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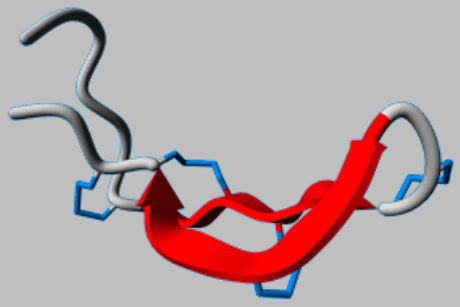

Reference values for serum Hepcidin-25

Reference ranges for serum hepcidin (nM) per 5-year age group for men and women in a reference population[1] as measured by weak cation exchange time-of-flight mass spectrometry (WCX-TOF MS)[2,3,4].

Reference levels for the WCX-TOF MS method are recalculated from those of our ELISA method[1], 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[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 International[4]. Results for heparine plasma, EDTA plasma, citrate plasma and serum do not differ from each other.

	Men (n=1066)					**Women** (n=882)*,#				
				95% reference range					95% reference range	
Age, years	N	(%)	Median	P2.5	P97.5	N	(%)	Median	P2.5	P97.5
18-24	10	(1)	5.6	0.8	11.6	21	(2)	1.2	< 0.5	6.6
25-29	16	(2)	5.2	< 0.5	16.1	28	(3)	1.5	< 0.5	7.0
30-34	18	(2)	4.4	< 0.5	16.7	24	(3)	2.0	< 0.5	13.8
35-39	22	(2)	3.8	< 0.5	12.8	36	(4)	1.6	< 0.5	10.4
40-44	19	(2)	6.4	< 0.5	11.6	65	(7)	2.6	< 0.5	16.0
45-49	76	(7)	3.6	< 0.5	13.9	110	(12)	1.7	< 0.5	9.4
50-54	106	(10)	4.2	< 0.5	14.6	140	(16)	3.1	< 0.5	15.1
55-59	173	(16)	4.6	< 0.5	16.6	129	(15)	5.2	< 0.5	14.3
60-64	179	(17)	4.7	< 0.5	15.1	137	(16)	5.0	< 0.5	18.2
65-69	186	(17)	5.6	< 0.5	14.7	95	(11)	5.2	< 0.5	15.0
70-74	133	(12)	5.2	< 0.5	17.9	62	(7)	5.4	< 0.5	25.5
75-79	99	(9)	4.0	< 0.5	17.0	16	(2)	5.7	0.8	19.4
80-84	22	(2)	4.0	1.7	13.3	10	(1)	7.6	< 0.5	12.7
≥85	7	(1)	7.2	1.7	13.5	9	(1)	3.9	< 0.5	16.2
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

[1]Galesloot TE, Vermeulen SH, Geurts-Moespot AJ, Klaver SM, Kroot JJ, van Tienoven D, Wetzels JF, Kiemeney LA, Sweep FC, den Heijer M, Swinkels DW. Serum hepcidin: reference ranges and biochemical correlates in the general population. *Blood* 2011; **117**: e218-25.

[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, *et al*. 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.**

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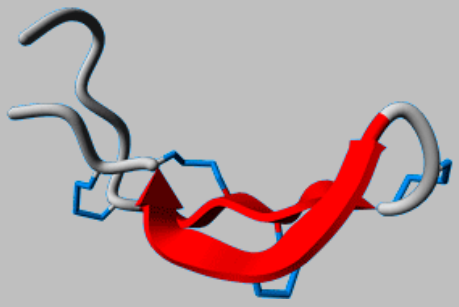

Reference values for [Hepcidin-25/Ferritin] and [TSAT/Hepcidin-25] ratios

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

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