Biological Variation of Holo-Transcobalamin in Elderly Individuals, Andrew McCaddon,1 Peter Hudson,2 Cherie McCracken,3 Richard Ellis,4 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; * address correspondence to this author at: Gardden Road Surgery, Rhoslanerchrugog, 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 methylnalonyl-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 “holo-TC”. 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 holo-TC in clinical samples (1–3). Although information exists for “between-person” variations in holo-TC concentrations, (2, 4), very few data exist regarding its “within-person” variability (5). Such knowledge will be essential for studies of diseases potentially associated with low concentrations of holo-TC, such as Alzheimer disease (6). We therefore examined the between- and within-person variability and within-assay variability of holo-TC 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 (7). Because valid estimates of the components of variation can be obtained from a relatively small number of participants (7), 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, t10 = 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. The same individual (a trained nurse) collected venous blood samples from fasted and seated individuals at the same time of day (between 0800 and 0930). On the last two sampling occasions, blood was also collected 3–4 h postprandially. All sample collection tubes were from a single batch.

Sample handling and storage at ~30°C were performed according to a preset fixed protocol; samples were processed and stored within 1 h of venipuncture. To minimize analytical variation, we assayed all samples from each individual in a single batch. A single analyst (R.E.) assayed all of the samples with the same instrument, reagents, calibrators, and quality-control materials. Each sample was analyzed twice. Because the manufacturer’s guidelines (Axis-Shield) already recommend assaying each sample in duplicate, to achieve this, we assayed two pairs of replicates. Each assay had one duplicate of two concentrations of manufacturer’s quality-control material at the beginning and end of the assay.

The Axis-Shield assay is a competitive protein-binding assay. Samples are pretreated with magnetic particles coated with monoclonal antibodies to human TC. When a magnetic separation rack is used, holo-TC is retained after the haptocorrin-containing supernatant is discarded. The magnetic particles are treated with a reducing and denaturing reagent to release free B12, which is then measured by means of a Co-57-labeled tracer and porcine intrinsic factor binder. Using calibrators prepared from recombinant human holo-TC, one can measure the concentration of holo-TC in each sample.

To analyze a complete set of samples from two participants in the same assay, the number of tubes used was increased from the maximum reagent-set size of 100 to 118. Reagents from two reagent sets of the same lot number were pooled to accommodate this increased assay size. On the day of each assay, samples were thawed at room temperature and thoroughly mixed. The positions of the duplicates from each participant were randomized to minimize any potential inherent positional or time-related difference within the assay.

One person defaulted after donating six samples. Six sets of replicates were incomplete because of limited volumes of serum and were rejected. Five sets of replicates containing a single noticeably discrepant concentration (115, 389, 237, 1, and 3 pmol/L, respectively) were also rejected.

A generalized estimating equation model (module GEE of the statistical programming language “R”) (8) was used to study the age dependence of holo-TC (9). Holo-TC decreased with age: holo-TC = 109.6 pmol/L – 0.82 × age [95% confidence interval (CI) of regression parameter, −1.55 to −0.07; P = 0.02].

Means and ranges of participants’ fasting and nonfasting holo-TC values are shown in Fig. 1. Data from participant 4 was rejected according to Reed’s criterion: the difference between the extreme value and the next highest (or lowest) value exceeded one-third of the range of all values (7). In the remaining individuals, females had higher holo-TC concentrations than males (Table 1; 95% CI for difference between means, 9.7–16.9 pmol/L; Student t-test, t243 = 6.6; P <0.0001). Holo-TC concentrations were, on average, just over 2 pmol/L higher in fasting samples (collected between 0800 and 0930) compared with nonfasting samples (collected between 1100 and 1230; Table 1 and two-way ANOVA, with “partici-
imprecision were higher than those reported by Ulleland et al. (2) and Loikas et al. (4), perhaps because of the increase in assay size to accommodate complete sample sets from two participants within the same assay. Additional information on the performance of the assay may be derived from a comparison of CV₀ and CVₐ. It has been suggested that the “desirable” specification for an assay should be that the analytical variation is less than one-half of the within-participant biological variation (CV₂CV₀) (10). This compares with the definition of “optimum” performance as CV₂<0.25CV₀ and “minimum” performance as CV₂<0.75CV₀. In our experience, the performance of the assay approximated desirable specification with CV₂=0.52CV₀.

Our finding of an intraparticipant variation for holo-TC of 16% in fasting participants is comparable to a value of 16% calculated over a 3-month period in a substudy of the Western Norway B-vitamin intervention trial (5). Similarly, our finding of higher holo-TC in females compared with males has been reported in other studies (4, 11). The finding of an age-related decrease in holo-TC has been noted in some, but not all, studies (4, 12).

It was perhaps surprising that holo-TC concentrations were lower in postprandial samples. In a study of diurnal variation of holo-TC, Bjorkesten et al. (13) observed increasing holo-TC concentrations between 0600 and 1200 followed by a subsequent decrease. Our results might reflect the time of day that samples were collected rather than a fasting/nonfasting effect per se.

Holo-TC reference intervals were recently determined from a sample of 303 healthy adults and elderly (4). Holo-TC, however, has a relatively low II of 0.51. When the II is low, particularly when it is <0.6, the dispersion of values for any individual will span only a small part of any reference interval. Conversely, an II of 1.4 is considered the critical value at which the distribution of values from a single individual covers much of the entire distribution (14). One device for increasing the II is by stratification of reference intervals, perhaps by gender. Stratification of our data by gender yielded an increase of II to 1.07 in females and a decrease to 0.38 in males (Table 1). Holo-TC reference intervals may, therefore, be of limited use, as is true for many other biological measures (10).

Their diagnostic utility in conjunction with other markers of B₁₂ status remains to be explored (1). A change in an individual’s holo-TC concentration is probably more significant than the determination of absolute concentrations. Indeed, Nexo et al. (5) have recently shown that

### Table 1. Components of variation of holo-TC values in fasting and nonfasting males (M) and females (F).

<table>
<thead>
<tr>
<th></th>
<th>CV₀ %</th>
<th>CVᵣ %</th>
<th>CVₐ %</th>
<th>II</th>
<th>RCV (P = 0.05)</th>
<th>RC</th>
<th>n</th>
<th>Mean TC (95% CI), pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>8.2</td>
<td>16</td>
<td>35</td>
<td>0.51</td>
<td>48.8</td>
<td>0.79</td>
<td>11</td>
<td>38.4 (36.4–40.5)</td>
</tr>
<tr>
<td>Nonfasting</td>
<td>7.3</td>
<td>16</td>
<td>46</td>
<td>0.38</td>
<td>48.5</td>
<td>0.87</td>
<td>10</td>
<td>36.3 (32.2–40.3)</td>
</tr>
<tr>
<td>Fasting (M)</td>
<td>7.3</td>
<td>16</td>
<td>46</td>
<td>0.38</td>
<td>48.5</td>
<td>0.87</td>
<td>6</td>
<td>32.1 (29.2–35.0)</td>
</tr>
<tr>
<td>Fasting (F)</td>
<td>8.4</td>
<td>15</td>
<td>16</td>
<td>1.07</td>
<td>47.8</td>
<td>0.47</td>
<td>5</td>
<td>45.4 (43.3–47.6)</td>
</tr>
</tbody>
</table>

* RCV, RCV at P = 0.05; n, number of participants included in the analysis; Mean TC, overall mean holo-TC with 95% CI.

* Components of variation were not calculated for fasting samples because there were only two replicate samples per participant.
TC-related markers are early and responsive indicators of changes in vitamin B₁₂ status. Some indication of the size of what constitutes a significant change in serial holo-TC values is provided by the calculation of RCVs. The present study indicates that holo-TC values would need to change by nearly 50% for an investigator to be 95% certain that one value was significantly different from a previous value.

Regression dilution describes the attenuation in a regression coefficient when a single measured value of a covariate is used instead of the usual or mean value over a period of time. The simple method of adjusting regression coefficients for this dilution arises out of measurement error theory and is easily implemented (15). In the case of a single covariate (simple linear regression), correction for regression dilution is achieved by multiplying the regression coefficient by a correction factor. The correction factor is simply the inverse of the RC. For holo-TC, it is 1.26, comparable to a value of 1.14 obtained under the conditions used, this particular holo-TC assay reagent set provided the holo-TC assay reagent sets.

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References

Age- and Sex-related Reference Values for Serum Adhesion Molecule Concentrations in Healthy Individuals: Intercellular Adhesion Molecule-1 and E- Selectin, Anne Ponthieux, Bernard Herbeth, Suzanne Droesch, Daniel Lambert, and Sophie Visvikis [Institut National de la Santé et de la Recherche Médicale (INSERM) U 525, 30 rue Lionnois, 54000 Nancy, France, and Centre de Médecine Préventive, 2 rue du Doyen Jacques Pariset, 54500 Vandoeuvre-lès-Nancy, France; *address correspondence to this author at: INSERM Unité 525, 30 rue Lionnois, 54000 Nancy, France; fax 33-03-83-32-13-22, e-mail Sophie.visvikis@cmp.u-nancy.fr]

Intercellular adhesion molecule-1 (ICAM-1) and E-Selectin, and L-Selectin are cellular adhesion molecules involved in the recruitment of leukocytes on the activated vessel wall during inflammation (1) and play an important role in the early stages of atherosclerosis and its complications (2). Thus, the measurement of soluble adhesion molecules in serum may have diagnostic relevance in many inflammatory diseases (3). A profile of soluble adhesion molecule concentrations may allow better therapeutic decisions in inflammatory and autoimmune disorders, infection, cancer, and cardiovascular pathologies and may also aid in the prediction of cardiovascular events (4, 5). However, the use of these markers in clinical practice depends critically on knowledge of their reference values.

The purpose of the present study was to establish age-and sex-specific reference intervals for serum concentrations of soluble ICAM-1 and E-, P-, and L-Selectin in healthy children (4–17 years) and adults (18–55 years).

Blood samples were taken from healthy individuals (157 boys and 146 girls 4–17 years of age and 245 men and 250 women 18–55 years of age) who were members of the Stanislas cohort (6). Participants were of French origin (Vosges and Meurthe et Moselle); free from serious and/or chronic illnesses, especially cardiovascular, hepatic, or renal diseases; and were not on treatment with lipid-lowering drugs. Volunteers with aspartate aminotransferase, alanine aminotransferase, or γ-glutamyltransferase activities >200 U/L, apolipoprotein E concentrations >200 mg/L, orosomucoid or haptoglobin...