

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) as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS)^{1,2,3}. 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 International⁴. Results for heparine plasma, EDTA plasma, citrate plasma and serum do not differ from each other.

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

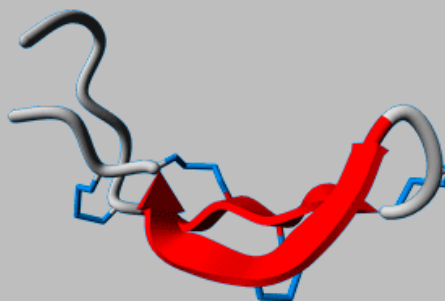
Hepcidin (nM)		95% CI	
N	Median	P2.5	P97.5
292	3.8	0.99	12.9

Hepcidin (nM)				95% CI	
Age (months)	N	(%)	Median	P2.5	P97.5
3-12	111	(38)	4.3	1.2	13.5
13-18	62	(21)	4.0	0.8	12.7
19-24	43	(15)	2.6	0.9	12.4
25-30	38	(13)	3.6	0.8	22.9
31-36	38	(13)	4.1	0.3	15.4

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	3.8	0.9	12.5
Female	85	4.2	0.8	14.8

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	3.2*	0.9	12.0
12.00-17.00 pm	122	4.7*	1.2	14.8

*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

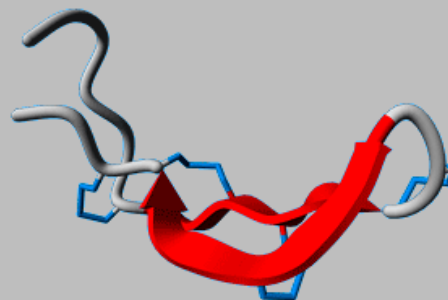
Hepcidin/ferritin ratio (pmol/ μ g)		95% CI	
N	Median	P2.5	P97.5
292	178.5	46.8	519.4

Hepcidin/ferritin (pmol/ μ g)				95% CI	
Age (months)	N	(%)	Median	P2.5	P97.5
3-12	111	(38)	184.6	53.6	549.5
13-18	62	(21)	154.0	57.5	508.5
19-24	43	(15)	164.0	34.9	450.4
25-30	38	(13)	187.9	30.1	690.4
31-36	38	(13)	181.4	16.1	484.1

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

Hepcidin/ferritin (pmol/ μ g)			95% CI	
Gender	N	Median	P2.5	P97.5
Male	207	156.5*	41.2	515.9
Female	85	221.2*	74.9	599.0

*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	172.1	44.8	520.2
12.00-17.00 pm	122	185.0	47.6	552.4

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 Laarakkers CM, Wiegerinck ET, Klaver S, Kolodziejczyk M, Gille H, Hohlbaum AM, Tjalsma H, Swinkels DW. Improved mass spectrometry assay for plasma hepcidin: detection and characterization of a novel hepcidin isoform. *PLoS ONE* 2013; **10**: e75518.
- 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.
- ⁴ Diepeveen LE *et al.* Provisional standardization of hepcidin assays: creating a traceability chain with a primary reference material, candidate reference method and a commutable secondary reference material. *Clin Chem Lab Med* 2018, Nov **29**.