Correlations between holo-transcobalamin II, holo-haptocorrin, and total B_{12} in serum samples from healthy subjects and patients

S N Wickramasinghe, S Fida

Abstract

***Aims*—To study the correlations between total vitamin B_{12}(B_{12}), holo-haptocorrin, and holo-transcobalamin II (holo-TCII) concentrations in human sera; the association between reduced holo-TCII concentrations and macrocytosis attributable to B_{12} deficiency.**

***Methods*—Serum samples from 38 healthy volunteers, 113 patients with normal total serum B_{12} concentrations and 93 patients with low total serum B_{12} were studied. Holo-TCII was removed from whole serum by adsorption with amorphous precipitated silica, and both whole serum and adsorbed serum were assayed for B_{12} using the Becton Dickinson vitamin B_{12} [^{57}Co] radioassay kit.**

***Results*—In all three groups of subjects studied there were strong correlations between the logarithms of the total serum B_{12} and the holo-haptocorrin concentrations with regression coefficients between 0·884 and 0·967. By contrast, the correlations between the logarithms of the total serum B_{12} and holo-TCII concentrations were weaker, especially in the patients with normal or low total serum B_{12}, for whom the regression coefficients were 0·491 and 0·391, respectively. Analysis of the clinical records of a proportion of the patients studied indicated that there were many more patients with low holo-TCII concentrations than with haematological disturbances related to B_{12} deficiency.**

***Conclusions*—The total serum B_{12} concentration is a relatively poor indicator of holo-TCII concentrations and, therefore, of the ability of serum to deliver B_{12} to tissues. Additional information regarding B_{12} values can therefore be gleaned from measuring holo-TCII concentrations in the serum. Low holo-TCII concentrations, however, are an early sign of negative B_{12} balance and are frequently unassociated with haematological abnormalities caused by B_{12} deficiency.**

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The main vitamin B_{12} (cobalamin) binding proteins in the serum are haptocorrin (transcobalamins I and III) and transcobalamin II (TCII). Haptocorrin does not seem to be involved in delivering vitamin B_{12} (B_{12}) to cells; this function is performed by TCII.[1] Despite this, most of the B_{12} in serum is bound to haptocorrin—that is, it is found as holo-haptocorrin—and in normal subjects only 6–20% is bound to TCII—that is, it is found as holo-TCII.[2 3] Herbert *et al* have suggested that B_{12} deficiency develops in three overlapping stages[4]: (1) early negative B_{12} balance in which holo-TCII concentrations are reduced, holo-haptocorrin and total serum B_{12} concentrations are normal, and there are no biochemical, haematological, or neurological consequences of B_{12} deficiency; (2) B_{12} depletion in which holo-TCII and holo-haptocorrin concentrations are reduced but there are no biochemical, haematological, or neurological consequences of B_{12} deficiency; and (3) B_{12} deficiency in which, in addition to reduced holo-TCII and holo-haptocorrin concentrations, there are increasing biochemical, haematological, or neurological abnormalities attributable to B_{12} deficiency. These authors have further proposed that a proper evaluation of the B_{12} concentration of an individual must include measurements of both total serum B_{12} as well as holo-TCII concentrations.[5 6] In this study we investigated the latter contention by studying the correlations between total serum B_{12}, holo-haptocorrin, and holo-TCII concentrations in sera from 38 healthy volunteers and 206 patients, 93 of whom had low total serum B_{12} concentrations.

Methods

Sera from 38 healthy adult meat eaters of both sexes, 113 adult patients with normal total serum B_{12} concentrations, and 93 adult patients with low total serum B_{12} concentrations were studied. The sera had been separated from clotted venous blood within 4 hours of venesection and stored at -20°C for up to one month. Total serum B_{12} concentrations were remeasured in all sera using the Becton Dickinson vitamin B_{12} [^{57}Co] radioassay kit (Becton Dickinson, Immunodiagnostics, New York, USA); in this kit the vitamin B_{12} binding protein is porcine intrinsic factor and the contaminating R proteins are blocked by a large excess of vitamin B_{12} analogues.

Holo-TCII was removed from whole serum by a modification of the method recommended by Das *et al*.[6] A slurry containing 3 g synthetic amorphous precipitated silica (Sipernat 283 LS) (PQ Corporation, Valley Forge, Philadelphia, USA) in 20 ml of dis-

Correlation coefficients (r) for relations between total serum B_{12}, holo-haptocorrin, and holo-TCII in various groups of subjects studied

Coordinates					
X	*Y*	*Group**	*N*	*r*	*p Value*
Log holo-haptocorrin	Log total serum B_{12}	A	38	0·967	< 0·001
		B	113	0·933	< 0·001
		C	93	0·884	< 0·001
		D	244	0·972	< 0·001
Log total serum B_{12}	Log holo-TCII	A	38	0·715	< 0·001
		B	113	0·491	< 0·001
		C	93	0·391	< 0·001
		D	244	0·720	< 0·001
Log holo-haptocorrin	Log holo-TCII	A	38	0·523	< 0·001
		B	113	0·228	< 0·008
		C	93	0·001	NS
		D	244	0·576	< 0·001

*A: healthy volunteers; B: patients with normal total serum B_{12}; C: patients with low total serum B_{12}; D: all cases studied (A + B + C).

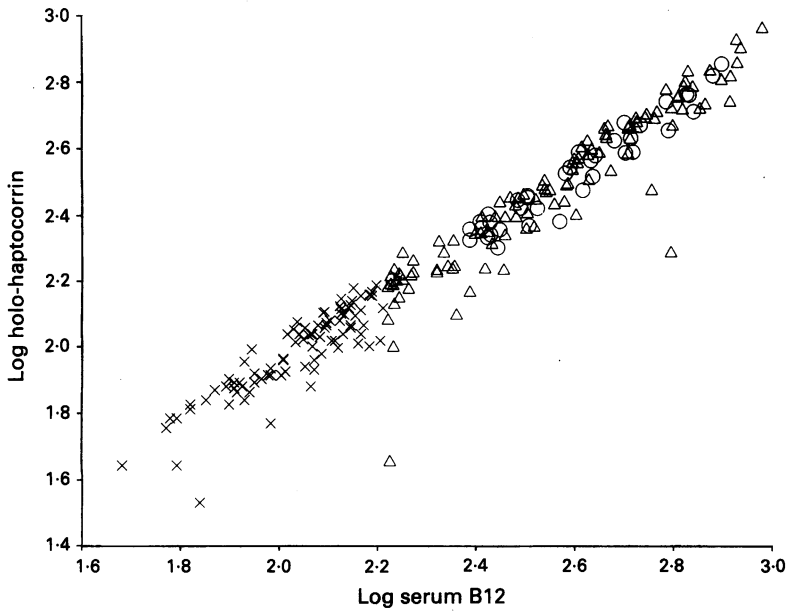

Figure 1 Correlation between the logarithms of the holo-haptocorrin and total serum B_{12} values in the three groups of subjects studied: ○ healthy volunteers; △ patients with normal total serum B_{12}; × patients with low total serum B_{12} values.

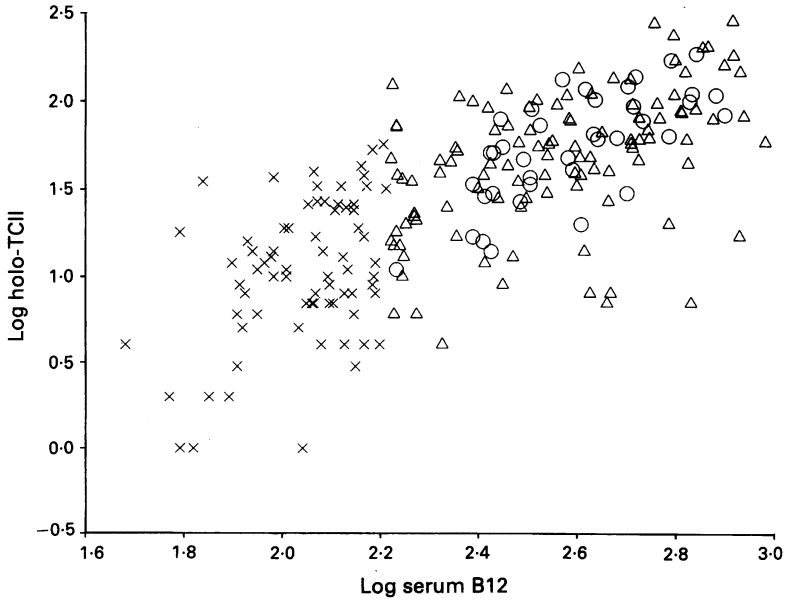

Figure 2 Correlation between the logarithms of the total serum B_{12} and holo-TCII concentrations in the three groups of subjects studied: ○ healthy volunteers; △ patients with normal total serum B_{12}; × patients with low total serum B_{12} values.

tilled water was prepared and stored at 4°C. The holo-TCII was adsorbed by adding 100 μl of the slurry containing 3 mg silica to 500 μl serum, vortexing the mixture, and leaving it at room temperature for 10 minutes. The mixture was then centrifuged at 5000 × *g* for 10 minutes and the supernatant fluid assayed for B_{12} using the Becton Dickinson radioassay kit according to the manufacturer's instructions. The value obtained represents the holo-haptocorrin concentration. The holo-TCII concentration was determined by subtracting the holo-haptocorrin concentration from the total serum B_{12} concentration.

Results

As in the case of total serum B_{12},[7] holo-haptocorrin and holo-TCII concentrations had a log-normal distribution. Therefore, the data were subjected to logarithmic transformation for the derivation of median values and 95% reference ranges and for the study of various correlations.

In our laboratory the median value and the 95% reference range for total serum B_{12} measured in 100 healthy adults by the radioassay kit were, respectively, 336 and 165–684 ng/l.[7] The corresponding values for holo-haptocorrin in the 38 healthy volunteers in the present study were 322 ng/l and 151–682 ng/l and for holo–TCII they were 57 and 13–244 ng/l, respectively.

Figure 1 and the table show that there were strong correlations between the logarithm of the holo-haptocorrin concentration and the logarithm of the total serum B_{12} concentration in all three groups of individuals studied. All patients with low total serum B_{12} had low holo-haptocorrin concentrations. Figure 2 and the table show the weaker correlations that existed between the logarithms of the total serum B_{12} and the holo-TCII concentrations. In fact, 34 of the 93 patients with low total serum B_{12} had normal holo-TCII concentrations and 13 of the 113 patients with normal total serum B_{12} (six with a total serum B_{12} value between 165 and 200 ng/l and seven with a value of >200 ng/l) had low holo-TCII concentrations. In healthy volunteers and patients with normal total serum B_{12} concentrations, the lowest correlation coefficients were observed for the relation between the logarithms of the holo-haptocorrin and the holo-TCII concentrations and in patients with low total serum B_{12} concentrations no correlation was found between these two variables (fig 3 and table).

None of the 13 patients with normal total serum B_{12} and low holo-TCII concentrations and only one of the 34 patients with low total serum B_{12} and normal holo-TCII concentrations had a high mean corpuscular volume (MCV) attributable to B_{12} deficiency. The exception was a patient with pancreatitis, obstructive jaundice, macrocytic anaemia, megaloblastic erythropoiesis, a deoxyuridine suppressed value of 40%, a total serum B_{12} of 62 ng/l, a holo-TCII concentration of 18 ng/l

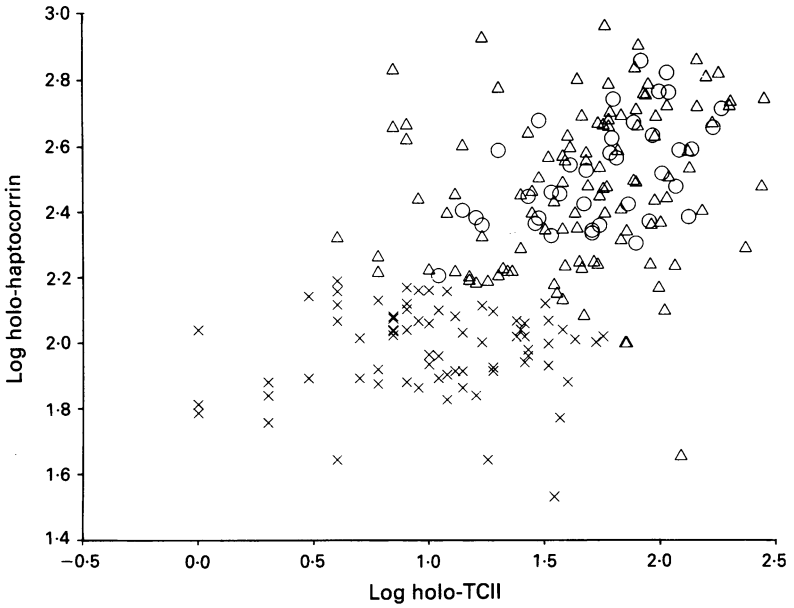

Figure 3 Correlation between the logarithms of the holo-TCII and holo-haptocorrin values in the three groups of subjects studied: ○ healthy volunteers; △ patients with normal total serum B_{12}; × patients with low total serum B_{12} values.

and an abnormal Schilling test (part I) result of 1·6%. At the time of writing, the case notes of 30 of the 59 patients with low total serum B_{12} and low holo-TCII concentrations had been analysed in detail; only nine of these 30 patients had an abnormal deoxyuridine suppressed value or a high MCV attributable to B_{12} deficiency.

Discussion

In healthy volunteers, patients with normal total serum B_{12} concentrations, and patients with low total serum B_{12} concentrations, there were strong significant correlations between holo-haptocorrin and total serum B_{12} concentrations. In these groups 93·5%, 87·0%, and 78·1%, respectively, of the variability in the logarithm of the total serum B_{12} concentration could be explained by its correlation with the logarithm of the holo-haptocorrin concentration. Thus a falling total serum B_{12} concentration indicates a falling holo-haptocorrin concentration and, presumably, a falling hepatic B_{12} store. By contrast, there was a considerably weaker correlation between total serum B_{12} and holo-TCII concentrations: the correlation was best in the healthy volunteers in whom 51·1% of the variation in the logarithm of the total serum B_{12} could be accounted for by its correlation with the logarithm of the holo-TCII concentration and worst in the patients with low total serum B_{12} concentrations in whom the corresponding figure was only 15·3%. Therefore, low total serum B_{12} concentrations do not necessarily indicate reduced holo-TCII concentrations and, consequently, a reduced supply of B_{12} to tissues. Indeed, in the present study, 36·6% of patients with low total serum B_{12} concentrations had normal holo-TCII concentrations and 11·5% of patients with normal total serum B_{12} had low holo-TCII concentrations. The weakness of the correlation between total serum B_{12} and holo-TCII concentrations observed in this investigation may account for the finding of normal total serum B_{12} in some patients with biochemical or clinical manifestations of B_{12} deficiency and low serum B_{12} values in other patients who do not seem to have the harmful effects of B_{12} deficiency.[8 9]

In the present investigation, low holo-TCII concentrations were found in a number of patients without macrocytosis. Furthermore, there were many more patients with low holo-TCII concentrations than with haematological disturbances attributable to B_{12} deficiency. These observations confirm the conclusion from two previous studies that low holo-TCII concentrations are an early sign of subnormal vitamin B_{12} absorption and negative vitamin B_{12} balance. In one study reduced holo-TCII concentrations were found one month after the last injection of 1000 μg cyanocobalamin in haematologically normal patients with treated pernicious anaemia.[5] In the other study low holo-TCII values were found in the presence of normal total serum B_{12} and normal serum homocysteine concentrations in patients with AIDS.[4] As low holo-TCII values may be found in the absence of haematological abnormalities, the serum concentration of holo-TCII at which tissue cells sustain the effects of B_{12} deficiency may vary from individual to individual and from tissue to tissue.

Our data support the model of developing B_{12} deficiency proposed by Herbert and his colleagues and their view that a proper study of B_{12} values should include not only measurements of total serum B_{12} but also of holo-TCII concentration.[4–6] Additional studies are needed to define the clinical importance of diagnosing and treating patients with early negative B_{12} balance.

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