The Risk of Too Much Iron: Normal Serum Ferritin Levels May Represent Significant Health Issues

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Abstract *While iron is an essential element involved in many biological processes, it is also well known to be a source of reactive oxygen species trough the Fenton reaction, and the result can be oxidative stress and cellular, deoxyribonucleic acid (DNA), vascular and organ damage. Iron is bound mostly by haemoglobin and ferritin and serum ferritin levels are generally regarded as a measure of body iron stores even though serum levels constitute only a small fraction of total ferritin. Ferritin is involved in iron homeostasis and appears also to be a marker for reactive iron even though the iron it sequesters is not reactive. The currently used laboratory reference range for normal serum ferritin typically covers from the 5th to the 80th or 90th population percentile and is gender dependent. However, there is considerable evidence that within this range adverse effects of iron are implicated, which impact the development and progression of a number of common disorders. There is also considerable data indicating that lowering ferritin levels within the normal range to values corresponding to near iron depletion produces beneficial results for a number of diseases. In addition, oxidative DNA damage is strongly and significantly associated with ferritin levels within the normal reference range with no apparent threshold. It is hypothesized that optimum ferritin levels are at the low end of the normal reference range near the threshold for anaemia. Failure to measure ferritin and respond to results above this suggested optimum may do a disservice to patients. Either blood donation or phlebotomy is very effective in achieving these levels.*

Introduction

It is well known that both low and high iron levels raise a number of health issues. Elevated iron levels can be dangerous due to the production of highly reactive species that can produce oxidative stress, damage cells and cause deoxyribonucleic acid (DNA), organ and vascular damage (Fenton chemistry). Thus, the postulated association with the chronic diseases such diabetes, cardiovascular disease, the metabolic syndrome and chronic kidney and liver disease.[1,2] Elevated iron levels are also implicated in aging and neurological problems.[3,4] However, there is a key issue. What constitutes significantly elevated levels?

Humans possess essential iron-handling processes for uptake, export, serum transport, and storage.[5,6] The cellular cytoplasm contains variable amounts of highly reactive free iron, known as the labile iron pool (LIP). The LIP is thought to be composed of Fe(II) and Fe(III) weakly bound to phosphates, organic acids or glutathione. Ferritin is also present in the cytoplasm, storing iron when it is plentiful and releasing it to the LIP when needed. Ferritin, is a large, hollow macromolecule built with two proteins. A complex regulatory system controls its biosynthesis.[7] It oxidizes Fe(II) and sequesters the Fe(III) in its cavity in large amounts, thus rendering the iron inactive. Thus while stored iron is regard-

ed as unreactive, the sequestering and secreting actions give ferritin a central role by interacting with the LIP and thus the pathophysiology associated with reactive iron.

While many of the fundamental biological aspects of ferritin are still unclear,[8] it is generally acknowledged that serum ferritin levels are an important marker for iron body stores in healthy individuals. It is second only to haemoglobin in the amount of bound iron. Ferritin is a versatile macromolecule having not only a role within the cytoplasm involving iron homeostasis, but may act both as an anti-oxidant and pro-oxidant. In addition, ferritin is involved in other functions related to inflammation, cellular and neurological development and angiogenesis. Serum ferritin, which represents only a small fraction of total ferritin, appears to be a marker for levels of active iron.[8,9]

The multiplicity of physiologic processes involving ferritin and in particular, its role as an acute phase reactant, has caused some to question the use of serum ferritin as a marker for the risk of various disorders.[10,11] However, as will be discussed, risk of incidence of various disorders correlates with ferritin levels in a large number of studies with significant numbers of participants and a diversity of disorders suggesting that this may not be a serious confounding factor. More importantly, there are also a number of disorders where lowering "normal" ferritin by blood removal from above, near or even below the population mean to near iron depletion (threshold for anaemia) produces significant improvements in clinical manifestations and markers. This reinforces the hypothesis of iron as a potentially causal factor with serum ferritin acting as a reliable marker of available reactive iron.[12]

Humans have no regulatory mechanism for the excretion of iron in excess of what is appropriate for normal physiological processes. Thus dietary intake, especially heme iron, can cause a gradual increase in stored iron. Premenopausal women have a mechanism for iron loss (approximately a liter per year) that maintains ferritin levels significantly below that of men, but after menopause the levels approach but rarely equal that of older men.

In sharp contrast to blood lipids and measures of glucose metabolism, ferritin does not appear to be a common marker included in the set of blood tests normally ordered in the typical clinical setting. Furthermore, there is limited justification for the upper normal limits which are typically and rather arbitrarily set at the population 80th to 90th percentiles. There are in fact legitimate questions concerning risks associated with iron levels between the mean or even below it and the upper limit of normal, and there are a number of studies where a threshold is observed within the normal reference range above which risk of a disorder becomes significant. In addition, and what is probably more important, there is an equally large body of data indicating significant benefit accruing from lowering ferritin levels starting at levels near the upper reference range value all the way to well below the mean population value and ending at close to the onset of anaemia. These data allow a critical appraisal of current reference levels and what in fact might be optimum levels.

Ferritin Levels and Reference Ranges Regarded as Normal

There is some variation in the upper reference limits for ferritin which constitute the thresholds for concern. In what follows, the units for ferritin, ng/mL, will be omitted. Consider the following reference ranges:

- Reported on Medline (US): Male (M) 12-300; female (F) 12-150
- Mayo Clinic: (M) 24-336; (F) 11-307
- UK: (M age 20-69) 30-400; (F age 17-60) 15-150
- Ontario, Canada, from laboratory reports: (M) 22-322; (F) 10-291
- Adams and Barton,[13] when discussing the diagnosis hyperferritinaemia indicate elevated ferritin levels are >300 for men and >200 for women.

The variation of serum ferritin levels in the US with age and gender can be obtained from the Third National Health and Nutrition Examination Survey (NHANES III).[14] For Caucasian men, the mean serum ferritin at age 17-19 is about 60 and by age 30-39

has a plateau at about 150 where it remains until about age 60 when a steady decline to about 90 by age 90 is observed. For women, the value is quite constant at around 30 until after menopause and then increases to about 80 by age 60 and then gradually increases to about 100 at age 80-90. These means or 50th percentile numbers representative of populations as a whole are considerably smaller than the upper limits of the normal range given above, which more closely corresponds to the 90th percentile for white populations from NHANES III.[15] If this rather arbitrary approach had been used for total cholesterol, for ages above 45, the 90th percentile for men yields 266 vs. < 200 mg/dL considered desirable, and for LDL the same percentile yields 184 while 70-100 mg/dL is considered desirable. The reason is, of course, that data pointed to graded risk throughout the range of population values.

Ferritin levels are very population dependent. For example, in an elderly population in Spain consuming a variant of the Mediterranean diet, the mean ferritin levels were 107 for men and 68 for women.[16] Furthermore, in a comparison of elderly men from either northern Europe (Zutphen) or the Mediterranean south (Crete), the mean ferritin levels were 134 and 70, respectively. The men from Crete also had consistently lower levels of indicators of oxidative stress, higher antioxidant capacity and higher concentrations of major antioxidants than men from Zutphen. These differences, including ferritin, may partly account for the significantly lower rates of coronary heart disease and greater longevity observed in the men in Crete compared to those from Zutphen.[17]

Iron overload is generally defined in terms of the degree of saturation of transferrin rather than the value of serum ferritin. Measured as a percentage of saturation, > 50% in women and > 60% in men are regarded as evidence of iron overload. The principal cause of iron overload is either hereditary haemochromatosis or rarely Wilson's disease. An imbalance between intake and excretion is a common cause of elevated ferritin levels without iron overload but can also be associated with liver disease, alcohol abuse and chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, bacterial infections, or iron related cataract syndrome. In the case of this latter disorder, ferritin reduction by phlebotomy is contraindicated.[13] Elevated ferritin levels are found in the sera of many cancer patients, and higher levels correlate with poor clinical outcomes.[18]

The data provided below suggests that there is in fact an association between the risk of developing a number of disorders and ferritin levels, and that the reference ranges in use ignore this. Indeed, even the 50th percentile numbers may be far from optimum. In the studies reviewed below, most of the thresholds for risk are obtained from the ferritin levels in the quartile or quintile where odds ratios reached statistical significance.

Association and Thresholds of Ferritin Levels and the Risk of Various Diseases

• From five studies in a recent systematic review, threshold ferritin levels for increased risk of incidence of type 2 diabetes (T2DM) were for women 86, 107,122, 134, 150 and for men 184,209, 215, 229, 300.[19]

• A study with a 17 year follow-up found in men aged 42 to 60, the risk for T2DM began to markedly increase at a ferritin level of 185.[20]

• The ferritin threshold for the increased risk of any coronary artery calcium was >257 in a study of over 12,000 men.[21]

• In a study of men and postmenopausal women, a ferritin threshold of >200 was associated with an increase in risk of a first heart attack.[22]

• A study classified coronary heart disease (CHD)-positive patients as having one or more coronary arteries with ≥ 50% blockage. Comparison of ferritin levels revealed that those CHD-positive had on average ferritin levels of 121 vs. 73 for those CHD-negative by this measure.[23]

• A study of ferritin levels as a risk factor for developing the metabolic syndrome found a threshold of 212 for postmenopausal women.[24]

• At a ferritin threshold of >137, increased risk of ischemic stroke was found in a study of

postmenopausal women.[25]
• A ferritin threshold of >145 to 164 was found for increased risk of acute ischemic stroke transforming to a haemorrhagic stroke in older men and women.[26]
• Inspired by the fact that iron overload can cause cardiomyopathy, a large study examined the association between ferritin levels and laboratory measured cardiovascular fitness (CVF) in young men. The likelihood of the absence of CVF, adjusted for numerous potential confounders, became significantly apparent at a ferritin threshold of >150.[27]
• Significant risk of middle-aged men developing hypertension, defined as systolic BP ≥ 140/90 mm Hg, had a ferritin threshold level > 146.[28]
• A study of the relationship between increased ferritin, oxidative stress and insulin resistance in 151 healthy men revealed no threshold, only continuous increases in markers with ferritin levels from the first tertile (≤97) to the third (≥180). The correlations remained strong and significant after adjustment for inflammation.[29]

While there is admittedly the possibility of confounding in some of the above associations, the ferritin lowering studies below suggest otherwise.

Ferritin Thresholds for Benefit in Iron Lowering Studies

• In a randomized prospective trial, iron reduction in male smokers with peripheral arterial disease (PAD) reduced the risk of death or nonfatal heart attack such that the number needed to treat to prevent one acute event with phlebotomy (from Kaplan-Meier plots) was only 8 over 5 years, a very low number rarely encountered in clinical studies. Significant benefits were also seen for all-cause mortality, non-fatal myocardial infarction (MI) and stroke The initial and final mean ferritin levels were 125 and 84. In a larger study in which the above was imbedded, unequivocal benefits were found for iron reduction in younger individuals 43-61 years of age for all-cause mortality, non-fatal MI and stroke when phlebotomy reduced ferritin levels to < 70.[30,31]
• In a group of patients who were either diabetic or carbohydrate intolerant, lowering mean ferritin from 272 to 45 resulted in an increase in HDL and reductions in blood pressure, triglycerides, fasting blood glucose and an improved oral glucose tolerance test.[32]
• A controlled trial involving phlebotomy which decreased ferritin levels from a mean of 188 to 105 for a group of men and women with the metabolic syndrome, found a decrease in systolic blood pressure from 149 to 131 mm Hg with no change in a control group. Blood glucose, glycosylated haemoglobin (HbA1c) and heart rate were also significantly decreased.[33]
• Use of the oral prescription chelator, deferiprone, over 9 months in patients with non-diabetic kidney disease reduced ferritin from 144 to 59 and resulted in significant clinical improvements.[34]
• In a trial using phlebotomy in patients with PAD, a reduction in mean ferritin levels from 122 to 74 resulted in a significant reduction in the incidence of visceral malignancy.[35]
• In a study of 10 healthy individuals with initially low ferritin, 500 mL withdrawal of blood resulted in a drop on average levels from 75 to 38 and a significant improvement in the results of a glucose tolerance test one month later.[36]

Comparison of both the above thresholds for risk and the baseline ferritin levels from which lowering produced benefit reveals an inconsistency with the commonly used reference range values regarded as normal. This illustrates the major point of this review. Even when the baseline ferritin level is a below the population mean, significant benefit still derives from phlebotomy. The upper reference range values for normal appear way too high, and in fact, the above results suggest that the 50th percentile numbers are also too high. Some of the studies presented below reinforce this latter observation, leading to the hypothesis that the lower the better may be a justifiable goal as long as lowering does not induce anaemia.

Iron Stores Reductions and Diabetic Complications

Advanced glycation end products are thought to play a role in the complications of

diabetes, and the basic biochemistry involves reactive oxygen species including those attributed to iron activity.[37] Studies on humans are limited. A 9 month study on T2DM patients using deferiprone, an oral iron chelator, reduced ferritin levels from 144 to 59 and decreased the mean albumin/creatinine ratio from 187 to 25 mg/L.[34] In addition, a study involving the progression of diabetic nephropathy used a polyphenol–enriched, low-iron carbohydrate-restricted diet over 4 years. There was no significant change in HbA1c, but there was an absolute decrease of 18% in the incidence of serum creatinine doubling and an absolute decrease of 18% in both mortality and end-stage kidney disease (number needed to treat over 4 years for either was 6).[38] Iron chelation due to the polyphenols in the diet was probably partly responsible for reduced ferritin from 325 to 53 and the benefits observed.

Iron Reduction and Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) starts with simple hepatic steatosis and can progress to non-alcoholic steatohepatitis (NASH). One hypothesis for the pathogenesis of this disorder is the so-called two-hit model where the first hit involves insulin resistance, visceral obesity and increased hepatic steatosis. The second hit involves one of a number of possible insults which lead to increased oxidative stress and liver inflammation. The increased deposition of iron as the disorder progresses suggests it is involved in the second hit, given its role in producing ROS as well as other pathogenic effects including altered insulin singling and lipid metabolism. Iron may also be involved in the initial development of steatosis.[39] High ferritin levels (threshold of 1.5 times the upper limit of normal or 450 for men, 300 for women) have been found independently associated with advanced hepatic fibrosis.[40] The following iron depletion studies are thus of interest.

• The effect of phlebotomy on insulin resistance in a group of patients with NAFLD and strongly elevated ferritin levels found a significant reduction in insulin resistance (homeostasis model of assessment - insulin resistance decreased from 4.81 to 3.12) when ferritin levels were reduced from 438 to 52. Alanine aminotransferase (ALT) decreased from a mean of 58.1 to near normal 34.3 IU/L.[41]

• A study examining the effect of phlebotomy involved 42 T2DM or carbohydrate intolerant subjects including 8 also diagnosed with NAFLD based on elevated ALT and ultrasound evidence of steatosis. DNA testing was used to exclude patients with haemochromatosis . The NAFLD and non-NAFLD groups had baseline mean ferritin levels of 299 and 220 respectively. Phlebotomy produced near iron depletion (ferritin 31-15) and ALT fell from 61 to 32 IU/L in the NAFLD group whereas there were insignificant ALT changes observed in the NAFLD-free group. Favourable metabolic changes associated with ferritin declines were seen in fasting insulin and the oral glucose tolerance test even though there were no changes in medication. Stronger effects were observed in the NAFLD group.[42]

• In a study where ferritin levels were manipulated with diet, 12 patients with NASH were placed on a calorie, fat and iron restricted diet. The baseline mean ferritin levels were 280 initially and 128 at 6 months of intervention. ALT levels decreased from 104 to 42 IU/L over the same period. Large changes were also seen in aspartate aminotransferase (AST) levels. Both males and females had similar baseline ferritin levels which means that the women had on average ferritin levels above the gender specific upper limits of normal, but not by very much.[43]

Iron and Oxidative DNA Damage

Urinary8-hydroxydeoxyguanosine(8-OHdG) is a reliable and frequently used biomarker of systemic oxidative DNA damage.[44,45] Given such a marker, the obvious question concerns correlation with body iron stores. Two studies have addressed this important question.

Hori et al[46] studied over 500 healthy Japanese aged 21-67. The correlations between 8-OHdG and ferritin measured by Spearmen rank correlation coefficients were 0.47, 0.76 and 0.73 for men overall, women aged less than 50 and women 50 years or

older, respectively. These strong correlations were essentially unchanged after adjustment for potential confounders. Subjects exhibited ferritin levels from near iron depletion to around 300 for men and 100 for women.

An earlier study by Nakano et al[45] found similar results. In a study of over 2500 healthy individuals age between 22 and 89 there was a smooth, almost linear 2.5 fold increase in 8-OHdG for men as ferritin ranged from 10 to about 300. For women, 8-OHdG was increased by a factor of 3 for ferritin levels ranging from below 9 to 160.

These results suggest no threshold and are consistent with a study of vascular function where when two groups, both with low ferritin levels (52 vs. 17), were compared, flow mediated vascular dilation was significantly greater in the very low ferritin group.[47] It is also consistent with the study described above where ferritin levels correlated with oxidative stress and insulin resistance with no apparent threshold.[29]

These are very important results since they not only indicate a strong dependence of DNA oxidative stress on ferritin levels as a marker for active iron, but the ferritin levels span the entire reference range for normal and these were healthy individuals. Thus, throughout the normal reference ranges for both genders, iron as measured by ferritin appears to be a continuously increasing risk factor for DNA damage. It is also highly significant that the threshold for iron associated DNA damage appears to be just above the level of near iron depletion level.

Two related studies are of interest. In one, lowering ferritin with phlebotomy has been found to reduce 8-OHdG in patients with chronic hepatitis C. The mean ferritin level was 259 at baseline and after phlebotomy, dropped to around 10 at 4 months and was 7.1 at 6 years. At 4 months, 8-OHdG as measured by two methods dropped to half the baseline value and at 6 years corresponded to that of normal controls. At and after about 1.5 years, ALT levels were normalized.[48]

A second study from this research group examined the impact of reducing iron stores to a near depletion on the development of hepatoacellular carcinoma (HCC) from chronic hepatitis C.[49] At baseline the mean ferritin level was 371 (range 77-1180). Phlebotomy reduced iron levels rapidly to < 11 and it was held at near this value for 12 years. The incidence of HCC in the phlebotomy group was 11.4% whereas in a control group it was 32.5%. This yields a number needed to treat of 5 to prevent over 12 years one progression to HCC.

What is the Optimum Ferritin Level?

It is clear from the cited studies that the serum ferritin thresholds for the appearance of risk and the baseline values from which lowering produces benefit are mostly well below the upper reference values for normal, and in fact more closely correspond to population 50th percentiles. However, lowering ferritin to levels far below the 50th percentile population values produces benefit associated with the severity of disorders which can be influenced by iron. In some studies, this is observed even when the baseline level for lowering is already quite low. Furthermore, low ferritin levels indicating benefit achieved by phlebotomy are in the range of that found in premenopausal women who are well known to exhibit very low rates of cardiovascular disease, differences which in fact may not be related to estrogen, as is commonly believed.[50] The DNA oxidative stress studies strongly support the view that the optimum ferritin level is that representing near iron depletion.

Overall, the answer to this question appears to be lower the better, provided anaemia is not the result. This observation urgently needs detailed study with controlled long-term follow-up studies. It challenges two widely held beliefs, namely that ferritin anywhere in the normal reference range should not cause concern and that normal means no enhanced risk from active iron. The evidence that this is wrong appears in fact to be compelling.

Influence of Blood Donation or Phlebotomy on Ferritin Levels

Blood donation typically removes 450-500 mL per visit. Phlebotomy sessions are

generally similar. A frequently cited number is 30 ng/mL decreases in ferritin per donation. **Table 1 (**below) illustrates this in a large sample of Danish men.

The results for zero donations is similar to modern results and on average 3 to 4 donations per year will result in a ferritin level below 100 with a median representing near iron depletion.

Chelation, the Alternative to Phlebotomy or Blood Donation

Oral chelation has been a common approach to iron overload for patients having pathological levels, and several prescription drugs are available, but these are not without side effects.[52] However, ferritin levels involved in most of the studies discussed above are nowhere near those encountered in pathological iron overload. Iron lowering therapy for haemochromatosis is generally initiated at a ferritin level of 1,000.

There are a number of "natural" iron chelators. N-acetylcysteine is in fact a standard therapy for treating pediatric pathological iron overload even in infants.[53,54] Green tea polyphenols,[55] silymarin (silybin, milk thistle extract),[56-59] and quercetin[60-62] all have documented success in iron chelation. These chelators also act to eliminate other toxic metals, although for mercury it may help to add selenium and alpha-lipoic acid to N-acetylcysteine, but the evidence is anecdotal.[63] Curcumin was recently found to be a very good iron chelator.[64] A randomized controlled trial demonstrated the effectiveness of curcumin in significantly improving markers of glucose metabolism in T2DM, possibly partly due to iron chelation.[65] However, clinical studies to directly examine application of natural oral chelation in this context appear non-existent. The extent to which these oral chelators remove desirable or essential minerals also appears unknown, but caution and supplementation would appear prudent.

Conclusion

The reference ranges for normal ferritin levels span approximately the range from greater than the 5th percentile to less than the 80th to 90th percentile. This arbitrary approach is not used when the risk dependence on marker level is believed to be well established. It has been show that the threshold for risk for a number of different disorders begins considerably below the upper normal limit of the ferritin reference range, and benefits accrue from lowering ferritin from initial values, frequently in the range of average or lower, to very low final values.

It appears that ferritin screening can be justified. Lowering ferritin levels can be accomplished with a high level of effectiveness by blood donation, which is free, safe, has monitoring for anaemia and is virtually without side effects. When disallowed by the blood donation services, ferritin lowering can be accomplished by office- or clinic-based phlebotomy.

Table 1. Danish study of the influence of blood donation of serum ferritin levels (ng/mL) in men, 30-66 years of age.[51]

Donation History Per Year	Ferritin Median 5-95 pct*	Ferritin Range
0	137	46-396
2	44	17-122
3	38	14-110
4	31	12-91

* 5th to 95th percentile

This review suggest the hypothesis that optimum adult serum ferritin levels in the context of health issues may be in the range of 20-40 for women and 50-70 for men. Adequately powered studies are needed to address this issue in the context of the chronic diseases where the existing studies are mostly too small and in some cases inconsistent.[2] However, examining this hypothesis would be lengthy, costly, and unlikely to find support from the pharmaceutical industry. An alternative but not ideal start would be retrospective studies based on large managed care databases merged with blood donor clinic data due to the absence of historical ferritin data. Catchment areas are generally small enough to make this possible. Data collection would include current serum ferritin levels and other pertinent current data presumably available as well as complete medical and even prescription history. Large cohorts would be necessary to capture a significant number of individuals with consistently frequent donation. The consistently positive results obtained with phlebotomy will no doubt encourage more and better intervention studies, which could considerably enhance the evidence base.

Competing Interests

The author declares that he has no competing interests.

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