Circadian Variation in Serum CrossLaps Concentration Is Reduced in Fasting Individuals

To the Editor:

Accurate assessment of bone resorption may be of significant value for selecting women for antiresorptive therapy, monitoring of therapeutic response in treated individuals, and predicting fracture risk (1). This goal may be facilitated by measurement of biochemical markers of bone metabolism. The Serum CrossLaps™ One Step ELISA measures fragments derived from the C-terminal telopeptide of collagen type I. This assay has been shown to provide a sensitive and specific index of bone resorption (2, 3).

An important issue for the use of biochemical markers is biological variability. This has been reported to be relatively high for specific markers of bone resorption. A recent letter by Wichers et al. (4) reports on the circadian variation in serum CrossLaps concentration in young males. Similar to what has been found for other specific markers of bone resorption, they report a pronounced circadian variation in serum CrossLaps concentration, with a nocturnal peak and a daytime nadir (5, 6). The amplitude of the variation was reported to be 60–66% of the mean values observed over the 24-h observation period. The six study subjects were allowed to eat and drink throughout the experiment.

In this letter, I show that fasting can have a pronounced effect on the circadian variation of markers of bone resorption. Fig. 1 shows the circadian variation in serum CrossLaps concentration in 11 premenopausal women who were subjected to a randomized crossover study. In one part of the study, the women fasted overnight (2200–0800) before initiation of the study, but were then allowed to eat and drink without restriction during the 24-h study period. In the other part of the study, they fasted from 10 h before the study and then 24 h throughout the study. Serum samples were obtained at 3-h intervals throughout the study and measured in the Serum CrossLaps One Step ELISA. The results are shown as the average percentage of the individual mean (± SE) for the two groups (Fig. 1). In fasting individuals, the average variation was ± 13.6%, whereas the variation under nonfasting conditions was ± 34%. The maximum changes from the 24-h mean were 20.3% and 44.8% for fasting and nonfasting women, respectively. Especially pronounced was the change in serum CrossLaps concentration seen in nonfasting women in the period from 0800 to 1100. Wichers et al. (4) also reported a similar pronounced decrease in this time interval for nonfasting men. However, the change in serum CrossLaps concentration in this time interval in fasting women was significantly lower.

From these results, we can conclude that the circadian variation of serum CrossLaps concentration is significantly lower in fasting than in nonfasting individuals. Thus, measurement of serum CrossLaps in morning fasting samples significantly reduces individual variability of the marker compared with measurement of serum samples from nonfasting individuals. Morning samples from individuals who have been fasting overnight are easily obtained in a clinical setting because this practice is used for several other common laboratory measures.

Wichers et al. (4) are right to conclude that control of sampling time is important in reducing individual variability in specific measures of bone resorption, but an equally or more important issue is to obtain samples from fasting individuals.

References

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Premetrological Variation of Thyrotropin, Thyroxine (Non-Protein Bound), and Triiodothyronine Concentrations in Serum

To the Editor:

The premetrological (preanalytical) phase is a recognized potential source of variation (1) that includes the variation attributable to sample collection, handling, centrifugation, and storage conditions. It is widely accepted that premetrological varia-
tion is negligible if all the processes included are standardized appropriately (2). However, there are few laboratories, if any, that can demonstrate this assumption in real day-to-day laboratory work. The aim of this study was to quantify the premetrological variation of some biochemical measurements related to the thyroid gland.

Blood specimens were obtained from 20 volunteers from our laboratory staff. For each individual, two almost simultaneous phlebotomies (one in each arm) were performed by two different phlebotomists withdrawing blood from the antecubital veins into evacuated tubes containing no additives but having a gel barrier as serum separator (Becton Dickinson). Each pair of phlebotomists was different for each individual. The tube of blood obtained by the first phlebotomist was sent 30 min after the first phlebotomy; the second phlebotomist was sent 30 min after the withdrawal; the tube of blood obtained by the second phlebotomist was sent 30 min after the first phlebotomy, to mimic day-to-day differences. The tubes were centrifuged at 1500g for 12 min at 10 °C in either of the two centrifuges we have for this purpose. The centrifuged tubes were loaded into the Elecsys 2010 analyzer (Roche Diagnostics) as customary, and the concentrations of thyrotropin (thyroid-stimulating hormone), thyroxine (non-protein bound; also called free T4), and triiodothyronine (also called total T3) in serum were measured. Additionally, all measurements were repeated in one randomly chosen serum sample of each pair.

For each quantity, the analytical variance was calculated as:

\[ s_{C}^2 = \frac{\sum (x - y)^2}{2n} \]

Where \( s_{C}^2 \) is the analytical variance (within-run metrological variance plus premetrological variance); \( x \) and \( y \) are the results obtained for different samples from the same individual; and \( n \) is the number of individuals.

For each quantity, the metrological within-run variance was calculated as:

\[ s_{Mu}^2 = \frac{\sum (z - z')^2}{2n} \]

Where \( s_{Mu}^2 \) is the metrological within-run variance; \( z \) and \( z' \) are the replicate results; and \( n \) is the number of individuals.

Finally, the premetrological variation was obtained by subtracting \( s_{Mu}^2 \) from \( s_{C}^2 \). The CVs corresponding to premetrological variation, calculated using the mean of the 60 measurements done for each quantity, are shown in Table 1.

For serum thyroxine (non-protein bound) and triiodothyronine, the premetrological variation was negligible; however, for serum thyrotropin, the CV was not null. We conclude that the premetrological CV for thyrotropin measurements should be taken into account when estimating the uncertainty of measurement (3) of this analyte.

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References


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Response to “Increased Creatine Kinase MB and Cardiac Troponin T with Normal Cardiac Troponin I in Metastatic Alveolar Rhabdomyosarcoma”

To the Editor:

We wish to comment on the case reported recently by Isotalo et al. (1). Two different cardiac troponin I (cTnI) assays were used: the AxSYM (Abbott) and the Opus Plus (Dade Behring). The title of the letter claimed that the cTnI value was normal. This is not consistent with the data presented.

The AxSYM values for cTnI of 1.2 (day 1), 0.8 (day 2), and 1.1 µg/L (2 weeks) are not normal. The limit of detection for the AxSYM is 0.3 µg/L, with a quoted upper reference limit of 0.4 µg/L and an acute myocardial infarction cutoff of 2.0 µg/L (2). The patient’s values fall within the range for detectable and prognostically significant myocardial damage.

The main conclusion that Isotalo et al. (1) make is that the increased cardiac troponin T (cTnT) and creatine kinase MB (CK-MB) concentrations are the result of release from a non-cardiac source related to abnormal expression of these proteins. They did not perform experiments to detect evidence of abnormal cTnI or CK-MB expression in the tumor bi-

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Table 1. CVs corresponding to premetrological variation at physiological concentrations (CVPM).

<table>
<thead>
<tr>
<th>Serum component</th>
<th>Mean concentration</th>
<th>( s_{G}^2 )</th>
<th>( s_{Mu}^2 )</th>
<th>CVPM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyrotropin</td>
<td>2.00 IU/L</td>
<td>0.077 IU/L</td>
<td>0.055 IU/L</td>
<td>2.7</td>
</tr>
<tr>
<td>Thyroxine (non-protein bound)</td>
<td>18.28 pmol/L</td>
<td>0.557 pmol/L</td>
<td>0.532 pmol/L</td>
<td>0.9</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>1.85 nmol/L</td>
<td>0.072 nmol/L</td>
<td>0.071 nmol/L</td>
<td>0.6</td>
</tr>
</tbody>
</table>

\( s_{G}^2 \), within-run metrological standard deviation plus premetrological standard deviation.

\( s_{Mu}^2 \), within-run metrological standard deviation.