mean peak BNPs p concentration was lower in this group. Again, the differences did not reach statistical significance by ANOVA (hsTnT, \( P = 0.104 \); BNPs p, \( P = 0.290 \); NT-proBNP, \( P = 0.324 \).

There was a statistically significant relationship between the absolute change (\( \delta \)) in the hsTnT concentration and the cumulative dobutamine dose (\( r^2 = 0.26; P = 0.015 \)) in the CAD patients. There was no corresponding correlation with \( \delta \) BNPs p or \( \delta \) hsTnT in the CAD group (\( r^2 = -0.028; P = 0.562 \)), and there was no significant correlation between \( \delta \) NT-proBNP and \( \delta \) BNPs p (\( r^2 = 0.083; P = 0.317 \)).

To our knowledge, this study is the first to demonstrate the patterns of release of TnT with a high-sensitivity assay during DSE testing. Our data suggest stepwise increments in DSE-induced increases in plasma hsTnT and BNPs p in healthy volunteers and CAD patients. CAD patients with inducible ischemia also received the highest dobutamine doses, a finding that must be considered. In contrast with hsTnT and NT-proBNP, the release kinetics for BNPs p indicate that it is a much more dynamic marker. The reasons for the attenuated release of BNPs p in individuals with echo-cardiographically positive test results are unclear. In view of the small sample size, this observation requires verification. If genuine, it is possible that BNPs p release mechanisms are more susceptible than troponin to ischemic preconditioning, or there may be a depletable pool of this peptide.

Given the results of this pilot study, we propose that both exaggerated cardiac troponin release and attenuated BNPs p release in patients with inducible ischemia during DSE warrant further investigation with a larger sample size—and with longer follow-up—to establish whether specific threshold biomarker responses correspond to worse ischemia and/or a worse prognosis.

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References


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Reference Intervals for and Validation of Recalibrated Immunoassays for Trypsinogen-1 and Trypsinogen-2

To the Editor:

Serum trypsinogen assays are used as diagnostic and prognostic tools for cystic fibrosis and acute pancreatitis (AP) (1, 2). Calibrator stability is a challenge in these assays, because trypsinogen readily auto-activates and subsequently autodegrades. Furthermore, the reference
intervals described thus far have been based on samples from a limited number of individuals (1,3). We recalibrated a new immunoassay for trypsinogen-2 and a previously described assay for trypsinogen-1 (1) with stable calibrators (2,4) and report reference intervals for these 2 analytes in serum.

We have produced new monoclonal antibodies and developed a time-resolved immunofluorometric assay for trypsinogen-2, as previously described (1) and report reference intervals for these 2 analytes in serum.

Trypsinogen-2 was stable in serum for at least 7 days at room temperature (CV, 8%–15%), at least 5 weeks at 4 °C (CV, 7%–17%), and at least 6 weeks at −20 °C (CV, 8%–13%). The immunoreactivity did not decrease after 6 freeze–thaw cycles repeated at 1-week intervals (CV, 7%–13%). Recombinant trypsinogen-2 calibrators were stable in assay buffer for 28 days at 4 °C and −20 °C (CVs, 8%–17% and 7%–14%, respectively).

The effect of a breakfast meal on serum trypsinogen-1 and -2 concentrations was studied in 21 volunteers among the laboratory staff (1 man, 20 women). Blood samples were drawn within 1 week before and after a regular Finnish breakfast that consists of some of these: coffee, tea, milk, juice, bread, cheese, ham, porridge, cereals, or yogurt. The breakfast had no effect on the trypsinogen-2 concentration, but trypsinogen-1 concentrations were slightly higher (5.5%; P = 0.0349, paired t-test).

Reference intervals were established with serum samples from 197 healthy volunteers. Samples from men and women were separately divided into age groups comprising 19 to 27 participants. In the age group 18–30 years, the concentrations of trypsinogen-1—but not of trypsinogen-2—were significantly lower in men than in women (P = 0.0015, Mann–Whitney U-test; Table 1). Trypsinogen-2 concentrations were significantly lower in men and women 18–30 years of age than in older (31–50 years) volunteers (P = 0.0354). Despite this finding, we combined sex and age groups for calculating reference intervals. In adults, the central 95% reference interval (5) was higher for trypsinogen-1 (13.0–46.2 μg/L) than for trypsinogen-2 (3.8–17.4 μg/L). The trypsinogen-1 concentrations are in line with those obtained in previous studies (1,3); however, serum trypsinogen-2 concentrations reported for various assays have shown greater variation (1,3). The form of the trypsinogen-2 calibrator used is the most likely source of these differences.

### Table 1. Trypsinogen-1 and -2 concentrations in serum from healthy volunteers.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Trypsinogen-1</th>
<th>Trypsinogen-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median, μg/L</td>
<td>Range, μg/L</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–30 years</td>
<td>27</td>
<td>19.6</td>
</tr>
<tr>
<td>31–50 years</td>
<td>26</td>
<td>22.9</td>
</tr>
<tr>
<td>51–70 years</td>
<td>26</td>
<td>25.0</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>20</td>
<td>26.4</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–30 years</td>
<td>27</td>
<td>25.3</td>
</tr>
<tr>
<td>31–50 years</td>
<td>25</td>
<td>24.9</td>
</tr>
<tr>
<td>51–70 years</td>
<td>25</td>
<td>27.9</td>
</tr>
<tr>
<td>&gt;70 years</td>
<td>21</td>
<td>26.8</td>
</tr>
</tbody>
</table>
We also analyzed trypsinogen-1 and -2 immunoreactivities in serum samples from 40 patients with mild AP and 22 patients with severe AP (2). The median trypsinogen-1 concentration for patients with mild AP was 253 μg/L (95% CI, 105–361 μg/L), whereas that for trypsinogen-2 was 522 μg/L (95% CI, 377–1047 μg/L). For patients with severe AP, the corresponding median concentrations were 364 μg/L (95% CI, 187–523 μg/L) and 1074 μg/L (95% CI, 661–1261 μg/L). The area under the ROC curve for differentiating between AP (n = 62) and healthy volunteers (n = 197) was 0.93 for trypsinogen-1 and 1.00 for trypsinogen-2. The values for the area under the curve for differentiating between mild and severe disease were 0.65 and 0.68, respectively.

In conclusion, we produced stable calibrators and used them to calibrate immunoassays for trypsinogen-1 and trypsinogen-2 and established serum reference intervals for these 2 analytes. The reference intervals for trypsinogen-2 were lower than for our earlier method, which used calibrators prepared from tumor-associated trypsinogen-2 (1). Our limited study of patients with AP confirms that trypsinogen-2 is a diagnostically sensitive and specific marker for the diagnosis of AP. It will be important to determine the commutability of these calibrators in other assays.

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