Reference values for serum Hepcidin-25

Reference ranges for serum hepcidin (nM) per 5-year age group for men and women in a reference population as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS). Reference levels for the WCX-TOF MS method are recalculated from those of our ELISA method, based on the regression line: (ELISA - 1.00)/1.52 = WCX-TOF MS that was derived from the results obtained by both methods for the same samples without hepcidin isoforms. All values are determined using secondary reference material for hepcidin assays, which value is assigned by a primary reference material, allowing traceability to the internationally recognized Système International. Results for heparine plasma, EDTA plasma, citrate plasma and serum do not differ from each other.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>N (%)</th>
<th>Median</th>
<th>95% reference range</th>
<th>N (%)</th>
<th>Median</th>
<th>95% reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>10 (1)</td>
<td>5.6</td>
<td>0.8</td>
<td>21 (2)</td>
<td>1.2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>25-29</td>
<td>16 (2)</td>
<td>5.2</td>
<td>&lt;0.5</td>
<td>28 (3)</td>
<td>1.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>30-34</td>
<td>18 (2)</td>
<td>4.4</td>
<td>&lt;0.5</td>
<td>24 (3)</td>
<td>2.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>35-39</td>
<td>22 (2)</td>
<td>3.8</td>
<td>&lt;0.5</td>
<td>36 (4)</td>
<td>1.6</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>40-44</td>
<td>19 (2)</td>
<td>6.4</td>
<td>&lt;0.5</td>
<td>65 (7)</td>
<td>2.6</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>45-49</td>
<td>76 (7)</td>
<td>3.6</td>
<td>&lt;0.5</td>
<td>110 (12)</td>
<td>1.7</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>50-54</td>
<td>106 (10)</td>
<td>4.2</td>
<td>&lt;0.5</td>
<td>140 (16)</td>
<td>3.1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>55-59</td>
<td>173 (16)</td>
<td>4.6</td>
<td>&lt;0.5</td>
<td>129 (15)</td>
<td>5.2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>60-64</td>
<td>179 (17)</td>
<td>4.7</td>
<td>&lt;0.5</td>
<td>137 (16)</td>
<td>5.0</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>65-69</td>
<td>186 (17)</td>
<td>5.6</td>
<td>&lt;0.5</td>
<td>95 (11)</td>
<td>5.2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>70-74</td>
<td>133 (12)</td>
<td>5.2</td>
<td>&lt;0.5</td>
<td>62 (7)</td>
<td>5.4</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>75-79</td>
<td>99 (9)</td>
<td>4.0</td>
<td>&lt;0.5</td>
<td>16 (2)</td>
<td>5.7</td>
<td>0.8</td>
</tr>
<tr>
<td>80-84</td>
<td>22 (2)</td>
<td>4.0</td>
<td>1.7</td>
<td>10 (1)</td>
<td>7.6</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>≥85</td>
<td>7 (1)</td>
<td>7.2</td>
<td>1.7</td>
<td>9 (1)</td>
<td>3.9</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

All 1066 (100) 4.7 <0.5 15.5 882 (100) 3.8 <0.5 15.4

*Pre-menopausal women (age <55; n=424)
Median = 2.1 nM
P2.5 = 0.1 nM (<0.5 nM)
P97.5 = 13.0 nM

*Post-menopausal women (age >=55; n=458)
Median = 5.2 nM
P2.5 = 0.2 nM (<0.5 nM)
P97.5 = 16.5 nM

Reference values for [Hepcidin-25/Ferritin] and
[TSAT/Hepcidin-25] ratios

Hepcidin values as given on page 1 should, like other hormones, be interpreted in the context of other indices of iron metabolism. For instance, in the absence of inflammation a low Hepcidin-25/ferritin ratio may be consistent with the presence of hereditary hemochromatosis or an iron loading anemia. On the other hand, a low transferrin saturation(TSAT)/Hepcidin-25 ratio may be consistent with Iron Refractory Iron Deficiency Anemia (IRIDA) due to a defect in the TMPRSS6 gene.

WCX-TOF MS – [Hepcidin-25/ferritin] ratios

Men (n=1064)
- Median = 28.2 pmol/µg
- P2.5 = 3.1 pmol/µg
- P97.5 = 92.7 pmol/µg

Pre-menopausal women (age <55; n=424)
- Median = 37.6 pmol/µg
- P2.5 = 3.2 pmol/µg
- P97.5 = 176.4 pmol/µg

Post-menopausal women (age >=55; n=458)
- Median = 42.7 pmol/µg
- P2.5 = 9.6 pmol/µg
- P97.5 = 150.9 pmol/µg

WCX-TOF MS – [TSAT/Hepcidin-25] ratios

Men (n=1059)
- Median = 6.9 %/nM
- P2.5 = 1.6 %/nM
- P97.5 = 243.0 %/nM

Pre-menopausal women (age <55; n=422)
- Median = 13.2 %/nM
- P2.5 = 1.9 %/nM
- P97.5 = 312.9 %/nM

Post-menopausal women (age >=55; n=457)
- Median = 5.4 %/nM
- P2.5 = 1.4 %/nM
- P97.5 = 69.6 %/nM