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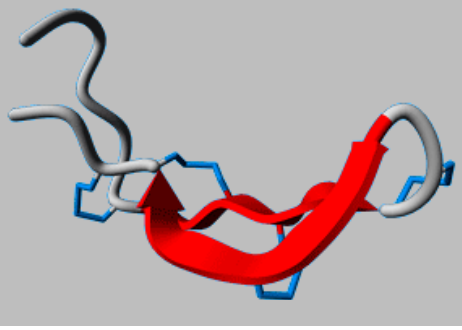


# WCX-TOF MS reference values for serum Hepcidin-25 in children aged 6 months to 3 years

Reference ranges for serum hepcidin (nM) and hepcidin/ferritin ratio (pmol/µg) in children (6 months to 3 years old) in the normative population (N=292)[1] as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS)[2,3,4].

**Reference ranges for hepcidin**

- are similar for boys and girls and for the 6 months age categories.
- are different for samples collected before and after 12.00 pm.

**Reference ranges for hepcidin/ferritin ratio**

- are similar for the 6 months age categories and time of blood sampling
- are different for boys and girls
- are 6-8 fold elevated compared to those obtained for adults.

## Hepcidin

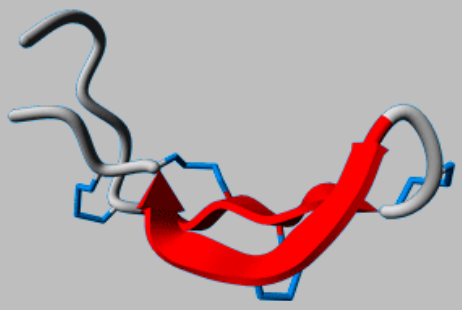
| **Hepcidin (nM)** | | **95% CI** | |
|---|---|---|---|
| N | Median | P2.5 | P97.5 |
| 292 | 3.6 | 0.94 | 12.2 |

| **Hepcidin (nM)** | | | | **95% CI** | |
|---|---|---|---|---|---|
| Age (months) | N | (%) | Median | P2.5 | P97.5 |
| 3-12 | 111 | (38) | 4.1 | 1.1 | 12.8 |
| 13-18 | 62 | (21) | 3.8 | 0.8 | 12.0 |
| 19-24 | 43 | (15) | 2.5 | 0.9 | 11.8 |
| 25-30 | 38 | (13) | 3.4 | 0.8 | 21.7 |
| 31-36 | 38 | (13) | 3.9 | 0.3 | 14.6 |

Not significantly different between age categories at the 0.05 level: p=0.183, according to Kruskal-Wallis test.

| **Hepcidin (nM)** | | | **95% CI** | |
|---|---|---|---|---|
| **Gender** | N | Median | P2.5 | P97.5 |
| Male | 207 | 3.6 | 0.9 | 11.9 |
| Female | 85 | 4.0 | 0.8 | 14.0 |

Not significantly different between boys and girls at the 0.05 level: p=0.225, according to Mann-Whitney U test.



| Hepcidin (nM) | | | 95% CI | |
|---|---|---|---|---|
| **Time of sample collection** | N | Median | P2.5 | P97.5 |
| <12.00 pm | 170 | 3.0* | 0.9 | 11.4 |
| 12.00-17.00 pm | 122 | 4.5* | 1.1 | 14.0 |

*Significantly different between categories of sample collection time at the 0.05 level: p=0.000, according to Mann-Whitney U test.

## Hepcidin/ferritin ratio

| Hepcidin/ferritin ratio (pmol/µg) | | 95% CI | |
|---|---|---|---|
| N | Median | P2.5 | P97.5 |
| 292 | 169.3 | 44.4 | 492.5 |

| Hepcidin/ferritin (pmol/µg) | | | | 95% CI | |
|---|---|---|---|---|---|
| **Age (months)** | N | (%) | Median | P2.5 | P97.5 |
| 3-12 | 111 | (38) | 175.1 | 50.8 | 521.1 |
| 13-18 | 62 | (21) | 146.0 | 54.5 | 482.2 |
| 19-24 | 43 | (15) | 155.5 | 32.9 | 427.1 |
| 25-30 | 38 | (13) | 178.2 | 28.5 | 654.7 |
| 31-36 | 38 | (13) | 172.0 | 15.3 | 459.1 |

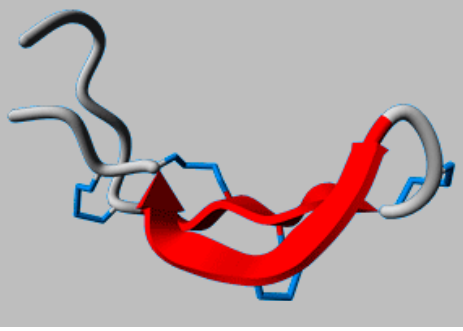
Not significantly different between age categories at the 0.05 level: p=0.798, according to Kruskal-Wallis test.

| Hepcidin/ferritin (pmol/µg) | | | 95% CI | |
|---|---|---|---|---|
| **Gender** | N | Median | P2.5 | P97.5 |
| Male | 207 | 148.4* | 39.1 | 489.2 |
| Female | 85 | 209.8* | 71.4 | 568.0 |

*Significantly different between boys and girls at the 0.05 level: p=0.000, according to Mann-Whitney U test.

| Hepcidin/ferritin (pmol/µg) | | | 95% CI | |
|---|---|---|---|---|
| **Time of sample collection** | N | Median | P2.5 | P97.5 |
| <12.00 pm | 170 | 163.2 | 42.3 | 493.3 |
| 12.00-17.00 pm | 122 | 175.4 | 45.1 | 523.8 |

Not significantly different between categories of sample collection time at the 0.05 level: p=0.665 according to Mann-Whitney U test.



## References

[1] Uijterschout L, Swinkels DW, Domellöf M, Lagerqvist C, Hudig C, Tjalsma H, Vos R, van Goudoever JB, Brus F. Serum hepcidin measured by immunochemical and mass-spectrometric methods and their correlation with iron status indicators in healthy children aged 0.5-3 y. *Pediatric Research* 2014; **76:** 409-414

[2] Swinkels DW, Girelli D, Laarakkers C, Kroot J, Campostrini N, Kemna EH, Tjalsma H. Advances in quantitative hepcidin measurements by time-of-flight mass spectrometry. *PLoS ONE* 2008; **3**: e2706.

[3] Kroot JJ, Laarakkers CM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, van Ede AE, Peters HP, van Dongen-Lases E, Wetzels JF, Sweep FC, Tjalsma H, Swinkels DW. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. *Clin Chem* 2010; **56**: 1570-1579.

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