

Supplementary Information

Increased levels of circulating Iron-Albumin complexes in peripheral arterial disease patients

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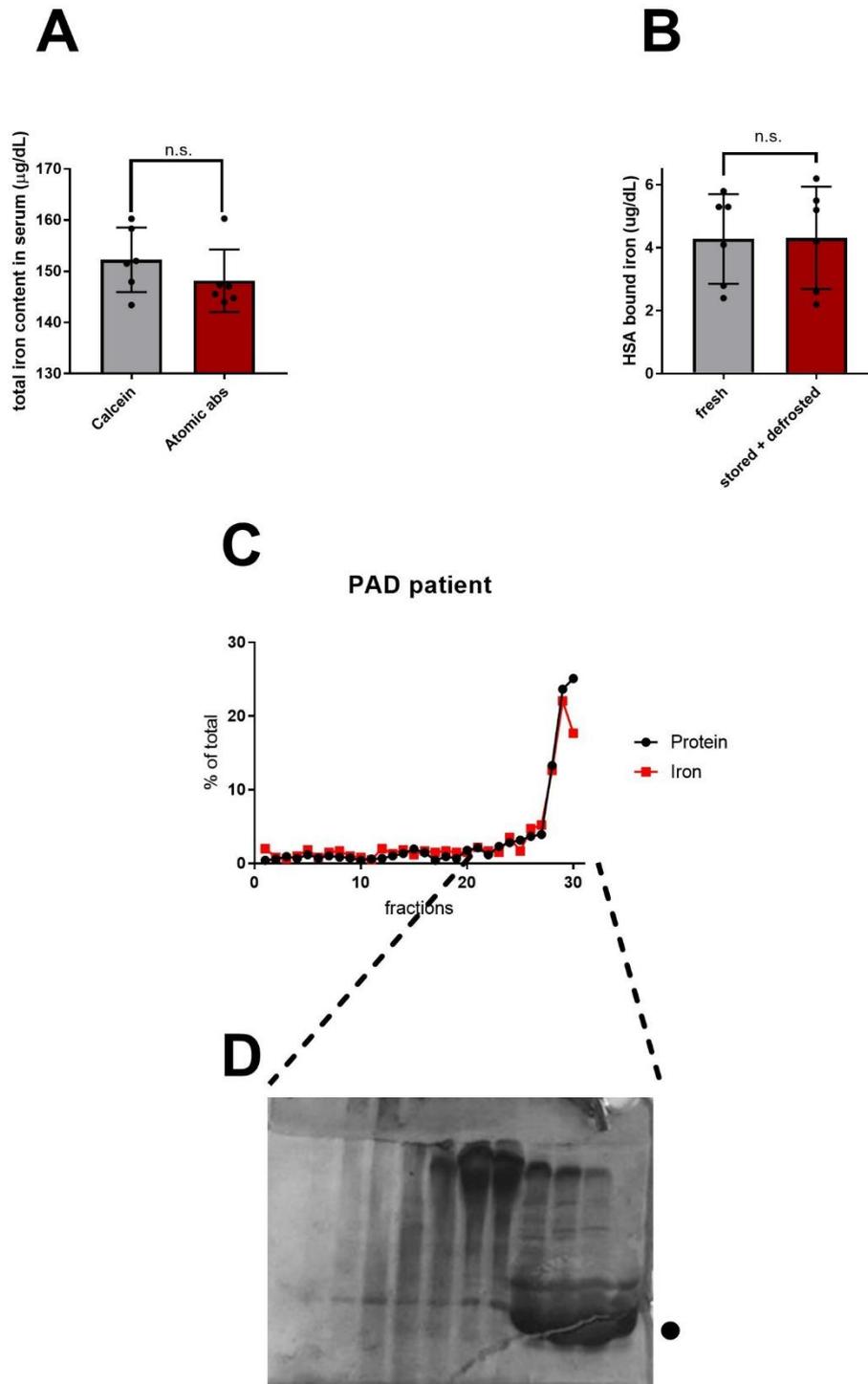


Figure S1. Supplementary Methodological Information. (A) Comparison between iron measurement by calcein (gray bar) and atomic absorbance spectroscopy (red bar) ($n=6$, n.s. non statistically different). Despite less sensitive (limit of detection of $45.8 \mu\text{g/dL}$ and 2 ppb for calcein and atomic absorbance spectroscopy, respectively) calcein quenching directly correlates to total iron concentration. (B): Effect of freezing and defrosting on immunoisolation of NTBI-HSA complex. Amount of iron (calcein method) present in HSA immunisolated from serum samples subjected (red bar) or not (gray bar) to freezing and defrosting ($n=6$, n.s. non statistically different). (C-D): Panels C and D show exemplificative results obtained for gel-filtration of serum from one of the PAD patients on Enrich SEC 70-75 HR 10/300 column. For each elution fraction, iron content (red squares, calcein method) and protein content (black dots, Bradford methods) are indicated. In panel D, Coomassie staining of non-reducing SDS-PAGE resolved elution fractions of sample shown in C (● indicates HSA).