dependent on renal function. In renal patients with a glomerular filtration rate below 36 ml/min, a significantly lower mean level of MMA was detected within the highest tertile of HoloTC concentration, compared to the lowest tertile. Thus, in renal patients, a higher serum concentration of circulating HoloTC is required to deliver sufficient amounts of HoloTC into the cells. **Conclusion:** Our data support the concept that the measurement of HoloTC and MMA provides a better index of cobalamin status than the measurement of total vitamin B12. HoloTC is the most sensitive marker, followed by MMA. The use of HoloTC and MMA enables us to differentiate between storage depletion and functional vitamin B12 deficiency. Renal patients have a higher requirement of circulating HoloTC. In renal dysfunction, HoloTC cannot be used as a marker of vitamin B12 status.

Holo transcobalamin as an Indicator of Dietary Vitamin B12 Deficiency

Zouë Lloyd-Wright¹, Anne-Mette Hvas², Jan Møller³, Tom A. B. Sanders^{1,a} and Ebba Nexø²

¹ Nutrition Food and Health Research Centre, King's College London, Franklin-Wilkins Bldg., 150 Stamford St., Waterloo, London SE1 9NN, United Kingdom; ² Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; ³ Department of Clinical Biochemistry, SKS, Aarhus University Hospital, DK-8200 Aarhus N, Denmark

^a address correspondence to this author at: fax 44-207-848-4171, e-mail tom.sanders@kcl.ac.uk

We report that serum holo transcobalamin (HoloTC) compares favorably with serum vitamin B12 for identifying vegans likely to have vitamin B12 deficiency as judged by measurements of the metabolites methylmalonic acid (MMA) and homocysteine (tHcy). We also report that measurement of HoloTC may possibly replace combined testing with serum vitamin B12, MMA, and tHcy in this population.

Recently, two new markers for vitamin B12 deficiency, HoloTC (TC saturated with vitamin B12) and the related TC saturation (the fraction of total TC present as HoloTC), have been introduced (1)(2)(3). Approximately 30% of circulating B12 is attached to TC, whereas the major part of B12 is attached to another protein, haptocorrin. Because only B12 attached to TC (HoloTC) is able to enter all the cells of the body, HoloTC may be a more useful marker than total B12 in serum.

We compared HoloTC with the tests currently used for diagnosis of vitamin B12 deficiency, i.e., B12, MMA, and tHcy, in vegan men, whose diets are devoid of food of animal origin (and thus low in vitamin B12) and who thus are susceptible to developing B12 deficiency.

Homocysteine in the context of cobalamin metabolism and deficiency states

Briddon A

Neurometabolic Unit, Department of Clinical Biochemistry, National Hospital for Neurology and Neurosurgery, London, UK. anthony.briddon@uclh.org

It is becoming increasingly clear that serum vitamin B12 (cobalamin) concentration is a dubious indicator of functional B12 status and, in contrast to long-standing convention, correlates poorly with haematological indices. This, in turn, has led to poorly defined reference intervals for serum B12. Patients presenting with neurological disturbance due to B12 deficiency are at risk of not being diagnosed if total reliance is placed on serum B12 levels and haematological parameters. Plasma homocysteine remethylation is uniquely placed at the metabolic end-point of B12 metabolism such that plasma total homocysteine is proving to be a sensitive marker of functional B12 status. Studies also show that plasma homocysteine correlates better with holotranscobalamin than serum B12. It is suggested that clinicians should cease to be guided by surrogate haematological markers when more specific tests of B12 deficiency, such as holotranscobalamin and total homocysteine, exist. These tests demand greater prevalence in routine diagnostic use.

Biological Variation of holotranscobalamin in Elderly Individuals

Andrew McCaddon^{1,a}, Peter Hudson², Cherie McCracken³, Richard Ellis⁴
and Anne McCaddon¹

¹ University of Wales College of Medicine, Division of General Practice, Wrexham LL13 7YP, UK; ² Department of Pathology, Wrexham Maelor Hospital, Wrexham LL13 7TD, UK;

³ University Department of Psychiatry, Royal Liverpool University Hospital, Liverpool L69 3GA, UK; ⁴ University Hospital of Wales, Cardiff CF14 4XW, UK

^a address correspondence to this author at: Gardden Road Surgery, Rhosllanerchrugog, Wrexham, North Wales LL14 2EN, UK; fax 44-0-1978-845782, e-mail andrew@mccaddon.demon.co.uk

Vitamin B12 is a water-soluble molecule essential for mammalian intracellular metabolism. Its two metabolically active forms, methyl-cobalamin and 5-deoxyadenosylcobalamin, are coenzymes in the reactions catalyzed, respectively, by methionine synthase and methylmalonyl-CoA mutase.

There are two vitamin B12 carrier proteins in serum, haptocorrin and transcobalamin (TC). Haptocorrin binds the majority of serum B12 but, unlike TC, does not deliver the vitamin to metabolically active cells. Only 5–20% of serum B12 is bound to TC as “HoloTC”. Current laboratory assays determine total serum B12 concentrations and are relatively poor indicators of the ability of serum to deliver the vitamin to tissues.

Methods are now available to measure HoloTC in clinical samples. Although information exists for “between-person” variations in HoloTC concentrations, very few data exist regarding its “within-person” variability. Such knowledge will be essential for studies of diseases potentially associ-

ated with low concentrations of HoloTC, such as Alzheimer disease. We therefore examined the between- and within-person variability and within-assay variability of HoloTC concentrations in healthy elderly volunteers in the fasting and nonfasting states.

The study received local research ethics committee approval and followed an established protocol aimed at minimizing various preanalytical factors that can influence the results of clinical laboratory tests. Because valid estimates of the components of variation can be obtained from a relatively small number of participants, six males and six females age 65 years were recruited. Their mean age was 82.5 years (range, 65–99 years). Ages were not significantly different between males and females (Student t-test, $t_{10} = 1.4$; $P = 0.2$). The participants were all maintaining their usual lifestyles and not taking any medication. Ten samples of venous blood were collected at 14-day intervals from each participant over a 5-month period. ... (report truncated).

Effect of TCN2 776C > G on vitamin B12 cellular availability in end-stage renal disease patients

Fodinger M, Veitl M, Skoupy S, Wojcik J, Rohrer C, Hagen W, Puttinger H, Hauser AC, Vychytil A, Sunder-Plassmann G

Institute of Medical and Chemical Laboratory Diagnostics; and Division of Nephrology and Dialysis, Department of Medicine III, University of Vienna, Austria

Background: Transcobalamin II is a serum protein that transports vitamin B12 from the intestine to the tissues. This complex, holotranscobalamin II, may reflect vitamin B12 availability in the body. Conflicting data exist with regard to the effect of a polymorphism in the gene coding for transcobalamin II, TCN2 776C>G, on transcobalamin II levels in the general population, which in turn may affect holotranscobalamin II, vitamin B12, as well as total homocysteine (tHcy) plasma levels. The effect of TCN2 776C>G on vitamin B12 cellular availability in dialysis patients is unknown. **Methods:** We examined the effect of TCN2 776C>G on holotranscobalamin II, vitamin B12, and tHcy plasma concentrations in 120 dialysis patients. **Results:** Holotranscobalamin II levels were normal or supranormal in all patients and showed a linear association with albumin ($r = 0.205$, $P = 0.025$) and with vitamin B12 ($r = 0.778$, $P = 0.001$), but not with age, creatinine, body mass index, tHcy, ln-tHcy, vitamin B6, plasma folate, and red blood cell folate concentration. TCN2 776C>G showed no effect on holotranscobalamin II, vitamin B12, and tHcy concentration [one-way analysis of variance (ANOVA), post-hoc Scheffe test]. Multiple linear regression analyses showed that albumin and B12 are independently associated with holotranscobalamin II, whereas TCN2 776C>G and MTHFR 677C>T had no effect. Independent predictors of ln-tHcy included creatinine, red blood cell folate, and the MTHFR 677TT genotype. There was also an effect of the TCN2 776CC genotype on ln-tHcy levels in this multivariate analysis, however, that deserves cautious interpretation because there was no effect of TCN2 genotypes by ANOVA and Scheffe test [median ln-tHcy concentrations according to TCN2 genotypes (micromol/L): CC, 3.22; CG, 3.30; GG, 3.23]. **Conclusion:** TCN2 776C>G does not influence holotranscobalamin II or vitamin B12 levels, and has no major effect on tHcy concentrations of end-stage renal disease patients.

The Transcobalamin (TC) Codon 259 Genetic Polymorphism Influences HoloTC Concentration in Cerebrospinal Fluid from Patients with Alzheimer Disease

Henrik Zetterberg^{1,a}, Ebba Nexö², Björn Regland³, Lennart Minthon⁴, Roberta Boson⁴, Mona Palmér¹, Lars Rymo¹ and Kaj Blennow^{1,5}

¹ Department of Clinical Chemistry and Transfusion Medicine; ³ Institute of Clinical Neuroscience, Psychiatry Section, and ⁵ Institute of Clinical Neuroscience, Department of Experimental Neuroscience, Sahlgrenska University Hospital, Göteborg University, S-413 45 Gothenburg, Sweden; ² Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; ⁴ Neuropsychiatric Clinic, Malmö University Hospital, S-205 02 Malmö, Sweden

^a author for correspondence: fax 46-31-828458, e-mail henrik.zetterberg@clinchem.gu.se

Two proteins bind vitamin B12 in plasma: haptocorrin (transcobalamin I) and transcobalamin (transcobalamin II; TC). The latter is the critical transporter that delivers vitamin B12 to peripheral tissues. TC carries one-third of the circulating B12 (HoloTC), but most TC is unsaturated (apo-TC). Polyacrylamide gel electrophoresis has revealed two common TC isotypes, M and X, and two rare variants, S and F that may influence the cellular availability of vitamin B12. The phenotypic variability is a multifactorial phenomenon that probably includes cell-type-specific processing of translated TC but the substitution of proline (P) for arginine (R) at codon 259 of the TC gene is the major determinant of the TC variability, at least in Caucasians, and affects TC concentrations in plasma. Most 259PP individuals have the TC M phenotype, whereas most 259RR individuals have the X phenotype.

In agreement with previously published studies, we found no associations between the TC P259R polymorphism and concentrations of tHcy, vitamin B12, or folate, but we did find a significant relationship of the polymorphism with total TC in plasma. The lower concentrations of total TC in both plasma and CSF in 259PR and 259RR individuals suggest that the 259R allele impairs TC expression, stability, and/or metabolism. We were, however, unable to confirm the previously reported association between the 259R allele and lower concentrations of HoloTC in plasma. Nevertheless, HoloTC in CSF was reduced in patients with the 259PR and 259RR genotypes, showing that the polymorphism indeed affected HoloTC concentrations in CNS. HoloTC concentrations in plasma and CSF were highly correlated, which is compatible with the notion that all HoloTC in CSF originates from plasma and needs to pass the blood–brain barrier to enter the CNS. ... (report truncated).

Vitamin B12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians

Herrmann W, Schorr H, Obeid R, Geisel J

Central Laboratory, Department of Clinical Chemistry, Saarland University Hospital, Homburg/Saar, Germany. kchwher@uniklinik-saarland.de

Background: Vegetarians have a lower intake of vitamin B12 than do omnivores. Early and reliable diagnosis of vitamin B12 deficiency is very important. **Objective:** The objective was to investigate vitamin B12 status in vegetarians and nonvegetarians. **Design:** The study cohort included 66 lactovegetarians or lactoovovegetarians (LV-LOV group), 29 vegans, and 79 omnivores. Total vitamin B12, methylmalonic acid, holotranscobalamin II, and total homocysteine concentrations were assayed in serum. **Results:** Of the 3 groups, the vegans had the lowest vitamin B12 status. In subjects who did not consume vitamins, low holotranscobalamin II (< 35 pmol/L) was found in 11% of the omnivores, 77% of the LV-LOV group, and 92% of the vegans. Elevated methylmalonic acid (> 271 nmol/L) was found in 5% of the omnivores, 68% of the LV-LOV group, and 83% of the vegans. Hyperhomocysteinemia (> 12 μ mol/L) was present in 16% of the omnivores, 38% of the LV-LOV group, and 67% of the vegans. The correlation between holotranscobalamin II and vitamin B12 was weak in the low serum vitamin B12 range ($r = 0.403$) and strong in the high serum vitamin B12 range ($r = 0.769$). Holotranscobalamin II concentration was the main determinant of total homocysteine concentration in the vegetarians ($\beta = -0.237$, $P < 0.001$). Vitamin B12 deficiency led to hyperhomocysteinemia that was not probable in the upper folate range (> 42.0 nmol/L). **Conclusions:** Vegan subjects and, to a lesser degree, subjects in the LV-LOV group had metabolic features indicating vitamin B12 deficiency that led to a substantial increase in total homocysteine concentrations. Vitamin B12 status should be monitored in vegetarians. Health aspects of vegetarianism should be considered in the light of possible damaging effects arising from vitamin B12 deficiency and hyperhomocysteinemia.

Low vitamin B12 status in confirmed Alzheimer's disease as revealed by serum holotranscobalamin

Refsum H, Smith AD

Department of Pharmacology, University of Oxford, Oxford, UK

Objective: To examine the possible association of holotranscobalamin, the active fraction of serum cobalamin, with Alzheimer's disease. **Methods:** 51 patients with pathologically confirmed Alzheimer's disease and 65 cognitively screened elderly controls were studied. Serum holotranscobalamin was measured by a new solid phase radioimmunoassay. **Results:** Geometric mean levels showed no significant case-control differences for serum total cobalamin, but lower levels of holotranscobalamin in Alzheimer's disease (41.1 pmol/l) than in controls (57.1 pmol/l) ($p < 0.001$). The odds ratio of Alzheimer's disease was significant for low holotranscobalamin but not for low total cobalamin. **Conclusions:** Disturbed cobalamin status is common in Alzheimer's disease and accordingly measurement of holotranscobalamin should be considered in the assessment of cognitively impaired patients.

RIA for serum holotranscobalamin: method evaluation in the clinical laboratory and reference interval

Loikas S, Lopponen M, Suominen P, Moller J, Irjala K, Isoaho R, Kivela SL, Koskinen P, Pelliniemi TT

Department of Clinical Chemistry, Turku University Central Hospital, Turku, Finland.
saila.loikas@tyks.fi

Background: Decreased serum holotranscobalamin (HoloTC) could be the earliest marker of cobalamin (Cbl) deficiency, but there has been no method suitable for routine use. We evaluated a new commercial HoloTC RIA, determined reference values, and assessed HoloTC concentrations in relation to other biochemical markers of Cbl deficiency. **Methods:** The reference population consisted of 303 individuals 22–88 years of age, without disease or medication affecting Cbl or homocysteine metabolism. In elderly individuals (≥ 65 years), normal Cbl status was further confirmed by total homocysteine (tHcy; $< 19 \mu\text{mol/L}$) and methylmalonic acid (MMA; $< 0.28 \mu\text{mol/L}$) concentrations within established reference intervals. HoloTC in Cbl deficiency was studied in a population of 107 elderly individuals with normal renal function. The Cbl deficiency was graded as potential (total Cbl $\leq 150 \text{ pmol/L}$ or tHcy $\geq 19 \mu\text{mol/L}$), possible (total Cbl $\leq 150 \text{ pmol/L}$ and either tHcy $\geq 19 \mu\text{mol/L}$ or MMA $\geq 0.45 \mu\text{mol/L}$), and probable (tHcy $\geq 19 \mu\text{mol/L}$ and MMA $\geq 0.45 \mu\text{mol/L}$). **Results:** The intra- and between-assay imprecision (CV) for the HoloTC RIA were 4–7% and 6–8%, respectively. A 95% central reference interval for serum HoloTC was 37–171 pmol/L. All participants ($n = 16$) with probable Cbl deficiency, 86% of those with possible, and 30% of those with potential Cbl deficiency had HoloTC below the reference limit ($< 37 \text{ pmol/L}$). The HoloTC correlated with total Cbl ($r(s) = 0.80$; $P < 0.0001$) and inversely with MMA ($r(s) = -0.52$; $P < 0.0001$). HoloTC concentrations were significantly ($P = 0.01$) higher in women than in men. **Conclusions:** The new HoloTC RIA is precise and simple to perform. Low HoloTC is found in individuals with biochemical signs of Cbl deficiency, but the sensitivity and specificity of low HoloTC in diagnosis of Cbl deficiency need to be further evaluated.

Vegetarian lifestyle and monitoring of vitamin B12 status

Herrmann W, Geisel J

Department of Clinical Chemistry – Central Laboratory, University Hospital of the Saarland, Bld. 40, D-66421 Homburg/Saar, Germany. kchwher@uniklinik-saarland.de

Vegetarians are at risk to develop deficiencies of some essential nutrients, especially vitamin B12 (cobalamin). Cobalamin occurs in substantial amounts only in foods derived from animals and is essential for one-carbon metabolism and cell division. Low nutritional intake of vitamin B12 may lead to negative balance and, finally, to functional deficiency when tissue stores of vitamin B12 are depleted. Early diagnosis of vitamin B12 deficiency seems to be useful because irreversible neurological damages may be prevented by cobalamin substitution. The search for a specific and sensitive test to diagnose vitamin B12 deficiency is ongoing. Serum vitamin B12 measurement is a widely applied standard method. However, the test has poor predictive value. Optimal monitoring of cobalamin status in vegetarians should include the measurement of homocysteine (HCY), methylmalonic acid (MMA), and holotranscobalamin II. Vitamin B12 deficiency can be divided into four stages. In stages I and II, indicated by a low plasma level of holotranscobalamin II, the plasma and cell stores become depleted. Stage III is characterized by increased levels of HCY and MMA in addition to lowered holotranscobalamin II. In stage IV, clinical signs become recognizable like macroovalocytosis, elevated MCV of erythrocytes or lowered haemoglobin. In our investigations, we have found stage III of vitamin B12 deficiency in over 60% of vegetarians, thus underlining the importance of cobalamin monitoring in this dietary group.

Cobalamin Status (Holotranscobalamin, Methylmalonic Acid) and Folate as Determinants of Homocysteine Concentration

Rima Obeid¹, Muhidien Jouma² and Wolfgang Herrmann^{1a}

¹ Department of Clinical Chemistry, University Hospital of the Saarland, 66421 Homburg/Saar, Germany; ² Department of Biochemistry/College of Pharmacy, Damascus University, Damascus, Syria

^a address correspondence to this author at: Department of Clinical Chemistry/Central Laboratory, University Hospital of the Saarland, Bldg. 40, Kirrberger Straße, D-66421 Homburg/Saar, Germany; fax 49-6841-1623109, e-mail kchwher@uniklinik-saarland.de

Concern has emerged in America about subtle cobalamin (Cbl; vitamin B12) deficiency, especially in at-risk population groups such as the elderly and vegetarians. An optimal test to diagnose vitamin B12 deficiency is still not available. The determination of total serum vitamin B12 has a low diagnostic accuracy. Measurements of homocysteine (HCY) and methylmalonic acid (MMA) have shown more specificity and sensitivity for subnormal Cbl status, but have disadvantages. HCY, for example, is also increased in folate and vitamin B6 deficiencies, as well as in renal insufficiency; in addition, MMA is expensive to measure, and it, too, increases in renal insufficiency. To improve specificity and sensitivity in diagnosis of vitamin B12 deficiency, holotranscobalamin (HoloTC) assays have been introduced. Because only transcobalamin II promotes the specific cellular uptake of Cbl, the Cbl subfraction attached to transcobalamin II represents the biologically active vitamin B12 fraction.

Our previous observations in Syrian individuals revealed a high prevalence of Cbl deficiency (~ 49%) when we used MMA as a metabolic marker for Cbl status. The present work was undertaken to further investigate the role of Cbl and folate status as determinants of hyperhomocysteinemia in Syrians.

We studied 222 patients [mean (SD) age, 52 years; 192 males and 30 females] with angiographically defined stenosis $\geq 50\%$ in at least one major coronary artery. Exclusion criteria included recent myocardial infarction (3 months), acute diseases, and vitamin usage. Blood samples were collected 1 day before the angiography, and the angiography results were followed after that. The control group included 101 apparently healthy non-vitamin users and 10 individuals (7 males and 3 females) who had no stenosis [mean (SD) control age, 46 years; total of 66 males and 45 females]. Only individual with creatinine concentrations within reference values were eligible for this study. Seventy-one percent of patients were hypertensive vs 25% of controls, 32% of patients had diabetes vs 11% of controls, and 23% of patients had never smoked vs 42% of controls. All participants gave informed consent. ... (report truncated).

Holo transcobalamin is an early marker of changes in cobalamin homeostasis. A randomized placebo-controlled study

Nexo E, Hvas AM, Bleie O, Refsum H, Fedosov SN, Vollset SE, Schneede J, Nordrehaug JE, Ueland PM, Nygard OK

Department of Clinical Biochemistry, AKH, Aarhus University Hospital, DK-8000 Aarhus C, Denmark. E.Nexo@dadlnet.dk

Background: We examined the effect of oral vitamin B12 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 B12 (0.4 mg), folic acid (0.8 mg), and vitamin B(6) (40 mg); (b) vitamin B12 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 B12 was approximately 10% for plasma total cobalamin, total TC, apo-TC, and apo-HC, and <20% for HoloTC and TC saturation. In B12-treated patients, the maximum change in concentrations was observed already after 3 days for total TC (–16%), HoloTC (+54%), and TC saturation (+82%). At this time HoloHC (+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 B12 treatment produces maximal effects on total TC, HoloTC, and TC saturation within 3 days, whereas maximal increases in HoloHC and plasma total cobalamin occur later. The results support the view that HoloTC is an early marker of changes in cobalamin homeostasis.

Modern approaches to the investigation of vitamin B12 deficiency

Ward PC

Department of Pathology and Laboratory Medicine, University of Minnesota, Duluth School of Medicine, 10 University Drive, Duluth, MN 55812, USA. path@d.umn.edu

The classic workup of a patient for possible PA is revisited in light of the vanishing Schilling test. The vagaries of testing for B12 and blocking antibodies are reexamined. The advantages and disadvantages of newer tests such as MMA and serum gastrin levels are catalogued. At this juncture in the evolution of new test strategies, there is a considerable controversy regarding the significance of high MMA levels in the face of normal B12 levels, particularly in the elderly. Hopefully, this controversy will soon be resolved and the newer crop of tests will be proven and accepted in the workplace. Still, the words of Alexander Pope spring to mind: "Be not the first by whom the new are tried, Nor yet the last to lay the old aside."

Quantification of Holotranscobalamin, a Marker of Vitamin B12 Deficiency

Ebba Nexo^{1a}, Anna-Lisa Christensen¹, Anne-Mette Hvas¹, Torben E. Petersen² and Sergey N. Fedosov²

¹Department of Clinical Biochemistry, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; ²The Laboratory for Protein Chemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

^aaddress correspondence to this author at: Department of Clinical Biochemistry, AKH, Aarhus University Hospital, Norrebrogade 44, DK-8000 Aarhus C, Denmark; fax 45-8949-3060, e-mail E.Nexo@dadlnet.dk

We report a new method for measurement of holotranscobalamin (HoloTC), in which magnetic beads coated with vitamin B12 (cobalamins) precipitate apo-transcobalamin (apoTC) and the HoloTC present in the supernatant is measured by ELISA.

Serum HoloTC denotes the part of vitamin B12 accessible for the cells of the body and is considered to be a sensitive marker of vitamin B12 deficiency. Serum HoloTC is not easily measured because it accounts for only approximately one-third of the circulating vitamin B12 and because the major part of TC circulates unsaturated with vitamin B12 (apoTC). In the few published attempts to measure HoloTC, TC is separated from the other vitamin B12-binding protein, haptocorrin, before quantification of vitamin B12. This allows a direct measurement of the cobalamins attached to TC or an indirect calculation of HoloTC from measurement of total plasma cobalamins and the plasma cobalamins not attached to TC. We have chosen another path. ... (report truncated).

Direct assay for cobalamin bound to transcobalamin (holotranscobalamin) in serum

Ulleland M, Eilertsen I, Quadros EV, Rothenberg SP, Fedosov SN, Sundrehagen E, Orning L

Axis-Shield ASA, Ulvenveien 87, PO Box 206 Okern, N-0510 Oslo, Norway

Background: Only cobalamin carried by transcobalamin (holotranscobalamin) is available for cellular uptake and hence is physiologically relevant. However, no reliable or accurate methods for quantifying holotranscobalamin are available. We report a novel holotranscobalamin assay based on solid-phase capture of transcobalamin. **Methods:** A monoclonal antibody specific for human transcobalamin with an affinity constant $>10^{10}$ L/mol was immobilized on magnetic microspheres to capture and concentrate transcobalamin. The cobalamin bound to transcobalamin was then released and assayed by a competitive binding radioassay. The quantification of holotranscobalamin was accomplished using calibrators composed of recombinant, human holotranscobalamin. **Results:** The assay was specific for holotranscobalamin and had a detection limit of 5 pmol/L. Within-run and total imprecision (CV) was 5% and 8–9%, respectively. The working range (CV < 20%) was 5–370 pmol/L. Dilutions of serum were linear in the assay range. The recovery of recombinant, human holotranscobalamin added to serum was 93–108%. A 95% reference interval of 24–157 pmol/L was established for holotranscobalamin in 105 healthy volunteers 20–80 years of age. For 72 of these sera, holohaptocorrin and total cobalamin were also determined. Whereas holohaptocorrin correlated well ($r^2 = 0.87$) with total cobalamin, holotranscobalamin correlated poorly ($r^2 = 0.23$) with total cobalamin or holohaptocorrin. **Conclusions:** The solid-phase capture assay provides a simple, reliable method for quantitative determination of holotranscobalamin in serum.

Measuring and Interpreting holo-transcobalamin (Holo-transcobalamin II)

Ralph Carmel

Department of Medicine, New York Methodist Hospital, Brooklyn, NY 11215, and Weill Medical College of Cornell University, New York, NY 10021, USA

address for correspondence: NY Methodist Hospital, 506 Sixth St., Brooklyn, NY 11215, USA. Fax 718-780-3259; e-mail rac9001@nyp.org

At one time, diagnosing cobalamin deficiency was fairly simple, usually involving a patient with clinical problems, and was usually settled by determining whether the serum cobalamin concentration was low or not. As reviewed elsewhere, things began to change after sensitive metabolic tests were introduced and as attention extended to the cobalamin status of asymptomatic persons. Metabolic studies confirmed that most low cobalamin concentrations in asymptomatic patients and seemingly healthy persons represented subclinical cobalamin insufficiency, but ~30–40% of these cobalamin concentrations did not represent insufficiency and thus could be considered “falsely low”. The diagnostic reliability of serum cobalamin was further challenged by metabolic demonstrations of “falsely normal” cobalamin concentrations; these too occurred most often, but not exclusively, in asymptomatic persons.

The search continues for the optimal test to diagnose deficiency because the metabolic tests also have disadvantages. Increased plasma total homocysteine is too nonspecific; methylmalonic acid determination, although posing fewer problems of specificity, is complex and expensive; and the deoxyuridine suppression test is too unwieldy for practical use.

Interest has been drawn to the possible benefit of measuring only the cobalamin attached to transcobalamin (TC; also called TC II; the TC-cobalamin complex is called HoloTC or HoloTC II) rather than the total cobalamin content of plasma. ... (report truncated).

Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians

Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, Ueland PM

Department of Pharmacology and the Locus for Homocysteine and Related Vitamins, University of Bergen, Bergen, Norway. helga.refsum@farm.uib.no

Background: In India, most people adhere to a vegetarian diet, which may lead to cobalamin deficiency. **Objective:** The objective was to examine indicators of cobalamin status in Asian Indians. **Design:** The study population included 204 men and women aged 27–55 y from Pune, Maharashtra, India, categorized into 4 groups: patients with cardiovascular disease (CVD) and diabetes, patients with CVD but no diabetes, patients with diabetes but no CVD, and healthy subjects. Data on medical history, lifestyle, and diet were obtained by interviews and questionnaires. Blood samples were collected for measurement of serum or plasma total cobalamin, holotranscobalamin (HoloTC), methylmalonic acid (MMA), and total homocysteine (tHcy) and hemetologic indexes. **Results:** MMA, tHcy, total cobalamin, and HoloTC did not differ significantly among the 4 groups; therefore, the data were pooled. Total cobalamin showed a strong inverse correlation with tHcy ($r = -0.59$) and MMA ($r = -0.54$). Forty-seven percent of the subjects had cobalamin deficiency (total cobalamin <150 pmol/L), 73% had low HoloTC (<35 pmol/L), 77% had hyperhomocysteinemia (tHcy >15 $\mu\text{mol/L}$), and 73% had elevated serum MMA (>0.26 $\mu\text{mol/L}$). These indicators of impaired cobalamin status were observed in both vegetarians and nonvegetarians. Folate deficiency was rare and only 2.5% of the subjects were homozygous for the MTHFR 677C \rightarrow T polymorphism. **Conclusions:** About 75% of the subjects had metabolic signs of cobalamin deficiency, which was only partly explained by the vegetarian diet. If impaired cobalamin status is confirmed in other parts of India, it may have important health implications.

Signs of impaired cognitive function in adolescents with marginal cobalamin status

Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, Ueland PM, Refsum H, van Staveren WA

Division of Human Nutrition and Epidemiology, Wageningen Agricultural University, Wageningen, The Netherlands

Background: Lack of cobalamin may lead to neurologic disorders, which have been reported in strict vegetarians. **Objective:** The objective of this study was to investigate whether cognitive functioning is affected in adolescents (aged 10–16 y) with marginal cobalamin status as a result of being fed a macrobiotic diet up to an average age of 6 y. **Design:** Data on dietary intake, psychological test performance, and biochemical variables of cobalamin status were collected from 48 adolescents who consumed macrobiotic (vegan type) diets up to the age of 6 y, subsequently followed by lactovegetarian or omnivorous diets, and from 24 subjects (aged 10–18 y) who were fed omnivorous diets from birth onward. Thirty-one subjects from the previously macrobiotic group were cobalamin deficient according to their plasma methylmalonic acid concentrations. Seventeen previously macrobiotic subjects and all control subjects had normal cobalamin status. **Results:** The control subjects performed better on most psychological tests than did macrobiotic subjects with low or normal cobalamin status. A significant relation between test score and cobalamin deficiency ($P = 0.01$) was observed for a test measuring fluid intelligence (correlation coefficient: -0.28 ; 95% CI: $-0.48, -0.08$). This effect became more pronounced ($P = 0.003$) within the subgroup of macrobiotic subjects (correlation coefficient: -0.38 ; 95% CI: $-0.62, -0.14$). **Conclusion:** Our data suggest that cobalamin deficiency, in the absence of hematologic signs, may lead to impaired cognitive performance in adolescents.

Holotranscobalamin – a sensitive marker of cobalamin malabsorption

Lindgren A, Kilander A, Bagge E, Nexø E

Department of Internal Medicine, Borås Central Hospital, Borås, Sweden

Background: No simple and reliable method of identifying patients with cobalamin malabsorption is available at present. The measurement of plasma holotranscobalamin, i.e. the metabolically active cobalamins bound to the transport protein transcobalamin, has been suggested as a means of fulfilling such criteria. **Design:** We describe a method that directly quantifies cobalamins attached to transcobalamin. The method is evaluated in patients referred for gastrointestinal examination because of suspected cobalamin malabsorption. **Results:** Of the 101 patients referred, all 48 with gastrointestinal conditions compatible with cobalamin malabsorption had plasma holotranscobalamin below 35 pmol L⁻¹ (interval of 35–160 pmol L⁻¹). None of the patients with plasma holotranscobalamin above the lower reference limit had conditions compatible with cobalamin malabsorption.

Conclusion: The values obtained for plasma holotranscobalamin showed a better correlation with possible malabsorption than the values obtained for plasma cobalamins. The specificity of the test, however, needs to be elucidated further.

Cobalamin pseudodeficiency due to a transcobalamin I deficiency

Adcock BB, McKnight JT

Department of Family Medicine, College of Community Health Sciences, University of Alabama, Tuscaloosa, USA

Cobalamin (vitamin B12) deficiency warrants appropriate evaluation because cobalamin is necessary in certain biochemical functions. R-binder deficiency, which causes low cobalamin levels, is a rare and benign pseudodeficiency. If not further evaluated by determining levels of methylmalonic acid and homocysteine, however, such a patient would be given unneeded treatment. We report a case in which a patient has an R-binder deficiency, specifically transcobalamin I deficiency, with a low vitamin B12 level but no true vitamin B12 deficiency.

Note: The terms transcobalamin I, R-binder, and haptocorrin are synonymous and all refer to the same circulating plasma vitamin B12 binding protein. This protein is now commonly referred to as haptocorrin. Transcobalamin (or previously transcobalamin II) is the other binding protein, responsible for cellular up-take of vitamin B12. Current vitamin B12 assays measure cobalamin bound to both proteins.

Transcobalamin II deficiency with methylmalonic aciduria in three sisters

Bibi H, Gelman-Kohan Z, Baumgartner ER, Rosenblatt DS

Pediatric Department, Clinical Genetic Institute Barzilai Medical Center, Ashkelon, Israel

Transcobalamin II (TC II) is a plasma protein that binds vitamin B12 (cobalamin, Cbl) and facilitates cellular Cbl uptake by receptor-mediated endocytosis. In autosomal recessive TC II deficiency, intracellular Cbl deficiency results in an early onset of megaloblastic anaemia that may be accompanied by neurological abnormalities. Inadequate treatment may lead to neurological abnormalities. We describe three sisters, the daughters of first cousins of Moroccan origin, with TC II deficiency requiring continuous and long-term vitamin B12 treatment. The diagnosis was suspected from the finding of low unsaturated vitamin B12 binding capacity and confirmed by absence of detectable TC II by radioimmunoassay and by inability of cultured fibroblasts to synthesize TC II.

Cobalamin (vitamin B12) and holo-transcobalamin changes in plasma and liver tissue in alcoholics with liver disease

Baker H, Leevy CB, DeAngelis B, Frank O, Baker ER

Department of Preventive Medicine and Community Health, University of Medicine and Dentistry, New Jersey Medical School, Newark 07107-3001, USA

Objective: We wanted to know if alterations in plasma cobalamin (B12) concentration and B12 carriers, e.g., holotranscobalamins (HoloTC), occur in blood and liver tissue from patients with severe alcoholic liver disease. Our purpose was to test the hypothesis that liver disease may disrupt B12 distribution. **Method:** Total B12, as well as B12 bound to transcobalamin I, II, III (HoloTC), were measured to determine their concentration in plasma and in liver tissue; *Poterochromonas malhamensis* – a protozoan reagent served to measure only metabolically active (true) B12. Total B12 as distributed in HoloTC in plasma and liver tissue of healthy subjects (controls) were compared to patients with severe alcoholic liver disease. **Results:** Severe liver disease initiates highly elevated B12 levels in plasma and a lowered liver tissue total B12 concentration. The percent of B12 distributed to HoloTC II is significantly depleted during liver disease. In contrast, HoloTC I and III are elevated in plasma during liver disease and contain more B12 than controls. Total B12 and B12 distributed to TC are lower in diseased liver tissue. **Conclusion:** Severe alcoholic liver disease involves leakage of total B12 from liver tissue into the plasma. HoloTC I and III concentration increases in plasma; this preserves the high plasma B12 from being excreted. However, plasma HoloTC II B12 distribution is decreased, indicating that there is a depression of exogenous B12 entering the plasma and tissues. In severe liver disease, liver tissue B12 binding and storage by TC is disrupted and causes B12 to leak out of the liver into the circulation. Eventually liver disease could produce enough severe tissue B12 deficits to cause metabolic dysfunction despite elevated plasma total B12. Elevation of plasma B12, accompanied by a lowering of HoloTC II distribution, seemed to be a useful index of liver disease severity suggesting preventive treatment.

Limited value of serum holotranscobalamin II measurements in the differential diagnosis of macrocytosis

Wickramasinghe SN, Ratnayaka ID.

Department of Haematology, Imperial College School of Medicine at St Mary's, London, UK

Aim: To study the value of serum holotranscobalamin II (HoloTCII) measurements in the differential diagnosis of macrocytosis. **Methods:** HoloTCII concentrations were measured in serum samples from 50 healthy non-vegetarian subjects and 30 patients with macrocytosis, using a technique based on the adsorption of HoloTCII with amorphous, precipitated silica. Deoxyuridine (dU) suppression tests were performed on the bone marrow cells of all the patients. Haematological diagnoses were made using standard criteria. **Results:** The causes of macrocytosis were cobalamin (Cbl) deficiency due to pernicious anaemia or following partial gastrectomy (10 patients), dietary folate deficiency with/without Cb1 deficiency (four patients), chronic alcoholism (four patients), myelodysplastic syndrome (five patients), treatment with methotrexate or azathioprine (three patients), and congenital dyserythropoietic anaemia (CDA) (four patients). Undetectable or low HoloTCII concentrations were found in all patients with Cb1 deficiency and in some or all patients from each of the other diagnostic categories. There was also no correlation between the dU suppressed value and the HoloTCII concentration: all 15 patients with high dU suppressed values and nine of 15 with normal dU suppressed values, including four patients with CDA, had low HoloTCII concentrations. **Conclusions:** Measurements of serum HoloTCII concentrations by the silica adsorption method are not of value in the differential diagnosis of macrocytosis. The finding of low serum HoloTCII concentrations in patients with macrocytosis due to causes other than Cb1 deficiency may result not only from a state of negative Cb1 balance but also from other factors, such as increased utilisation of HoloTCII as a consequence of erythroid hyperplasia.

Note: The method used to quantitate HoloTC resulted in undetectable or low HoloTC concentrations and as the authors noted, use of this *method* is not of value.

Cobalamin

Markle HV

Centenary Health Centre, Scarborough, Ontario, Canada

Cobalamin (vitamin B12) is an essential nutrient derived exclusively from bacterial sources. It is an essential cofactor for three known enzymatic reactions. Untreated deficiency, caused by either the autoimmune disease pernicious anemia or nutritional lack, results in a macrocytic anemia and/or subacute combined degeneration of the spinal cord and is eventually fatal. Cobalamin in serum is bound to two proteins, transcobalamin and haptocorrin. The former is responsible for the essential delivery of cobalamin to most tissues. Inadequate tissue availability of cobalamin results in increased concentration of methylmalonic acid and homocyst(e)ine due to inhibition of methylmalonyl-CoA mutase and methionine synthase, respectively. Strict vegetarians have long been known to be at risk of cobalamin deficiency, which develops insidiously over many years. It is now clear that a significant number of the elderly and HIV-positive individuals are also at increased risk of deficiency. Any individual with reduced ability to split cobalamin from food-protein may also become deficient even though intrinsic factor is present. Diagnosis of cobalamin deficiency has frequently relied on total serum cobalamin and the Schilling test. Newer approaches such as analysis of methylmalonic acid, homocyst(e)ine, holotranscobalamin, anti-intrinsic factor antibodies, and serum gastrin may provide more cost-effective testing, as well as identify those with a covert deficiency.

Long-term follow up of patients with transcobalamin II deficiency

Monagle PT, Tauro GP

Department of Laboratory Haematology, Royal Children's Hospital, Parkville, Victoria, Australia

Five cases of transcobalamin II deficiency presenting to our institution were reviewed. A delay in diagnosis often led to acute deterioration. Two patients have long term neurological sequelae. Minimal treatment in these patients may be dangerous. While haematological normality may be maintained, the adequate therapeutic dose of vitamin B12 to allow normal neurological development and function is not easily determined and damage sustained early in life may be irreversible.

The neurologic aspects of transcobalamin II deficiency

Hall CA

Stratton Veterans Affairs Medical Center, Albany, NY 12208, USA

Thirty-four symptomatic cases of inherited transcobalamin II (TCII) deficiency were analysed in order to determine the frequency and nature of neurologic manifestations. In no instance was there definite evidence of a neurologic disorder at the time of presentation as a young infant. One child of 2½ years transiently lost deep tendon reflexes at a time of suboptimal treatment. A syndrome of mental retardation and other neurologic manifestations was observed in three cases, all with the following in common: (1) an extended duration of illness of 2–17 years; (2) inadequate or no treatment with Cbl; (3) treatment with folic or folinic acid. TCII deficiency rarely if ever presents with neurologic manifestations. However, neurologic disorders can be produced subsequently by improper treatment.

Transcobalamin II deficiency: Case report and review of the literature

Kaikov Y, Wadsworth LD, Hall CA, Rogers PC

Department of Paediatrics, University of British Columbia, British Columbia Children's Hospital, Vancouver, BC, Canada

A male Caucasian infant presented at 6 weeks of age with failure to thrive, diarrhoea, macrocytic anaemia, and decreased IgG. He had normal serum B12 and folate levels. Serum cobalamin binding capacity showed no detectable transcobalamin II. Both parents showed levels consistent with a heterozygous state. The literature is extensively reviewed, and the importance of early diagnosis to prevent neurological dysfunction is stressed.

Subacute combined degeneration with high serum vitamin B12 level and abnormal vitamin B12 binding protein. New cause of an old syndrome

Reynolds EH, Bottiglieri T, Laundry M, Stern J, Payan J, Linnell J, Faludy J
Department of Neurology, King's College Hospital, Denmark Hill, London, UK

Subacute combined degeneration of the spinal cord due to vitamin B12 deficiency invariably has been associated with a low serum vitamin B12 level. We describe a young man who presented with a unique syndrome of subacute combined degeneration associated with high serum vitamin B12 level, low red blood cell vitamin B12 level, and an abnormal plasma vitamin B12-binding protein. Uptake of cobalamin by his leukocytes in vitro was inhibited by his own but not by normal control plasma. Intensive hydroxocobalamin (vitamin B12) treatment was associated with clinical and electrophysiologic recovery accompanied by normalization of mean corpuscular volume, red blood cell vitamin B12 level, plasma homocysteine, and urinary methylmalonic acid. The subacute combined degeneration was probably precipitated by treatment with folic acid as the significance of his high serum vitamin B12 level was not apparent when he first presented with megaloblastic anemia 3 years earlier. To our knowledge, this is the first example of neurologic disease associated with high serum vitamin B12 level and provides further evidence that sometimes a serum vitamin B12 level may not be a reliable guide to vitamin B12 deficiency.

Transcobalamin II deficiency presenting with methylmalonic aciduria and homocystinuria and abnormal absorption of cobalamin

Barshop BA, Wolff J, Nyhan WL, Yu A, Prodanos C, Jones G, Sweetman L, Leslie J, Holm J, Green R, et al.

Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA

An infant with deficiency of transcobalamin II (TCII) presented with virtually complete failure to thrive and life-threatening pancytopenia. Methylmalonic acid and homocystine were found in the urine. The concentration of B12 in the serum was 26 pg/ml. Fibroblasts derived from the patient failed to take up labeled cobalamin in the absence of a source of TCII. Uptake was normal in the presence of TCII. Treatment with parenteral cobalamin reversed the clinical and hematological manifestations of the disease but she developed glossitis when the interval between injections was lengthened. Intestinal absorption of ⁵⁷Co-cobalamin was less than 1% and remained abnormal when highly purified human intrinsic factor was given along with the labeled B12. Absorption improved when the labeled B12 was given together with rabbit TCII. The data suggest that TCII as well as intrinsic factor is required for transport of cobalamin from the intestine to the blood.

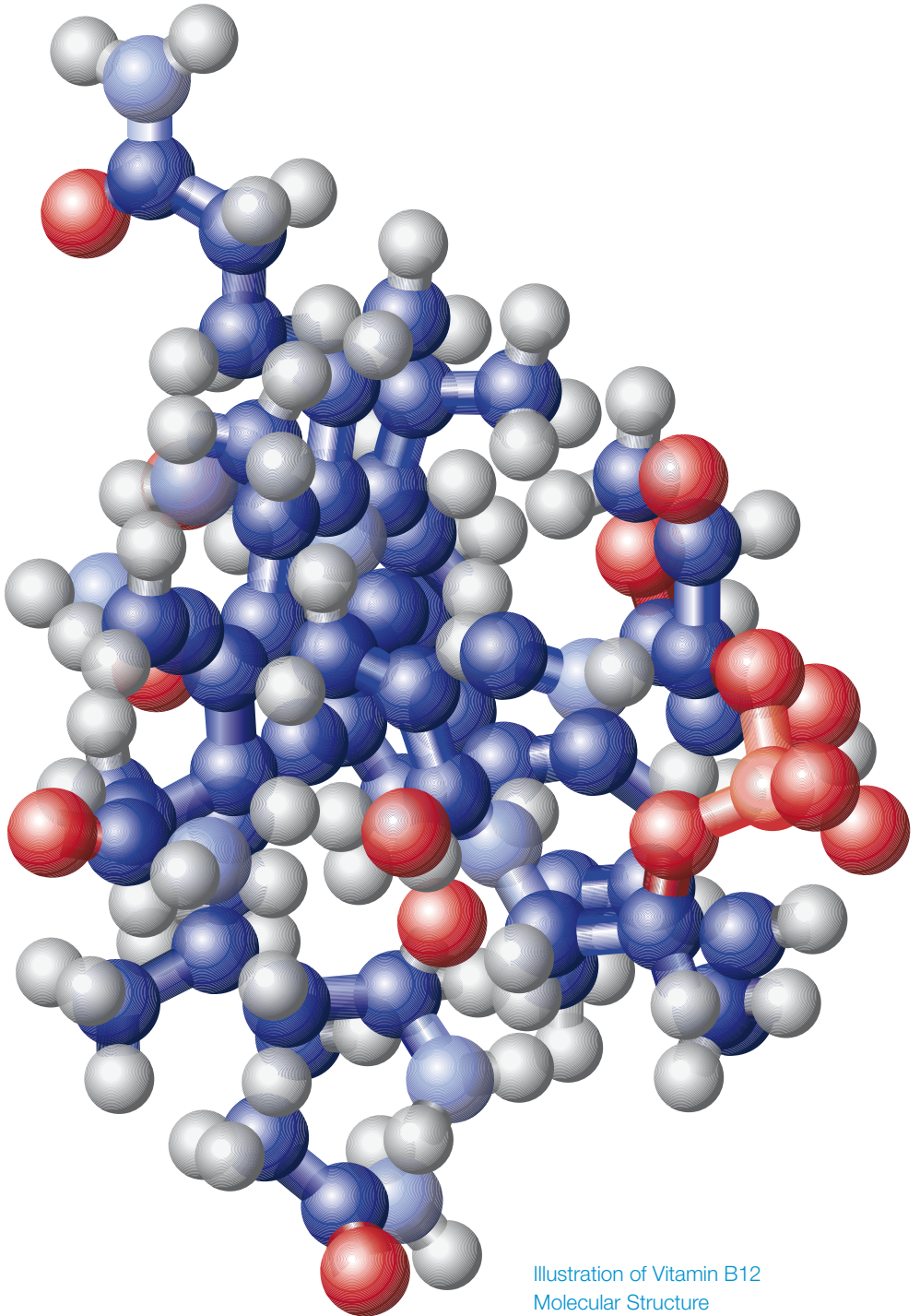


Illustration of Vitamin B12
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Axis-Shield plc
The Technology Park
Dundee DD2 1XA
UK
Tel. (+44) 1382 422 000
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