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#1 Biochemical analyses

The CGPL laboratory was DANAK accredited by the International Organization for Standardization (ISO) standards ISO 17025 followed by ISO 15189

Ferritin was assessed in serum by two commercially available assays, Cobas-Core II (Roche Diagnostics A/S, Hvidovre, Denmark) and ADVIA Centaur/CentaurXP (Bayer/Siemens Healthcare Diagnostics, Tarrytown, NY) according to the instruction of the manufacturers. For the Cobas-Core assay the interserial coefficient of variation percentage (CV%) was 2.4 % (at level 23 µg/L) and 5.5 % (at level 645 µg/L). For the Centaur/CentaurXP assay the CV% was 5.6 % (at level 72 µg/L), 5.8 % (at level 182 µg/L) and 5.8 % (at level 455 µg/L). The Centaur/CentaurXP assay was used after December 1, 2002 and until the end of study. Both assays were subjected to external quality control through participation in the LabQuality External Quality Assessment Scheme (LabQuality, Helsinki, Finland). The results confirmed the reliability of the assays. The assessment scheme included 12 distributions annually (each distribution comprised 1 sample) or 6 to 8 distributions annually (each distribution comprised 2 sample). The results from LabQuality (2002 to 2015) confirmed the reliability of the assays, and the results (n=270) deviated less than the target limits (± 15 %) set by LabQuality in 93 % of the cases. The mean deviation from the target set by LabQuality was 0.6 % (n=270).

Iron was assessed in serum by three commercially available assays, one on Olympus A600 (Olympus A/S, Denmark) and two assays (IRON and IRON II) on ADVIA Chemistry System 1650/2400 (Bayer®/Siemens®, Denmark) according to the instructions of the manufacturers. For the Olympus A600 assay the interserial coefficient of variation percentage (CV%) was 2.0 % (at level 20 µmol/L) and 1.5 % (at level 46 µmol/L). For the ADVIA Chemistry System IRON assay

the CV% was 3.2 % (at level 28 µmol/L) and 2.4 % (at level 40 µmol/L). For the IRON II assay the CV% was 3.8 % (at level 18 µmol/L) and 1.2 % (at level 43 µmol/L). The Olympus A600 assay was used until December 1, 2002. The ADVIA Chemistry System assays were use from December 2, 2002 to October 17, 2006 (IRON) and from October 18, 2006 until the end of study (IRON II). All results were made traceable to the NFKK Reference Serum X (Rustad et al. Scand J Clin Lab Invest 2004;64:271-284). Results obtained by the Olympus assay were adjusted by subtracting 1.3 µmol/L. The 1.3 µmol/L was determined by parallel analysis of 32 human serum samples during a period of 5 days in March 2002. The adjusted Olympus assay results and the IRON assay results were multiplied 1.1 to make the results traceable to NFKK Reference Serum X. The factor 1.1 was determined by parallel analysis of the Reference Serum and the Iron assay calibrator material. The relation between the corrected IRON and IRON II assays was: IRON II = 1.0003*IRON +1.6. The equation was determined by parallel analysis of 48 human serum samples during a period of 3 days in April and May 2006. The parallel analysis confirmed the standardization of the two assays. All three assays were subjected to external quality control through participation in the LabQuality External Quality Assessment Scheme (LabQuality, Helsinki, Finland). The results confirmed the reliability of the assays. The assessment scheme included 12 distributions annually (each distribution comprised 1 sample) or 4 distributions annually (each distribution comprised 2 sample). The results from LabQuality (2002 to 2015) confirmed the reliability of the assays, and the results (n=157) deviated less than the target limits (± 12 %) set by LabQuality in 92 % of the cases. The mean deviation from the target set by LabQuality was -5.9 % (n=147).

Transferrin was assessed in serum by two commercially available immune turbidimetric assays, Hitachi® 911 (Roche Diagnostics, Denmark) and ADVIA Chemistry System 1650/2400 (Bayer®/Siemens®, Denmark) according to the instructions of the manufacturers. For the Hitachi

assay the interserial coefficient of variation percentage (CV%) was 4.5 % (at level 22.4 µmol/L) and 3.5 % (at level 42.2 µmol/L). For the ADVIA Chemistry assay the CV% was 5.9 % (at level 27.2 µmol/L), and 4.2 % (at level 33.4 µmol/L). The ADVIA Chemistry assay was used after December 1, 2002 and until the end of the study. Both assays were subjected to external quality control through participation in the LabQuality External Quality Assessment Scheme (LabQuality, Helsinki, Finland). The results confirmed the reliability of the assays. The assessment scheme included 12 distributions annually (each distribution comprised 1 sample) or 4 distributions annually (each distribution comprised 2 sample). The results from LabQuality (2002 to 2015) confirmed the reliability of the assays, and the results (n=144) deviated less than the target limits (± 8 %) set by LabQuality in 89 % of the cases. The mean deviation from the target set by LabQuality was -1.8 % (n=135).

The transferrin saturation percentage was calculated from the results of iron and transferrin according to the formula: Transferrin saturation = Iron/(2*Transferrin)*100.

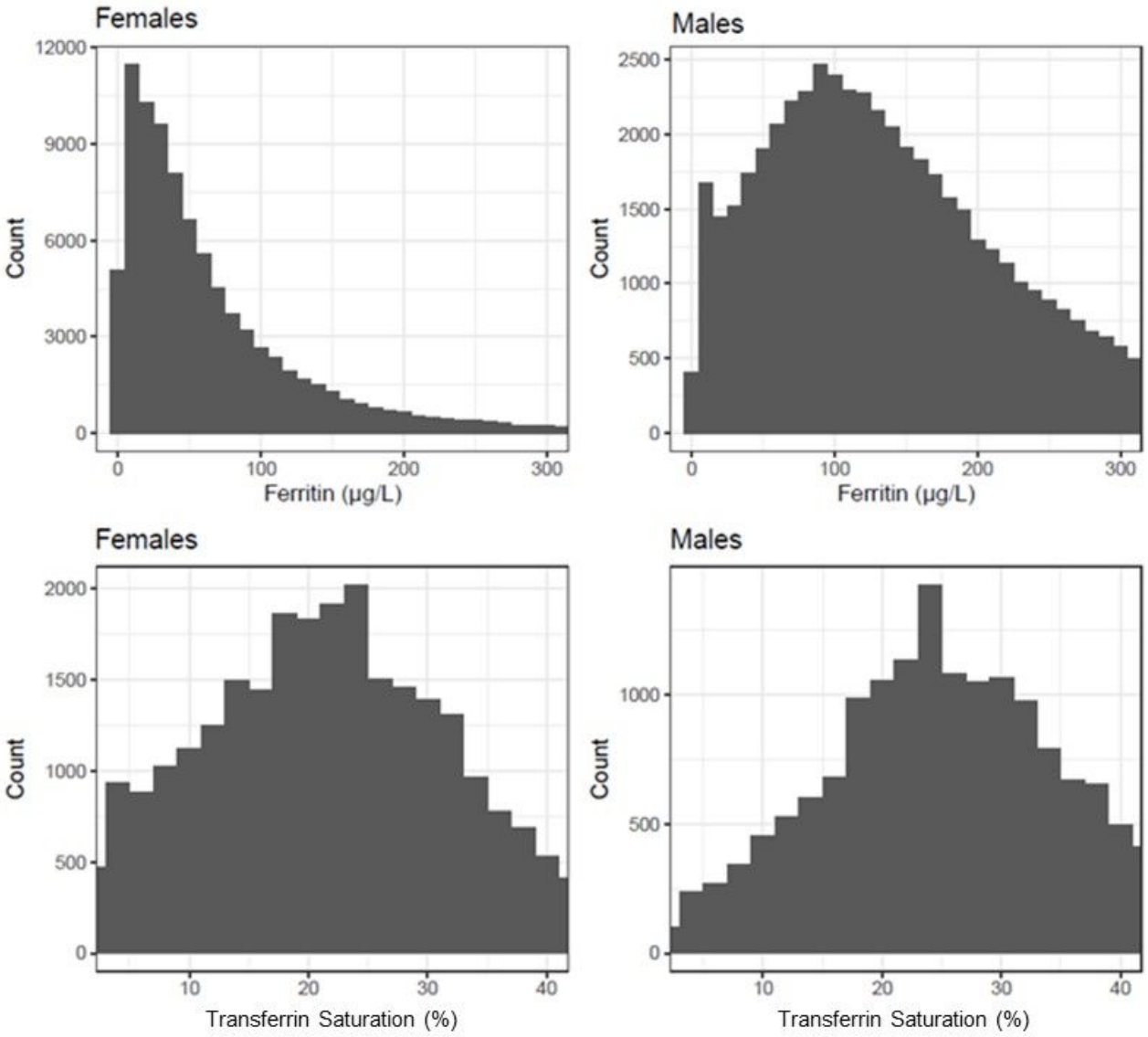
C-Reactive protein was assessed in serum by commercially available assays from Olympus A600 (Olympus A/S, Denmark) and ADVIA Chemistry System 1650/2400 (Bayer®/Siemens®, Denmark) according to the instructions of the manufacturers. Two ADVIA Chemistry assays were used, the C-Reactive Protein(CRP) and the C-Reactive Protein(CRP)_2. All the assays were standardized and results traceable to the international reference material CRM 470. For the Olympus assay, the interserial coefficient of variation percentage (CV%) was 5.1 % (at level 7 mg/L), 1.2 % (at level 19 mg/L) and 2.5% (at level 31.5 mg/L). For the Advia Chemistry CRP assay, the CV% was 12.7 % (at level 12 mg/L), 4.0 % (at level 24.7 mg/L) and 2.6 % (at level 98.6 mg/L). For the Advia Chemistry CRP_2 assay, the CV% was 1.6 % (at level 23.5 mg/L), 1.5 % (at level 75.5 mg/L) and 2.6 % (at level 86.1 mg/L). The CRP assays were subject to external quality

control through participation in the LabQuality External Quality Assessment Scheme (LabQuality, Helsinki, Finland). The assessment scheme included 12 distributions annually (each distribution comprised 1 sample) or 4 distributions annually (each distribution comprised 2 sample). The results (2002 to 2015) confirmed the reliability of the assays, and the results (n=145) deviated less than the target limits (± 15 %) in more than 97 % of the cases. The mean deviation from the target set by LabQuality was 0.8 % (n=112).

Low results were reported as < 3 mg/L (until December 1, 2002), < 5 mg/L (between December 2, 2002 and May 28, 2008) and < 4 (from May 29, 2008). High results were reported as > 300 mg/L (until December 1, 2002).

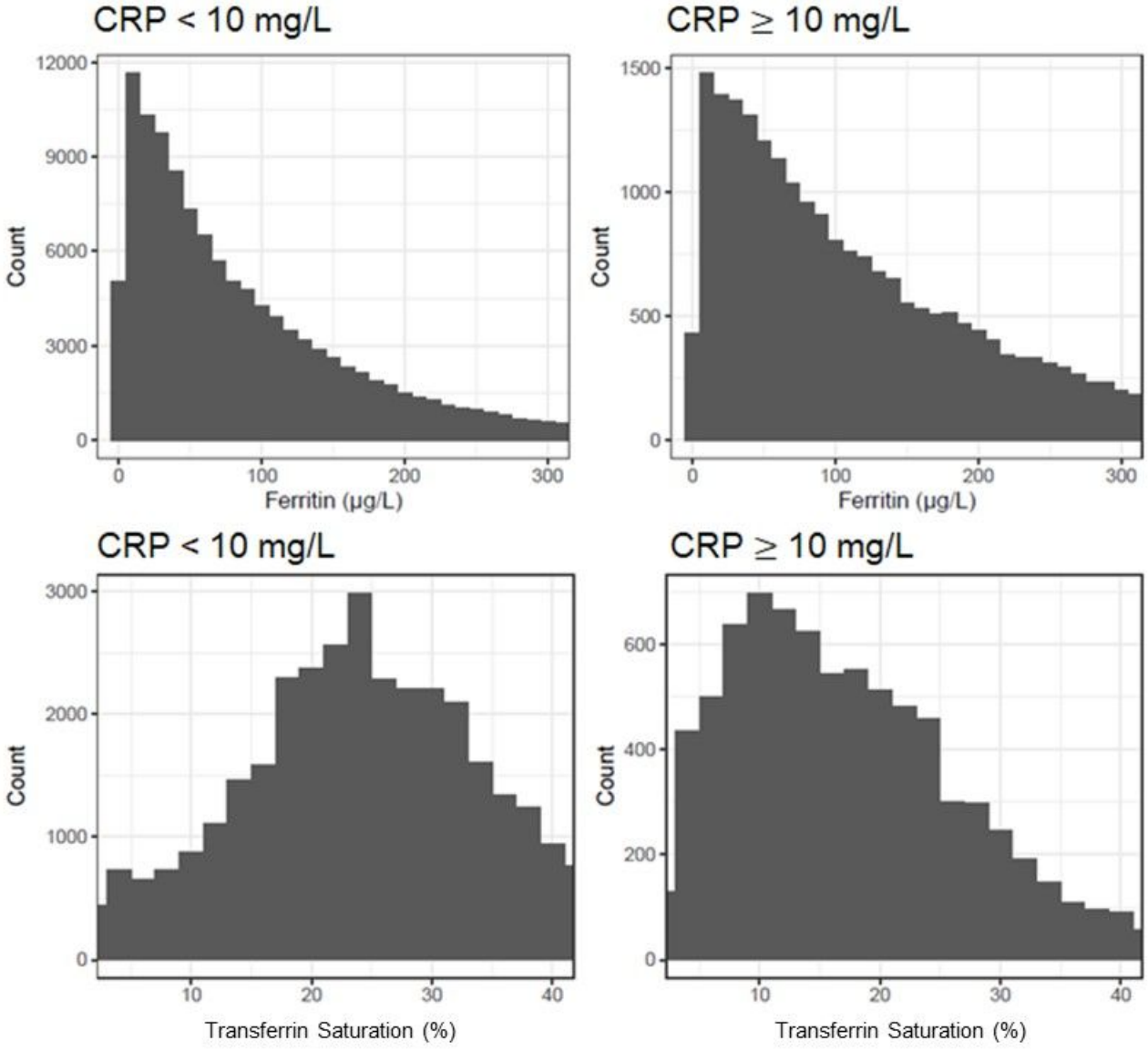
#2 Ferritin and Transferrin saturation levels by sex (figure S1)

The distribution of ferritin and Transferrin saturation stratified by sex.



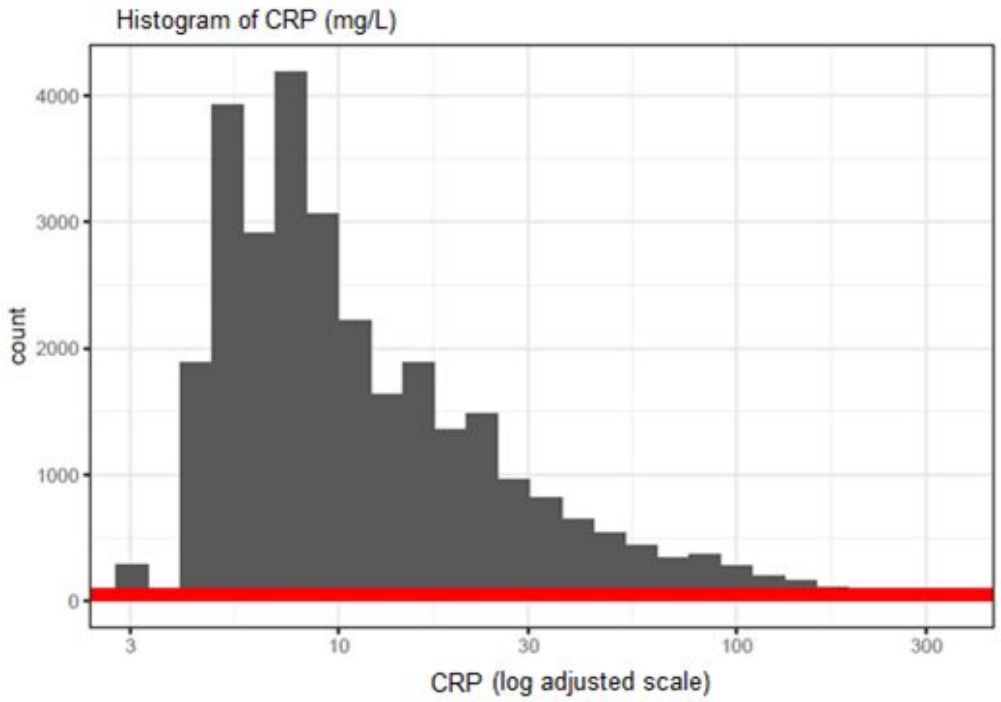
#3 Ferritin and Transferrin saturation levels by C-reactive protein (figure S2)

The distribution of ferritin and Transferrin saturation stratified by inflammation status.

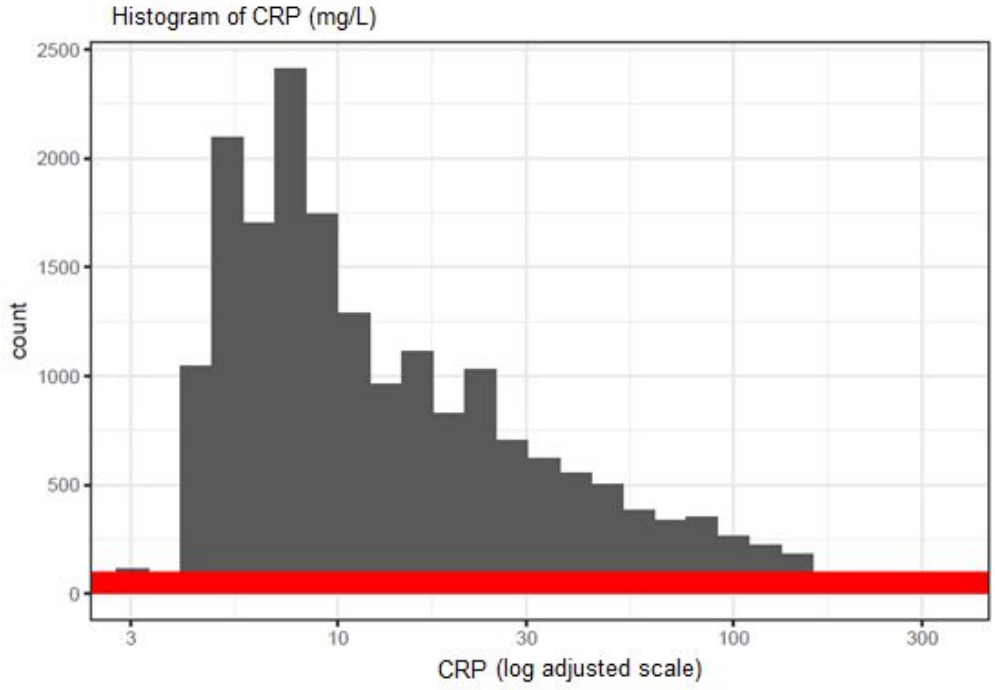


#4 C-reactive protein distribution by sex in the ferritin population (figure S3)

Females

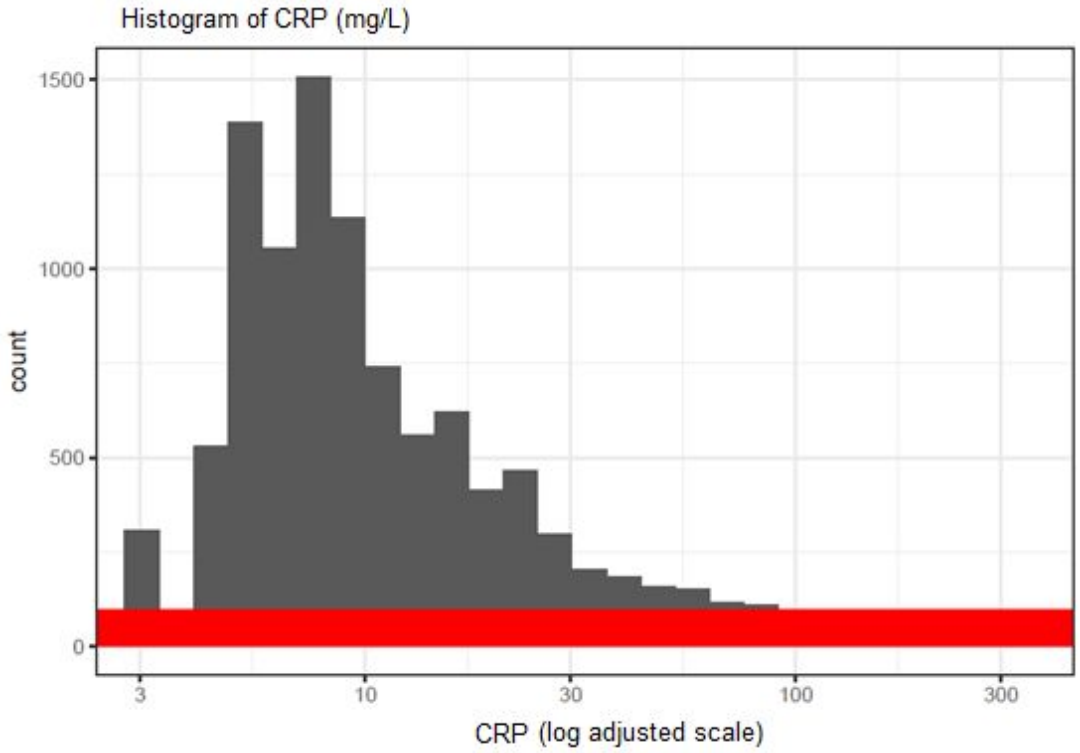


Males

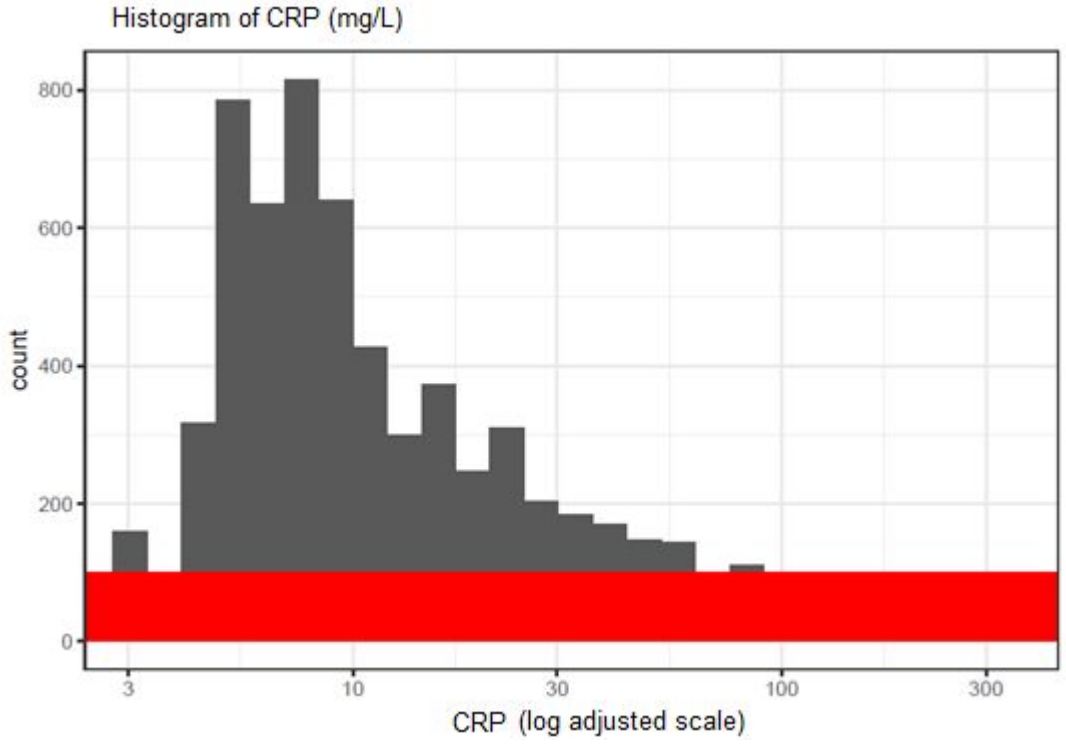


#5 C-reactive protein distribution by sex in the Transferrin saturation population (figure S4)

Females

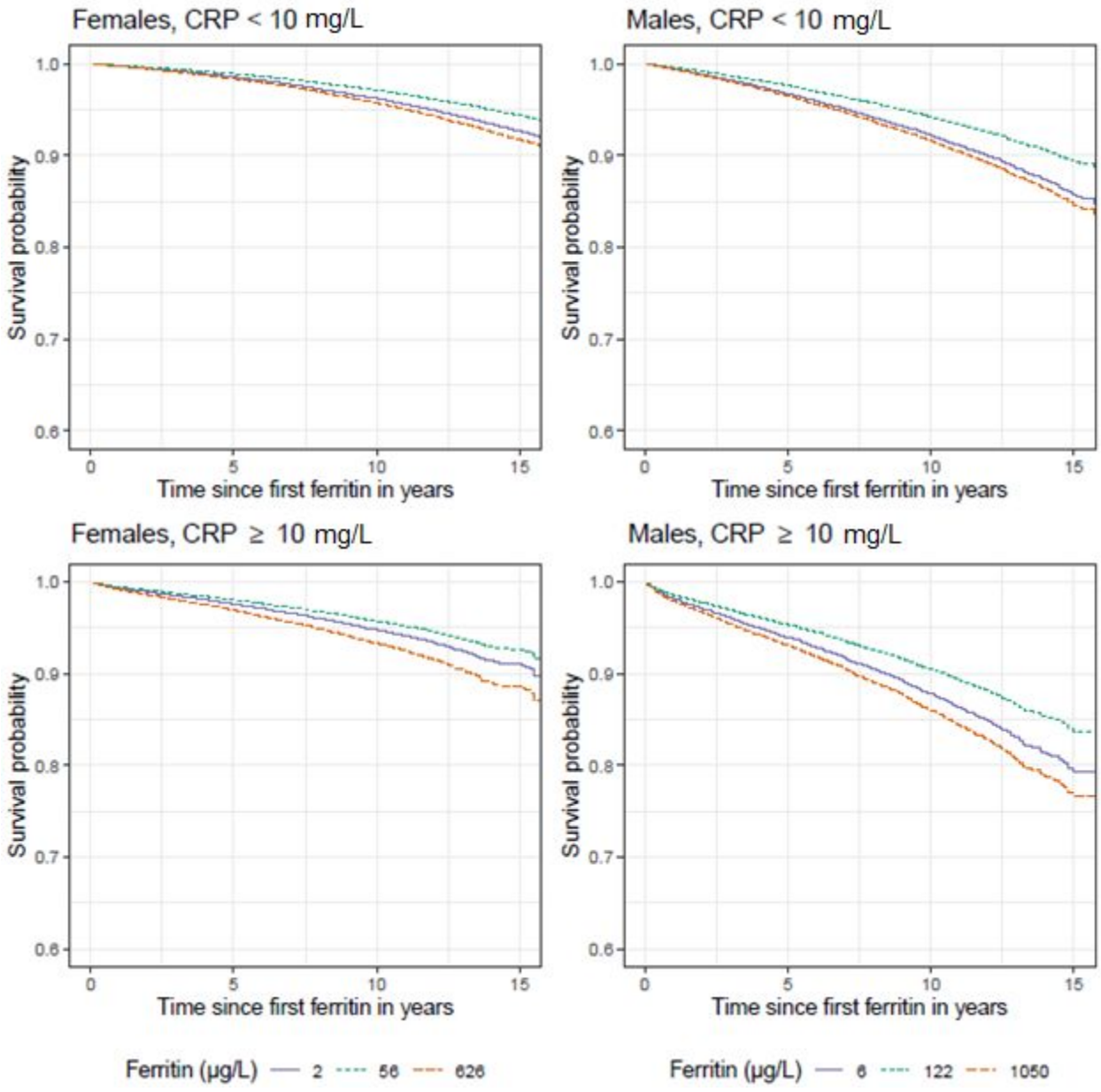


Males



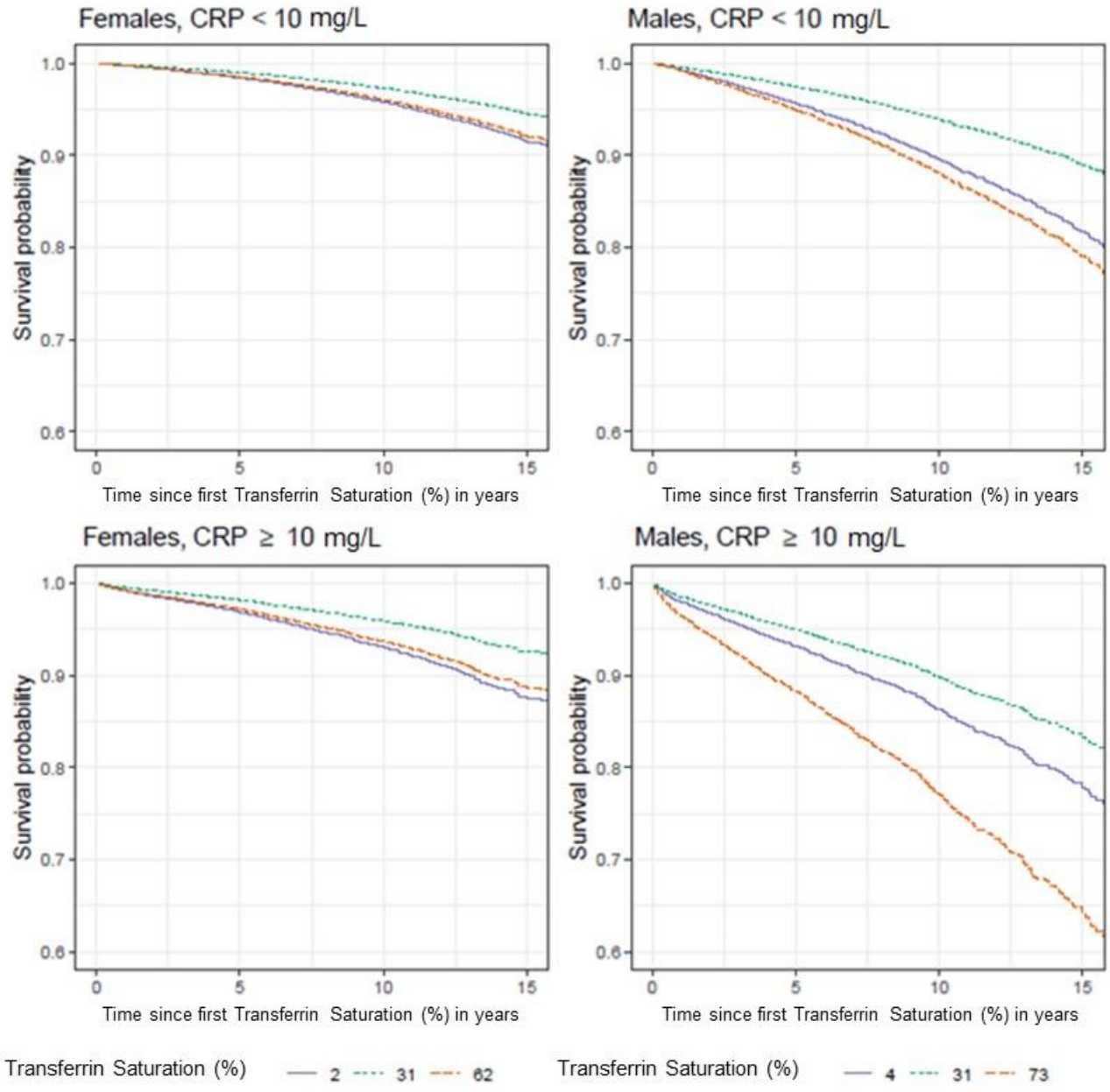
#6 Absolute survival probability ferritin (figure S5)

Figure 7 illustrates (based on the Cox model for ferritin) the predicted absolute survival probabilities for a standardized (meaning median age and CCI=0) male or female given absent or present inflammation. Survival probabilities are shown for the ferritin value associated with the lowest all-cause mortality, and for the 1st and 99th percentile values.

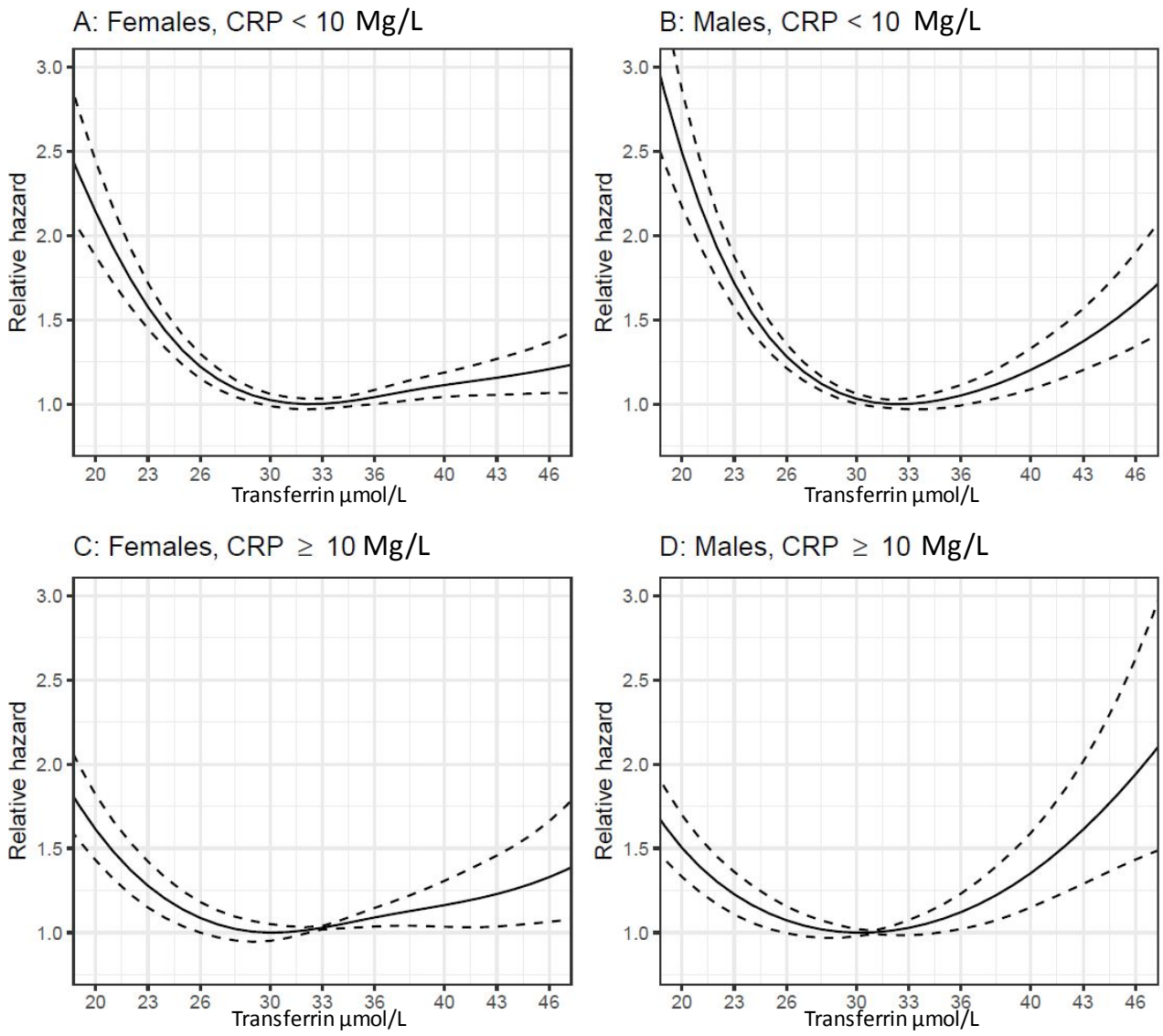


#7 Absolute survival probability Transferrin saturation (figure S6)

Figure 8 illustrates (based on the Cox model for Transferrin saturation) the predicted absolute survival probabilities for a standardized (meaning median age and CCI=0) male or female given absent or present inflammation. Survival probabilities are shown for the Transferrin saturation value associated with the lowest all-cause mortality, and for the 1st and 99th percentile values.



#8 Transferrin and all-cause mortality by sex and inflammation status (figure S7)



#9 Iron and all-cause mortality by sex and inflammation status (figure S8)