# Optimal Serum Ferritin Level Range: iron status measure and inflammatory biomarker

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## Abstract

This report provides perspectives concerning  dual roles of serum ferritin as a measure of both iron status and of inflammation. We suggest benefits of a lower range of serum ferritin as has occurred for total serum cholesterol and fasting blood glucose levels.  Observations during a prospective randomized study using phlebotomy in patients with peripheral arterial disease (PAD) offered unique insights into dual roles of serum ferritin both as an iron status marker and acute phase reactant. Robust positive associations between serum ferritin, interleukin 6 [IL-6], tissue necrosis factor-alpha (TNF-a), and high sensitivity C-reactive protein (hsCRP) were discovered.  Elevated serum ferritin and IL-6 levels associated with increased mortality and with reduced mortality at ferritin levels < 100 ng/mL. Epidemiologic studies demonstrate similar outcomes. Extremely elevated ferritin and IL-6 levels also occur in individuals with high mortality due to SARS-CoV-2 infection. Disordered iron metabolism reflected by a high range of serum ferritin level signals disease severity and outcomes.  Based upon experimental and epidemiologic data, we suggest testing the hypotheses that optimal ferritin levels for cardiovascular mortality reduction range from 20-100 ng/mL with percent transferrin levels from 20-50%, to

ensure adequate iron status and that ferritin levels above 194 ng/mL associate with all-cause mortality in population cohorts.

**Graphic abstract**

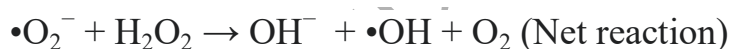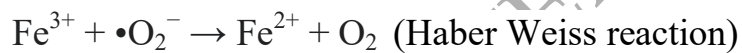**Ferritin: Dual iron status measure and inflammatory biomarker**
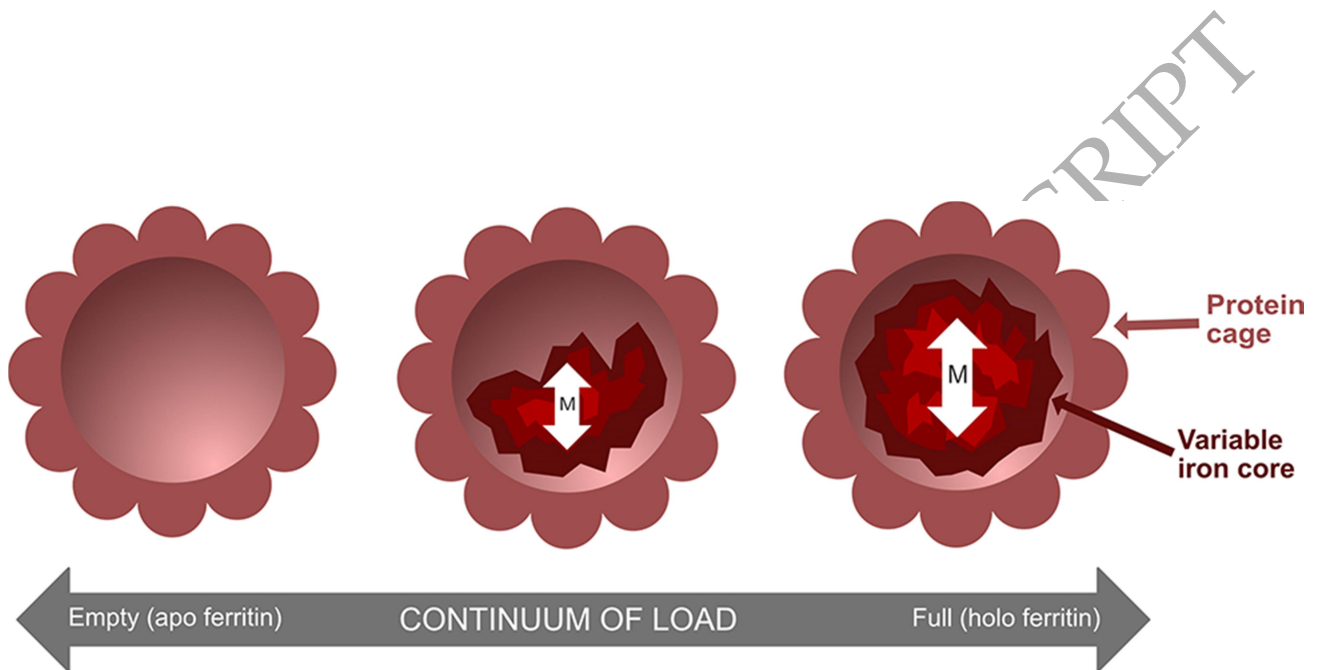


**Ferritin, a ~475-481 kilodalton spherical protein, can sequester varying amounts, up to 4500 ferric iron atoms, within a 80nm mineral core.**

**Ferritin in Iron Metabolism: overview**

Iron, essential for hemoglobin synthesis, functions within cells to comprise mitochondrial heme-iron-sulfur clusters, key components of proteins and enzymes involved in biological processes including respiration, nucleic acid replication and repair, metabolic reactions, and host defense. Although vital for life, iron is potentially toxic through catalytic generation of oxidative stress. The last two decades witnessed considerable progress in understanding iron status, body content and transfer kinetics. Intracellular iron contained within ferritin plays a critical transport role in cell metabolism (1). The superfamily of ferritins in all species resides at the center of iron and oxidative phosphorylation to confine ferrous iron within its core by initiating formation of caged iron oxide, $Fe_2O_3$ (2).

Fritz Haber and his student Joshua Weis in 1932 first advanced the notion that biologic iron in the presence of oxygen is toxic [3]. Subsequently, it was noted that oxidative stress toxicity could not be entirely caused by the slow kinetics of the Haber-Wiess reaction, but rather by the Fenton reaction [4]. Net postulated equations for iron damaging reactions are:

$$Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2 \text{ (Haber Weiss reaction)}$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH \text{ (Fenton reaction)}$$

$$\bullet O_2^- + H_2O_2 \rightarrow OH^- + \bullet OH + O_2 \text{ (Net reaction)}$$

Iron, essential for life, paradoxically damages cells due to generation of reactive oxygen species (ROS) in its reduced (Fe ++ form) [5]. The need exists *in vivo* to ensure that ferrous iron remains in liganded forms. Ferritin serves this purpose. Ferrous iron and ROS are important initiators and mediators of cell death in a variety of organisms and pathological situations [6,7]. Estimated iron intake ranges from 10-20 mg daily, while bodily iron loss from all sources is estimated to be 1-1.5 mg daily [8]. While no physiological pathway for excretion of excess iron exists (apart from menstrual blood loss in women), in the presence of iron excess, increased hepcidin production reduces intestinal iron absorption and iron macrophages release [1]. As will be further discussed, hepcidin, discovered in 2001,

serves as the main protective mechanism against the toxic potential of excess ferrous iron (1).

Ferritin, the primary iron intracellular cytosolic storage protein, releases iron during iron deficiency and stores excess iron during overload [8,9]. Laufberger, in 1937, first isolated a novel protein, ferritin, from horse spleen containing up to 23% by dry weight of iron [9, 10]. Shortly thereafter, ferritin was discovered in human serum (11). In 1972, using an immunoradiometric assay, Addison et al. measured ferritin in human serum [12}. To determine the relationship between serum ferritin levels and total body iron stores, serum ferritin levels were examined in a normal healthy population, in patients with iron deficiency, and those with iron overload. Serum ferritin levels were clearly elevated in patients with iron overload and decreased in patients with iron deficiency diseases [13,14].

Ferritin, a ~475-481 kilodalton spherical protein, is capable of sequestering up to 4500 ferric iron atoms within its 80nm mineral core. Cells with high iron levels are rich in L chain ferritin representing the long-term storage function for L chain ferritin.  H-rich ferritins are believed more active in iron metabolism and kinetics [15].  Emerging evidence suggests an extensive role for ferritin extending beyond its iron storage functional capacity [16]. Mitochondrial ferritin plays an essential role in cellular metabolism. Human mitochondrial ferritin incorporates iron, thus modulating cellular iron metabolism [17]. Extracellular iron taken up by cells, is transported to mitochondria, where it functions as a cofactor essential for enzyme function, oxidation-reduction reactions, energy production, DNA synthesis and repair, and other cellular processes [17].

While serum ferritin levels correlate with iron stores and inflammation both, its exact pathogenic contribution to disease processes, cell damage, and oxidative stress at any singular time point remains undefined. Serum ferritin levels also correlate with other phenotypic signals including erythrocyte morphology [6, 18}.  More research is needed to account for the serum ferritin level paradox as representing total bodily iron content, inflammation, or both. Elevated ferritin levels correlate with biomarkers of cell damage; with associated biomarkers of oxidative stress notably interleukin 6 (IL-6); with high sensitivity C-reactive protein (hsCRP); and presence and/or the severity of chronic disease processes. Elevated serum ferritin levels might represent cell damage when an excess of unliganded catalytic ferrous iron could be the actual cause of cell damage

or disease itself [19]. While it is possible to measure iron content within the ferritin protein moiety using optical dispersion, this important determination has yet to be done clinically [20]. Clinical measurement of iron content of serum ferritin, as will be discussed, could further clarify serum ferritin's significance as a measure of overall iron status or as a response to inflammation.

   **The hormone** hepcidin is central to iron transport and utilization [21, 22, 23]. Increased hepcidin production elicits increased iron to be placed into storage and there liganded by intracellular ferritin. Plasma iron level, in turn, regulates hepcidin homeostasis. Increasing plasma iron levels stimulate hepcidin production, blocking dietary intestinal iron absorption [23]. By contrast, during iron deficiency, hepcidin production decreases to enhance intestinal iron absorption and macrophage iron release.  The core activities of erythropoiesis require high levels of iron use (24). Here, hepcidin suppression activates release of stored iron by hepatocytes and macrophages and increases intestinal iron absorption. Macrophages and round cells, stimulated by the inflammatory process, release myriad cytokines, most prominently interleukin-6 (IL-6), the primary inducer of hepcidin expression promoting protective hypoferremia [25]. Inflammation-associated increases in the levels of IL-6 [26], and other cytokines, including bone morphogenetic protein (BMP), results in increased hepcidin production causing hypoferremia and anemia [1, 24, 25, 26, 27]. Hypoferremia represents a strategic host defense limiting or denying iron availability to invading microorganisms. When hepcidin levels increase during inflammatory states, serum iron falls due to decreased intestinal iron absorption and iron trapping within macrophages, liver cells. The result is anemia of chronic disease. By contrast, during iron deficiency hepcidin falls, thus enhancing intestinal iron absorption and cellular iron release.


   Values of ferritin and hepcidin considered to be within "normal" ranges vary considerably as provided by differing clinical laboratories. Table 1 provides representative reference values for ferritin (28,29,30,31,32), transferrin (28,29,30,31) % transferrin saturation, (33,34,35) and hepcidin (36,37,38,39).  Transferrin, a blood-plasma glycoprotein, dominates iron transport in the blood to tissues including the liver, spleen, and bone marrow.  The percent of transferrin saturation indicates the level of serum iron divided by the total iron-

binding capacity of available transferrin, reflecting iron transport and sequestration.

Table 1 shows widely varying ranges for ferritin and hepcidin and differing ranges between men and women. Ferritin and hepcidin values characteristically are lower for women as compared to men due to menstrual blood loss.

## Table 1.

Representative reference values for Ferritin, Transferrin, % Transferrin Saturation and Hepcidin

| Ferritin | Adults: 30-360 ng/mL<br><br>Munoz M, Villar I, García-Erce JA. (28) | Adult Male: 30 - 400 ng/mL<br><br>Adult Female: 13 - 150 ng/mL<br><br>University North Carolina 2020: Reference Ranges: Iron (29) | Adult Male: 30 - 400 ng/mL<br><br>Adult Female: 13 - 150 ng/mL<br><br>University of Iowa 2020: Department of Pathology Laboratory Services. (30) | Adult Male: 12-300 ng/mL<br><br>Adult Female: 12-150 ng/mL<br><br>Columbia Southern University Laboratory Values. (31) | Adult Women:<br><br>12 ng/mL to 250 ng/mL<br><br>Mackie S and Winkleman JW.(32) |
|---|---|---|---|---|---|
| Transferrin | Adults: 200-360 mg/dL<br><br>Munoz M, Villar I, García-Erce JA. (28) | Adults: 200-380 mg/dL<br><br>University North Carolina 2020: Reference Ranges: Iron (29) | Adults: 200-360 mg/dL<br><br>University of Iowa 2020: Department of Pathology Laboratory Services. (30) | Adults: 200-350 mg/dL<br><br>Columbia Southern University Laboratory Values. (31) | |
| %Transferrin Saturation | Adults: 15% to 50%<br><br>Health Encyclopedia University of Rochester (33) | Adult Male: 20-50%<br>Adult Female: 15-50%<br><br>Mosby's Diagnostic & Laboratory Test Reference. 14th (34) | Adult Male: 15-50%<br>Adult Female: 12-45%<br><br>Iron overload. In Hematology. Fourth edition. Company, 1990, pp 482-505. (35) | | |
| Hepcidin | Adults : n=120 1.23 – 36.46 ng/mL (mean 9,25 ± 6,45 ng/mL) Men=6- Women=60 (p Men vs women: p<001<br><br>Pre-menopausal n= 38 | Adult Male: N=1066 Median 7.8nM/L (21.75ng/ml)<br><br>Adult Female: N=882 Median 6.5 nM/L (18.13ng/mL) | All N=40 Adults: N=40: 60.99 ng/mL<br><br>Male: N=21 18.227-81.541 ng/mL<br><br>Female: N=19 17.186–91.237 ng/mL | Adult Male: 29-254 ng/mL<br>Adult Female: 17-286 ng/mL | |

| | | | | |
|---|---|---|---|---|
| | 5.51 ± 2.8 ng/mL Post-menopausal n= 22 7.29 ± 3.59 ng/mL p<0.05 Journal of IMAB - Annual Proceeding (36) | Serum hepcidin: reference ranges and biochemical correlates in the general population doi: 37) | Int J Blood Transfus Immunohematol (38) | Intrinsic Lifesciences 2020: Normal serum hepcidin in adult (39) |

The wide biologic variation in "normal" serum ferritin range suggests a need to reexamine measurement techniques as well as their clinical significance.  Widely varying biomarker values may not reflect states of optimal or healthy iron metabolism. From a clinical perspective, using current normal limits for ferritin based on sampling asymptomatic individuals may be too high. Many "normals" appear to be at higher risk for adverse events, and lowering accepted normal levels of serum ferritin, while at the same time ensuring absence of iron deficiency may be advantageous, just as demonstrated in lowering of past recommended values for total cholesterol and fasting glucose levels.

## Phlebotomy, Ferritin Levels and Clinical Outcomes in Peripheral Arterial Disease (PAD).

The VA Cooperative Study, CSP 410, The Iron and Atherosclerosis Study (FeAST), a prospective, randomized, controlled single blinded clinical trial of iron reduction, used graded phlebotomy in participants with (PAD) (www.clinicaltrials.gov, Identifier NCT00032357) [40]. This intervention uncovered a paradox concerning the significance of ferritin levels as measures of iron status and inflammation. The study tested the hypothesis that improved clinical outcomes would be achieved by reducing ferritin to levels typical of children and pre- menopausal women ($\sim$ 25 to 60 ng/ml) [40]. The study included 24 Veterans Medical Centers with 1,277 mostly male Veterans, average age 67 years. Its primary outcome was all cause mortality; its secondary outcome was combined death plus non-fatal myocardial infarction (MI) and stroke. Based on ferritin level, calibrated phlebotomy was performed using the following formula:  Ferritin ng/mL -25 x 10 = ml of blood to be removed by phlebotomy [40]. The study began in 1999 and concluded in 2005. It is important to note that hepcidin effects on iron metabolism were not reported when this study was initiated; therefore, the effects this key

biomarker and also the effects of guideline-initiated statin administration for PAD were not among the initial planned variables.

The original 2007 JAMA publication abstract indicated lack of overall effect of phlebotomy on outcomes [40]. However, within the text analyses, according to randomization variables at entry, noted improved outcomes with iron reduction in younger ages by quartile for the secondary endpoint (p for interaction =0.004) along with a favorable effect in smokers (p for interaction 0.006). When analyzed as a continuous variable using the Cox proportional hazards regression model and log relative hazard plots, age interacted nonlinearly with iron reduction by phlebotomy, demonstrating favorable effects upon both primary (p=0.04) and secondary (p<0.001) outcomes. The Cox model showed improved primary (HR 0.47, 95% CI 0.24-0.90, p=0.02) and secondary (HR 0.41, 95% CI 0.24-0.68, p<0.001) outcomes in youngest age quartile of participants (43 to 61 years) randomized to phlebotomy as compared to controls. The primary investigator, Leo R Zacharski, MD believed that interactions between age and body iron levels and smoking masked possible beneficial effects of iron reduction in the overall cohort.

Reanalysis of age effects and ferritin levels, published in the American Heart Journal in 2011, showed that phlebotomy interventions significantly improved primary and secondary outcomes in youngest age quartile participants, as displayed using Kaplan-Meier plots [41]. These findings comport with findings in young Finnish men with an average age of 32 +/-8 years, where moderately elevated ferritin (200-500ng/ml) correlated with an elevated coronary risk profiles [42]. Mean follow-up ferritin levels (MFFL) in the FeAST cohort showed a decrease with increasing entry age in controls. Older age (p=0.026) and higher ferritin levels (p<0.001) at entry predicted poorer compliance with phlebotomy and rising MFFL in iron reduction participants. Phlebotomy produced greater ferritin reduction in younger participants. Improved outcomes with lower MFFL occurred in iron reduction patients for both primary (HR 1.11, 95% CI 1.01-1.23, p=0.028) and secondary (HR 1.10, 95% CI 1.0-1.20, p=0.044) outcomes, and for the entire cohort: primary PAD outcome (HR 1.11, 95% CI 1.01-1.23, p=0.037). Improved outcomes occurred with MFFL below versus above the median of the entire cohort means: primary outcome HR 1.48,

95% CI 1.14-1.92, p=0.003; secondary outcome HR 1.22, 95% CI 0.99-1.50, p=0.067 [41].


FeAST investigators also reported that lower ferritin levels associated with lower cancer risk [43]. The study excluded participants with a history of visceral malignancy within the preceding five years. Data collected on occurrence of new visceral malignancy and cause-specific mortality including death from cancer prospectively over a four-and-a-half-year follow-up revealed diagnosis of a new visceral malignancy in 60 control and 38 phlebotomized participants, a 37% (HR 0.63; 95% CI = 0.42 - 0.95, p = 0.026) decrease in risk associated with iron reduction [43]. Reduced cancer risk with iron reduction was also confirmed on time-to-event analysis (HR = 0.65; 95% CI = 0.43 - 0.97, p = 0.036). Decreased risk was observed for other tumor types. Iron reduction participants had lower cancer - specific mortality and lower all-cause mortality in participants diagnosed with cancer (HR = 0.39; 95% CI = 0.21 - 0.72, p = 0.003 and HR = 0.49; 95% CI = 0.29 - 0.83, p = 0.009 respectively), compared to control participants. MFFL during follow-up in participants randomized to iron reduction and who developed cancer were comparable to levels in control participants ($t93$ = 0.8, p = 0.428). The MFFL in phlebotomy participants developing cancer was 127 ng/mL, 95% CI = 71.2 -183.0. The MFFL was significantly lower in participants not developing cancer, 76.4 ng/mL, 95% CI = 71.4 - 81.4, p = 0.017) [43].

Serial biomarker measures obtained prospectively from a cohort of 100 FeAST participants from the Veterans Affairs (VA) Sierra Nevada Health Care System (SNHCS) showed statistically significant links between ferritin and inflammatory biomarkers, uncovering consistent inflammatory cytokine signatures in participants with PAD [44]. Notably, phlebotomy interventions reduced elevated levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF-a) coincident with serum ferritin reduction [44]. Significant associations between ferritin, inflammatory cytokines, and high-sensitivity C-reactive protein (hsCRP) were observed. Ferritin levels positively correlated with IL-6 and hsCRP levels. Notably, ferritin and IL-6 levels were significantly higher in participants who died as compared to survivors in control and intervention groups, irrespective of whether or not phlebotomy had

been applied [44]. Statin administration tracked in this cohort also associated with reduced ferritin levels, independently of the phlebotomy intervention, highlighting statin effects upon ferritin levels reflecting reduction in inflammatory responses associated with improved outcomes. These findings supported a biologic rationale for our further study of ferritin's role in atherosclerosis [45,46].

Baseline inflammatory markers, including cytokines, C-reactive protein (CRP), and ferritin in the FeAST PAD participants within the SNHCS taking statins were then compared with participants not taking statins within the SNHCS Cohort (45, 46). Inflammatory markers in the serum of 47 participants not taking statins, and a healthy cohort of 21 medication-free men were compared with 53 participants receiving statins at study entry. Healthy subjects had significantly lower levels of TNFa-R1, IL-6, and CRP. TNF-a R1 averaged 2.28 ng/mL versus 3.52 ng/mL, p = .0025; IL-6 averaged 4.24 pg/mL versus 16.61 pg/mL, p = .0008; and CRP averaged 0.58 mg/dL versus 0.92 mg/dL, p = .0192. A higher level of IL-6 was detected in statin users versus those not taking statins: 19.47 pg/mL versus 13.24 pg/mL, p = .0455, IL-6. This finding associated with significantly higher proportion of diabetic PAD. A nexus between statins and ferritin reduction was noted. Statins increased high-density lipoprotein to low-density lipoprotein ratios while reducing ferritin levels possibly by non-interacting mechanisms. Notably, favorable clinical outcomes and reduced mortality associated with lower ferritin levels but not with improved lipid ratios in this elderly PAD population, average age 67+/-9 years (47).

Subsequent analyses of FeAST data showed provocative interactions between ferritin levels, smoking, diabetes, and race. Iron reduction in smokers clearly associated with improved primary and secondary outcomes in smokers but not in non-smokers based upon comparative Kaplan-Meier curve analyses. Smokers, in comparison to nonsmokers, required more blood removal by phlebotomy to achieve comparable serum ferritin reductions. Phlebotomy related outcomes clearly favored smokers over non-smokers. Ferritin levels ranging from 70 to 79 ng/mL associated with lower mortality along with lower inflammatory markers, mainly IL-6. More iron was removed from the smokers as compared to non-smokers based on phlebotomy cumulative blood volumes [48].

FeAST data were further analyzed to define ferritin and percent transferrin saturation (%TSAT) levels associated with Type 2 diabetes (T2D) and cardiovascular disease (CVD) risk (49). Smoothing plot curves compared continuous quantitative ferritin, hemoglobin, and %TSAT levels over a broad range of observed values. Inflection points in the curves were compared to serum ferritin and %TSAT levels. Increased T2D and CVD risk was observed with increasing ferritin level and decreased %TSAT levels a finding also noted in prior epidemiologic studies (49, 50). Increased serum ferritin levels up to ~ 80 ng/mL and %TSAT up to ~ 25 %TSAT paralleled increasing hemoglobin levels associated with minimal T2D and CVD risk. Displaced Loess trajectories showed lower hemoglobin levels in diabetics as compared to non-diabetics. Ferritin levels up to ~100ng/mL paralleled proportionately increasing %TSAT levels up to ~ 55% TSAT corresponding to further limitation of T2D and CVD risk. Ferritin levels over 100 ng/mL did not associate directly with hemoglobin levels but did coincide with increased T2D and CVD risk. The study conclusions suggested modified normal ranges for ferritin ranging from ~15 ng/mL up to ~ 80-100 ng/mL and %TSAT ranging from ~ 20% up to ~ 50-55%. Values within these ranges improved the predictive value of these biomarkers indicating lowered risk of T2D [49]. By contrast, in established diabetes in PAD participants, Zacharski et al reported that diabetics and non-diabetics had consistently comparable iron measures during follow-up [50]. However, Loess analysis of paired ferritin and hemoglobin, and paired ferritin and glucose levels in diabetics randomized to phlebotomy showed that higher ferritin levels associated with lower hemoglobin and higher glucose levels.

**Racial Disparities noted during the FeAST Study**

The FeAST trial uncovered racial differences in iron measures and clinical outcomes related to iron reduction. Elevated ferritin levels clearly contributed to differing health outcomes. At entry, Black participants had higher ferritin, lower red cell measures, while exhibiting differing ferritin and percent transferrin saturation (%TSAT) phlebotomy responses. Statin use in Whites associated with significantly lower and more favorable ferritin (p=. 003) and %TSAT levels (p=<.001). By contrast, in Black statin takers, ferritin (p=.410) and %TSAT (p=.334) remained in a relatively unfavorable range in

comparison to statin non-users. HDL/LDL ratios associated with statin use also differed in Black as compared to White participants (44, 53, 55): White statin non-users: = 0.43+/- 0.301 vs. Black non-statin users 0.456+/-0.199 ( p=.002); White statin users: 0.459+/- 0.208 vs. Back statin users: 0.436 +/-0.162 (p=.662).

Lower hematocrit levels overall during six-year follow-up in Blacks resulted in fewer phlebotomies, less ferritin reduction, (p=.035) and 32% less iron removed as compared to White participants randomized to phlebotomy. Improved primary (all-cause mortality) and secondary (death plus non-fatal myocardial infarction and stroke) outcomes among participants correlated with lower ferritin levels (p=.005 and p=.053, respectively) and higher %TSAT levels (p=.001 and p=.001 respectively). These associations were not observed in Black participants. This analysis suggests that variant iron metabolic effects might be contributory to racial health outcomes. Personalized intervention strategies based upon racial backgrounds and race-specific clinical trial designs were suggested [51].

Further analysis demonstrated racially variant interactions between hemoglobin, ferritin, and %TSAT levels [52]. Lower hemoglobin and %TS levels and higher ferritin levels occurred in Black compared to White participants within specific cardiovascular disease risk categories. Ferritin levels approximating 80 ng/mL related to higher hemoglobin levels in White but not Black participants. Higher %TSAT levels with ferritin levels above 80ng/mL in White participants were blunted in Black participants. Ferritin/%TSAT ratios were significantly higher in Black than White participants. Differing iron metabolism and iron toxic effects may, in part, contribute to disparities in health outcomes between Whites and Blacks, while clearly acknowledging well documented differences in health care access for minorities [53,54]. Overall, this prospective interventional phlebotomy study for iron reduction improved primary and secondary outcomes in the youngest quartile of PAD participants; lower levels of ferritin associated with lower cancer risk; and data subset analyses exposed provocative interactions between ferritin levels, smoking, diabetes, and race.

Prior epidemiologic observations support racial differences observed during the prospective FeAST study. Serum ferritin levels examined by age, sex, and race, and values were compared with the percent transferrin saturation in 20,040 individuals >17 years of age from the third National Health and Nutrition Examination Survey (NHANES III) database in 2000 [55]. Body iron stores, then believed to be reflected by serum ferritin levels, rose in the late teens in men and after menopause in women. The increase in ferritin level was more rapid and ferritin levels were consistently higher in both instances for Blacks than Whites of comparable age and sex.

The mean results of laboratory measurements, including hemoglobin, mean corpuscular volume (MCV), serum transferrin saturation (%TSat), serum ferritin, and white blood cell count of African Americans reportedly differed from those of Whites in another epidemiologic study published in 2005 [56]. Anonymous samples and laboratory data from 1491 African American and 31,005 White subjects, approximately equally divided between men and women, were analyzed. The hematocrit, hemoglobin, mean corpuscular volume (MCV), and white blood cell counts of African Americans were lower than those of Whites, while serum ferritin levels were higher in Blacks than in Whites. When iron-deficient patients were eliminated from consideration, the differences in hematocrit, hemoglobin, and MCV among women were less. The -3.7-kilobase alpha-thalassemia deletion accounted for about one third of the difference in the hemoglobin levels of African Americans and Whites, but neither sickle trait nor elevated creatinine levels showed significant associations. Overall, 19.8% of African American women would have been classified as "anemic" compared with 5.3% of Whites women. Among men, the figures were 17.7% for African Americans and 7.6% for Whites. Excluding iron-deficient or thalassemic subjects, the difference narrowed to 6.1% and 2.77% and to 4.29% and 3.6%, respectively.

Overall, these observations imply that the same reference standards for hemoglobin, hematocrit, MCV, and TS and white blood cell count may not apply for differing ethnic groups [51,52,55,56). These differences have important therapeutic implications in the use of supplemental iron. In some cases of anemia due to chronic disease, intravenous iron administration is detrimental, for example individuals

with chronic kidney disease [57]. By contrast, for individuals with chronic heart failure, iron deficiency is common and intravenous iron administration is clearly beneficial [58].

Genetics exert profound effects on iron metabolism. Ferroportin Q248H polymorphism associated with increased serum ferritin levels in Sub-Saharan Africans and African Americans [59]. The frequency of ferroportin Q248H polymorphism was found to be higher in African American men with elevated serum ferritin levels as compared to those with normal serum ferritin levels. However, these differences were not observed among African American women. Men with elevated serum ferritin were three times more likely to have Q248H polymorphism than women with elevated serum ferritin [59].

## Protective effects of Menstruation on CVD Prevalence

As early as 1976, The Framingham Study revealed that natural menopause and surgically induced menopause associated with a two-fold increase in CVD [60]. In 1981 Sullivan suggested that increased CVD prevalence related to oxidative stress due to increased iron stores as represented by elevated serum ferritin levels [61]. The FeAST study intended to test the hypothesis that lowering ferritin to levels of 25ng/ml., as occurs in healthy menstruating women, would be of benefit in PAD. Ferritin levels in this range, using the study protocols were not achieved. However, ferritin levels of ~76ng/ml in study participants indicated clear clinical benefit in both intervention and control cohorts. This finding reinforces the observation that premature cessation of normal menses has a deleterious effect on CVD prevalence in women [62, 63, 64]. The data also suggest that a lower range of ferritin levels likely signals benefit in both men and women.

## Elevation of Ferritin and Cytokine level in COVID 19

The coronavirus 2 (SARS-CoV-2; COVID-19) pandemic, presently spreading globally, exhibits varying mortality rates among those affected. Respiratory failure is the leading cause of mortality in this disease, while multi-organ failure including brain lesions occur and persist in so called "long haul patients". Mounting evidence links accelerated pathogenesis in gravely ill COVID-19 patients to a hyper-

inflammatory state. Extreme hyper-ferritinemia with strikingly elevated levels of IL- 6 identifies individuals with significantly increased mortality risk [65]. In 2020 over 438 publications listed in PubMed highlight ferritin and inflammatory cytokines as prognostic indicators in COVID 19. The role of iron and inflammation in SARS-CoV-2 infection could be elucidated using sequential investigation of serum iron markers obtained during the course of this illness for later systematic analysis. Frozen sera stored for eight years in repository at SNHCS proved useful in uncovering sequential associations in ferritin and inflammatory cytokines and outcomes during the FeAST study. The use of serum repositories offers similar advantages in delineating these biomarker relationships during the course of COVID 19. High early baseline or early course elevated ferritin levels in Blacks, obesity, metabolic syndromes, and diabetes possibly presage increased mortality during SARS-CoV-2 infections [43, 44, 51, 66]. These biomarker measures are of value not only for prognostic use but also for assessing efficacy of intervention strategies involving IL 6 iron-hepcidin signaling sequences (1).

Severe Sars-CoV-2 infection shares clinical and laboratory features with certain designated hyperferritinemic syndromes including macrophage activation syndrome (MAS), adult-onset Still's disease (AOSD), catastrophic anti-phospholipid syndrome (CAPS), and septic shock. All exhibit exceedingly elevated serum ferritin levels associated with hyper-inflammatory cytokine storm and multi-organ failure [67,68]. The similarities between severe COVID-19 and hyperferritinemic syndromes suggest that severe COVID-19 is a fifth member of this spectrum of hyperferritemic inflammatory diseases [68]. Excessive or misplaced molecular iron and inflammation appear to drive a number of pathologic processes in acute and chronic diseases. In some manner, yet unknown, while inflammation and iron dysmetabolism appear to interact in bidirectional relationships. Although the iron withholding BMP-IL-6 hepcidin ferroportin defense system acts to prevent accumulation of redox-active free iron, myriad genetic, behavioral, and environmental factors appear capable of overwhelming this defense system [1, 69].

Serum ferritin, an accepted biomarker of iron status, is also an indicator of inflammatory cytokine responses. Regardless of

underlying causes, elevated serum ferritin exhibits a significant monotonic positive relationship to mortality clearly shown in a large population Danish group study as shown in figure 1 (70**).** Associations of ferritin levels above 193mg/dl with cardiovascular and all-cause mortality in men has shown in a British longitudinal study with conflicting results in females at much lower ferritin levels (71).  Many patients with elevated ferritin levels may not have iron overload, but have common conditions associated with "reactive" high ferritin levels. These include liver disease, e.g. non-alcoholic steatohepatitis, or viral hepatitis; excessive alcohol intake; acute and chronic inflammatory states; infections; malignancy; renal failure; metabolic syndrome, or less commonly, thyrotoxicosis and acute myocardial infarction (72). Ferritin, (*possibly signaling catalytic iron excess… italics RD*) as leakage products of damaged cells, likely impacts clotting in COVID-19 patients, (and other inflammatory conditions) where pathological clotting is a crucial comorbidity as described by Venter et al (73)

In defined clinical contexts, elevated serum ferritin is useful for diagnosis of iron overload, while its degree of reduction marks treatment efficacy. These clinical entities include iron overload, occurring for example in hemochromatosis or transfusion iron overload. In these instances, ferritin levels increase to approach 1000 ng/mL. These conditions respond dramatically to reduction of ferritin levels using calibrated phlebotomy based upon serum ferritin levels and selected iron chelators [74, 75].   Serum ferritin levels, for example ~200-300ng/mL considered to be normal, do appear to associate with chronic disease including the anemias of chronic disease, atherosclerosis, and metabolic syndrome and obesity.  Levels in this "normal" range require more scrutiny.  In a previously ignored study of 140 patients, measurement of ferritin iron content reportedly separates serum ferritin associated with excess bodily iron stores from iron poor ferritin due to inflammation  (76, 77) figure 2. The method used in this report  measured and purified  serum ferritin, then destructively measure inorganic iron in the isolate –  by chelation and colorimetry – a complex procedure not currently available in  routine clinical laboratories. Recently, Gupta et al have reported the use of spectroscopy as an alternative (20)

The type and distribution of ferritin H and L chains in human sera may also provide insights into serum ferritin as a signal of abnormal iron metabolism in relation to iron status and relationships to systemic inflammatory responses [6, 78]. In spite of these considerations, the most commonly used cost-effective clinical tool for iron store assessment remains determination of serum ferritin level and transferrin saturation (79).
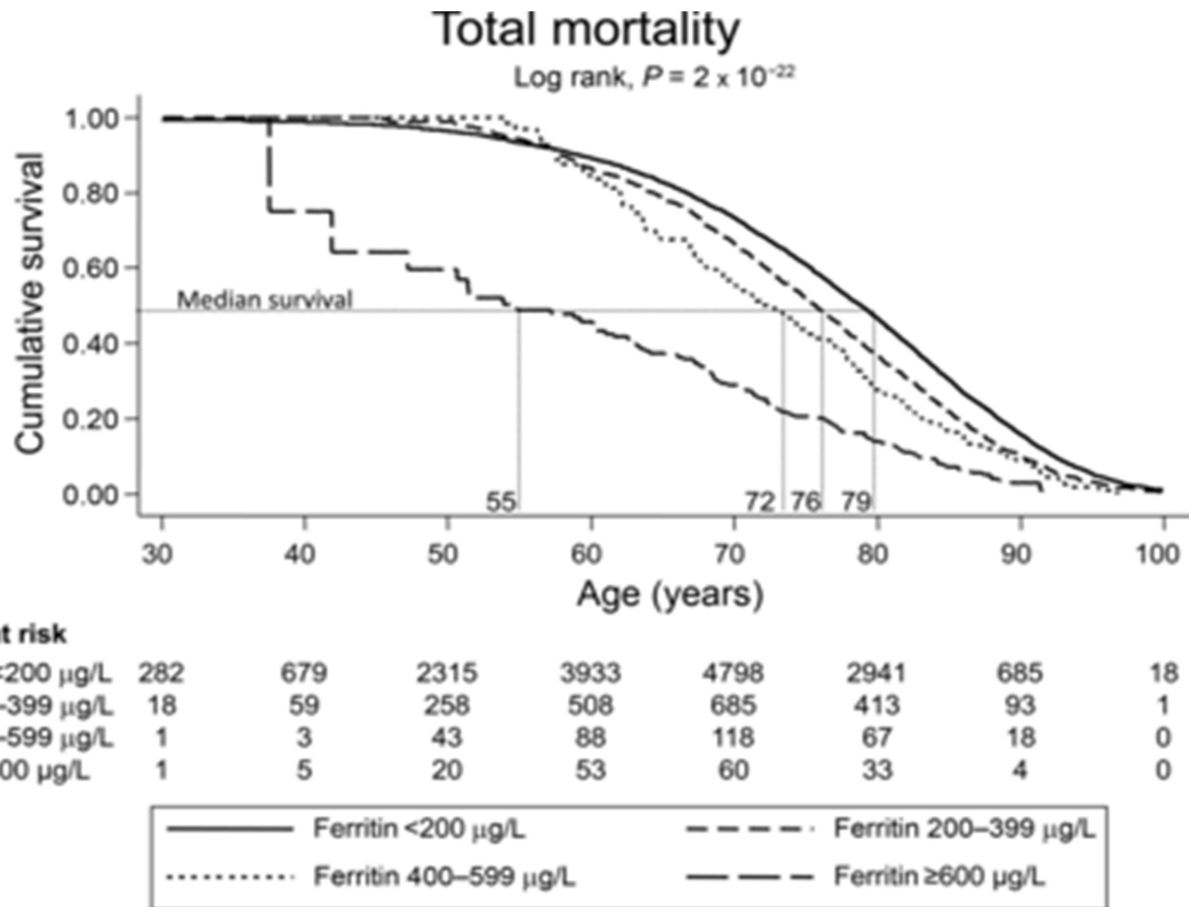
**Conclusions/Ways forward**

The striking absence of an accurate measure of total body iron itself is shown by wide estimates of this measure in reports [25-40%] cited as authoritative [1, **78, 80**]. Table 1 illustrates significant variations in 'normal' serum ferritin and hepcidin ranges. Observed wide ranges and differing values cited as normal require clarification and harmonization to determine true 'normal' or optimal ranges. Inflammatory disease states associated with iron dysmetabolism offer important therapeutic targets. These include iron reduction using chelators and iron binding proteins such as lactoferrin as suggested by Kell et al (81). Antibiotic siderophores of the tetracycline class recently have been shown to have therapeutic actions on a variety of inflammatory conditions including early acute Covid 19 as well as prevention of its neuroinflammatory complications (82, 83). Overall, interventions in the inflammatory responses triggered by the BMP 6, IL 6, hepcidin sequences, as suggested by Camaschella et al, offer a range of therapeutic options yet to be fully exploited (1). Key to these efforts, measurement techniques could be improved to assess accuracy of ferritin measurement, where biased analytic methods have been reported [84]. Future sequential measurements of hepcidin, total body iron, transferrin, total iron binding capacity, and heme oxygenase could provide better understanding of clinical signals conveyed by a higher range of serum ferritin.

Future studies of hepcidin, total body iron, transferrin, total iron binding capacity, and heme oxygenase may provide better understanding of clinical signals conveyed by higher ranges of serum ferritin. At this time, based upon validated mortality and morbidity data associated with ferritin levels, we suggest testing the hypotheses that optimal ferritin values for CVD benefit range from 20 up to-100 ng/mL, with %TSAT ranging from 20-50% to ensure adequate iron status and

that overall mortality, based on epidemiologic studies, associates with ferritin levels greater than 194 ng/dl.

**Figure 1: Cumulative survival by plasma ferritin concentrations.**



## Total mortality

Log rank, $P = 2 \times 10^{-22}$

**Number at risk**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ferritin <200 µg/L | 282 | 679 | 2315 | 3933 | 4798 | 2941 | 685 | 18 |
| Ferritin 200–399 µg/L | 18 | 59 | 258 | 508 | 685 | 413 | 93 | 1 |
| Ferritin 400–599 µg/L | 1 | 3 | 43 | 88 | 118 | 67 | 18 | 0 |
| Ferritin ≥600 µg/L | 1 | 5 | 20 | 53 | 60 | 33 | 4 | 0 |

*Clin Chem*, Volume 60, Issue 11, 1 November 2014, Pages 1419–1428, https://doi.org/10.1373/clinchem.2014.229013 with permission

**Figure 2: The percentage iron by weight in 1 ml of serum ferritin in patients with iron overload and inflammation adapted with permission Stem Cells, Wiley (76)**
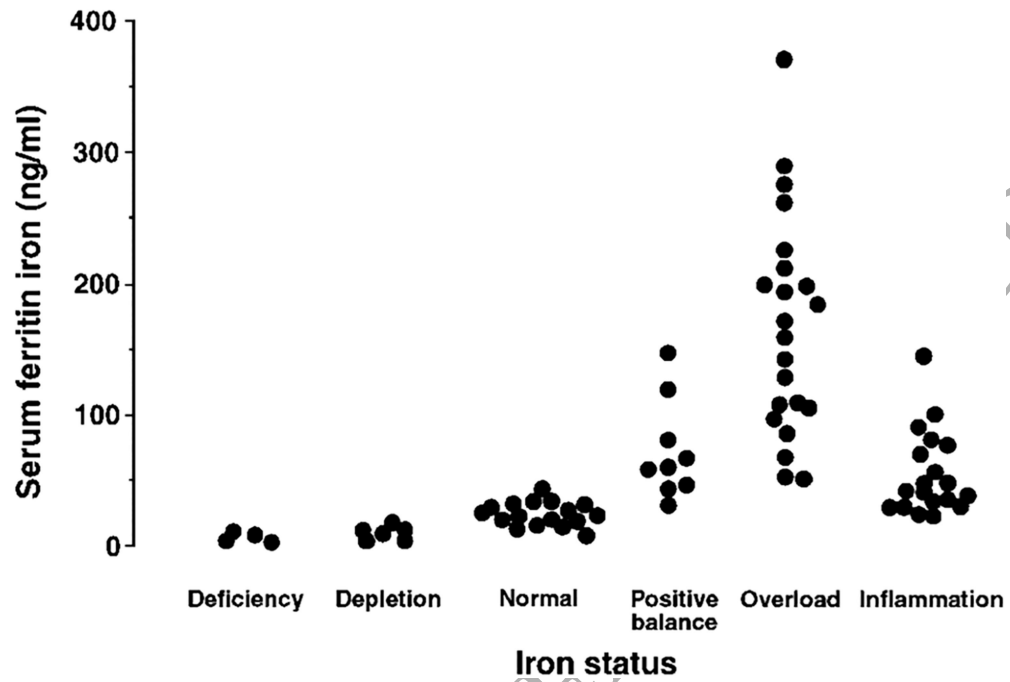
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**Data Availability** *The data underlying this article will be shared on reasonable request to the corresponding author*

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