A sex difference in serum cobalamin and transcobalamin levels1–3

F Fernandes-Costa, S van Tonder, and J Metz

ABSTRACT In healthy young adults, serum levels of cobalamin, unsaturated and total cobalamin binding capacity, and transcobalamin (TC) II are significantly higher in females, while TC III is higher in males. These findings have relevance to the reference values in normals for these tests. Am J Clin Nutr 1985;41:784–786.

KEY WORDS Cobalamins, vitamin B12 binders

Introduction

Measurement of serum cobalamin (vitamin B12) is a well established index of cobalamin nutrition, and in recent years, quantification of the cobalamin binding proteins, the transcobalamin (TC) is assuming increasing clinical significance. In establishing reference values for these tests, data on sex differences are of obvious relevance. Many of the published studies of serum cobalamin and TC levels in normal subjects have ignored the possibility of a sex difference. The evidence from studies that have considered the sex of the test subjects has been conflicting, some workers reporting higher values for serum cobalamin and TC's in females, others failing to find a sex difference.

In view of these conflicting reports and the availability of newer methods to quantitate three TC proteins, we have re-examined the questions of a sex difference in serum levels for cobalamin and TCs. In this study, particular care was taken to exclude subjects in whom factors that might influence the serum levels of cobalamin and TC such as iron or folate deficiency, oral contraceptives, vitamin supplements etc, were operative.

Materials and methods

Subjects studied

The studies were approved by the Committee for Research on Human Subjects of the University of the Witwatersrand. All subjects gave informed consent and the workers adhered to the provisions of the Declaration of Helsinki. A total of 77 males and 82 females were studied. The subjects were all young adult Caucasoid volunteers, and the ages (18–36 years) of the male and female groups were comparable.

The subjects were all laboratory, medical, or nursing staff. All were in apparent good health with normal blood counts, serum cobalamin, folate, transferrin and iron levels, and normal red cell folate concentration.

None of the subjects was taking medication except patent analgesics, and subjects taking vitamin supplements or oral contraceptive agents were specifically excluded. The subjects were not lactating and had not been pregnant in the six-month period prior to the study.

Collection of blood samples

Blood was taken by antecubital venepuncture, without stasis, using vacuum tubes ('Vacutainer', Becton Dickinson, Rutherford, NJ; or 'Venoject', Jintan Terumo, Tokyo). The blood was taken in the non-fasting state between 8 AM and 1 PM. Blood in plain glass tubes was allowed to clot in a 37°C waterbath for not less than one hour and then centrifuged after which one ml aliquots of serum were transferred to sterile tubes using plastic disposable pipettes and stored at −20°C. All tests were performed within one month of collection. Once an aliquot of serum had been thawed, it was not refrozen, but discarded.

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Laboratory methods

Radioactive cobalamin was obtained from the Radiochemical Center Amersham, as ²⁵²Co-vitamin B₁₂ (specific activity 180–300 μCi/μg). The serum cobalamin concentration was measured by the radioisotope dilution method of Green et al (1) in which chicken serum is used as the source of cobalamin binding protein. The unsaturated cobalamin binding capacity (UBBC) was measured by the method of Gottlieb et al (2) and TC’s were quantified by the method of Jacob et al (3) in which TC II is adsorbed to a micro-fine precipitate of silica and TC I and TC III separated by batch elution from DEAE cellulose.

The total cobalamin binding capacity (TBBC) was calculated from the formula:

\[ \text{TBBC (pg cobalamin/ml)} = \text{serum cobalamin} + \text{UBBC}. \]

Results

The results are detailed in Table 1. Females had a significantly higher mean serum cobalamin level than males. The mean value for females (819.2 pg/ml) was 172 pg/ml higher than that for males (640.0 pg/ml), and the difference is highly significant (p < 0.001).

The mean UBBC value in females (1125.2 pg/ml) was significantly higher than in males (1021.8 pg/ml; p < 0.05). As would be expected from the results for serum cobalamin and UBBC, females had significantly higher mean TBBC values than males.

TC I levels were not significantly different in male and female subjects. However, the mean TC II value of 856.9 pg/ml for females was significantly higher than the for males (713.1 pg/ml; p < 0.001). Males had significantly higher mean TC III levels than females (males 218.6 pg/ml, females 179.9 pg/ml; p < 0.001).

Discussion

Higher levels for serum cobalamin in females compared with males was reported by Low-Beer et al (4), Metz et al (5), Fleming (6) and Jacob et al (3), but in the latter two studies the differences were not statistically significant. Hom and Ahluwalia (7), Rosner and Schreiber (8) and Scott et al (9) were unable to detect any sex differences in serum cobalamin levels. The published reports of TC levels are also conflicting. Low-Beer et al (4), Scott et al (9) and Jacob et al (3) found higher values for UBBC in females, but Kumar et al (10) could find no such difference. However, the latter workers added only 500 pg radio-labeled cobalamin per ml serum, a dose which is insufficient to saturate the UBBC of most normal subjects, and thus they could have missed the possible sex difference. When some of the individual TC’s were assayed individually Sonneborn et al (11) found no sex differences in TC III levels and Jacob et al (3) reported that TC II levels were higher in females.

The present study of 159 healthy subjects is the largest in which a possible sex difference has been examined. Factors which could possibly influence serum cobalamin or TC levels or both, such as iron nutrition, folate deficiency, use of oral contraceptive agents, other medication and recent pregnancy, were specifically excluded. It is possible that failure to consider these factors, all of which are either more common or exclusive to females, may account for the failure of some of the previous studies to detect sex differences in serum cobalamin and TC’s. Scott et al (9) did exclude the possibility of folate deficiency and intake of medication, but the study included 22 subjects only.

We have shown a clear sex difference in serum cobalamin and TC levels, females having significantly higher levels of cobalamin, UBBC, TBBC and unsaturated TC II, while males had higher levels of unsaturated

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<tr>
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<th>Male</th>
<th>Female</th>
<th>t</th>
<th>p</th>
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<tbody>
<tr>
<td>Cobalamin</td>
<td>647.0±180.5</td>
<td>819.3±117.8</td>
<td>5.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UBBC</td>
<td>1021.8±288.7</td>
<td>1125.2±318.4</td>
<td>2.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TBBC</td>
<td>1669.3±392.2</td>
<td>1944.7±459.9</td>
<td>4.07</td>
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</tr>
<tr>
<td>Transcobalamin I</td>
<td>90.4±28.4</td>
<td>88.7±29.1</td>
<td>0.37</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Transcobalamin II</td>
<td>713.1±288.2</td>
<td>856.9±281.1</td>
<td>3.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transcobalamin III</td>
<td>218.6±77.4</td>
<td>179.9±64.1</td>
<td>3.42</td>
<td>&lt;0.001</td>
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</table>
TC III. The mechanisms underlying these differences are not immediately apparent. Serum cobalamin levels may be affected by the animal protein content of the diet, and by the efficacy of absorption of cobalamin from the diet. All subjects in this study were consuming an average Western diet; although not specifically measured, we have no evidence that the dietary intake of animal protein was greater in the female subjects. Similarly, there is no evidence that females absorb more cobalamin from the diet than males. It is unlikely that the higher serum cobalamin levels in females reflect larger body stores of the vitamin, for females have a greater propensity to develop cobalamin-deficient megaloblastic anemia than males (12).

The sex difference in serum cobalamin levels is most likely related to the difference in serum TC concentrations, which in turn is probably associated with hormonal factors. However, the exact mechanism remains unexplained, since pharmaceutical doses of female hormones raise the UBBC (13) but lower the serum cobalamin levels.

The relationship between serum cobalamin levels and the TC’s is a complex one. This was not examined in the present study in which only unsaturated binding protein and not total binder was measured.

References